**Single-atom nanozymes**

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Conventional nanozyme technologies face formidable challenges of intricate size-, composition-, and facet-dependent catalysis and inherently low active site density. We discovered a new class of single-atom nanozymes with atomically dispersed enzyme-like active sites in nanomaterials, which significantly enhanced catalytic performance, and uncovered the underlying mechanism. With oxidase catalysis as a model reaction, experimental studies and theoretical calculations revealed that single-atom nanozymes with carbon nanoframe–confined FeN5 active centers (FeN5 SA/CNF) catalytically behaved like the axial ligand–coordinated heme of cytochrome P450. The definite active moieties and crucial synergistic effects endow FeN5 SA/CNF with a clear electron push–effect mechanism, as well as the highest oxidase-like activity among other nanozymes (the rate constant is 70 times higher than that of commercial Pt/C) and versatile antibacterial applications. These suggest that the single-atom nanozymes have great potential to become the next-generation nanozymes.

**RESULTS**

**Synthesis and characterization of FeN5 SA/CNF**

For the synthesis of FeN5 SA/CNF, we first designed a host-guest structure of MOF-encapsulated iron phthalocyanine (FePc) (FePc@Zn-MOF) and then pyrolyzed the precursor at 900°C under N2 atmosphere to obtain single-atom nanozymes. Our previous work has demonstrated that the square planar FeN4 sites would be retained during the calcination of iron porphyrin and FePc, but the monodispersed sites tend to agglomerate into nanoparticles in the absence of substrate support (21–29). During the carbonization process, secondary building units of nitrogenous organic linkers transformed into pyridinic N carbon nanoframes and Zn ions were evaporated. Meanwhile, the isolated FeN4 sites within the confine-

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The morphology and structure of FeN\textsubscript{5} SA/CNF were characterized with a number of techniques. Representative scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images reveal that the fusiform FePc@Zn-MOF is the dominant product with uniform morphology (fig. S2). The average length and diameter of the precursor are 400 and 200 nm, respectively. The hollow cavities and porous shells endow FePc@Zn-MOF with high specific surface areas and abundant hierarchical nanopores (fig. S3B). The x-ray diffraction (XRD) patterns indicate that the crystal structure of Zn-MOF undergoes no significant change after in situ encapsulating FePc (fig. S3A). The x-ray diffraction (XRD) patterns indicate that the crystal structure of Zn-MOF undergoes no significant change after in situ encapsulating FePc (fig. S3A). The Fourier transform infrared (FTIR) spectrum of FePc@Zn-MOF indicates the successful encapsulation of FePc (fig. S4). FeN\textsubscript{5} SA/CNF obtained from pyrolysis of FePc@Zn-MOF precursor at 900°C has maintained its original morphology and porous property (fig. 1, B to D, and fig. S5, A to C). The Brunauer-Emmett-Teller surface area of FeN\textsubscript{5} SA/CNF reaches 1407 m\textsuperscript{2} g\textsuperscript{-1}, and the average pore diameters are 0.8 and 3.4 nm (figs. S5D and S6). The high-resolution TEM (HRTEM) images, selected-area electron diffraction, and XRD patterns exhibit no observable particles or characteristic crystal peaks, excluding the formation of nanoparticles (figs. S5E and S7A). Furthermore, atomic-resolution scanning TEM (STEM) Z-contrast images of FeN\textsubscript{5} SA/CNF confirm the existence of atomically dispersed Fe atoms over the carbon nanosheets, and the observation of multiple regions indicates that only individual Fe atoms are present in FeN\textsubscript{5} SA/CNF (fig. 1, E and F, and fig. S7B). The electron energy-loss spectroscopy (EELS) mapping images show...
that the Fe and N atoms are homogeneously distributed throughout the whole domain, indicating the generation of Fe–N sites in three-dimensional matrices (Fig. 1G and fig. S5F). We used inductively coupled plasma–mass spectrometry (ICP-MS) and elementary analysis to identify the loadings of Fe [1.2 weight % (wt %)] and N (4.8 wt %) elements, respectively.

