Highly selective generation of singlet oxygen from dioxygen with atomically dispersed catalysts†

Wenjie Ma,‡ac Junjie Mao,‡d Chun-Ting He,‡‡d‡‡ Leihou Shao,‡ Ji Liu,‡
Ming Wang,‡ac Ping Yu‡ac and Lanqun Mao‡*ab

Singlet oxygen (1O2) as an excited electronic state of O2 plays a significant role in ubiquitous oxidative processes from enzymatic oxidative metabolism to industrial catalytic oxidation. Generally, 1O2 can be produced through thermal reactions or the photosensitization process; however, highly selective generation of 1O2 from O2 without photosensitization has never been reported. Here, we find that single-atom catalysts (SACs) with atomically dispersed MN4 sites on hollow N-doped carbon (M1/HNC SACs, M = Fe, Co, Cu, Ni) can selectively activate O2 into 1O2 without photosensitization, of which the Fe1/HNC SAC shows an ultrahigh single-site kinetic value of 3.30 \times 10^{15} \text{ min}^{-1} \text{ mol}^{-1}, representing top-level catalytic activity among known catalysts. Theoretical calculations suggest that different charge transfer from MN4 sites to chemisorbed O2 leads to the spin-flip process and spin reduction of O2 with different degrees. The superior capacity for highly selective 1O2 generation enables the Fe1/HNC SAC as an efficient non-radiative therapeutic agent for in vivo inhibition of tumor cell proliferation.

Introduction

Dioxygen (O2) occupies a critical position in a great variety of oxidation reactions involved in both chemical reactions and biological processes.1–6 In aerobic biology, O2-related oxidation reactions are achieved by a series of evolutionary metalloenzymes such as cytochrome P450 through the interaction with O2, resulting in the formation of reactive oxygen species (ROS) such as singlet oxygen (1O2), hydroxyl radical (·OH) and superoxide anion (O2−) or reactive metal-O2 intermediates including metal superoxo, (hydro)peroxo, and metal-oxo species.7–11 Among the active species, 1O2 with an unoccupied π∗ orbital has strong electronegativity and unique reactivity and selectivity to serve as a synthetic reagent, attracting enormous interest from both fundamental studies and practical application fields.12–14 To this end, increasing attention has been drawn to the development of efficient approaches to highly selective generation of 1O2.15–22

Conventional approaches to 1O2 production mainly include thermal processes using enzymatic or chemical reactions and photosensitization of O2 with rationally designed photosensitizers.23–26 However, the production of 1O2 through these approaches is always accompanied by other ROS generation. In addition, thermal processes often require harsh conditions such as rigorous pH and particular solvents.27,28 Despite the simplicity and controllability of the photosensitization route, it faces inherent limitations like poor selectivity of 1O2, photobleaching of sensitizers, and difficulty in large-scale production.29–31 Therefore, development of new approaches to highly selective production of 1O2 is of great significance not only in the investigation of 1O2-related biological and physical processes but also in the development of new materials and biological tools with 1O2.

Recently, single-atom catalysts (SACs) have shown considerable potential in industrial chemical processing and photo/electrochemical energy conversion with high catalytic activity and unique selectivity due to their excellent properties like maximum atom utilization efficiency, well-defined active centers, and tunable coordination environment.32–34 Among the developed SACs, metal-nitrogen (MNx) sites embedded in carbon skeletons represent a significant series of SACs with excellent catalytic activity toward oxygen-related reactions including the oxygen reduction reaction and advanced oxidation processes, benefitting from precise tuning of electronic structures of active sites.35–38 Thus, MNx SACs provide great possibilities and opportunities in the design and fabrication of...
selective catalysts for $^1$O$_2$ production. However, despite some investigations on the capacity of MN$_x$ SACs toward oxygen activation with non-selective ROS production, the atomic engineering of MN$_x$ SACs is still needed to optimize the metal–O$_2$ interaction to achieve selective $^1$O$_2$ generation.

