

A heterovalent single-atom bioorthogonal nanozyme engineered via metal-phenolic networks for potent antimicrobial therapy

Shuhan Zhang¹, Zihao Deng¹, Zhiyan Zhao¹, Chen Wang¹, Congcong Huang², Chuanqi Zhao², Yujun Song³, and Jingsheng Niu¹✉

¹Henan Institute of Advanced Technology, College of Chemistry, Zhengzhou University, Zhengzhou 450001, China

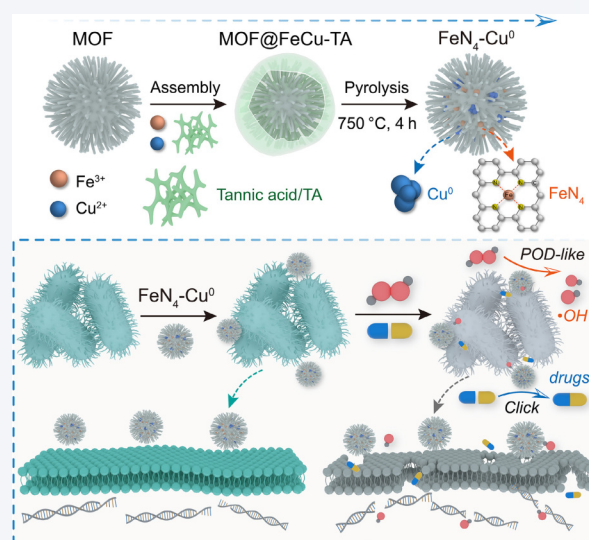
²Laboratory of Chemical Biology and State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

³State Key Laboratory of Analytical Chemistry for Life Science, College of Engineering and Applied Sciences, Nanjing University, Nanjing 210023, China



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ABSTRACT: Single-atom nanozymes (SAzymes) are emerging as superior alternatives to conventional nanozymes for antibacterial therapy and other biomedical applications, owing to their well-defined structures, unambiguous catalytic sites and exceptional catalytic activities. However, constructing tailored multicomponent SAzymes with mixed-valence state catalytic units remains challenging. Herein, we present a heterovalent FeN₄-Cu⁰ bioorthogonal nanozyme engineered from metal-phenolic networks. This unique architecture compartmentalizes distinct catalytic functions: The FeN₄ single atoms exhibit high peroxidase-like activity to catalyze the generation of hydroxyl radicals ($\cdot\text{OH}$), while the Cu⁰ clusters activate antimicrobial molecules via click chemistry. Meanwhile, the spiky morphology of FeN₄-Cu⁰ potentiates the antibacterial efficacy by physically trapping bacteria, thereby enabling the localized synergistic sterilization through reactive oxygen species and activated drugs. Combined experimental and mechanistic studies demonstrate that this system achieves remarkable antibacterial efficacy by disrupting the bacterial membrane structure, inhibiting biosynthesis and metabolism, inducing oxidative stress, and disrupting energy metabolism. This work provides a feasible strategy for constructing customizable single-atom bioorthogonal nanozymes with mixed oxidation-state catalytic units.



KEYWORDS: single-atom nanozyme, heterovalent, antimicrobial, metal-phenolic, click chemistry

1 Introduction

The escalating prevalence of pathogenic bacteria, coupled with the expanding problem of multidrug-resistant strains caused by the misuse of antibiotics, poses a major threat to global public health [1]. Traditional antibiotic strategies are challenged by their diminishing efficacy. Reactive oxygen species (ROS)-generating nanozymes have emerged as a promising class of artificial

antibiotics, due to their ability to exhibit broad-spectrum antimicrobial activity while evading bacterial resistance [2]. However, the further potential application of nanozyme-based antibiotics is hindered by several intrinsic limitations, including insufficient catalytic activity, the short lifespan, and the limited diffusion range of ROS [3–5].

Single-atom nanozymes (SAzymes) have developed rapidly and are widely applied in biomedicine and nanomedicine due to their impressive catalytic activity and unambiguous catalytic sites and mechanisms [6]. SAzymes demonstrate superior enzyme-mimicking activities compared to previously reported nanozymes, which can be attributed to their structural mimicry of the active catalytic centers of natural enzymes [7]. For instance, Fe-based SAzymes have been successfully employed in tumor catalytic

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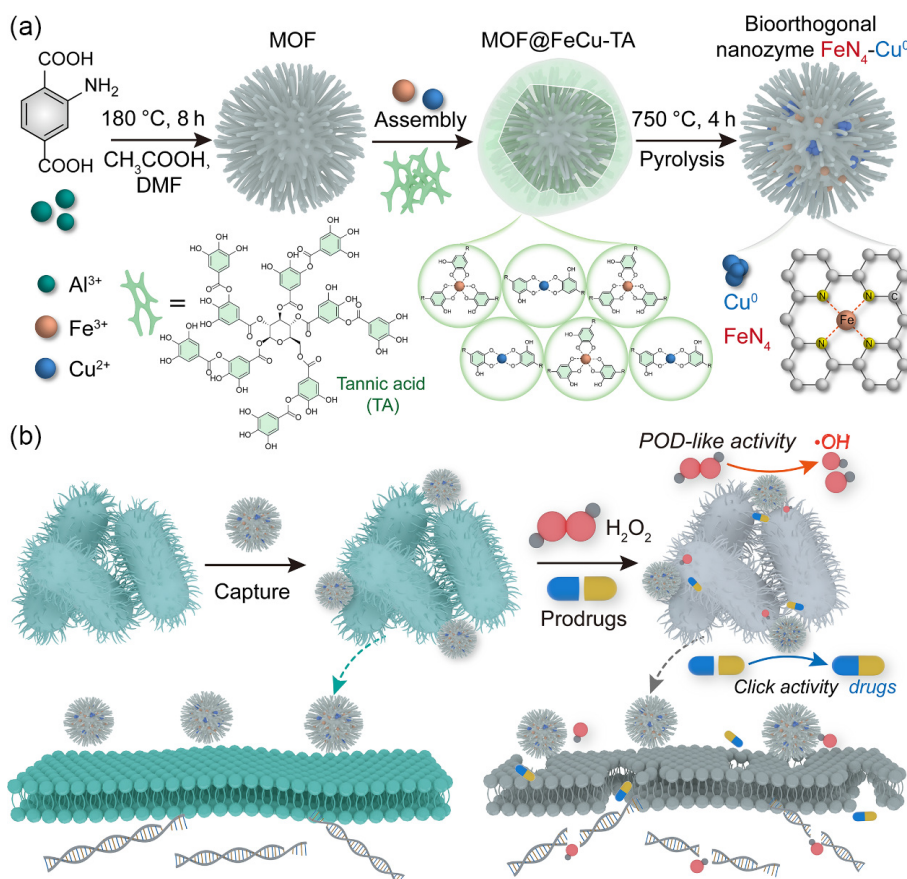
✉ Address correspondence to niujingsheng@zzu.edu.cn

therapy, antibacterial applications, antioxidant, and anti-inflammatory treatments [8–11]. Additionally, Cu-based SAzymes are being developed for oncotherapy, immunotherapy, and cuproptosis [12]. Meanwhile, Mn- and Pt-based SAzymes have shown promise in combating bacterial infections [13, 14]. Beyond these, other metal-based SAzymes (e.g., Ce, Pd, Zn, Ni, Co) have found applications in diverse areas such as cascade catalysis, myocardial ischemia-reperfusion, tumor proliferation inhibition, wound healing, and biomimetic catalysis (e.g., urate oxidase, cytochrome *c* oxidase and catalase mimics) [15–24]. However, the performance of these metal-centered SAzymes is currently primarily constrained by their limited catalytic functionality and insufficient enzymatic activity compared to their natural enzyme counterparts.

To enhance the catalytic activity of Fe-based single-atom nanozymes, multiple strategies have been strategically employed, including axial chlorine engineering, dimensionality engineering, and curvature engineering [25–27]. Furthermore, the concept of dual-atom nanozymes has been introduced to enhance catalytic activity [28]. However, current research on dual-atom nanozymes remains largely confined to the synergistic enhancement of redox enzyme-like activity via the coexistence of two metal atoms, with the expansion of catalytic diversity remaining largely unexplored [29, 30]. Although single-atom/cluster complexes have been applied in the field of catalysis, they usually enhance catalytic efficiency rather than expand catalytic functionality [31]. Moreover, the

synthesis typically involves a complex process of sequentially forming single atoms and clusters [32, 33]. To date, the development of SAzymes capable of integrating two distinct chemical species in different oxidation states for divergent catalytic functions remains a fundamental challenge.

Metal-phenolic engineering has been widely employed in diverse coating systems, including cell protection, bacterial encapsulation, and surface modification [34–36]. Owing to the broad applicability of metal ions, this approach holds great promise for constructing SAzymes on suitable supports. Specifically, a limited number of prior studies have reported the synthesis of Fe SAzymes employing metal-phenolic chemistry [37, 38]. Inspired by these advances, we herein construct a $\text{FeN}_4\text{-Cu}^0$ bioorthogonal nanozyme based on metal-phenolic engineering (Scheme 1). This system comprises two distinct valence states, namely FeN_4 and Cu^0 , which serve as catalytic functional units exhibiting peroxidase-like and click chemistry activities, respectively. Moreover, the use of a spiky metal-organic framework (MOF) as the substrate enabled the creation of a $\text{FeN}_4\text{-Cu}^0$ microstructure possessing a sufficiently rough interface, thereby facilitating effective bacterial adhesion and localized synergistic sterilization by ROS and activated drugs. To the best of our knowledge, this study presents a pioneering strategy for fabricating a heterovalent single-atom bioorthogonal nanozyme antibacterial system through the pyrolysis of tannic acid-coordinated Fe/Cu precursors.