**Atomic structure analysis of FeN₅ SA/CNF**

We used x-ray photoelectron spectroscopy (XPS), x-ray absorption fine structure (XAFS), and Mössbauer spectroscopy to further characterize the atomic structures of FeN₅ SA/CNF. The XPS Fe 2p spectrum shows two peaks at binding energies of 711.2 eV (Fe 2p₃/₂) and 724.5 eV (Fe 2p₁/₂). The XPS N 1s spectrum can be principally deconvoluted into pyridinic N, which, derived from organic linkers, could serve as attachment sites for coupling the FeN₄ active centers (Fig. 2A and fig. S8, A to C). The Fe K-edge x-ray absorption near-edge structure (XANES) of FeN₅ SA/CNF, with Fe foil, Fe₂O₃, and FePc as references, is shown in Fig. 2B. The pre-edge peak at 7117 eV that appears in the samples is similar to FePc, suggesting that the FeN₅ SA/CNF samples contain a similar FeN₄ structure (24, 29).

Moreover, Fourier-transformed k³-weighted extended XAFS (FT-EXAFS) spectrum of FeN₅ SA/CNF at Fe K-edge presents a main peak at 1.55 Å, which is in accord with Fe–N scattering path, and no Fe–O bond (1.50 Å) or Fe–Fe bond (2.13 Å) is detected, indicating the formation of atomically dispersed Fe–N sites in FeN₅ SA/CNF (Fig. 2C). The corresponding EXAFS fitting of the first coordination shell was performed to determine the structural parameters and quantitative chemical configuration of Fe atoms (Fig. 2D). The coordination number of Fe atom is nearly five, which indicates the formation of Fe–N₅ moieties in FeN₅ SA/CNF. We used Mössbauer spectroscopy to investigate the electron structure and Fe coordination of FeN₅ SA/CNF based on the recoil-free absorption of γ rays by Fe⁵⁷ nuclei (fig. S8D). The Mössbauer spectrum is deconvoluted into three different doublets according to the isomer shift (δiso) and quadrupole splitting (ΔE_Q) values (table S1). The distinctive quadrupole doublet of the second doublet with the smallest δiso value of 0.13 mm s⁻¹ and the largest ΔE_Q value of 2.75 mm s⁻¹ can be assigned to a N-Fe³⁺N₄ medium-spin species with five coordinated rhombic monopyramidal structures (25, 29, 30). The relative absorption area (24.65%) of that second doublet indicates the high content of FeN₅ moieties in FeN₅ SA/CNF.

**Oxidase-like activity assays**

We determined the oxidase-like activities of FeN₅ SA/CNF by colorimetric assays. We used the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) as a model catalytic reaction to investigate the interaction of O₂ molecules with FeN₅ SA/CNF in various environments (Fig. 3A). We prepared a series of referenced catalysts through this general method with simply altering the pyrolytic temperature, organic ligand, and metal phthalocyanine (MPc) of the MPc@Zn-MOF precursor (figs. S9 to S15). The HRTEM and high-angle annular dark-field STEM (HAADF-STEM) images exhibit no observable particles, excluding the formation of nanoparticles in these catalysts. Furthermore, FT-EXAFS spectra of MN₅ SA/CNF at the K-edge present a main peak corresponding to the M–N scattering path, and metal bond is detected, indicating the formation of atomically dispersed M–N sites in these catalysts. We optimized the synthesis and oxidase-like activity test conditions of FeN₅ SA/CNF (figs. S16...
and S17). The oxidase-like activities of FeN$_5$ SA/CNF are highly dependent on the pyrolysis temperature. After pyrolyzing of the FePc@Zn-MOF precursor at 900°C, the optimum reconstitution of carbon nanoframes and FeN$_5$ active centers leads to a catalytic structure similar to oxidase and active sites more efficient and accessible to substrates. Thus, the FeN$_5$ SA/CNF exhibits higher oxidase-like activity than other FeN$_x$ SA/CNF and FePc. In addition, the FeN$_5$ SA/CNF can still retain at least 80 and 90% of the oxidase-like activity even when the test temperature is extended to 60°C and the catalysts undergo 21 hours of strong acid (alkali) treatment, respectively (Fig. 3C and fig. S17A). Compared to the time-dependent absorbance at 652 nm of FeN$_5$ SA/CNF in air-saturated buffer, the reaction rates of FeN$_5$ SA/CNF–catalyzed TMB oxidation show a significant increase in O$_2$-saturated conditions and a sharp decrease in N$_2$-saturated conditions. These suggest the intense catalytic action of FeN$_5$ SA/CNF in catalyzing O$_2$ reduction and that the oxidation rate of TMB is highly dependent on O$_2$ concentration (Fig. 3B and fig. S18).

