Diverse Metal Coordination Center in M-N-C Enabling Different Type of Enzyme-Mimic Reactions

Xinghua Chen, Lufang Zhao, Qing Zhou, Yuan Xu, Yongjun Zheng, Yanfei Shen, Songqin Liu, Yuanjian Zhang

Submitted date: 20/05/2020 • Posted date: 21/05/2020
Licence: CC BY-NC-ND 4.0
Citation information: Chen, Xinghua; Zhao, Lufang; Zhou, Qing; Xu, Yuan; Zheng, Yongjun; Shen, Yanfei; et al. (2020): Diverse Metal Coordination Center in M-N-C Enabling Different Type of Enzyme-Mimic Reactions. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.12337481.v1

The epoch-making breakthrough of nanoscience has brought new perspective to empower new generation of nanozymes with enzyme-like structure and further to propel the comprehending of the structure-property relationship. Here, we report that the regulation of metal coordination center in M-N-C nanozymes (M = Fe, Co, Mn, Ni, and Cu) greatly altered their biocatalytic activities so as to selectively drive different types of enzymatic reactions. It was revealed that the intrinsic selectivity in interaction and activation of ROS by different M-N$_x$ was the origin to promote disparate types of enzyme-like reactions. This work would open a new vista of nanozymes to selectively catalyze different types of reactions, enabled by mimicking the molecular structure of natural enzymes and a further modulation.

File list (2)

| File name       | Size          | View on ChemRxiv | Download file |
|-----------------|---------------|-------------------|---------------|
| Manuscript.pdf  | 624.19 KiB    | view on ChemRxiv  | download file |
| SI.pdf          | 1.42 MiB      | view on ChemRxiv  | download file |
Diverse Metal Coordination Center in M-N-C Enabling Different Type of Enzyme-Mimic Reactions

Xinghua Chen, Lufang Zhao, Qing Zhou, Yuan Xu, Yongjun Zheng, Yanfei Shen, Songqin Liu, and Yuanjian Zhang *

Jiangsu Engineering Laboratory of Smart Carbon-Rich Materials and Device, Jiangsu Province Hi-Tech Key Laboratory for Bio-Medical Research, School of Chemistry and Chemical Engineering, Medical School, Southeast University, Nanjing 211189, China. E-mail: Yuanjian.Zhang@seu.edu.cn

Abstract

The epoch-making breakthrough of nanoscience has brought new perspective to empolder new generation of nanozymes with enzyme-like structure and further to propel the comprehending of the structure-property relationship. Here, we report that the regulation of metal coordination center in M-N-C nanozymes (M = Fe, Co, Mn, Ni, and Cu) greatly altered their biocatalytic activities so as to selectively drive different types of enzymatic reactions. It was revealed that the intrinsic selectivity in interaction and activation of ROS by different M-Nx was the origin to promote disparate types of enzyme-like reactions. This work would open a new vista of nanozymes to selectively catalyze different types of reactions, enabled by mimicking the molecular structure of natural enzymes and a further modulation.
Introduction

Substance transformation engages in a diverse of processes, ranging from natural metabolism in livings to artificial industrial reactions and chemical sensing\textsuperscript{1-5}. Despite in various manners, those interconversions are often accelerated by catalysts. One great example is that metabolic enzymes precisely drive biological reactions in an incredibly efficiency thanks to the refined structures as a result of millions of years of evolution\textsuperscript{6-8}. Unfortunately, high cost and environment-dependent activity of enzymes limit their wide applications \textit{in vitro}. Hence, with rising interest in preparing robust and cost-effective alternatives, tremendous nanomaterials, such as Fe\textsubscript{3}O\textsubscript{4} nanoparticles have been confirmed with intriguing enzyme-like functions\textsuperscript{9-14}. To date, these nanozymes have been successfully applied to a broad of important applications, such as biosensing, bioimaging, tumor therapeutics and antibacterial\textsuperscript{15}. However, owing to the limited understanding of the structure-property relationships, modulating molecular structures of nanozymes to selectively catalyze different types of reactions still remain a major challenge.

As a typical N-coordinated transition metal-doped carbon (M-N-C), Fe-N-C have been widely explored in the past decades as a non-precious electrocatalyst, such as for O\textsubscript{2}, CO\textsubscript{2} and N\textsubscript{2} reduction\textsuperscript{16-18}. The sophisticated in situ/operando characterization techniques verified the critical role of Fe-N\textsubscript{x} moieties in promoting the electrocatalytic activities\textsuperscript{19-22}. Very recently, due to the similar activation of H\textsubscript{2}O\textsubscript{2} and molecular oxygen to generate reactive oxygen species (ROS), Fe-N-C was newly discovered having exceptional high (per)oxidase-like activity\textsuperscript{23-27}. Notably, distinct to most nanozymes that only have enzyme-like functions, Fe-N-C have additional heme-like Fe-N\textsubscript{x} moieties. In this context, M-N-C would be a promising platform to mimic different enzymatic reactions as the metalloporphyrin-like structure was modulable with different affinity to ROS, but few studies have been reported yet.

Herein, we report that the regulation of metal coordination center in M-N-C nanozymes (M = Fe, Co, Mn, Ni, and Cu) greatly altered their biocatalytic activities to selectively drive different types of enzymatic reactions. Both experiments and theoretic calculation revealed that the intrinsic selectivity in interaction and activation of ROS by different M-N\textsubscript{x} was the origin to promote disparate types of enzyme-like reactions. This work would open a new vista of
nanozymes to selectively catalyze different types of reactions, enabled by mimicking the molecular structure of natural enzymes and a further modulation.