Scheme 1 Construction and antimicrobial mechanism of the bioorthogonal nanozyme $\text{FeN}_4\text{-Cu}^0$. (a) Schematic illustration of the synthesis process of $\text{FeN}_4\text{-Cu}^0$. (b) The antimicrobial mechanism of $\text{FeN}_4\text{-Cu}^0$. The efficient POD-like activity of FeN_4 and the click activity of Cu^0 in $\text{FeN}_4\text{-Cu}^0$ trigger ROS burst and prodrug activation respectively, resulting in disruption of the bacterial cell membrane and damage to intracellular contents. The spiky microstructure further enhances the antibacterial efficacy by physically trapping bacteria, thereby localizing the action of *in situ* generated ROS and activated drugs.

2 Experimental

2.1 Preparation of MOF

The synthesis of MOF was slightly modified according to previous literature [36]. In brief, 2-aminoterephthalic acid (0.3 mmol), aluminum sulfate hydrate (0.1427 mmol) were dissolved in N,N-dimethylformamide (30 mL) at room temperature, transferred to a 50 mL Teflon-lined stainless-steel autoclave, and heated to 180 °C for 8 h. The obtained solutions were centrifuged at 8000 rpm for 10 min, and then were washed using deionized (DI) water and ethanol respectively. The MOF was obtained for further use through freeze-drying.

2.2 Preparation of MOF@FeCu-TA

The synthesis of MOF@FeCu-TA followed the steps described in the referenced literature with slight modifications. Firstly, the prepared MOF (2 mg) was dissolved in deionized water (4.85 mL). Subsequently, the tannic acid solution (24 mM) was added while stirring. Then, the previously dissolved $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (24 mM) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (24 mM) solutions were added to the above MOF solution drop by drop. After adding 10 μL of 1 M NaOH, the reaction continued to proceed under stirring for 10 min. Finally, the obtained solution was centrifuged at 8000 rpm for 10 min, and then washed three times with deionized water and absolute ethanol respectively. The MOF@FeCu-TA_n with different layers were obtained by repeating the above process. The MOF@FeCu-TA was obtained for further use through freeze-drying.

2.3 Preparation of FeN₄-Cu⁰

The above resulting MOF@FeCu-TA mixtures were dried at 60 °C in a vacuum oven. Subsequently, FeN₄-Cu⁰ was obtained after 4 h of pyrolysis treatment at 750 °C under Ar atmosphere with a heating rate of 5 °C/min.

3 Results and discussion

3.1 Synthesis and characterizations of MOF@FeCu-TA and FeN₄-Cu⁰

The design and synthesis process of FeN₄-Cu⁰ single-atom bioorthogonal nanozyme was shown in Fig. 1(a) and Fig. S1 in the Electronic Supplementary Material (ESM). Firstly, spherically spiky MOFs were employed as precursors for carbon and nitrogen, and were synthesized according to previously reported methods [39]. Scanning electron microscopy (SEM) demonstrated the successful synthesis of MOFs (Fig. 1(b) and Fig. S1(a) in the ESM). The average hydrodynamic diameter of MOFs, as measured by dynamic light scattering (DLS), was approximately 907 nm (Fig. 1(g)). Subsequently, in order to introduce Fe and Cu, a metal-phenolic network (MPN) composed of Fe³⁺, Cu²⁺ and tannic acid (TA) was coated on the surface of the MOF nanoparticles (abbreviated as MOF@FeCu-TA). SEM images confirmed that the synthesized MOF@FeCu-TA had a spherical spiky structure similar to that of MOF, with a hydrodynamic diameter of approximately 1048 nm, which was slightly higher than that of MOF (Figs. 1(c) and 1(h)). Finally, after pyrolysis for 4 h at 750 °C under argon atmosphere, MOF@FeCu-TA was transformed into FeN₄-Cu⁰. The morphology of FeN₄-Cu⁰ single-atom bioorthogonal nanozyme was first characterized by SEM and transmission electron microscopy

(TEM). FeN₄-Cu⁰ successfully maintained the spherically spiky morphology of the MOF nanoparticles (Figs. 1(d) and 1(e)). The hydrodynamic diameter of FeN₄-Cu⁰ was approximately 953 nm, which was smaller than that of MOF@FeCu-TA before thermal decomposition (Fig. 1(i)). High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) mapping analysis revealed that each element in FeN₄-Cu⁰ was uniformly distributed throughout the structure (Fig. 1(f) and Fig. S2 in the ESM). The zeta potential test results indicated that MOF was positively charged, while the zeta potentials of MOF@FeCu-TA and FeN₄-Cu⁰ reversed after the self-assembly process (Fig. 1(j)). This might be caused by the introduction of TA containing hydroxyl and carboxyl groups.

Next, the Fe and Cu contents in MOF@FeCu-TA and FeN₄-Cu⁰ were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES). The Fe and Cu contents in MOF@FeCu-TA were 1.16 wt.% and 1.36 wt.%, respectively, while those in FeN₄-Cu⁰ were 3.67 wt.% and 3.84 wt.% (Fig. 1(k), Table S1 in the ESM). The increase in the relative percentage of metals in FeN₄-Cu⁰ might be due to the departure of carbonized and vaporized organic elements. Furthermore, we conducted an analysis of the composition and elemental form of FeN₄-Cu⁰ by X-ray photoelectron spectroscopy (XPS). The total spectrum of FeN₄-Cu⁰ showed that it contained Fe, Cu, Al, N, C and O elements (Fig. 1(l)). The XPS survey indicated that the weight content of C, N, O, Al, Fe and Cu was computed to be 61.99%, 2.38%, 24.67%, 9.75%, 0.8% and 0.41% respectively (Table S2 in the ESM). The N 1s spectrum showed that N mainly exists in three configurations: pyridinic-N, pyrrolic-N, and graphitic-N (Fig. 1(m)). These N atoms were used to stabilize the Fe single atoms. The high-resolution Fe 2p spectrum of FeN₄-Cu⁰ showed two main peaks, located at 711.3 and 725.5 eV, corresponding to the Fe 2p_{3/2} and Fe 2p_{1/2} orbitals (Fig. 1(n)). The characteristic peaks of the high-resolution Cu 2p spectrum of FeN₄-Cu⁰ indicated the coexistence of Cu²⁺ and Cu⁰/Cu⁺ (Fig. 1(o)). In the C 1s and O 1s regions of the XPS spectra, no characteristic peaks of metal-based carbides and oxides were observed in all samples. The high-resolution C 1s spectrum showed four peaks, which could be classified as C=C, C-C, C-N, and C=O (Fig. S3 in the ESM). The successful synthesis of FeN₄-Cu⁰ was also verified by Fourier transform infrared (FT-IR) spectroscopy (Fig. S4 in the ESM). The spectrum showed characteristic bands at 1694.6 and 3490.8 cm⁻¹, assignable to the stretching vibrations of -C=O and -O-H groups, respectively. Additional bands observed at 1578.8 and 3385.5 cm⁻¹ corresponded to the -N-H vibrations. The structure of FeN₄-Cu⁰ was further confirmed by powder X-ray diffraction (XRD). The results showed that the characteristic peaks in the (002) and (111) directions were highly consistent with the main diffraction peaks of CuO, which might be attributed to the surface oxidation of Cu⁰ clusters (Fig. S5 in the ESM). All the above results confirmed the successful construction of FeN₄-Cu⁰.