Here, we demonstrate a new approach to highly selective generation of $^1$O$_2$ from O$_2$ without photosensitization with single transition metal atoms anchored on hollow N-doped carbon as atomically dispersed catalysts (M$_1$/HNC SACs, M = Fe, Co, Cu, Ni). The $^1$O$_2$ generation efficiency is highly related to the metal centers, following the sequence of Fe$_1$/HNC > Co$_1$/HNC > Cu$_1$/HNC > Ni$_1$/HNC. Among the catalysts examined, the Fe$_1$/HNC SAC with a single atomic motif of FeN$_4$ coordination shows the best kinetic value of 0.140 min$^{-1}$, exceeding those of Co$_1$/HNC (0.033 min$^{-1}$), Cu$_1$/HNC (0.019 min$^{-1}$) and Ni$_1$/HNC (0.016 min$^{-1}$), and the single-site kinetic value of the Fe$_1$/HNC SAC reaches up to as high as $3.30 \times 10^{10}$ min$^{-1}$ mol$^{-1}$, representing top-level catalytic activity among known catalysts. Density functional theory (DFT) calculations demonstrate that the selective $^1$O$_2$ generation capacity originates from significant charge transfer from MN$_4$ sites to chemisorbed O$_2$, leading to the spin-flip process and spin reduction of O$_2$ with the lowest value of 0.25 for O$_2$ adsorbed on the FeN$_4$ site. Based on the efficient production of $^1$O$_2$ enabled by the Fe$_1$/HNC SAC, we develop a non-radiative therapeutic platform for in vivo inhibition of tumor cell proliferation.

Results and discussion

To synthesize M$_1$/HNC SACs, metal acetylacetonate encapsulated zeolitic imidazolate framework-8 (M/ZIF-8) was first synthesized as the raw material to form core–shell composites by tannic acid (TA) coating. The core–shell precursors were then pyrolyzed at 900 °C under an Ar atmosphere to obtain M$_1$/HNC catalysts (Fig. 1a). Hollow N-doped carbon (HNC) was prepared with pure ZIF-8 without the encapsulation of metal acetylacetone as the control catalyst. X-ray diffraction (XRD) patterns of the catalysts show peaks for (002) and (101) planes of graphitic carbon, located at around 24° and 43°, respectively, and no observable peaks for metal-related nanoparticles were found (Fig. S1†), indicating the absence of metal nanoparticles in M$_1$/HNC catalysts. Raman spectra of the M$_1$/HNC SACs and HNC reveal similar intensity ratios of the D band to the G band (Fig. S2†), suggesting that the catalysts possess a similar structure of the defective carbon skeleton. In addition, the nitrogen sorption isotherm analysis of M$_1$/HNC SACs and HNC (Fig. S3†)
gave a similar Brunner–Emmet–Teller (BET) surface area at around 700 cm² g⁻¹, further indicating the structural similarity of the prepared catalysts. Transmission electron microscopy (TEM) and high-angle annular dark-field scanning TEM (HAADF-STEM) images show that Fe₁/HNC has a hollow polyhedral morphology with an average size of 200 nm and thickness of 10 nm and no metal nanoparticles were observed (Fig. 1b). The elemental mapping images show a homogeneous distribution of Fe, N, and C elements over the whole domain of the Fe₁/HNC SAC (Fig. 1c). Furthermore, the aberration-corrected HAADF-STEM image was obtained to confirm single atomic Fe in the Fe₁/HNC SAC. As shown in Fig. 1d, the bright dots highlighted with red circles clearly demonstrate that the atomically dispersed Fe atoms exist over N-doped carbon. Similarly, the morphology of other three M₁/HNC SACs and the atomic dispersion were confirmed by using TEM and HAADF-STEM images, all showing a hollow polyhedral structure and atomically dispersed metal atoms (Fig. S4–S6).