Results and discussion

Figure 1. (a) Synthetic processes for M-N-C nanozymes and the conjectural structure. (b, f) TEM, (c, g) high-resolution BF-STEM, (d, h) DF-STEM, and (e, i) the corresponding element mapping images of Fe$_{0.5}$-N-C (b-e) and Co$_{0.5}$-N-C (f-i) nanozyme.

The M-N-C nanozymes were synthesized via a high-temperature pyrolysis, followed by an acid-etching (Figure 1a). Taking preparation of Fe-N-C as an example, briefly, o-phenylenediamine (o-PD) and Fe (III) chloride were first stirred together with carbon black (CB) in 1 M HCl. The polymerization of o-PD was triggered by using ammonium persulfate in an ice bath. The final Fe-N-C was obtained by pyrolyzing the precursor at 900 °C under N$_2$ and acid-etching of metallic iron and iron oxide impurities by concentrated HCl. The other nanozymes with different transition metal species (i.e. Co, Mn, Ni, and Cu) and varied metal content (0.15 g, 0.5 g, 1.5 g
and 4.5 g metal salts for \(M_{0.15}, M_{0.5}, M_{1.5}, M_{4.5}\)-N-C, respectively) were prepared similarly, and denoted as \(M_x\)-N-C, where \(M\) referred to the transition metal species and \(x\) was used to distinguish samples prepared with different metal content in the precursor. As control, the N-C without any metals was also synthesized under the identical conditions except for no adding of metal salts.

The microstructures of M-N-C were firstly characterized by scanning electron microscope (SEM) images. Figure S1a-c showed that \(Fe_{0.5}\)-N-C mainly consisted of nanoparticles with an average size of 40-50 nm. Moreover, their size and shape did not significantly rely on the concentration of iron precursor. Similar features were also observed for other nanozymes with different transition metal species (i.e. Co, Mn, Ni, and Cu; Figure S2). As the size and morphology of nanozymes may influence their activity\(^9,28\), the negligible variation of these structure features would simply the disclosure of structure-properties relationship for M-C-N nanozymes.

The microstructure of Fe-N-C was further ascertained by transmission electron microscopy (TEM). Figure 1b and S3 revealed that all the Fe-N-C nanozymes were composed of characteristic disordered, turbostratic multilayer graphite domains of CB support and the secondary graphitic structure, presumably produced from o-PD during pyrolysis. Notably, there was no obvious metal nanoparticles or clusters in the nanocomposites. The \(N_2\) sorption isotherms (Figure S4a) and the pore size distribution imitated using a quenched solid density functional theory (QSDFT, Figure S4b) of all the Fe-N-C featured a typical texture with a majority of micropores (ca. 0.5-2 nm) and a minor mesopores (ca. 2-6 nm). These pores were much smaller than the diameter of the particle cavity observed by TEM images, indicative of a closed turbine-like graphite shell layer. The almost diminished mesoporous structure and significantly reduced Brunauer-Emmet-Teller (BET) specific surface area (Table S1) of all the Fe-N-C nanozymes with respect to that of CB support (Figure S4c-d) were also observed, suggesting the growth of a secondary graphene-like structure on the surface of graphene domains and inside mesopores of CB support. Therefore, we speculate that the highly accessible active moieties were produced mainly during pyrolysis, which would contribute critically to the improvement of the enzyme-like activity. Interestingly, the Co-N-C nanozymes exhibited a similar trend of structural development in view of TEM (Figure 1f, S5), HR-TEM images (Figure 1f, S5) and \(N_2\) sorption analysis (Figure S6).
To better understand the graphitic structures of M-N-C, the powder X-ray diffraction (XRD) patterns were measured. For instance, a broad (002) peak indicating the anarchy of layer stacking was observed in the XRD pattern of Fe_{0.5}-N-C (Figure S7), consistent with the HR-TEM images (Figure S3). As no obvious peaks of crystallized iron-related species were observed, iron was supposed to exist at the atomic level. By moderate increase of the iron concentration, the (002) peak (Figure S7) was practically not affected, but some weak crystallized iron and iron sulfide were noticed (Figure S7) in an extreme case, i.e. Fe_{4.5}-N-C. It suggested that a proper moderate metal concentration was essential herein for the preparation of atomically dispersed nanocomposites. A similar trend was also observed for the other M-N-C (M = Co, Mn, Ni, and Cu, Figure S8). Complementarily, the Raman spectra (Figure S9) were used to further confirm the disordered graphitic texture of M-N-C.

Figure 2. The deconvoluted high-resolution N 1s spectra of Fe_{0.5}-N-C (a) and Co_{0.5}-N-C (b) nanozyme.

For distinctly uncovering the state of doped metal sites of M-N-C nanozymes, we then resorted to the high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM). The homogeneous, abundant, isolated single Fe-N sites of Fe_{0.5}-N-C nanozyme were identified finally by synergistically comparing and analyzing the bright-field (BF) and dark-field (DF)-STEM coupled EDS mapping images, as shown in Figure 1c-e. The average size of Fe sites was ca. 1-1.5 Å based on the statistical analysis. Due to the accompanying appearance of Fe and N phases, it was speculated that the effective coordination structures were formed between Fe and N phases. The Co-N sites of Co_{0.5}-N-C nanozyme were also confirmed by the same method, showing in Figure 1g-i.
The electronic environment and relative content of doped elements within the near-surface region of M-N-C nanozymes were analyzed by X-ray photoelectron spectroscopy (XPS). The high-resolution N 1s spectra of Fe$_{0.5}$-N-C (Figure 2a) could be deconvoluted into porphyrin-like Fe-N coordination and/or amide (399.7 eV), as well as pyridinic- (398.3 eV), pyrrolic- (401 eV), graphitic- (402.3 eV), and oxidized- (403.5 eV) N species. Interestingly, the percentage of N centered at 399.7 eV of Fe$_{0.5}$-N-C (18.49 %) was significantly higher than that of N-C (14.45 %) and gradually increased with Fe content (Figure S10, Table S2), evidently verifying the formation of Fe-N coordination in Fe-N-C. A very similar M-N coordination structures of the other M-N-C (M = Co, Mn, Ni, and Cu) were also confirmed by deconvoluting the high-resolution N 1s spectra (Figure S11, S12) and the quantification analysis of the N percentage (Table S2). A detailed analysis of the metal 2p$_{3/2}$ shake-up photoemission lines (Figure S13, S14 and S15) offered more insights in the chemical state of the metallic species.