To determine the metal type and atomic structure of FeN₄-Cu⁰, we conducted measurements using a differential aberration-corrected high-angle annular dark-field scanning transmission electron microscope (AC-HAADF-TEM) (Figs. 2(a) and 2(b)). The AC-HAADF-TEM images showed that the crystal structure was almost completely absent, indicating that FeN₄-Cu⁰ had an amorphous structure. Moreover, isolated bright spots (indicated by red circles, Fe single atoms) and particle clusters (indicated by blue circles, Cu clusters) were observed experimentally (Fig. 2(a)),

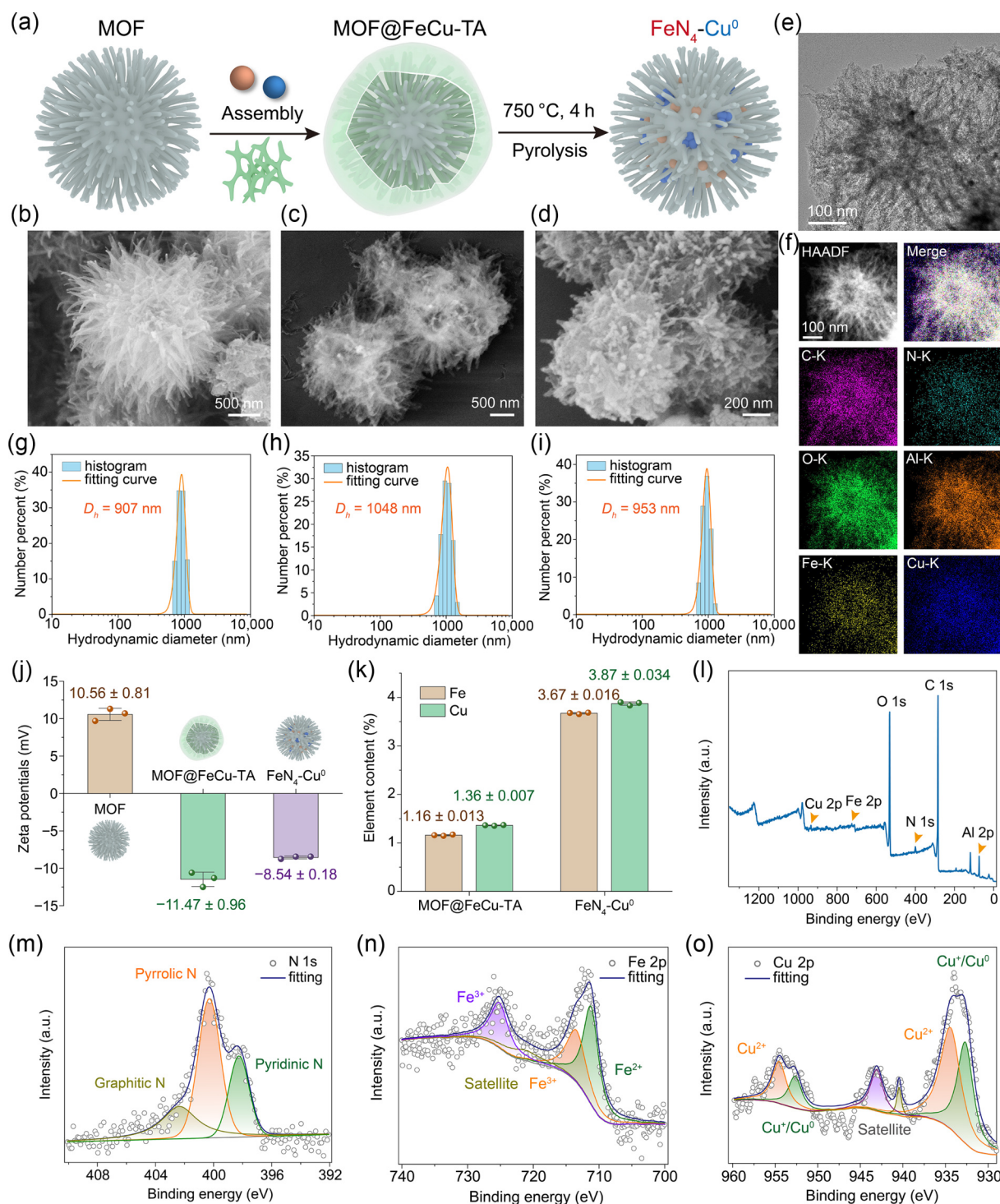


Figure 1 Preparation and characterization of the bioorthogonal nanozyme FeN₄-Cu⁰. (a) Synthesis illustration for FeN₄-Cu⁰ (Fe, yellow; Cu, blue; TA, green). SEM images of (b) MOF, (c) MOF@FeCu-TA, and (d) FeN₄-Cu⁰. (e) TEM image of FeN₄-Cu⁰. (f) TEM EDS mapping showing the distribution of C, N, O, Al, Fe, and Cu elements in FeN₄-Cu⁰. Hydrodynamic diameter distributions of (g) MOF, (h) MOF@FeCu-TA, and (i) FeN₄-Cu⁰. (j) The Zeta-potential of different nanoparticles: MOF, MOF@FeCu-TA, and FeN₄-Cu⁰. (k) The element content (%) of Fe and Cu in MOF@FeCu-TA and FeN₄-Cu⁰ tested by ICP-OES. (l) XPS full spectrum of FeN₄-Cu⁰. (m) N 1s, (n) Fe 2p, and (o) Cu 2p XPS spectra of FeN₄-Cu⁰.

suggesting that the two metal atoms present different dispersion states in FeN₄-Cu⁰. This result was also confirmed by the corresponding three-dimensional topographic atomic images obtained from the AC-HAADF-TEM images (Fig. S6 in the ESM).

To further investigate the precise coordination environment and oxidation states of Fe and Cu elements in FeN₄-Cu⁰, we conducted

X-ray absorption spectroscopy (XAS) analysis. The Fe K-edge X-ray absorption near-edge spectrum (XANES) showed that the absorption threshold lies between FeO and Fe₂O₃, indicating that it is in the +2 to +3 positive oxidation state (Fig. 2(c)). The Fourier transform (FT) *k*²-weighted extended X-ray absorption fine structure (EXAFS) spectra at the Fe K-edge of the sample showed a

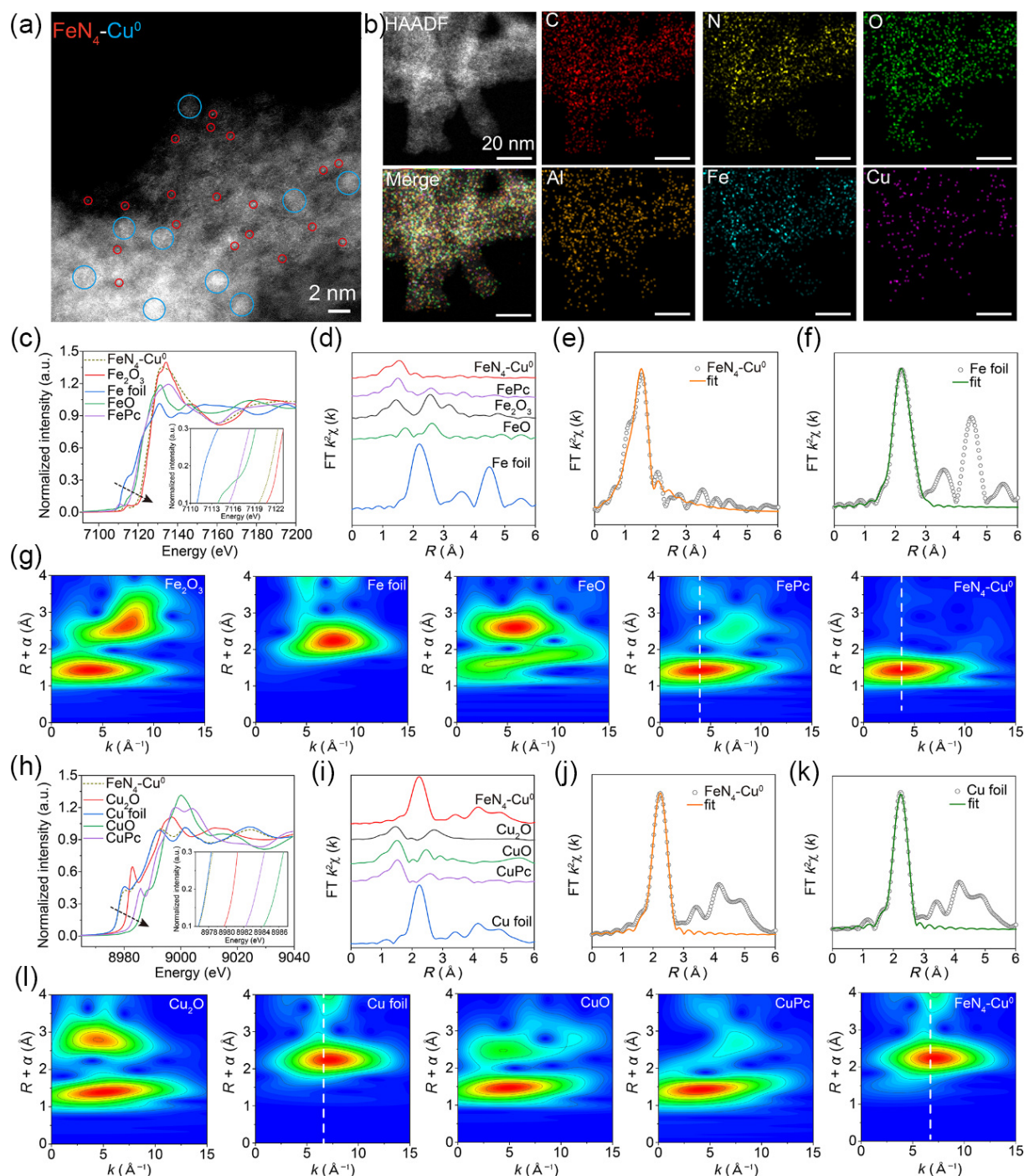


Figure 2 Atomic structural analysis of $\text{FeN}_4\text{-Cu}^0$. (a) Aberration-corrected HAADF-STEM image and (b) corresponding elemental mapping images of $\text{FeN}_4\text{-Cu}^0$. (c) XANES spectra of $\text{FeN}_4\text{-Cu}^0$ and the reference samples at Fe K-edge. (d) FT-EXAFS spectra of $\text{FeN}_4\text{-Cu}^0$ and the reference samples at Fe K-edge. The corresponding Fe K-edge EXAFS fitting curves of (e) $\text{FeN}_4\text{-Cu}^0$ and (f) Fe foil. (g) WT-EXAFS contour plots of $\text{FeN}_4\text{-Cu}^0$ and the reference samples at Fe K-edge. (h) XANES spectra of $\text{FeN}_4\text{-Cu}^0$ and the reference samples at Cu K-edge. (i) FT-EXAFS spectra of $\text{FeN}_4\text{-Cu}^0$ and the reference samples at Cu K-edge. The corresponding Cu K-edge EXAFS fitting curves of (j) $\text{FeN}_4\text{-Cu}^0$ and (k) Cu foil. (l) WT-EXAFS contour plots of $\text{FeN}_4\text{-Cu}^0$ and the reference samples at Cu K-edge.