Then, we systematically studied the oxidase-like activities of FeN$_4$ SA/CNF and Mn$_5$ SA/CNF where (M is Mn, Fe, Co, Ni, and Cu). As shown in Fig. 3D, the absorbance of oxidized TMB (oxTMB) immediately reaches 1.0 arbitrary unit (a.u.) in 1 min with the catalysis of FeN$_5$ SA/CNF in air-saturated buffer, the reaction rates of FeN$_5$ SA/CNF–catalyzed TMB oxidation show a significant increase in O$_2$-saturated conditions and a sharp decrease in N$_2$-saturated conditions. These suggest the intense catalytic action of FeN$_5$ SA/CNF in catalyzing O$_2$ reduction and that the oxidation rate of TMB is highly dependent on O$_2$ concentration (Fig. 3B and fig. S18).

The highest oxidase-like activity of 0.44 $\mu$M s$^{-1}$, which is 17 times higher than that of the FeN$_4$ SA/CNF (Fig. 3E). Furthermore, the experimental order of oxidase-like activity is FeN$_5$ SA/CNF > Mn$_5$ SA/CNF > CoN$_5$ SA/CNF > FeN$_4$ SA/CNF ≫ NiN$_5$ SA/CNF > CuN$_5$ SA/CNF, indicating that the central metal atom and axial five-N–coordinated structure are of equal importance for single-atom nanozymes. The synergistic effects of these factors give rise to the superior oxidase-like characteristics of FeN$_5$ SA/CNF. In addition, compared with most reported nanoparticles with oxidase-like characteristics, such as CeO$_2$, Fe$_3$O$_4$, MnO$_2$, CuO, Au, Pd, Pt, and prussian blue, FeN$_5$ SA/CNF exhibits oxidase-like activity that outperforms these nanozymes even with merely 1.2 wt % Fe content (fig. S19 and table S2); in particular, the catalytic rate constant ($k_{cat}$) of FeN$_5$ SA/CNF is 70 times greater than that of the commercial Pt/C (table S3). Moreover, to the best of our knowledge, the single-atom nanozymes of FeN$_5$ SA/CNF exhibit more than 30 to 1000 times greater oxidase-like catalytic rate constant than other conventional nanozymes (table S6). These indicate that the superior oxidase-like activities of Fe atoms are derived from the highly dispersed atomic sites and the intrinsic coordination structure. The inhibitive effect of TMB oxidation reaction by ascorbic acid (AA) makes the FeN$_5$ SA/CNF sensitive to the antioxidant. The oxidation rate of TMB gradually decreases with the increase of AA concentration, and there is a good linear relationship between the absorbance of oxTMB and AA concentration in the range of 0.1 to 10 $\mu$M with a limit of detection of 0.07 $\mu$M (fig. S20). In view of the high-oxidation catalytic activity and unique catalytic
pathway of oxidase-like characteristics, the single-atom nanozymes of FeN$_5$ SA/CNF would generate reactive oxygen species or oxidative stress during the catalytic reduction of oxygen, which were able to seriously impair the membrane integrity of bacteria and enhance antibacterial efficiency (31–33). To accurately evaluate the antibacterial activity of FeN$_5$ SA/CNF, we used in vitro antibacterial experiments. In comparison to the control group, the bacterial survival rates of *Escherichia coli* and *Staphylococcus aureus* cells exposed to FeN$_5$ SA/CNF were markedly reduced, indicating that the high oxidase-like activities of FeN$_5$ SA/CNF could significantly enhance the antibacterial activity. The fluorescence-based live-dead cell assays showed that most of the bacteria emitted red fluorescence with the staining of propidium iodide (PI) after incubating with FeN$_5$ SA/CNF, suggesting the occurrence of significant membrane damage. Furthermore, the destruction of the cell membrane in SEM images was in accord with the fluorescence assays, bacteria were severely damaged with loss of cell morphology, and extensive membrane damage resulted in the cell collapse (fig. S21). In addition, we conducted cytotoxicity experiments through comparing the effects of FeN$_5$ SA/CNF on the viability of normal colon mucosa (NCM460) cells. As a result, no obvious toxicity was observed in cell lines, even at the high concentration of FeN$_5$ SA/CNF (500 µg ml$^{-1}$) (fig. S22).