The atomic electronic structure and coordination configuration of metal species in M₁/HNC SACs were explored by X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) characterization experiments. Fig. 2a shows the XANES spectra of Fe₁/HNC at the Fe K-edge in comparison with those of references including Fe foil and Fe₂O₃. The peak position of Fe₁/HNC is situated between those of Fe₂O₃ and Fe foil, indicating that the valence state of Fe atoms is between 0 and +3. The Fourier-transform EXAFS spectrum of Fe₁/HNC shows only one peak at about 1.5 Å, ascribed to the first coordination shell of Fe–N, and no Fe–Fe peak like that of Fe foil at around 2.2 Å was observed (Fig. 2b), suggesting the inexistence of metal-related nanoparticles or clusters in Fe₁/HNC. In order to further investigate the atomic distribution of Fe in Fe₁/HNC, wavelet transform (WT) analysis of Fe K-edge EXAFS was carried out. As illustrated in Fig. 2c, only one intensity maximum at approximately 4.2 Å⁻¹ related to Fe–N coordination was observed from the WT contour plot of Fe₁/HNC, which is different from the contour WT plots of Fe foil and Fe₂O₃ with the intensity maximum corresponding to Fe–Fe contribution, elucidating that no Fe–Fe bond is present in Fe₁/HNC, but isolated Fe atoms exist. Moreover, the coordination configuration and structural parameters of Fe atoms were obtained from EXAFS fitting curves (Fig. 2d and S7†). As listed in Table S1,† the coordination number of Fe atoms and the bond length of Fe–N are 4.2 and 2.02 Å, respectively. From the above results, the atomic structure configuration of Fe₁/HNC was constructed (Fig. 2e). The local structure of other three M₁/HNC SACs was also confirmed with XANES and EXAFS spectra (Fig. S8–S10†), all possessing similar atomic configuration with Fe₁/HNC.

In order to systematically evaluate the oxidizing capacity of M₁/HNC SACs, the oxidation of colorless 3,3',5,5'-tetramethylbenzidine (TMB) to blue oxidized TMB (ox-TMB) with the characteristic adsorption at ca. 652 nm was used as the catalytic model reaction.⁴⁰–⁴³ Considering that the pyrolysis temperature has significant effect on the coordination microenvironment of SACs,⁴⁴–⁴⁵ the TMB oxidation activities of the catalysts prepared at different temperatures were explored. As shown in Fig. 3a, the catalyst obtained at 900 °C (denoted as Fe₁/HNC-900) exhibits

Fig. 2  XAFS analysis of the Fe₁/HNC SAC. (a) Normalized XANES spectra and (b) corresponding Fourier-transform EXAFS spectra at the Fe K-edge of Fe₁/HNC (red line), Fe foil (purple line), and Fe₂O₃ (blue line). (c) Wavelet transforms of the Fe K-edge EXAFS spectra for Fe₁/HNC, Fe foil, and Fe₂O₃. (d) Fourier-transform EXAFS spectrum and the corresponding fitting curve of Fe₁/HNC in R-space. (e) Schematic structure of Fe₁/HNC.
the highest catalytic activity towards TMB oxidation, and 900 °C was thus chosen to prepare other SACs. We next compared the catalytic activity of M1/HNC SACs with different transition metal centers toward TMB oxidation. The results displayed in Fig. 3b and S11† show that the activity trends follow the sequence of Fe1/HNC > Co1/HNC > Cu1/HNC > Ni1/HNC > HNC, indicating that the single metal atom center plays a vital role in the oxidation reaction. Among the catalysts investigated, Cu1/HNC and Ni1/HNC SACs exhibit quite low catalytic activity towards TMB oxidation like HNC. Comparatively, both Fe1/HNC and Co1/HNC SACs show a high catalytic oxidation rate, far surpassing those of Cu1/HNC and Ni1/HNC SACs. The significant difference in catalytic performance of M1/HNC SACs may arise from the binding strength of different MN4 centers with O species.46–48