dominant peak at $1.53 \text{ \AA}$, which could be attributed to the first layer scattering path of the Fe-N coordination. It was worth noting that no metal peaks corresponding to Fe-Fe or Fe-Cu scattering paths were observed at $2.2 \text{ \AA}$, indicating that there was no direct bonding between Fe and Cu atoms (Fig. 2(d)). Through further analysis of R space and K space, the coordination configuration of the Fe groups in $\text{FeN}_4\text{-Cu}^0$ was revealed. The best fitting results confirmed that the coordination number of the Fe-N bond was 4.34 ± 0.3 , verifying the

configuration of the FeN_4 structural unit (Figs. 2(e) and 2(f), and Fig. S7 and Table S3 in the ESM). The Fe K-edge k^2 -weighted EXAFS wavelet transformation showed that the maximum intensity was located at approximately $4.0 \text{ \AA}^{-1}$, which was very close to FePc but clearly different from Fe foil, confirming the existence of Fe-N coordination but no Fe-Fe coordination (Fig. 2(g)). The wavelet transform results at Fe K-edge further confirmed the separated distribution of the Fe element. The Cu K-edge XANES showed that

the absorption threshold of $\text{FeN}_4\text{-Cu}^0$ was very close to that of the Cu foil (Fig. 2(h)). The Cu K-edge FT k^2 -weighted EXAFS spectrum of $\text{FeN}_4\text{-Cu}^0$ showed absorption peaks that were very similar to those of the Cu foil (Fig. 2(i)). Moreover, through further curve analysis in R space and K space as well as the analysis of Cu K-edge k^2 -weighted EXAFS wavelet transformation (Figs. 2(j)–2(l), and Fig. S7 in the ESM), it was confirmed that the oxidation state of Cu in $\text{FeN}_4\text{-Cu}^0$ was 0. The above analysis, combined with the results of AC-HAADF-TEM, confirmed that in $\text{FeN}_4\text{-Cu}^0$, the Fe element was in the form of FeN_4 structure while the Cu element was in a zero-valence atomic clusters structure.

3.2 Peroxidase-like activity of $\text{FeN}_4\text{-Cu}^0$

The peroxidase (POD)-like activity of MOF@FeCu-TA and $\text{FeN}_4\text{-Cu}^0$ was characterized by catalyzing the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H_2O_2 (Fig. 3(a)). We investigated the effect of the number of metal-polyphenol coating layers on the POD activity. The catalytic oxidation of TMB by MOF@FeCu-TA (MOF@FeCu-TA_1 , MOF@FeCu-TA_2 , MOF@FeCu-TA_3 , MOF@FeCu-TA_4) was observed as shown in Figs. S8 and S9 in the ESM. MOF@FeCu-TA_1 exhibited higher catalytic performance. Therefore, in subsequent experiments, unless otherwise stated, MOF@FeCu-TA refers specifically to MOF@FeCu-TA_1 . At pH values ranging from 3 to 8, the absorbance spectra demonstrated that MOF@FeCu-TA and $\text{FeN}_4\text{-Cu}^0$, in the presence of H_2O_2 , oxidized the TMB substrate into its corresponding oxidation products (Figs. 3(b) and 3(c), and Figs. S10 and S11 in the ESM). Moreover, the higher intensity at 652 nm indicated that $\text{FeN}_4\text{-Cu}^0$ had superior POD-like activity compared to MOF@FeCu-TA (Figs. 3(b)–3(e)), and the reaction rate significantly accelerated as the concentration of $\text{FeN}_4\text{-Cu}^0$ increased (Fig. S12 in the ESM). Consistent with the typical behavior of POD-mimicking nanozymes, MOF@FeCu-TA and $\text{FeN}_4\text{-Cu}^0$ exhibited higher catalytic performance under acidic conditions (pH = 4) (Figs. 3(d) and 3(e), and Fig. S10 in the ESM). Therefore, under pH 4 condition, the enzyme catalytic kinetics was determined by changing the concentrations of H_2O_2 or TMB in the presence of MOF@FeCu-TA or $\text{FeN}_4\text{-Cu}^0$. The reactions exhibited typical Michaelis-Menten kinetic characteristics. The Michaelis-Menten constant (K_m) and the maximum reaction rate ($V_{\max}$) were obtained through the corresponding Lineweaver-Burk curves. The results of the steady-state kinetics method for studying the effect of H_2O_2 concentration on enzyme activity indicated that, compared with MOF@FeCu-TA ($V_{\max} = 4.672 \times 10^{-6} \text{ M}\cdot\text{s}^{-1}$, $K_m = 4.371 \text{ mM}$), $\text{FeN}_4\text{-Cu}^0$ exhibited a higher maximum initial velocity ($V_{\max} = 8.955 \times 10^{-6} \text{ M}\cdot\text{s}^{-1}$) and a higher Michaelis constant ($K_m = 9.260 \text{ mM}$) (Figs. 3(f)–3(j)). The results of the study on the effect of TMB concentration on enzyme activity indicated that, compared with MOF@FeCu-TA ($V_{\max} = 3.951 \times 10^{-6} \text{ M}\cdot\text{s}^{-1}$, $K_m = 0.1003 \text{ mM}$), $\text{FeN}_4\text{-Cu}^0$ exhibited a higher maximum initial velocity ($V_{\max} = 5.904 \times 10^{-6} \text{ M}\cdot\text{s}^{-1}$) and a smaller Michaelis constant ($K_m = 0.08168 \text{ mM}$) (Fig. S13 in the ESM). $\text{FeN}_4\text{-Cu}^0$ also displayed superior enzymology indexes across the other reported state-of-the-art POD-mimics that include metal oxides, metal nanoparticles, single-atom enzyme analogs, and metal-organic frameworks (Table S5 in the ESM). These results indicated that $\text{FeN}_4\text{-Cu}^0$ with POD activity exhibited more significant catalytic performance.

Phthalic acid (PTA) reacts with $\cdot\text{OH}$ to form 2-hydroxyphthalic acid, and this product exhibits characteristic fluorescence at 435 nm [40]. Therefore, the presence of $\cdot\text{OH}$ can be sensitively detected

through the fluorescence signal of 2-hydroxyphthalic acid. As shown in Fig. 3(k), in the $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$ group, the fluorescence intensity was much higher than that of the control group containing only H_2O_2 , indicating that this catalyst significantly promoted the generation of $\cdot\text{OH}$. Then, we employed electron spin resonance spectroscopy (ESR) to explore the reaction mechanism [41]. The experimental spectra indicated that the type of free radical generated in the reaction was the hydroxyl radical ($\cdot\text{OH}$), confirming that this radical was an active intermediate. The ESR signal intensity of $\text{FeN}_4\text{-Cu}^0$ catalyzing the generation of $\cdot\text{OH}$ was significantly higher than that of MOF@FeCu-TA (Fig. 3(l)), indicating that $\text{FeN}_4\text{-Cu}^0$ possessed more excellent POD-like activity.

3.3 Click-catalytic activity of $\text{FeN}_4\text{-Cu}^0$

Copper-catalyzed azide-alkyne cycloaddition (CuAAC) and copper-free click chemistry have both been widely applied in the fields of antibacterial and antitumor research [42–45]. We investigated whether $\text{FeN}_4\text{-Cu}^0$ could catalyze the click reaction between azide groups and alkyne functional groups [46]. As shown in Fig. S14 in the ESM, using the CuAAC model, we studied the click catalytic ability of $\text{FeN}_4\text{-Cu}^0$ in 3-azido-7-hydroxy coumarin and phenylacetylene. After 1 h of reaction at a monitoring wavelength of 475 nm, the $\text{FeN}_4\text{-Cu}^0$ group exhibited an ~ 1.7 -fold increase in fluorescence intensity compared to the catalyst-free control. This catalytic effect was also superior to that achieved by MOF@FeCu-TA under identical conditions (Fig. S14 in the ESM). Subsequently, the click catalytic activity of $\text{FeN}_4\text{-Cu}^0$ for prodrug activation was evaluated by liquid chromatography-mass spectrometry (LC-MS). The analysis confirmed the coexistence of both prodrugs (m/z 290.1709, 307.1759) in the LB medium. Upon addition of $\text{FeN}_4\text{-Cu}^0$, the catalytic generation of the antibacterial-active molecule (m/z 596.3777) from these inactive precursors was observed (Figs. 3(m)–3(o)), unequivocally demonstrating its efficient click catalytic activity. All of the above results indicated that $\text{FeN}_4\text{-Cu}^0$ possessed dual catalytic functions, arising from its distinct oxidation-state components, which confer excellent POD-like and click catalytic activities.