We used in vivo antibacterial experiments of the actual wound infection model on Balb/c mice to assess the practical antibacterial efficacy of FeN$_5$ SA/CNF. As shown in fig. S23, within the 4-day observation of the wound after *E. coli* infection and therapy, we observed the clear remission of ulceration and accelerated wound healing in the mice treated with FeN$_5$ SA/CNF compared to the control group, which coincided with the hematoxylin and eosin (H&E) staining of infected wounds. The staining results indicated that the keratinocytes migrated to the wound site from the normal tissue, and the epidermis gradually became complete and thick in the normal skin sections after treatment (34, 35). Overall, these results indicated that the synthesized FeN$_5$ SA/CNF were highly biocompatible bactericidal nanozymes.

Further research into the catalytic mechanism on FeN$_5$ SA/CNF was carried out by steady-state kinetic assay and electron paramagnetic resonance (EPR) analysis. We used typical Michaelis-Menten curves conducted by altering the concentration of TMB for calculating the Michaelis constant ($K_m$) through the Lineweaver–Burk equation (table S3). The low $K_m$ value of FeN$_5$ SA/CNF reveals the ultrahigh affinity of FeN$_5$ active centers toward TMB (fig. 3F and fig. S24), and the highest $k_{cat}/K_m$ value for FeN$_5$ SA/CNF indicates the much higher catalytic efficiency than other catalysts (36). Besides, high-valent Fe(IV)═O intermediate (compounds I or II iron) is suggested to be a plausible and essential active transient state in catalytic cycles of heme-containing enzymes, which generally exist in heme analogs as well (27). To verify whether the FeN$_5$ active site of FeN$_5$ SA/CNF generates an Fe(IV)═O intermediate as is seen in heme, we conducted EPR spectroscopy through the reaction of FeN$_5$ SA/CNF with excess phenylloxidine (PhIO) at 77 K. A typical rhombic marker signal at g ≈ 2.03, consistent with $\eta^2$-peroxo heme species, indicates the formation of the Fe(IV)═O intermediate and the similar reaction process with oxidase (fig. S25A). The oxidase-like activity of FeN$_5$ SA/CNF rapidly decreases against the addition of excess KSCN for the strong coordination of SCN$^-$ with FeN$_5$ moieties and the poisoning of FeN$_5$ SA/CNF. This demonstrates that atomically dispersed FeN$_5$ sites are actual active centers in these catalysts as well (fig. S25B).

**Theoretical evaluation on oxidase-like activity**

To elucidate the origin of the enhanced oxidase-like activity of FeN$_5$ SA/CNF, we performed density functional theory (DFT) calculations for the oxygen molecular reduction process on single-atom metal sites with TMB molecules as the reducing agent in acidic conditions (19, 27). The result is represented in terms of the change of free energy on the basis of the proposed four-electron pathway (Fig. 4A and fig. S26B). First, we build an FeN$_4$ structure in a graphene matrix coupled with pyridinic N as the optimized calculation model of FeN$_5$ SA/CNF (fig. S26A). In view of the negligible oxidase-like activity and infeasible transition state of NiN$_5$ SA/CNF and CuN$_5$ SA/CNF, here, we investigated the active oxidase-like catalysts of FeN$_5$ SA/CNF, MnN$_5$ SA/CNF, FeN$_5$ SA/CNF, and CoN$_5$ SA/CNF. The first step of O$_2$ adsorption determines the following activity of electron transfer from active centers to adsorbed intermediates. The calculated adsorption energy ($\Delta G_{O_2}$) suggests the different adsorption strength of O$_2$ on the adsorption configuration of these catalysts (Fig. 4B, fig. S25C, and table S4 and S5). For the highest $\Delta G_{O_2}$, the O–O distance of adsorbed $^*$O$_2$ on FeN$_5$ SA/CNF is much larger than that of free molecular O$_2$ (1.23 Å). This indicates that the strong