To verify whether O2 is the only substrate for the oxidation reaction, Fe1/HNC was selected as the representative catalyst to evaluate the TMB oxidation reaction under a N2 atmosphere. As shown in Fig. 3c, the absorbance intensity of ox-TMB in the N2-saturated B–R buffer (pH 4.0) is significantly lower than that under air-saturated conditions, indicating the indispensable role of O2 in the oxidation of TMB catalyzed by the Fe1/HNC SAC. In addition, as displayed in Fig. S12–S15,† the catalytic oxidation rate of ABDA with M1/HNC and HNC in the B–R buffer without scavengers (brown line) or with SOD (blue line), mannite (purple line), and NaN3 (red line) is depicted in Fig. 3d. The k value of the ABDA oxidation reaction with 5 μg mL⁻¹ M1/HNC and HNC in the B–R buffer (pH 4.0) is shown in Fig. 3e. The decrease in fluorescence intensity (Ex/Em = 380/433 nm) of ABDA with 5 μg mL⁻¹ Fe1/HNC in 5 min in the B–R buffer at different pH values is presented in Fig. 3f. TEMPO ESR signals in the absence (blue line) and presence of 20 μg mL⁻¹ Fe1/HNC in the B–R buffer (pH 4.0) are demonstrated in Fig. 3g. The EPR amplitude of Fe1/HNC + TEMP is depicted in Fig. 3i.
TMB oxidation reaction with M1/HNC (Fe1/HNC and Co1/HNC as examples) is dose- and pH-dependent.

It is generally known that the catalytic oxidation reactions with O2 are always accompanied with the generation of ROS like \( \cdot \text{OH}, \text{O}_2^-, \) and \( ^1\text{O}_2 \), thus, it is vital to identify the exact species produced during the process of the oxidation reaction by M1/HNC SACs. For this purpose, mannite, superoxide dismutase (SOD), and NaN3 were chosen as the ROS scavengers for \( \cdot \text{OH}, \text{O}_2^- \), and \( ^1\text{O}_2 \), respectively. Notably, only NaN3 exhibits efficient inhibition of TMB oxidation catalyzed by the Fe1/HNC SAC, while the others display no obvious effects on the oxidation reaction (Fig. 3d), suggesting that the Fe1/HNC SAC selectively activates O2 into \( ^1\text{O}_2 \). In consideration of photosensitization usually implemented to produce \( ^1\text{O}_2 \), the comparison of the Fe1/HNC-catalyzed TMB oxidation reaction under normal light conditions and in a dark environment was performed. As shown in Fig. 3e, a negligible difference in characteristic absorbance of ox-TMB was observed, indicating the striking generation of \( ^1\text{O}_2 \) without photosensitization. Collectively, highly selective generation of \( ^1\text{O}_2 \) from O2 can be achieved by using the Fe1/HNC SAC without the assistance of the externally applied stimulus.

To further validate the selective production of \( ^1\text{O}_2 \) from O2 enabled by M1/HNC SACs, an \( ^1\text{O}_2 \)-specific probe 9,10-anthracenediy-bis(methylene) dimalonic acid (ABDA) was adopted to selectively recognize and react with \( ^1\text{O}_2 \). ABDA is a fluorescent molecule with \( \lambda_{\text{ex}} \) and \( \lambda_{\text{em}} \) at ca. 380 nm and 433 nm, respectively, which turns to be a non-fluorescent endoperoxide product upon selective oxidation by \( ^1\text{O}_2 \) via a [4 + 2]-cycloaddition. With ABDA as the probe, we evaluated the catalytic efficiency of M1/HNC SACs in generating \( ^1\text{O}_2 \) from O2. As can be seen in Fig. 3f and S16,† the activity towards ABDA oxidation follows the rate sequence of Fe1/HNC (99.8%) > Co1/HNC (42.8%) > Cu1/HNC (30.9%) > Ni1/HNC (23.4%) > HNC (17.3%), which is consistent with the trends in TMB oxidation. Notably, Fe1/HNC displays the highest catalytic activity toward

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**Fig. 4** DFT calculations. PDOS of the M 3d (red line) and O 2p (blue line) orbitals of (a) FeN4, (b) CoN4, (c) CuN4 and (d) NiN4 sites adsorbed with O2. Calculated charge density differences of (e) FeN4, (f) CoN4, (g) CuN4 and (h) NiN4 centers adsorbed with O2. (i) Comparison of the DFT results for constructed MN4 structures.