All these results considered, simply regulating the type of transition metal salts, a series of M-N-C (M = Fe, Co, Mn, Ni, and Cu) with different single M-N centers were successfully prepared. Meanwhile, their geometry structure, carbon crystallinity and N-doping almost kept the same. Such unique configurations were highly envisioned to simplify the investigation of the distinct role of each M-N center in M-N-C nanozymes for selectively driving enzyme-like reactions.

As commonly used natural peroxidase substrates, 3,3’,5,5’-tetramethylbenzidine (TMB) and 3-aminophthalhydrazide (luminol), were selected as substrates in aqueous solution to explore the influence of the central metal of M-N-C nanozymes on the peroxidase-like activity and selectivity. In the first set of experiments, the activity of M$_{0.5}$-N-C for catalyzing the oxidation of TMB with H$_2$O$_2$ in HAc-NaAc buffer solution was assessed by monitoring the characteristic absorption peak at 652 nm of the oxidized TMB (TMB$_{ox}$). It was found that the steady-state kinetic of M$_{0.5}$-N-C in catalytic oxidation of TMB with H$_2$O$_2$ exhibited a typical Michaelis-Menten mechanism (Figure S16, Table S3) and obeyed a dependence to the iron content in the precursor (Figure S17), pH and temperature (Figure S18) within the scope of the investigation. For instance, the specific activity (SA, Figure S19) of Fe$_{0.5}$-N-C, defined as activity units per milligram of nanozyme, was calculated as 16.27 U/mg using the nanozyme activity standardization method, competitive to the state-of-the-art peroxidase-mimicking nanozymes. More interestingly, as
shown in Figure 3a, the initial reaction rates of the oxidation reaction catalyzed by Fe-N-C (6.08 μM/s) was far greater than that by the other M-N-C and N-C in a factor of up to 21-fold. Considering the similar particle size, morphology, carbon crystallinity, and even the element composition, it was speculated that the nature of the transition metals in M-N-C played a crucial role for the massive difference in the TMB oxidation catalytic activity.

**Figure 3.** (a) Initial velocity of the catalytic oxidation of 1 mM TMB with 100 mM H$_2$O$_2$ in the presence of 20 μg mL$^{-1}$ of CB, N-C and different M$_{0.5}$-N-C nanozymes in 0.1 M HAc-NaAc (pH 3.5). (b) Time evolution of chemiluminescence intensity at 425 nm for monitoring the catalytic oxidation of 2.5 mM luminol with 250 mM H$_2$O$_2$ in the presence of 50 μg mL$^{-1}$ CB, N-C and different M$_{0.5}$-N-C nanozymes in 0.01 M NaOH. Inset: photographs of the reactants before and after the catalytic oxidation. Effects of ROS scavengers on the oxidation of TMB (c) and luminol (d) with H$_2$O$_2$ catalyzed by Fe$_{0.5}$-N-C and Co$_{0.5}$-N in view of the typical absorption at 652 nm and CL emission intensity, respectively.
The activity of M-N-C for catalyzing the oxidation of luminol by H$_2$O$_2$ in alkaline solution was further investigated by detecting the chemiluminescent (CL) emission intensity. In the case of Co-N-C, the CL light emission, produced by the oxidation of luminol, was observable (Figure 3b, S20 and S21), even by naked eyes (Figure 3b inset). The recorded CL intensity in Figure 3b showed that the CL was rapidly triggered when luminol was injected into the solution containing Co$_{0.5}$-N-C and H$_2$O$_2$, and light intensity attenuated over time. It could be explained by the high catalytic activity of Co$_{0.5}$-N-C and the gradual consumption of luminol during the reaction, respectively. In contrast, the other M-N-C (M = Fe, Mn, Ni, Cu), metal-free N-C and CB did not exhibit any noticeable catalytic activity using the same method, indicating the nature of M-N center was the kernel of the catalytic activity.

To understand the completely opposite catalytic characteristic of Fe-N-C and Co-N-C in the two H$_2$O$_2$ involved redox reactions, the possible intermediate radical species, e.g. ROS, were firstly explored using trapping technique. As illustrated in Figure 3c, neither superoxide dismutase (SOD) nor mannitol that respectively scavenges superoxide and hydroxyl radical have any significant influences on the oxidation of TMB. It depicted the superoxide and hydroxyl radical as a result of redox of H$_2$O$_2$ were not produced in the Fe-N-C catalyzed TMB oxidation. In contrast, interestingly, the superoxide radical was predominately observed in the Co-N-C catalyzed luminol oxidation by H$_2$O$_2$, shown in Figure 3d. Thus, the highly catalytic selectivity in oxidation of TMB and luminol was strongly correlated to the different M-N centers that had a variable affinity to ROS.