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![Fig. 4. Theoretical investigation of oxidase-like activity over FeN$_5$ SA/CNF. (A) Proposed reaction pathways of O$_2$ reduction to H$_2$O with optimized adsorption configurations on FeN$_5$ SA/CNF. The gray, blue, purple, red, and white balls represent the C, N, Fe, O, and H atoms, respectively. (B) Free energy diagram for oxygen reduction reaction on single-atom enzyme mimics with TMB as reductant in an acidic medium.](http://advances.sciencemag.org/)
compared with the square planar FeN$_4$ SA/CNF, the additional axial-
like activity is FeN$_5$ SA/CNF $>$ MnN$_5$ SA/CNF $>$ CoN$_5$ SA/CNF $>
$ CNF is. According to these energetics, the calculated order of oxidase-
zymes are the origin of superior oxidase-
central metal atom and the steric configuration of single-atom nano-
Therefore, the DFT calculations unambiguously identify that the
adsorption of O$_2$ and activation of the O$\cdash$O bond, thereby restricting
the reaction rate and oxidative-like activity. In addition, MnN$_5$
SA/CNF and CoN$_5$ SA/CNF have moderate $\Delta$G$_{O2}$, but the higher
G$_{O}^\alpha$ of CoN$_5$ SA/CNF results in the high-energy barrier of the third
step (*OOH + H$^+$ + e$^-$ $\rightarrow$ *O + H$_2$O), thus MnN$_5$ SA/CNF are better
to cleave the O$\cdash$O bond of adsorbed *OOH than CoN$_5$ SA/
CNF is. According to these energetics, the calculated order of oxidase-
like activity is FeN$_5$ SA/CNF $>$ MnN$_5$ SA/CNF $>$ CoN$_5$ SA/CNF $>
$ FeN$_5$ SA/CNF, coinciding with the experimental results. To sum up,
compared with the square planar FeN$_5$ SA/CNF, the additional axial-
coordinated N atom gives FeN$_5$ SA/CNF a strong electron push
effect, which activates O$_2$ and favors the cleavage of the O$\cdash$O bond,
thus promoting the oxidative capability of the Bronsted$-$basic ad-
sorbed o xo species toward acquiring acidic hydrogens from substrates
(TMB) en route to the oxidation of the substrates (19, 26, 27).
Meanwhile, the synergistic effects between the central metal Fe atom
and axial N-coordinated structure effectively optimize the free energy
of each transition state on FeN$_5$ SA/CNF under moderate conditions.
Therefore, the DFT calculations unambiguously identify that the
central metal atom and the steric configuration of single-atom nano-
zymes are the origin of superior oxidase-like activity.

**DISCUSSION**

In summary, we have discovered a new class of single-atom nano-
zymes with atomically dispersed enzyme-like active sites in nano-
materials. The maximized atomic utilization efficiency and well-defined
active moieties significantly enhanced the catalytic performance relative to conventional nanzymes and uncovered the underlying
mechanism. With oxidase catalysis as a model reaction, both experi-
mental studies and theoretical calculations revealed that the syn-
thesized single-atom nanzymes of FeN$_5$ SA/CNF catalytically behaved
like the axial-coordinated hemes of oxidoreductases. The electron
push-effect mechanism and crucial synergistic effects endow FeN$_5$
SA/CNF with the highest oxidase-like activity among other nano-
zymes; in particular, the catalytic rate constant of FeN$_5$ SA/CNF is
70 times greater than that of the commercial Pt/C. Meanwhile, FeN$_5$
SA/CNF exhibited broad-spectrum bactericidal properties in vitro
and efficient wound disinfection in vivo. The present results suggest
that the defined single-atom nanzymes provide a new perspective to
the catalytic mechanism and rational design of nanzymes and ex-
hibit great potential to become the next-generation nanzymes.