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| MN4  | O2, Ads. Energy (eV) | O-O Bond Length (ads) | Bader Charge of O2 (ads) | Hirshfeld Spin of M | Hirshfeld Spin of O (ads) | Hirshfeld Spin of O (ads) |
|------|----------------------|-----------------------|--------------------------|---------------------|--------------------------|--------------------------|
| FeN4 | -1.039               | 1.284                 | -0.44                    | 1.89                | 0.31                     | 1.25                     |
| CoN4 | -0.841              | 1.269                 | -0.43                    | 0.41                | 0.03                     | 1.46                     |
| CuN4 | -0.272              | 1.235                 | -0.21                    | 0.53                | 0.11                     | 0.91                     |
| NiN4 | -0.244              | 1.233                 | -0.18                    | 0                   | 0.11                     | 1.82                     |
ABDA oxidation with a rate of nearly 100%, indicative of more efficient ¹⁰O₂ generation catalyzed by Fe₁/HNC in comparison with other SACs. In addition, the kinetic profiles of ABDA oxidation were examined to assess ¹⁰O₂ generation capacities of M₁/HNC SACs by calculating the oxidation rate of ABDA with pseudo-first-order approximation. As shown in Fig. S17, an exponential decrease in the fluorescence intensity of ABDA against the reaction time was observed and the pseudo-first-order kinetic constants (k) values were obtained from the linear relationship. The results shown in Fig. 3g reveal that Fe₁/HNC catalyzes the generation of ¹⁰O₂ for ABDA oxidation with a k value of 0.140 min⁻¹, considerably higher than that of Co₉/HNC (0.033 min⁻¹), Cu₉/HNC (0.019 min⁻¹) and Ni₉/HNC (0.016 min⁻¹), further highlighting the critical role of different single metal atom coordination. The single-site kinetic constant (k_{single-site} value) of the Fe₁/HNC SAC was further evaluated to be as high as 3.30 × 10⁻¹⁰ mol⁻¹ min⁻¹ due to the high catalytic efficiency with a low metal content of 0.17 wt% determined via inductively coupled plasma optical emission spectrometry (ICP-OES). Besides, the Fe₁/HNC-enabled selective generation of ¹⁰O₂ was investigated with different concentrations of the catalyst and in reaction media with different pH values (Fig. 3h and S18†). As shown in Fig. S19, the ABDA oxidation reaction shows a dose-dependent behavior. In addition, the generation of ¹⁰O₂ for ABDA oxidation is peculiarly prone to occur under acidic conditions.

To further confirm the selective generation of ¹⁰O₂ enabled by Fe₁/HNC, colorimetric and fluorescent probes for other ROSs were utilized, thereinto, ρ-phth acids (PTA) and iodonitrotetrazolium chloride (INT) were selected to detect `OH, and O₂⁻, respectively. PTA, as a widely used `OH probe, can be selectively hydroxylated upon reaction with `OH to form 2-hydroxy terephthalic acid with strong fluorescence at λ_{ex}/λ_{em} = 315/400 nm. INT is a specific probe for O₂⁻, which can be reduced by O₂⁻ to generate the colored formazan product with the maximum absorption at 510 nm. As shown in Fig. S20, no obvious changes in the fluorescence intensity of PTA or absorbance of INT were observed in the oxidation reaction, demonstrating negligible generation of `OH and O₂⁻ but highly selective production of ¹⁰O₂ during the O₂ activation process. Furthermore, electron spin resonance (ESR) characterization using 2,2,6,6-tetramethylpiperidine (TEMP) as the ¹⁰O₂ trapping agent and 5-tert-butoxycarbonyl-5-methyl-1-pyrroline N-oxide (BMPO) as the trapping agent for `OH and O₂⁻ were also conducted to give more direct evidence of the produced ROSs. Fig. 3i shows the typical 1 : 1 : 1 triplet ESR signal for 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) formed due to the ¹⁰O₂ generation in the presence of the Fe₁/HNC SAC. However, there are no observable signals when BMPO was used as the trapping agent (Fig. S21†), indicating that negligible `OH and O₂⁻ are produced in the process of Fe₁/HNC-catalyzed O₂ activation.