Figure 4. Proposed mechanism for the oxidation TMB and luminol with H$_2$O$_2$ catalyzed by Fe-N-C (a) and Co-N-C (b), respectively.
Inspired by the mechanism of reaction of H₂O₂ with horseradish peroxidase (HRP)³³-³⁵, the metalloporphyrin-like structure of Fe-N-C and the strong TMB and H₂O₂ dependent reaction rate, we speculated that the Fe-N-C catalyzed oxidation of TMB with H₂O₂ in acidic condition through a competition between peroxidase- and catalase-like properties³⁶, depended by the substrate. To verify this conjecture, the catalase-like property of M-N-C (M = Fe, Co) was studied by measuring the produced oxygen in 0.1 M HAc-NaAc buffer solution. As shown in Figure S22a, the O₂ formation rate was greatly improved with the catalysis of Fe-N-C, while it decreased significantly when TMB was further added. In contrast, Co-N-C showed a negligible activity in catalytic decomposition of H₂O₂.

The catalase-like property of Co-N-C in alkaline condition was also investigated in the catalyzed oxidation of luminol. As shown in Figure S22b, O₂ was detected in the solution simultaneously containing H₂O₂ and Co-N-C. Nevertheless, the O₂ yield was obviously inhibited when luminol, a stronger electron donor, was introduced to the solution. In contrast, at the identical conditions, Fe-N-C showed a much lower activity in the catalytic decomposition of H₂O₂; meanwhile, the O₂ yield was negligible influenced by introducing luminol (Figure S22b). In this context, we speculated that Co-N-C catalyzed oxidation of luminol with H₂O₂ also through the competition mechanism but variable in ROS affinity.

Briefly, As shown in the proposed mechanism (Figure 4a), H₂O₂ firstly bound to N coordinated Fe³⁺ in Fe-N-C to form Fe³⁺-superoxo species. Then, O-O cleavage of H₂O₂ to release a H₂O molecule with the oxidation of Fe³⁺ to Fe⁵⁺=O. Whether Fe⁵⁺=O was reduced by a second H₂O₂ to form O₂ or TMB to form TMBox was determined by their electron donor ability. Similarly, in the Co-N-C catalytic redox reaction, HO₂⁻ firstly bound to N coordinated Co²⁺ in Co-N-C forming Co³⁺-superoxo species. Then, the O-O cleavage of HO₂⁻ to release a OH⁻ with the oxidation of Co²⁺ to Co⁴⁺=O. Lastly, whether Co⁴⁺=O was reduced by a second HO₂⁻ to form O₂ or luminol to form an oxidized product was relied on their reducing ability. Based on the above experimental results, we speculated that the ability of M-N-C (M= Fe, Co) to adsorb and activate H₂O₂ (or HO₂⁻) played an important role on producing active intermediate M = O (i.e. Fe⁵⁺=O, Co⁴⁺=O). The electron withdrawing ability of M = O from substrate molecule was essential for substrate oxidation.
Consequently, a rational design strategy of nanozymes for driving the types of enzymatic reactions is to regulate the nitrogen coordinated metal center with different affinity to ROS.

Conclusion

In summary, different types of enzymatic reactions were driven by regulating metal coordination center in M-N-C nanozymes (M = Fe, Co, Mn, Ni, and Cu) nanozymes at the molecular level through pyrolysis strategy. Taking the most widely studied peroxidase reactions as models, we found that Fe-N-C could efficiently catalyze the oxidation of TMB with the Initial velocity of 6.08 μM/s, which was far greater than that by the other M-N-C and especially by Co-N-C in a factor of up to 19-fold. While Co-N-C had the activity of catalyzing oxidation of luminol and the other M-N-C has not been found to have obvious activity. The structural analysis of M-N-C nanozymes proved that metal center was the kernel of the distinct catalytic capacity. It was revealed that the intrinsic selectivity in interaction and activation of ROS by different M-Nx was the origin to promote disparate types of enzyme-like reactions.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21775018, 21675022), the Natural Science Foundation of Jiangsu Province (BK20160028), the Open Funds of the State Key Laboratory of Electroanalytical Chemistry (SKLEAC201909), and the Fundamental Research Funds for the Central Universities. We thank doctor Haibo Ma and doctoral candidate Yaping Wen (Nanjing University) for help discussion of reaction mechanism.
Reference

(1) Petersen, M. C.; Vatner, D. F.; Shulman, G. I. Regulation of hepatic glucose metabolism in health and disease. *Nat. Rev. Endocrinol.* 2017, 13, 572-587.

(2) Chen, J. G.; Crooks, R. M.; Seefeldt, L. C.; Bren, K. L.; Bullock, R. M.; Daresbourg, M. Y.; Holland, P. L.; Hoffman, B.; Janik, M. J.; Jones, A. K.; Kanatzidis, M. G.; King, P.; Lancaster, K. M.; Lymar, S. V.; Pfommm, P.; Schneider, W. F.; Schrock, R. R. Beyond fossil fuel-driven nitrogen transformations. *Science* 2018, 360, 873.

(3) Mayer, M.; Baeumner, A. J. A Megatrend Challenging Analytical Chemistry: Biosensor and Chemosensor Concepts Ready for the Internet of Things. *Chem. Rev.* 2019, 119, 7996-8027.

(4) Sheldon, R. A.; Pereira, P. C. Biocatalysis engineering: the big picture. *Chem. Soc. Rev.* 2017, 46, 2678-2691.

(5) Lee, S. Y.; Kim, H. U.; Chae, T. U.; Cho, J. S.; Kim, J. W.; Shin, J. H.; Kim, D. I.; Ko, Y. S.; Jang, W. D.; Jang, Y. S. A comprehensive metabolic map for production of bio-based chemicals. *Nat. Catal.* 2019, 2, 18-33.