**MATERIALS AND METHODS**

**Materials**

2,2'$^\prime$-Bipyridine-5,5'$^\prime$-dicarboxylic acid (H$_2$bpdc, >97%) was pur-
chased from Ark Pharm Inc. 1,1'$^\prime$-Biphenyl-4,4'$^\prime$-dicarboxylic acid
(H$_2$bpdc, 97%), lauric acid (LA, 98%), N,N-dimethylecetamide
(DMAC, 98%), zinc acetate dihydrate [Zn(OAc)$_2$, 98%], and poly-
vinyi pyrrolidone (PVP K-30, 99%) were purchased from Sigma-
Aldrich. Manganese(II) phthalocyanine (MnPc, 97%) and nickel(II)
phthalocyanine (NiPc, 97%) were purchased from Alfa Aesar. FePc
(97%), cobalt(II) phthalocyanine (CoPc, 95%), copper(II) phthal-
ocyanine (CuPc, 99%) and TMB (98%) were purchased from Aladdin
(Shanghai, China). All chemicals were used as received without any
further purification.

**Methods**

**Synthesis of FeN$_5$ SA/CNF**

The FePc@MOF precursor was prepared via a one-step hydrothermal
synthesis with FePc molecules in situ encapsulating in [Zn(bpdc)
(DMAC)]$_n$. In a typical synthesis, 50 mg of H$_2$bpdc, 20 mg of PVP,
20 mg of LA, and 2 mg of FePc were dissolved in 20 ml of DMAC,
followed by sonication and vigorous stirring for 2 hours, and
then 150 mg of Zn(OAc)$_2$ was added to the solution with stirring
for an additional 1 min. The mixed solution was transferred into a
40-ml Teflon-lined high-pressure vessel and heated at 100°C for
12 hours in an electric oven before it was cooled to room tempera-
ture. The product was collected by centrifugation and washed several
times with DMAC-ethanol solution. After drying in a vacuum oven,
the precursor was placed in the center of a quartz tube furnace and an-
nealed at 900°C for 2 hours under of N$_2$ flow to obtain FeN$_5$ SA/CNF.
The synthesis of CoN$_5$ SA/CNF, NiN$_5$ SA/CNF, CuN$_5$ SA/CNF, and
MnN$_5$ SA/CNF was similar to that of FeN$_5$ SA/CNF, except that FePc
was replaced by equal moles of the corresponding MPc. FeN$_5$ SA/
CNF was synthesized by simply replacing H$_2$bpdc with H$_2$bpdc and
without using PVP while keeping the other reaction parameters the
same. The as-obtained product was treated with 0.1 M HCl for
2 hours to remove unvolatile zinc and then washed several times
with deionized water and dried for further characterization.