3.4 In vitro bacterial capture capacity and antibacterial activity

Having established the potent ROS-generating and click catalytic activities of $\text{FeN}_4\text{-Cu}^0$, we next evaluated its antibacterial efficacy *in vitro* using *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) as representative Gram-positive and Gram-negative bacterial strains, respectively. The short lifespan and limited diffusion range of ROS necessitate close proximity for efficient bactericidal action. We therefore initially assessed the bacterial adhesion capability of $\text{FeN}_4\text{-Cu}^0$ using *E. coli* as a model. As shown in the figure, only approximately 1.7×10^8 colony-forming units (CFU) of *E. coli* was adhered to the surface of MOF@FeCu-TA , while approximately 2.6×10^8 CFU of *E. coli* remained on the surface of $\text{FeN}_4\text{-Cu}^0$ (per 1 mg) (Fig. S15 in the ESM). The quantitative analysis by ICP-OES confirmed that $\text{FeN}_4\text{-Cu}^0$ can enhance bacterial adhesion compared to MOF@FeCu-TA , possibly due to the weakening of negative charge after pyrolysis.

In the subsequent experimental studies, the samples were divided into seven groups: PBS, $\text{FeN}_4\text{-Cu}^0$, Prodrugs (abbreviated as Pro), H_2O_2 , $\text{FeN}_4\text{-Cu}^0 + \text{Pro}$, $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$, and $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$. Firstly, under the fixed concentrations of H_2O_2 and Pro, the

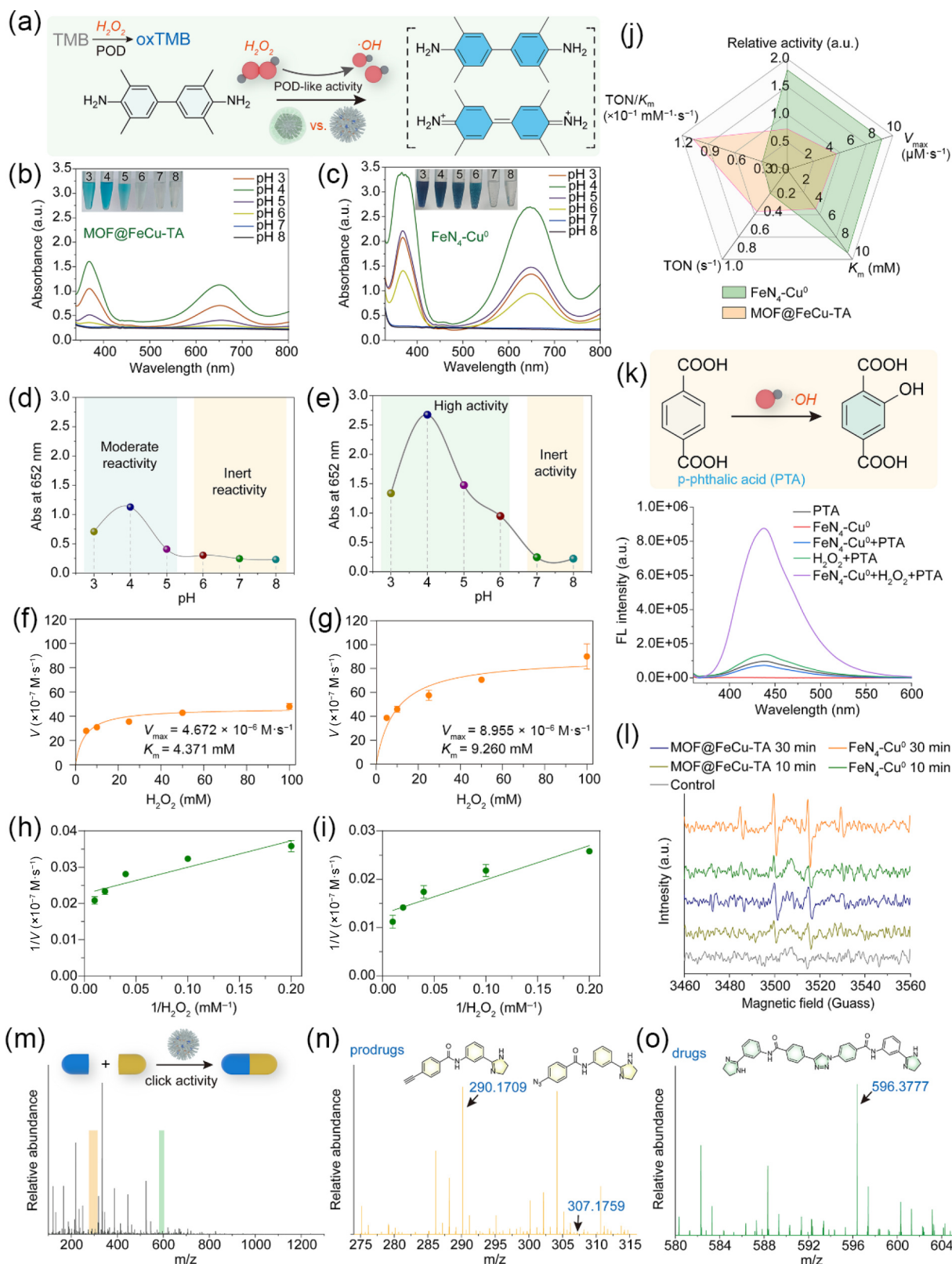


Figure 3 POD-like activity and click catalytic ability of FeN₄-Cu⁰. (a) Schematic of TMB chromogenic reaction catalyzed by MOF@FeCu-TA and FeN₄-Cu⁰. Absorption curves of TMB chromogenic reaction catalyzed by (b) MOF@FeCu-TA and (c) FeN₄-Cu⁰ at pH 3–8. Absorbance at 652 nm of the ox-TMB obtained by the (d) MOF@FeCu-TA and (e) FeN₄-Cu⁰ catalysts in PBS buffer at pH 3–8. Typical Michaelis-Menten curves for (f) MOF@FeCu-TA and (g) FeN₄-Cu⁰. Double-reciprocal plots for determining the kinetic constants of (h) MOF@FeCu-TA and (i) FeN₄-Cu⁰. (j) Kinetic parameters in MOF@FeCu-TA and FeN₄-Cu⁰. (k) Fluorescence spectra of TAOH monitored the ability of FeN₄-Cu⁰ to catalyze H₂O₂ to produce -OH at pH 4. (l) ESR spectra of MOF@FeCu-TA and FeN₄-Cu⁰ in the system containing H₂O₂, 5,5-dimethyl-1-pyrroline 1-oxide (DMPO) and PBS (pH = 4). (m) The LC-MS analysis of the conversion of the prodrugs to the drugs in LB medium via click activity in the presence of FeN₄-Cu⁰. (n) The yellow area in panel (m) confirmed the presence of the prodrugs. (o) The green area in panel (m) confirmed that the drugs were produced.

minimum inhibitory concentration (MIC) of $\text{FeN}_4\text{-Cu}^0$ for different strains was determined. As shown in Fig. S16 in the ESM, the experimental results indicated that $\text{FeN}_4\text{-Cu}^0$ almost completely inhibited the growth of *S. aureus* and *E. coli* at concentrations of approximately 80 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively. The antibacterial ability of $\text{FeN}_4\text{-Cu}^0$ was further verified by the agar plate counting method. As shown in Fig. S17 in the ESM, within the MIC range, the inhibition rates of $\text{FeN}_4\text{-Cu}^0 + \text{Pro}$ and $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$ were approximately 60% against both strains, while $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ showed significantly inhibitory effects on both strains, with an inhibition rate exceeding 95%, demonstrating excellent antibacterial properties. Furthermore, bacterial growth curves monitored at 600 nm showed that after 6 h, $\text{FeN}_4\text{-Cu}^0$, Pro, and H_2O_2 had almost no inhibitory effect. The inhibitory effects of $\text{FeN}_4\text{-Cu}^0 + \text{Pro}$ and $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$ on *E. coli* were slightly weaker than those on *S. aureus*, while $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ had excellent inhibitory effects on both *E. coli* and *S. aureus*, which was consistent with the results of the plate experiment (Figs. 4(a)–4(d), and Fig. S17 in the ESM). We further verified the inhibitory effect of the combined system of $\text{FeN}_4\text{-Cu}^0$, Pro, and H_2O_2 against the drug-resistant bacterium methicillin-resistant *Staphylococcus aureus* (MRSA) through MIC determination and growth curve analysis (Fig. S18 in the ESM). In addition, agar plate diffusion tests were conducted for different treatments co-cultured with bacteria. As shown in Figs. 4(e)–4(h), the inhibition zones of the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ group were significantly larger than those of the other groups, which also proved the excellent antibacterial effect of our system. To further investigate the *in vitro* antibacterial effect, we used the live/dead staining method to assess the bactericidal effect. As shown in Figs. 4(i)–4(l), minimal bactericidal effect was observed in the PBS, $\text{FeN}_4\text{-Cu}^0$, Pro, and H_2O_2 groups, as nearly all bacteria were alive with only a few dead bacteria. In the $\text{FeN}_4\text{-Cu}^0 + \text{Pro}$ and $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$ groups, there were both dead and live bacteria. In contrast, the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ group exhibited a complete elimination of bacteria, with no viable bacteria detected.