(6) Garcia-Viloca, M.; Gao, J.; Karplus, M.; Truhlar, D. G. How enzymes work: Analysis by modern rate theory and computer simulations. *Science* 2004, 303, 186-195.

(7) Stillier, J. B.; Kerns, S. J.; Hoemberger, M.; Cho, Y. J.; Otten, R.; Hagan, M. F.; Kern, D. Probing the transition state in enzyme catalysis by high-pressure NMR dynamics. *Nat. Catal.* 2019, 2, 726-734.

(8) Studer, S.; Hansen, D. A.; Pianowski, Z. L.; Mittl, P. R. E.; Debon, A.; Guffy, S. L.; Der, B. S.; Kuhlman, B.; Hilvert, D. Evolution of a highly active and enantiospecific metalloenzyme from short peptides. *Science* 2018, 362, 1285.

(9) Gao, L. Z.; Zhuang, J.; Nie, L.; Zhang, J. B.; Zhang, Y.; Gu, N.; Wang, T. H.; Feng, J.; Yang, D. L.; Perrett, S.; Yan, X. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat. Nanotech.* 2007, 2, 577-583.

(10) Komkova, M. A.; Karyakina, E. E.; Karyakin, A. A. Catalytically Synthesized Prussian Blue Nanoparticles Defeating Natural Enzyme Peroxidase. *J. Am. Chem. Soc.* 2018, 140, 11302-11307.

(11) Fan, K. L.; Xi, J. Q.; Fan, L.; Wang, P. X.; Zhu, C. H.; Tang, Y.; Xu, X. D.; Liang, M. M.; Jiang, B.; Yan, X. Y.; Gao, L. Z. In vivo guiding nitrogen-doped carbon nanozyme for tumor catalytic therapy. *Nat. Commun.* 2018, 9, 1440.

(12) Wang, X. Y.; Gao, X. J. J.; Qin, L.; Wang, C. D.; Song, L.; Zhou, Y. N.; Zhu, G. Y.; Cao, W.; Lin, S. C.; Zhou, L. Q.; Wang, K.; Zhang, H. G.; Jin, Z.; Wang, P.; Gao, X. F.; Wei, H. e(g) occupancy as an effective descriptor for the catalytic activity of perovskite oxide-based peroxidase mimics. *Nat. Commun.* 2019, 10, 704.
(13) Wu, J. X.; Wang, X. Y.; Wang, Q.; Lou, Z. P.; Li, S. R.; Zhu, Y. Y.; Qin, L.; Wei, H. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). *Chem. Soc. Rev.* **2019**, *48*, 1004-1076.

(14) Huang, Y. Y.; Ren, J. S.; Qu, X. G. Nanozymes: Classification, Catalytic Mechanisms, Activity Regulation, and Applications. *Chem. Rev.* **2019**, *119*, 4357-4412.

(15) Jiang, D. W.; Ni, D. L.; Rosenkrans, Z. T.; Huang, P.; Yan, X. Y.; Cai, W. B. Nanozyme: new horizons for responsive biomedical applications. *Chem. Soc. Rev.* **2019**, *48*, 3683-3704.

(16) Chung, H. T.; Cullen, D. A.; Higgins, D.; Sneed, B. T.; Holby, E. F.; More, K. L.; Zelenay, P. Direct atomic-level insight into the active sites of a high-performance PGM-free ORR catalyst. *Science* **2017**, *357*, 479-483.

(17) Gu, J.; Hsu, C. S.; Bai, L. C.; Chen, H. M.; Hu, X. L. Atomically dispersed Fe3+ sites catalyze efficient CO2 electroreduction to CO. *Science* **2019**, *364*, 1091.

(18) Wang, Y.; Cui, X. Q.; Zhao, J. X.; Jia, G. R.; Gu, L.; Zhang, Q. H.; Meng, L. K.; Shi, Z.; Zheng, L. R.; Wang, C. Y.; Zhang, Z. W.; Zheng, W. T. Rational Design of Fe-N/C Hybrid for Enhanced Nitrogen Reduction Electrocatalysis under Ambient Conditions in Aqueous Solution. *ACS Catal.* **2019**, *9*, 336-344.

(19) Zitolo, A.; Goellner, V.; Armel, V.; Sougrati, M. T.; Mineva, T.; Stievano, L.; Fonda, E.; Jaouen, F. Identification of catalytic sites for oxygen reduction in iron- and nitrogen-doped graphene materials. *Nat. Mater.* **2015**, *14*, 937.

(20) Wang, W.; Jia, Q. Y.; Mukerjee, S.; Chen, S. L. Recent Insights into the Oxygen-Reduction Electrocatalysis of Fe/N/C Materials. *ACS Catal.* **2019**, *9*, 10126-10141.

(21) Wagner, S.; Auerbach, H.; Tait, C. E.; Martinaiou, I.; Kumar, S. C. N.; Kubel, C.; Sergeev, I.; Wille, H. C.; Behrends, J.; Wolny, J. A.; Schunemann, V.; Kramm, U. I. Elucidating the Structural Composition of a Fe-N-C Catalyst by Nuclear- and Electron-Resonance Techniques. *Angew. Chem. Int. Ed.* **2019**, *58*, 10486-10492.

(22) Sahraie, N. R.; Kramm, U. I.; Steinberg, J.; Zhang, Y. J.; Thomas, A.; Reier, T.; Paraknowitsch, J. P.; Strasser, P. Quantifying the density and utilization of active sites in non-precious metal oxygen electroreduction catalysts. *Nat. Commun.* **2015**, *6*, 8618.