**Instrumentation**

TEM images were characterized by a JEM-2010 operating at 200 kV.
SEM images were measured with an XL30 ESEM FEG SEM (Philips,
Netherlands) operating with an accelerating voltage of 20 kV. The
atomic-resolution HAADF-STEM images were obtained by using a
Titan 80-300 STEM operated at 300 kV, equipped with a probe
spherical aberration corrector. XRD patterns were collected on a D8
ADVANCE (Bruker AXS, Germany) diffractometer using Cu K$_\alpha$
radiation. XPS measurements were conducted on an ESCALAB MKII
spectrometer (VG Co., UK) with Al K$_\alpha$ x-ray radiation as the x-ray
source for excitation. The nitrogen adsorption/desorption isotherms
were obtained with an ASAP 2020 Physisorption Analyzer (Micro-
metrics Instrument Corporation). The metal loadings of the cata-
lysts were measured by ICP-MS, which were obtained by a Thermo
Scientific iCAP6300 (Thermo Fisher Scientific, USA). X-band EPR
spectroscopy was conducted on a Bruker spectrometer equipped with
an Oxford ESR-910 liquid helium cryostat. The microwave frequency
was calibrated with a frequency counter and the magnetic field with
a nuclear magnetic resonance gauss meter. Typical experimental
parameters were as follows: 77 K, 9.853 GHz, a microwave power of
10.8 mW, a modulation of 100 kHz, and a modulation amplitude of
10 G. XAFS spectra operated at 2.5 GeV with a maximum current
of 250 mA were obtained at the 1W1B station at the Beijing Syn-
chrotron Radiation Facility (P. R. China). XAFS measurements at
the Fe, Co, Ni, Cu, and Mn K-edges were performed in fluorescence
mode using a Lycite detector. All samples were tableted as disks with
a diameter of 13 mm and a thickness of 1.0 mm using graphite powder
as binder. The acquired EXAFS data were processed according to the
standard procedures using the ATHENA module implemented in the IFEFFIT software packages. The EXAFS spectra were obtained by subtracting the post-edge background from the overall absorption and then normalizing with respect to the edge jump step. Then, $\chi(k)$ data in the $k$-space ranging from 2.6 to 12.6 Å$^{-1}$ were Fourier-transformed to real (R) space using hanning windows ($\Delta k = 1.0$ Å$^{-1}$) to separate the EXAFS contributions from different coordination shells. The $^{57}$Fe Mössbauer spectrum was carried out on a Topologic 500A spectrometer driving with a proportional counter at room temperature. The radioactive source was $^{57}$Co (Rh) moving in a 500A spectrometer driving with a proportional counter at room temperature. The radioactive source was $^{57}$Co (Rh) moving in a constant acceleration mode. Data analyses were performed assuming a Lorentzian line shape for computer folding and fitting. The components of iron phases were identified based on their Mössbauer parameters including $\delta_{iso}$, $\Delta E_Q$, magnetic hyperfine field, and relative area of Fe ions.

### Oxidase-like activity

Oxidase activities were determined by colorimetric assays. Ten microliters of catalysts (0.5 mg ml$^{-1}$) and 20 µl of 20 mM TMB were added into a 1.5-ml tube containing 970 µl of sodium acetate–acetic acid buffer [100 mM (pH 4.0)]. The catalytic oxidation of TMB (oxTMB) was studied by measuring the absorption changes of the oxidized form of TMB at $\lambda_{max} = 652$ nm ($\varepsilon = 39,000$ M$^{-1}$·cm$^{-1}$). Unless otherwise stated, oxidase activities were carried out in an air-saturated buffer. To explore the optimal conditions of the oxidation of TMB with single-atom nanomolecules, a range of temperatures (20° to 60°C) and pH values (2.0 to 9.0) for the reaction were measured under the same conditions mentioned above. The Michaelis-Menten constant was calculated using Linweaver-Burk plots of the double reciprocal of the Michaelis-Menten equation $v = v_{max} \times [S]/(K_m + [S])$, where $v$ is the initial velocity, $v_{max}$ is the maximal reaction velocity, $[S]$ is the concentration of substrate, and $K_m$ is the Michaelis constant. The catalytic rate constant ($k_{cat}$) defined as $k_{cat} = v_{max}/[E]$ was calculated, where [E] is the molar concentration of metal in single-atom nanomolecules.

### Colorimetric detection of AA

Ten microliters of FeN$_5$ SA/CNF (0.5 mg ml$^{-1}$) were added into a 1.5-ml tube containing 970 µl of sodium acetate–acetic acid buffer [100 mM (pH 4.0)]. Then, 20 µl of 20 mM TMB with varying concentrations of AA (0.1 to 50 µM) was added into the solution. The catalytic oxidation of substrates was studied by measuring the absorption changes of the oxTMB at $\lambda_{max} = 652$ nm ($\varepsilon = 39,000$ M$^{-1}$·cm$^{-1}$). The colorimetric detection of AA was carried out in an air-saturated buffer.

### Bacterial culture and antibacterial experiments

Monoclonies of E. coli and S. aureus on the solid Luria Bertani (LB) agar plates were transferred to 20 ml of liquid LB broth [yeast extract, 5 g liter$^{-1}$; tryptone, 10 g liter$^{-1}$; and NaCl, 5 g liter$^{-1}$ (pH 7.0)] and grown at 37°C for 12 hours at a rotation of 200 rpm. Then, the bacteria were diluted with phosphate-buffered saline (PBS) solution [10 mM (pH 5.5)] to 10$^7$ colony-forming units (CFU) ml$^{-1}$ and mixed with FeN$_5$ SA/CNF (100 µg ml$^{-1}$) for 40 min. Both E. coli and S. aureus bacteria were stained with PI (10 µg ml$^{-1}$) for 15 min in the dark and washed twice with PBS. The membrane-impermeant PI would only enter through damaged bacterial membranes; thus, it was used to label dead bacteria with red fluorescence. The live and dead bacterial cells were then visualized with a fluorescence microscope. The concentration of bacteria was monitored spectrophotometrically by measuring the optical density at 600 nm (OD$_{600}$).