In order to investigate the antibacterial mechanism, the morphological integrity of bacteria following treatment with $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ was evaluated using SEM. As shown in Fig. S19 in the ESM, there were significant differences in the bacterial morphology between the control group and the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ group. The bacteria in the control group had a smooth and intact surface, without any obvious morphological changes. Conversely, the bacteria treated with $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ showed wrinkled surfaces, demonstrating potent bactericidal efficacy via membrane disruption-induced bacterial death. Subsequently, we used the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe to further detect the intracellular ROS levels of the bacteria. The results indicated that the $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$ and $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treatments induced a significant increase in ROS within both bacterial strains compared to other groups (Fig. S20 in the ESM), suggesting that $\cdot\text{OH}$ triggered a strong oxidative stress response. Furthermore, propidium iodide (PI) fluorescence flow cytometry analysis revealed that $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treatment severely compromised bacterial membrane integrity, as evidenced by a markedly increased PI internalization rate (Fig. S21 in the ESM).

In addition to the planktonic bacteria model, we also tested the anti-biofilm performance of $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$. As shown in Fig. S22 in the ESM, the group treated with $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ showed a significant reduction in biofilms. In conclusion, $\text{FeN}_4\text{-Cu}^0$

+ Pro + H_2O_2 exhibited outstanding antibacterial effects by disrupting the bacterial cell membrane and wall, activating prodrugs via click chemistry, and enhancing intracellular oxidative stress through its POD-like activity.

3.5 Underlying antibacterial mechanism *in vitro*

To deeply explore the mechanism of sterilization, we conducted RNA sequencing analysis on *E. coli* treated with $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$, and compared the differentially expressed genes (DEGs) between the experimental group and the control group (PBS). The gene transcription analysis of *E. coli* from the control group and the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ group showed that there were 3901 commonly expressed genes in both groups, and 722 genes were only expressed in the experimental group (Fig. 5(a)). Principal component analysis revealed the differences in the transcriptome profiles between the control group and the experimental group (Fig. 5(b)); $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treatment significantly regulated the gene expression of *E. coli*. As shown in the volcano plot, compared with the control group, the experimental group had 2920 differentially expressed genes (1569 upregulated and 1351 downregulated) (Fig. 5(c)). To reveal the functional categories of DEGs, we conducted enrichment analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO). The GO analysis revealed that the differentially expressed genes in *E. coli* induced by the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treated group were associated with multiple biological processes, cellular components, and molecular functions. Some redox enzymes and amino acid metabolism processes were affected (Fig. 5(d)).

The KEGG pathway enrichment analysis revealed that the energy metabolism, redox homeostasis, and amino acid metabolism of bacteria were significantly affected, including oxidative phosphorylation, phosphotransferase system, arginine, proline, alanine, aspartate, glutamate metabolism, and glycolysis, etc. (Fig. 5(e)). The heat map of differentially expressed genes further showed that multiple key genes in the oxidative phosphorylation pathway were downregulated after $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treatment, significantly inhibiting aerobic respiration and ATP synthesis, resulting in suppressed energy metabolism, reduced growth rate, and possibly triggering metabolic compensation or stress responses (Fig. S23 in the ESM). Arginine, as an essential amino acid for protein synthesis, has multiple metabolic functions. It can be converted into proline and glutamate, which are precursors of key substances such as nitric oxide, creatine, polyamines, and urea, playing important roles in cell proliferation, physiological regulation, and stress responses [47]. In this study, multiple genes involved in arginine biosynthesis (*proB*, *proC*, *adiA*, *speF*) were significantly downregulated after $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treatment (Fig. S24 in the ESM). Additionally, glycolysis can rapidly generate ATP by decomposing glucose into lactate or pyruvate in the cytoplasm [48]. The downregulation of glycolysis confirmed that $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ hindered bacterial energy supplementation (Fig. S25 in the ESM), indicating that $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ could effectively inhibit the biological activity of bacteria. The main regulated GO terms were presented in a circular format (Fig. 5(f)). Figure 5(g) provided a detailed illustration of the representative genes in the above pathways. The membrane and transmembrane transport of bacteria were also affected, with the outer membrane and passive transmembrane transporter activity pathways being affected, proving that $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ disrupted the normal physiological activities of bacterial membranes to a certain extent [49].

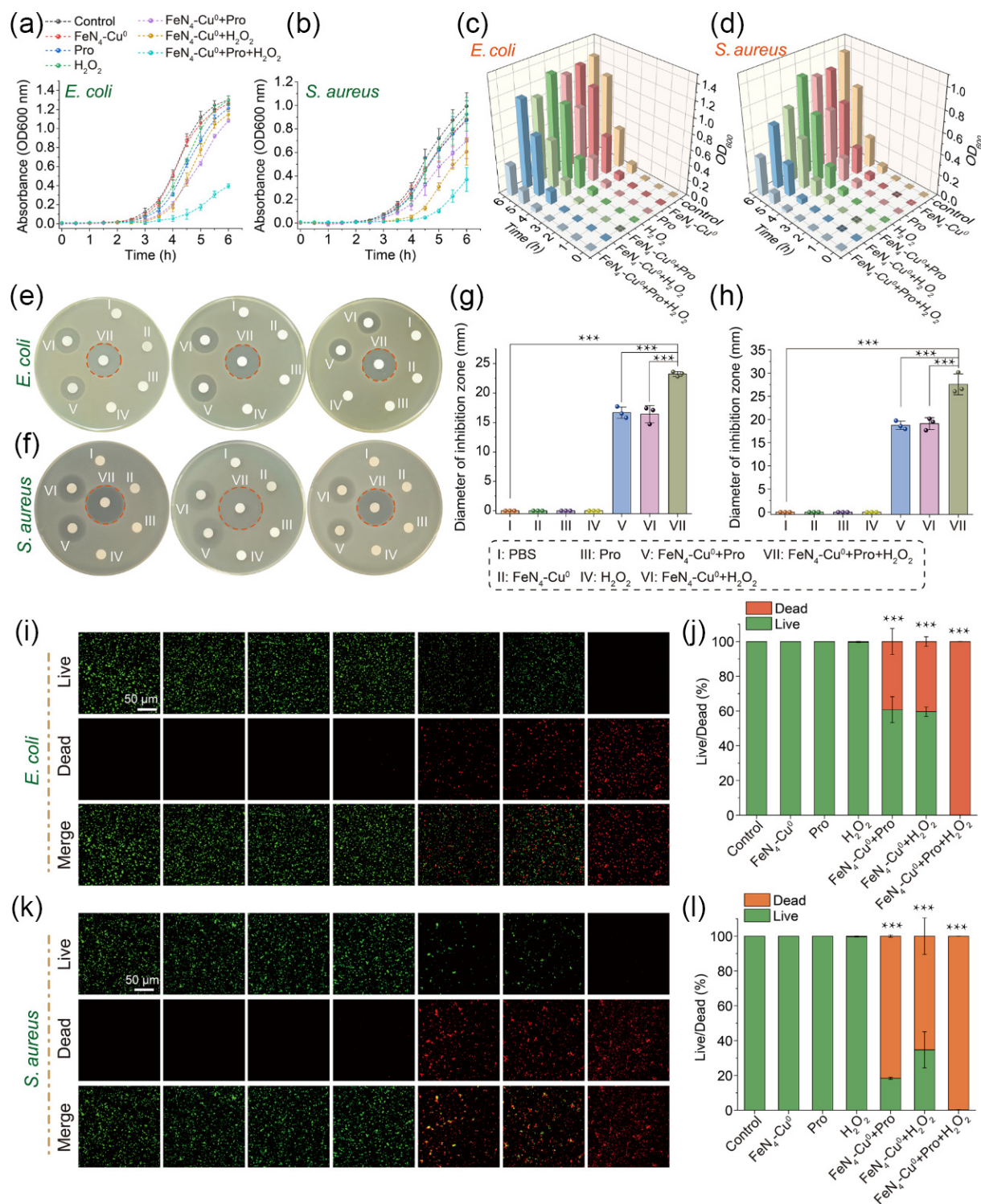


Figure 4 Antibacterial activity of FeN₄-Cu⁰ *in vitro*. (a)–(d) Real-time OD₆₀₀ values for the control, FeN₄-Cu⁰, Pro, H₂O₂, FeN₄-Cu⁰+Pro, FeN₄-Cu⁰+H₂O₂, and FeN₄-Cu⁰+Pro+H₂O₂ against *E. coli* and *S. aureus*. The inhibition zones of (e) *E. coli* and (f) *S. aureus* after different treatments (*n* = 3). Quantification of inhibition zone diameter after 24 h co-incubation with (g) *E. coli* and (h) *S. aureus*, respectively. Live/dead bacterial staining images of (i) *E. coli* and (k) *S. aureus* after incubating with different samples, with green and red fluorescence representing live and dead bacteria. (j) and (l) Ratio of live/dead bacteria as depicted in panels (i) and (k), with red and green columns indicating dead and live bacteria. *P* values are assessed by unpaired Student's two-side *t* test analysis (****P* < 0.001).