(23) He, F.; Mi, L.; Shen, Y. F.; Mori, T.; Liu, S. Q.; Zhang, Y. J. Fe-N-C Artificial Enzyme: Activation of Oxygen for Dehydrogenation and Monooxygenation of Organic Substrates under Mild Condition and Cancer Therapeutic Application. *ACS Appl. Mater. Inter.* **2018**, *10*, 35327-35333.

(24) Huang, L.; Chen, J. X.; Gan, L. F.; Wang, J.; Dong, S. J. Single-atom nanozymes. *Sci. Adv.* **2019**, *5*, 5.

(25) Jiao, L.; Xu, W. Q.; Yan, H. Y.; Wu, Y.; Liu, C. R.; Du, D.; Lin, Y. H.; Zhu, C. Z. Fe-N-C Single-Atom Nanozymes for the Intracellular Hydrogen Peroxide Detection. *Anal. Chem.* **2019**, *91*, 11994-11999.
(26) Wang, Y.; Zhang, Z. W.; Jia, G. R.; Zheng, L. R.; Zhao, J. X.; Cui, X. Q. Elucidating the mechanism of the structure-dependent enzymatic activity of Fe-N/C oxidase mimics. *Chem. Commun.* **2019**, *55*, 5271-5274.

(27) Lu, M. J.; Wang, C.; Ding, Y. Q.; Peng, M. H.; Zhang, W.; Li, K.; Wei, W.; Lin, Y. Q. Fe-N/C single-atom catalysts exhibiting multienzyme activity and ROS scavenging ability in cells. *Chem. Commun.* **2019**, *55*, 14534-14537.

(28) Ge, C. C.; Fang, G.; Shen, X. M.; Chong, Y.; Wamer, W. G.; Gao, X. F.; Chai, Z. F.; Chen, C. Y.; Yin, J. J. Facet Energy versus Enzyme-like Activities: The Unexpected Protection of Palladium Nanocrystals against Oxidative Damage. *ACS Nano* **2016**, *10*, 10436-10445.

(29) Sun, Y. Y.; Silvioli, L.; Sahraie, N. R.; Ju, W.; Li, J. K.; Zitolo, A.; Li, S.; Bagger, A.; Arnarson, L.; Wang, X. L.; Moeller, T.; Bernsmeyer, D.; Rossmeisl, J.; Jaouen, F.; Strasser, P. Activity-Selectivity Trends in the Electrochemical Production of Hydrogen Peroxide over Single-Site Metal-Nitrogen-Carbon Catalysts. *J. Am. Chem. Soc.* **2019**, *141*, 12372-12381.

(30) Artyushkova, K.; Serov, A.; Rojas-Carbonell, S.; Atanassov, P. Chemistry of Multitudinous Active Sites for Oxygen Reduction Reaction in Transition Metal-Nitrogen-Carbon Electrocatalysts. *J. Phys. Chem. C* **2015**, *119*, 25917-25928.

(31) Jiang, B.; Duan, D. M.; Gao, L. Z.; Zhou, M. J.; Fan, K. L.; Tang, Y.; Xi, J. Q.; Bi, Y. H.; Tong, Z.; Gao, G. F.; Xie, N.; Tango, A.; Nie, G. H.; Liang, M. M.; Yan, X. Y. Standardized assays for determining the catalytic activity and kinetics of peroxidase-like nanozymes. *Nat. Protoc.* **2018**, *13*, 1506-1520.

(32) Shah, S. N. A.; Khan, M.; Rehman, Z. U. A prolegomena of periodate and peroxide chemiluminescence. *Trac-trend. Anal. Chem* **2020**, *122*, 115722.

(33) Rodriguez-Lopez, J. N.; Lowe, D. J.; Hernandez-Ruiz, J.; Hiner, A. N. P.; Garcia-Canovas, F.; Thorneley, R. N. F. Mechanism of reaction of hydrogen peroxide with horseradish peroxidase: Identification of intermediates in the catalytic cycle. *J. Am. Chem. Soc.* **2001**, *123*, 11838-11847.

(34) Campomanes, P.; Rothlisberger, U.; Alfonso-Prieto, M.; Rovira, C. The Molecular Mechanism of the Catalase-like Activity in Horseradish Peroxidase. *J. Am. Chem. Soc.* **2015**, *137*, 11170-11178.

(35) Wirstam, M.; Blomberg, M. R. A.; Siegbahn, P. E. M. Reaction mechanism of compound I formation in heme peroxidases: A density functional theory study. *J. Am. Chem. Soc.* **1999**, *121*, 10178-10185.

(36) Ghosh, A.; Mitchell, D. A.; Chanda, A.; Ryabov, A. D.; Popescu, D. L.; Upham, E. C.; Collins, G. J.; Collins, T. J. Catalase-Peroxidase Activity of Iron(III)-TAML Activators of Hydrogen Peroxide. *J. Am. Chem. Soc.* **2008**, *130*, 15116-15126.
Supporting Information

Diverse Metal Coordination Center in M-N-C Enabling Different Type of Enzyme-Mimic Reactions

Xinghua Chen, Lufang Zhao, Qing Zhou, Yuan Xu, Yongjun Zheng, Yanfei Shen, Songqin Liu, and Yuanjian Zhang *

Jiangsu Engineering Laboratory of Smart Carbon-Rich Materials and Device, Jiangsu Province Hi-Tech Key Laboratory for Bio-Medical Research, School of Chemistry and Chemical Engineering, Medical School, Southeast University, Nanjing 211189, China. E-mail: Yuanjian.Zhang@seu.edu.cn
Materials.