### Morphology observation of bacteria

After the antibacterial assessment, four typical groups of the bacterial suspensions were dropped on silicon wafers and fixed with 2% glutaraldehyde-containing PBS solution for 4 hours at 4°C. Then, the bacteria were dehydrated by sequential treatments with 30, 50, 70, 90, and 100% of ethanol for 10 min, respectively. Dried bacteria were sputter-coated with gold for field-emission SEM images.

### In vitro cytotoxicity experiments

NCM460 cells were used for the investigation of the cell viabilities. NCM46 cells were grown in normal Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. The in vitro cytotoxicity was measured by a standard Cell Counting Kit-8 (CCK-8) assay. The cells were incubated in a 96-well plate (about 5000 cells per well, eight wells for each concentration) for 24 hours in a humidified incubator (37°C, 5% CO$_2$), then each well was washed with PBS (10 mM, pH 7.4), and FeN$_5$ SA/CNF in different concentrations (0, 100, 200, 300, 400, and 500 µg ml$^{-1}$) were added. Then, the cells were incubated for 24 hours. CCK-8 was subsequently added to each well, and the plate was kept in the incubator for another 1.5 hours. Last, cell viability was evaluated by the absorbance at 450 nm of each well by a microplate reader (SpectraMax M2, MDC, USA). Cells without FeN$_5$ SA/CNF were used as control, and the culture medium without cells but with CCK-8 were considered as background. Cell viability was calculated relative to the control cells.

### In vivo mice wound model

To evaluate the antibacterial effects of FeN$_5$ SA/CNF in vivo, the injury model was built. Female Balb/c mice (6 to 8 weeks and 17 to 21 g) were randomly divided into two groups (five mice per group). A wound about 16 mm$^2$ was obtained by surgical procedure on the back of each mouse after anesthesia. The wounds were then infected by 20 µl of E. coli bacterial suspension (1 $\times$ 10$^7$ CFU ml$^{-1}$). After 12 hours, 10 µl of FeN$_5$ SA/CNF (100 µg ml$^{-1}$) and PBS solutions were dropped on the wound area at the corresponding groups. The wounds of infection were recorded and photographed within 4 days. After 4 days, the wound tissues were harvested from the mice for analysis after euthanasia. To determine the amount of bacteria, the wound tissues were placed in 1 ml of PBS and homogenized, and then, the solutions were cultured in liquid LB broth at 37°C for 24 hours at a rotation of 200 rpm before measuring the OD$_{600}$. Animal studies were conducted in compliance with the guidelines of the Institutional Animal Care and Use Committee. The mice were discarded according to the standard approved protocol after we finished the experiment.

### Histological analysis

The wound tissues in different groups were fixed in neutral buffered formalin, processed routinely into paraffin, sectioned into about 4-µm slices, and stained with H&E. The samples were examined under a digital microscope.

### Computational studies

The spin-unrestricted calculations in this study were performed with the package DMol$^3$ in Materials Studio 17.1 at the DFT level (40, 41). The generalized gradient-corrected Perdew-Burke-Ernzerhof functional (42), along with a double numerical basis set including p-polarization function, was applied for all calculations. The core treatment of MN$_5$C (M = Fe, Co, and Ni) and FeN$_4$C is the density functional semicore pseudopotential, which includes some degree of relativistic correction. A dispersion-corrected DFT scheme was used to describe the van der Waals interaction. The solution
SUPPLEMENTARY MATERIALS

Fig. S1. The structures of cytochrome P450, horseradish peroxidase, and catalase and the corresponding active center.

S1. The structures of cytochrome P450, horseradish peroxidase, and catalase and the corresponding active center.

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