We further investigated the antibacterial mechanism of FeN₄-Cu⁰ + Pro + H₂O₂ against *S. aureus*. RNA sequencing analysis revealed a total of 1855 co-expressed genes between the control group and the FeN₄-Cu⁰ + Pro + H₂O₂ group, while 82 genes were uniquely expressed in the control group and 50 genes were uniquely

expressed in the bacterial treatment group (Fig. S26(a) in the ESM). Correlation analysis confirmed the reliability of the sequencing results (Fig. S26(b) in the ESM). Furthermore, a volcano plot intuitively displayed the differences in gene expression levels between the two groups and their statistical significance (Fig. S27 in

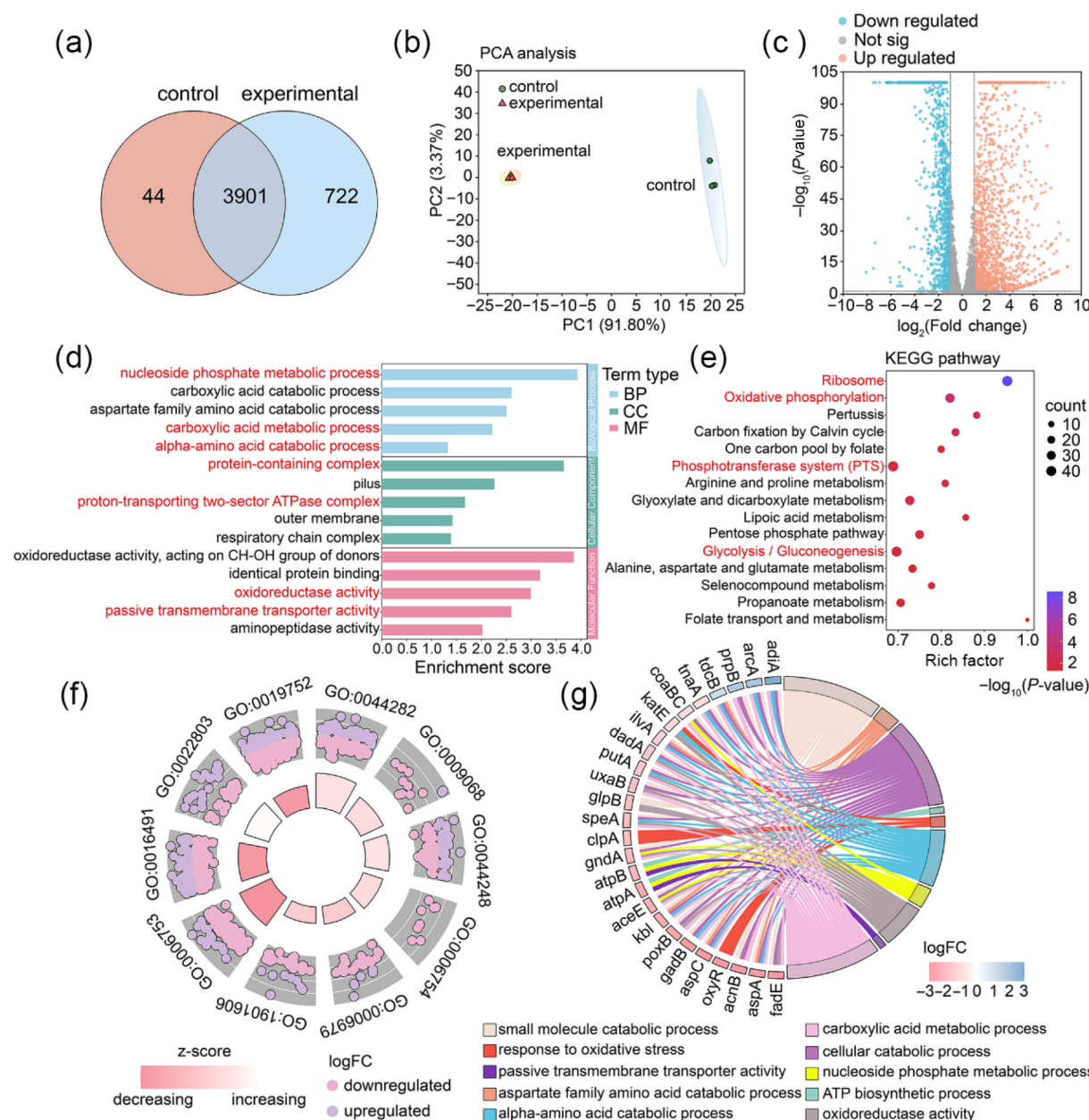


Figure 5 RNA-sequence analysis of *E. coli* after the treatment of $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ (experimental group). (a) Venn plot comparing differential gene expressions on the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ systems and controls to the *E. coli*. (b) PCA analysis. (c) DEGs volcano plot. The blue and red dots indicate DEGs that were downregulated and upregulated. (d) GO enrichment evaluations of all DEGs (BP: biological process; CC: cellular component; MF: molecular function). (e) KEGG enrichment analysis of all DEGs. (f) Circular diagram of GO terms. (g) Enriched chord diagram obtained through GO analysis.

the ESM). Moreover, GO and KEGG enrichment analyses showed that $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ inhibited transmembrane transport activity, compromised bacterial membrane integrity, and subsequently induced a strong oxidative stress response and energy metabolism imbalance in the bacteria, ultimately achieving efficient bactericidal effects (Figs. S28 and S29 in the ESM). To visually depict the transcriptional regulation of these key pathways, we performed heatmap visualization analysis of the differentially expressed genes (Figs. S30 and S31 in the ESM). The results showed that a series of core genes involved in the aforementioned pathways related to amino acid metabolism, energy metabolism, and membrane transport were suppressed, further supporting the multi-target antibacterial mechanism of this treatment at the molecular level.

In a word, $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ causes oxidative stress and energy metabolism imbalance in both *E. coli* and *S. aureus* by disrupting the bacterial membrane structure, inhibiting bacterial biosynthesis and metabolism, thereby achieving a significant antibacterial effect.

3.6 Biosafety assay of $\text{FeN}_4\text{-Cu}^0$

Before conducting the animal experiments, we first evaluated the biological safety of $\text{FeN}_4\text{-Cu}^0$ at the cellular and blood levels. Firstly, we verified the biosafety of $\text{FeN}_4\text{-Cu}^0$ in red blood cells (RBCs), mouse embryonic fibroblast cells (NIH 3T3), and human umbilical vein endothelial cells (HUVECs) through hemolysis experiments, cell counting kit-8 (CCK-8) cell viability assay kits, and live/dead cell staining methods. The hemolysis rates of different

concentrations of $\text{FeN}_4\text{-Cu}^0$ were all below 5%, and the hemolysis rates in different systems were also below 5% (Figs. S32 and S33 in the ESM), indicating good safety of the red blood cell membrane. The survival rates of NIH 3T3 cells co-cultured with different concentrations of $\text{FeN}_4\text{-Cu}^0$ were all above 90% (Fig. S34 in the ESM), and different systems showed good cell compatibility after co-culture with NIH 3T3 cells for 1 and 2 days (Fig. S35 in the ESM). Similarly, HUVECs also exhibited excellent survival rates under the different treatment groups and concentrations (Fig. S36 in the ESM). The results of live/dead cell staining further confirmed the excellent cell compatibility (Fig. S37 in the ESM). Additionally, the scratch test of L929 cells confirmed the ability of $\text{FeN}_4\text{-Cu}^0$ to promote cell migration (Figs. S38 and S39 in the ESM). These experiments demonstrated that $\text{FeN}_4\text{-Cu}^0$ has good biological safety and potential ability to promote wound healing.

3.7 In vivo antibacterial activity assay

The *in vivo* antibacterial efficacy of $\text{FeN}_4\text{-Cu}^0$ was evaluated using a mouse skin wound model infected with *E. coli* (Fig. 6(a)). After treatment with $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ every other day, the infected wounds in the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ group were rapidly repaired and almost completely disappeared. By day 9, the relative wound area in this group was reduced to 19.0%. In comparison, the $\text{FeN}_4\text{-Cu}^0 + \text{Pro}$ and $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$ groups also promoted tissue healing, yielding relative wound areas of 42.1% and 34.8%, respectively, which remained substantially smaller than that of the control group (62.4%) (Figs. 6(b) and 6(c), and Fig. S40 in the ESM). The rapid wound healing promoted by $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treatment was attributed to the synergistic action of click chemistry-mediated drug generation and POD-like catalytic production of ROS from H_2O_2 . Furthermore, bacterial CFU counts of the wound tissues were consistent with the observed healing trends (Figs. 6(d) and 6(e)).