To gain in-depth insight into the mechanism of selective ¹⁰O₂ generation enabled by M₁/HNC SACs, DFT calculations were performed to explore the electronic structure of the active sites and their interactions with O₂. In accordance with the atomic structural analysis, the models of M₁/HNC SACs adsorbed with O₂ were constructed and optimized. The adsorption energy of O₂ on the Fe₁ site in the Fe₁/HNC SAC was calculated to be −1.039 eV, which is less than those of other M₁/HNC SACs (Fig. 4i), illustrating the strong interaction between the Fe₁ site and O₂. In order to further elucidate the electronic interactions between MN₄ sites and absorbed O₂, the projected density of states (PDOS) for M 3d and O 2p was calculated. As shown in Fig. 4a–d, more hybridization between the PDOS for Fe 3d orbitals and O 2p orbitals can be evidently observed in contrast with those for Cu₉ or Ni₉ centers. Meanwhile, the Fe 3d orbitals shift toward the Fermi level upon O₂ adsorption (Fig. S22–S25†), further demonstrating the occurrence of O₂ activation on FeN₄. In addition, the Bader charge analysis and charge density difference analysis (Fig. 4e–h) reveal distinct charge transfer with 0.44 e from the FeN₄ site to O₂, leading to the elongation of the O–O bond with a length from 1.22 Å in free O₂ to 1.284 Å in O₂ adsorbed on the FeN₄ site (Fig. 4i), which is longer than those on other MN₄ sites. The longest O–O bond of O₂ upon adsorption on the FeN₄ site manifests the strongest interaction between the FeN₄ site and O₂. The transferred electrons will occupy the half-filled antibonding π*(O₂) orbitals of O₂ and give rise to the reduction of the Hirshfeld spin value of O₂ from 1 in free O₂ to 0.25, suggesting that a significant spin-flip process occurs for chemisorbed O₂ on the FeN₄ site. The spin value of O₂ upon chemisorption on the FeN₄ site (0.25) is close to that of ¹⁰O₂ (0) and relatively lower than those on other MN₄ sites, elucidating the superior capacity of the FeN₄ site toward the generation of ¹⁰O₂. Given the above, the charge transfer from the MN₄ site to adsorbed O₂ contributes to different spin-flip degrees of O₂, thus bringing about a discrepancy in their capacities toward selective ¹⁰O₂ generation.

Having demonstrated the high efficiency of selective ¹⁰O₂ generation without photosensitization from molecular O₂ enabled by the Fe₁/HNC SAC, we then employed the Fe₁/HNC SAC as a therapeutic agent for suppressing tumor cell growth. To improve the dispersibility and biocompatibility of Fe₁/HNC, an amphiphatic molecule DSPE-PEG2000 was modified on the surface of Fe₁/HNC to form PEGylated Fe₁/HNC (denoted as P-Fe₁/HNC) through the hydrophobic interaction. The cellular uptake of P-Fe₁/HNC was visualized by confocal laser scanning microscopy (CLSM) imaging with fluorescein isothiocyanate (FITC)-labeled P-Fe₁/HNC. As displayed in Fig. 5a and S26, the bright green fluorescence and the black dots in cytosol clearly indicate the efficient internalization of P-Fe₁/HNC in HeLa cells.

Next, the inhibition effect of P-Fe₁/HNC on cell proliferation was systematically explored by the cell viability test with a standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] assay. The results show that the treatment of HeLa cells with PEGylated HNC (P-HNC) without single Fe atomic centers exhibits a negligible effect on the proliferation of HeLa cells with cell viability exceeding 90% even at a concentration of P-HNC as high as 0.3 mg mL⁻¹ (Fig. 5b), indicating the excellent biocompatibility and low cytotoxicity of P-HNC. In striking contrast, an obvious decrease in cell viability of HeLa cells to less than 50% upon the treatment of P-Fe₁/HNC with a low concentration of 0.06 mg mL⁻¹ was observed and the cell viability decreases with increasing P-Fe₁/HNC concentration.
providing direct evidence for the antiproliferation effect on HeLa cells.