o-Phenylenediamine (o-PD), ammonium persulfate (APS), iron(III) chloride hexahydrate (FeCl$_3$ · 6H$_2$O), cobalt(II) chloride hexahydrate (CoCl$_2$ · 6H$_2$O), manganese(II) chloride tetrahydrate (MnCl$_2$ · 4H$_2$O), nickel(II) chloride hexahydrate (NiCl$_2$ · 6H$_2$O), copper(II) chloride dihydrate (CuCl$_2$ · 2H$_2$O), hydrochloric acid (HCl), hydrogen peroxide (H$_2$O$_2$, 30%), acetic acid (HAc), sodium acetate trihydrate (NaAc · 3H$_2$O), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO) and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Aminophthalhydrazide (luminol) and mannitol were purchased from Aladdin Chemistry Co., Ltd. (China). 3,3',5,5'-tetramethylbenzidine (TMB) and superoxide dismutase (SOD, from bovine erythrocytes, $\geq 3,000$ units/mg protein) were purchased from Sigma-Aldrich (USA). Carbon black (Ketjenblack EC 600 JD, CB) was obtained from Akzo Nobel N.V. (Netherlands). All chemicals were of analytical grade and used without further purification unless otherwise specified. Except that the solvent used in TMB solution was DMSO, the solvent used in other solutions or dispersions was water. Ultrapure water (18.2 MΩ cm) used in all the experiments was obtained from a Direct-Q 3 UV pure water purification system (Millipore, USA).

Synthesis of M-N-C nanozymes.

The M-N-C nanozymes were synthesized via a high-temperature pyrolysis, followed by an acid-etching. Taking preparation of Fe$_0.5$-N-C as an example, briefly, o-phenylenediamine (o-PD, 3 g) and iron (III) chloride hexahydrate (0.5 g) were first stirred together with carbon black (CB, 1.5 g) in 1 M HCl (75 mL). The polymerization of o-PD was triggered by using ammonium persulfate (APS, 7.5 g) in an ice bath. The final Fe-N-C was obtained by pyrolyzing the precursor (3 g) at 900 °C under N$_2$ and acid-etching of metallic iron and iron oxide impurities by concentrated hydrochloric acid (30 mL). The other nanozymes with different transition metal species (i.e. Co, Mn, Ni, and Cu) and varied metal content (0.15 g, 0.5 g, 1.5 g and 4.5 g metal salts for M$_{0.15}$, M$_{0.5}$, M$_{1.5}$, M$_{4.5}$-N-C, respectively) were prepared similarly, and denoted as M$_x$-N-C, where M referred to the transition metal species and x was used to distinguish samples prepared with different metal content in the precursor. As control, the N-C without any metals was also synthesized under the identical conditions except for no adding of metal salts.
Characteristic of M-N-C nanozymes.

The scanning electron microscopy (SEM) images were obtained from a FEI Inspect F50 (FEI, USA). The transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HR-TEM) images were taken on a Tecnai G2 T20 (FEI, USA) and a JEM-2100 (JEOL, Japan) at an accelerating voltage of 200 kV. The N$_2$ adsorption–desorption (77 K) isotherms were measured by an Autosorb-iQ (Quantachrome, USA). The X-Ray diffraction (XRD) patterns were collected by an Ultima IV (Rigaku, Japan). The Raman spectra were performed on a DXR (Thermo Fisher, USA). The high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) images were investigated by HF5000 (Hitachi, Japan) at an accelerating voltage of 200 kV. The X-ray photoelectron spectroscopy (XPS) was carried out on an ESCALAB 250XI electron spectrometer (Thermo Fisher, USA) with monochromatic Al Kα X-rays (hν = 1486.6 eV) as the excitation source, and the peak positions were corrected by the C 1s peak at 284.6 eV. The UV-Vis absorption spectra were recorded by a Cary 100 UV-is (Agilent, Singapore). The time-dependent light intensity spectra were tracked with a QE Pro (Ocean Optics, Singapore). The generated oxygen was measured by a specific oxygen electrode on JPSJ-605 (Leici, China). The photographs were acquired using a D7100 (Nikon, Japan).

Peroxidase-like activity and kinetic assay of M-N-C nanozymes.

The catalytic oxidation of TMB with H$_2$O$_2$.

The activity of M-N-C for catalyzing the oxidation of TMB with H$_2$O$_2$ was assessed by monitoring the time dependent absorbance (at 652 nm for the TMB$_{ox}$) change via the kinetic mode of a Cary 100 UV-is (Agilent, Singapore). Typically, the M-N-C nanozyme (2 mg mL$^{-1}$, 10 μL) was added firstly into 0.1 M HAc-NaAc buffer solution (pH 3.5 , 990 μL) containing TMB (100 mM in DMSO, 10 μL) and H$_2$O$_2$ (10 M, 10 μL) then started the test quickly at room temperature. The initial reaction velocity was caculated as follow:

$$v = \frac{\Delta A}{\Delta t} \times \frac{1}{l}$$
Where \( v \) is initial reaction velocity; \( \Delta A/\Delta t \) is the initial rate of change in absorbance at 652 nm \( s^{-1} \); \( \varepsilon \) is the molar absorption coefficient of TMB \( (\varepsilon_{652 \text{ nm}} = 39,000 \ M^{-1} \ cm^{-1}) \); \( l \) is the path length of light traveling in the cuvette \( (\text{cm}) \).