To evaluate the therapeutic effect of $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ on infected wounds from a histological perspective, hematoxylin and eosin (H&E) and Masson staining tests were conducted. H&E staining demonstrated several features of delayed wound healing in the untreated group, including excessive infiltration of inflammatory cells, delayed formation of granulation tissue, poor epithelial regeneration, and insufficient tissue remodeling. Treatment resulted in a significant improvement in these parameters (Fig. 6(f)). It is notable that after 10 days of treatment with $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$, the skin regeneration effect was significant, and hair follicles and sebaceous glands recovered significantly. This was consistent with the wound healing speed shown in Fig. 6(b). The wound lengths of the control, $\text{FeN}_4\text{-Cu}^0$, Pro, and H_2O_2 groups decreased at a significantly slower rate. In contrast, the wound length of the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ group shortened rapidly to approximately 2 mm, which was better than $\text{FeN}_4\text{-Cu}^0 + \text{Pro}$ and $\text{FeN}_4\text{-Cu}^0 + \text{H}_2\text{O}_2$, and significantly better than the control, $\text{FeN}_4\text{-Cu}^0$, Pro, and H_2O_2 groups (Fig. 6(g)). The complete healing of the wound also included the repair of the dermis. Through measurement of dermal thickness, it was found that the dermis in the $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ treated group was thicker, indicating a more stable healing process and good tissue repair (Fig. 6(h)). In contrast, the thickness of the newly formed epidermis was closer to that of normal skin tissue, indicating a more balanced healing response. This thinner epidermis helps prevent the formation of thick crusts or keloids, which could

otherwise damage the normal function of the regenerated tissue (Fig. 6(i)) [50]. Additionally, collagen can serve as the extracellular matrix required for tissue regeneration (indicated in blue by Masson staining), and the process of collagen synthesis and aggregation in the wound could be clearly observed. The $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ group showed a higher level of collagen deposition during the healing process, with a value that was 3.5 to 4.7 times higher than that of the other groups (Figs. 6(f) and 6(j)). Clearly, $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ significantly accelerated the regeneration and remodeling process of bacterial-infected wounds.

The 10-day study on mice showed that during the entire treatment period, the mice's body weight showed a slight increase (Fig. S41 in the ESM). Additionally, we conducted detection and analysis on the blood samples collected from the mice after the treatment, quantifying RBCs, hemoglobin (HGB), platelets (PLTs), white blood cells (WBCs), lymphocytes (Lym), and neutrophils (Neu) (Fig. S42 in the ESM). These results indicated no statistically significant differences among all groups, and all hematological parameters remained within the established physiological ranges. At the same time, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea (UREA), and creatinine (CRE) were all within the normal range (Fig. S43 in the ESM). After 10 days of treatment, histological analysis of the major organs such as the heart, liver, spleen, lungs, and kidneys showed no obvious damage or inflammatory lesions (Fig. S44 in the ESM). These results demonstrated the excellent biocompatibility of $\text{FeN}_4\text{-Cu}^0 + \text{Pro} + \text{H}_2\text{O}_2$ throughout the entire treatment period.

4 Conclusions

In summary, we report here a heterovalent $\text{FeN}_4\text{-Cu}^0$ bioorthogonal nanozyme with integrated distinct catalytic activities for potent antimicrobial therapy through a facile metal-phenolic strategy. This catalytic system is composed of tailored multicomponent units in different oxidation states, which exert catalytic effects through functionally distinct sites. Among them, the FeN_4 single atoms are responsible for generating $\cdot\text{OH}$ by POD-like activity, while Cu^0 clusters activate antibacterial molecules via click chemistry. Benefiting from the spiky morphology of $\text{FeN}_4\text{-Cu}^0$, effective physical bacterial capture can be achieved, thereby enabling the localized synergistic sterilization through reactive oxygen species and activated drugs. This work establishes a paradigm for developing multicomponent SAzymes with multi-catalytic capabilities, paving the way for expanding their functional scopes and application potentials.

Electronic Supplementary Material: Supplementary material (materials, measurements, SEM images, FT-IR, XRD of $\text{FeN}_4\text{-Cu}^0$, time-dependent absorption curves of TMB, CuAAC reaction, Agar plate assay, SEM images of bacteria capture, transcriptome analysis of *S. aureus*, CCK-8 assay and ^1H NMR data) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94908851>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and/or the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

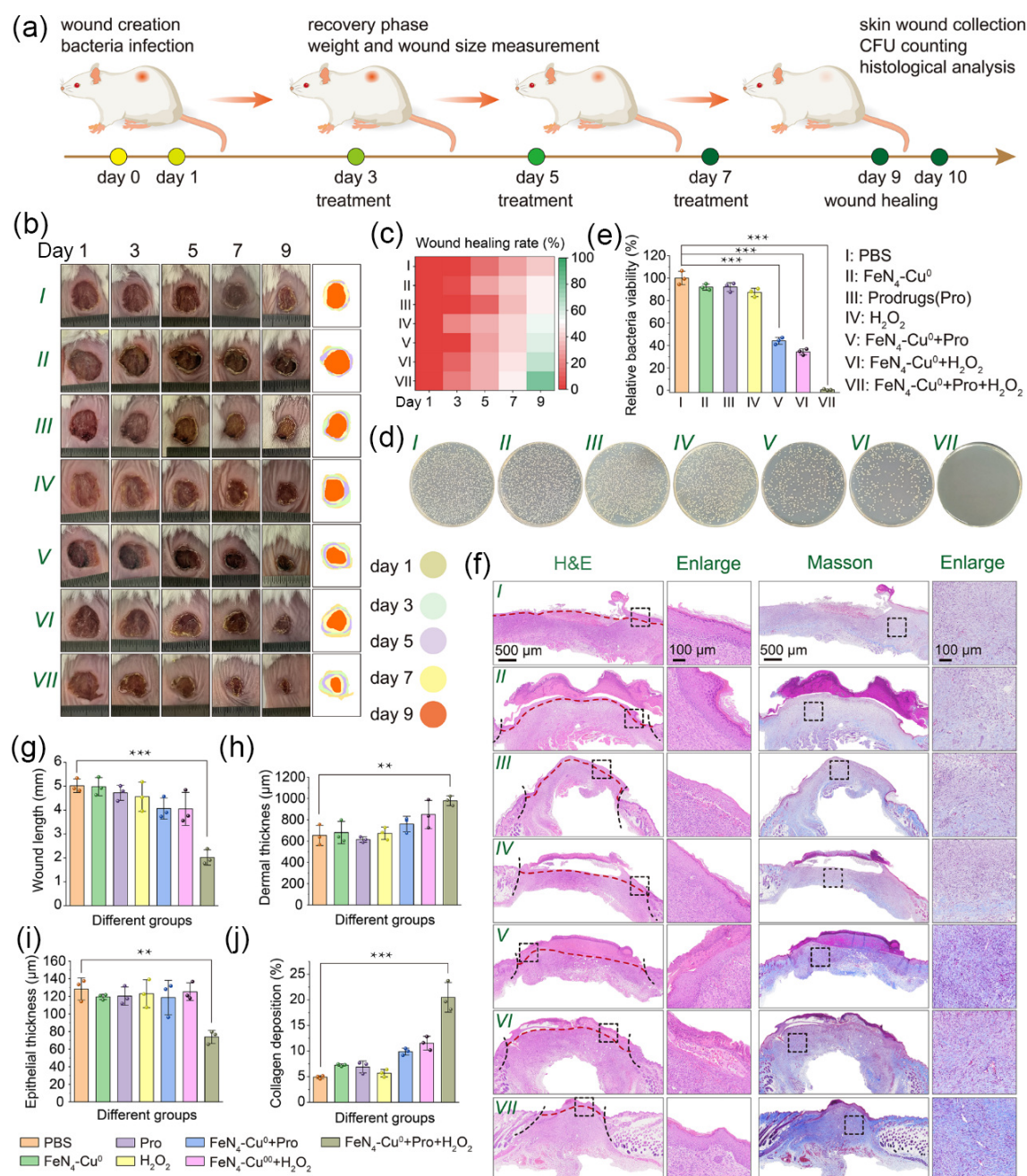


Figure 6 *In vivo* therapeutic effect of FeN₄-Cu⁰+Pro+H₂O₂ on *E. coli*-infected wounds. (a) Schematic diagram of the establishment of the *E. coli* wound infection model and therapeutic pathway. (b) Photographs of infectious wounds area after administration of diverse treatments on days 1, 3, 5, 7 and 9. (c) Wound healing rate of different groups over time. (d) Photographs and (e) quantitative analysis of bacterial colonies obtained from wound tissues after various treatments. (f) H&E and Masson staining of different groups. (g) Wound length in different groups. (h) Dermal thickness in different groups. (i) Relative epithelial thickness in different groups. (j) Collagen deposition (%) in different groups. *P* values are assessed by unpaired Student's two-side *t* test analysis (***P* < 0.01, ****P* < 0.001).

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

S. H. Z.: Writing – original draft, conceptualization, methodology, investigation, data curation. Z. H. D. and Z. Y. Z.: Conceptualization, validation, data curation, visualization. C. W.: Methodology, investigation, visualization. C. C. H.: Investigation, data curation. C. Q. Z. and Y. J. S.: Writing – review & editing, validation, supervision. J. S. N.: Writing – review & editing, funding acquisition, resources, validation, project administration, conceptualization.

Informed consent

Not applicable.

Ethics statement

All animal experiments were conducted in compliance with the guidelines of the care and use of Laboratory Animals of Zhengzhou University, Zhengzhou, China (Ethics No. ZZUIRBGZR2023-1378).

Use of AI statement

None.

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