In the meantime, the cytotoxicity of P-Fe₁/HNC was further investigated by co-staining HeLa cells with calcein-AM (green fluorescent dye for viable cell staining) and propidium iodide (PI, red fluorescent dye for dead cell staining) and visualized with CLSM imaging. As exhibited in Fig. 5c and S27† in comparison with the group treated with PBS or P-HNC showing bright green fluorescence, cancer cells treated with P-Fe₁/HNC show strong red fluorescence, indicating the high cytotoxicity against HeLa cells of Fe₁/HNC SACs. According to the results demonstrated above, such capacity of killing cancer cells was deemed to originate from the intracellular oxidative stress induced by Fe₁/HNC SAC-enabled selective production of $^{1}\text{O}_2$, which was distinctly confirmed with a cell-permeable ROS-sensitive probe ($2',7'$-dichlorofluorescein diacetate, DCFH-DA). Obviously, cancer cells treated with P-Fe₁/HNC show the brightest green fluorescence of DCF (Fig. 5d and S28†), indicative of most $^{1}\text{O}_2$ generation enabled by Fe₁/HNC. In order to further demonstrate the cytotoxicity against HeLa cells of the Fe₁/HNC SAC from the oxidative damage induced by the generation of $^{1}\text{O}_2$, ascorbic acid (AA) as an antioxidant was used to pre-incubate HeLa cells. As shown in Fig. 5e, the pre-incubation of AA can efficiently reduce the cytotoxicity of the Fe₁/HNC SAC through the elimination of generated $^{1}\text{O}_2$ with a concentration-dependent behavior.

The antitumor effect of the Fe₁/HNC SAC in vivo was also investigated with HeLa tumor-bearing mice as a model. When the tumor volume reached about 100 mm³, the mice were randomly divided into three groups and intravenously injected
with PBS, P-HNC, and P-Fe/HNC, respectively. As shown in Fig. 5f, the mice treated with P-HNC exhibit a similar tumor growth trend with that of PBS, indicating that HNC had no obvious therapeutic effect on suppressing tumor growth. In contrast, the group treated with P-Fe/HNC displays a relatively higher tumor inhibition effect with the reduction of the tumor size to 46.5% in 12 days compared with the control group, which was attributed to the high capacity of the Fe/HNC SAC for \( ^1\text{O}_2 \) generation within the tumor site. Moreover, photographs of the excised tumors further confirmed the good anti-tumor effect of Fe/HNC (Fig. S29†). In addition, no obvious changes of the body weight of mice were observed during the experimental period upon administration of different catalysts, indicating the favorable biocompatibility of the Fe/HNC SAC (Fig. S5g). Further blood biochemical analysis also implied the good biosafety of these catalysts (Fig. S30†).

Conclusions

In summary, we have demonstrated that atomically dispersed MN4 sites on hollow N-doped carbon can be used for the selective generation of strong oxidizing \( ^1\text{O}_2 \) from \( \text{O}_2 \) without photosensitization. The as-prepared M4/HNC SACs show different \( ^1\text{O}_2 \) generation efficiencies in the sequence of Fe/HNC > Co/HNC > Cu/HNC > Ni/HNC. Among the developed catalysts, the Fe/HNC SAC shows the best kinetic value of 0.14 min\(^{-1}\) and a single-site kinetic value of \( 3.30 \times 10^{10} \text{ mol}^{-1}\text{min}^{-1} \), originating from the spin-flip process and spin reduction of \( \text{O}_2 \) to 0.25 induced via significant charge transfer with 0.44 e from the FeN4 site to \( \text{O}_2 \). More importantly, the Fe/HNC SAC with superior \( ^1\text{O}_2 \) generation capacity has been successfully utilized as an efficient non-radiative therapeutic agent for inhibiting tumor cell proliferation in vitro and in vivo. We believe that this finding would provide a facile method for the selective production of \( ^1\text{O}_2 \), opening up a new avenue for the development of functional SACs in biomedical applications.

Data availability

All experimental and computational data is available in the ESL†

Author contributions

L. M., W. M. and J. M. conceived the idea for the project. W. M. and J. M. designed the experiments and conducted material synthesis, structural characterization, performance test and data analysis. C. H. carried out the DFT calculations. L. S. contributed to the animal experiments. J. L. and M. W. helped in the cell experiments. P. Y. contributed to the discussion of the catalytic part. W. M. drafted the manuscript, and L. M. finalized the manuscript. All authors discussed and commented on the manuscript.

Conflicts of interest

There are no conflicts to declare.

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