The steady-state kinetic assay of Fe\(_{0.5}\)-N-C was performed under above condition with varied volume of TMB \( (0, 1, 2, 4, 6, 10, 15, 20, 30, 40 \ \mu\text{L}) \) or \( \text{H}_2\text{O}_2 \) \( (0, 0.5, 1, 2, 4, 6, 8, 10, 20 \ \mu\text{L}) \) solution, respectively. The Michaelis-Menten constant (e.g. \( K_m \) and \( v_{\text{max}} \)) was calculated as follow based on the Michaelis-Menten saturation curve:

\[
v = \frac{v_{\text{max}} \times [S]}{K_m + [S]}\]

Where \( v \) is the initial reaction velocity; \( v_{\text{max}} \) is the maximal reaction rate that is observed at saturated substrate concentration; \( [S] \) is the concentration of the substrate and \( K_m \) is the Michaelis constant. In addition, the diverse \( \text{pH} \) \( (4.5 \text{ and } 5.5) \) and temperature \( (37 \ ^\circ\text{C} \text{ and } 60 \ ^\circ\text{C}) \) were also investigated in the same method for Fe\(_{0.5}\)-N-C.

The specific activity \( (SA) \) of Fe\(_{0.5}\)-N-C, defined as activity units per milligram of nanozyme, was carried out at the similar condition with a series of Fe\(_{0.5}\)-N-C dosage \( (2.5, 5, 7.5, 10, 12.5, 15 \ \mu\text{L}) \). One unit is defined as the amount of nanozyme that catalytically produces 1 \( \mu\text{mol} \) of product per minute at standard condition. The SA was obtained by the following formula:

\[
SA = \frac{V \times v}{[m]}\]

Where \( SA \) is the specific activity expressed in units per milligram \( (U \ \text{mg}^{-1}) \); \( V \) is the total volume of reaction solution \( (\mu\text{L}) \); \( v \) is initial reaction velocity \( (\mu\text{M} \ \text{min}^{-1}) \) and \( [m] \) is the nanozyme weight \( (\text{mg}) \) of each assay.

The catalytic oxidation of Luminol with \( \text{H}_2\text{O}_2 \).

The activity of M-N-C for catalyzing the oxidation of Luminol with \( \text{H}_2\text{O}_2 \) was investigated by tracking the time dependent chemiluminescent (CL) emission intensity (typically maximized at 425 nm for luminol) though the timing diagram mode of a QE Pro (Ocean Optics, USA). Briefly, M-N-C nanozyme \( (2 \ \text{mg mL}^{-1}, 50 \ \mu\text{L}) \), \( \text{H}_2\text{O}_2 \) \( (10 \ \text{M}, 10 \ \mu\text{L}) \) and Luminol \( (0.1 \ \text{M}, 50 \ \mu\text{L}) \) was added
successively into 0.01 M NaOH (1850 μL) under stirring at room temperature after the timing diagram mode was started. For Co$_{0.5}$-N-C, the different mixing orders (luminol, Co$_{0.5}$-N-C and H$_2$O$_2$ in sequence and luminol, H$_2$O$_2$ and Co$_{0.5}$-N-C in sequence) were also operated by the method.

**Free radical identification.**

The reactive oxygen species (ROS) participated in the oxidation of TMB and Luminol were investigated by comparing the reactivity in the absence or presence of ROS scavengers (SOD and mannitol scavenges superoxide and hydroxyl radical, respectively). In the catalytic oxidation of TMB, the M-N-C nanozyme (2 mg mL$^{-1}$, 5 μL) was added firstly into 0.1 M HAc-NaAc buffer solution (pH 3.5, 995 μL) containing SOD (2 mg mL$^{-1}$, 10 μL; or 10 μL, 1 M mannitol), TMB (100 mM in DMSO, 10 μL) and H$_2$O$_2$ (10 M, 10 μL) then started the test quickly at room temperature. In the catalytic oxidation of Luminol, SOD (2 mg mL$^{-1}$, 50 μL; or 50 μL, 1 M mannitol), M-N-C nanozyme (2 mg mL$^{-1}$, 50 μL), H$_2$O$_2$ (10 M, 10 μL) and luminol (0.1 M, 50 μL) was added successively into 0.01 M NaOH (1800 μL) under stirring at room temperature after the timing diagram mode was started.

**Catalase-like activity of M-N-C nanozyme.**

The catalase-like property of M$_{0.5}$-N-C (M=Fe, Co) was studied by measuring the produced oxygen via a specific oxygen electrode on JPSJ-605 (Leici, China) in 0.1 M HAc-NaAc buffer and 0.01 M NaOH. Briefly, H$_2$O$_2$ (10 M, 0.02 mL) was added into the buffer solution (9.98 mL) containing M$_{0.5}$-N-C (2 mg mL$^{-1}$, 0.01 mL, or absence of nanozyme for control) under stirring at room temperature and the dissolved oxygen concentration (mg L$^{-1}$) was recorded at different time. The influence of TMB and Luminol on the decomposition of H$_2$O$_2$ catalyzed by M$_{0.5}$-N-C were carried out at the identical conditions except that the buffer solution also contains TMB (0.1 M, 0.1 mL) and Luminol (0.1 M, 0.1 mL) in 0.1 M HAc-NaAc buffer and 0.01 M NaOH, respectively.
Figure S1. SEM images of Fe_{0.5}-N-C (a), Fe_{0.15}-N-C (b), Fe_{1.5}-N-C (c) and metal free N-C (d) nanozymes.

Figure S2. SEM images of Mn_{0.5}-N-C (a), Co_{0.5}-N-C (b), Ni_{0.5}-N-C (c) and Cu_{0.5}-N-C (d) nanozymes.
Figure S3. TEM (a, c, e) and high-resolution TEM (b, d, f) images of Fe$_{0.5}$-N-C (a, b), Fe$_{1.5}$-N-C (c, d) and Fe$_{4.5}$-N-C (e, f) nanozymes, respectively.

Figure S4. N$_2$ adsorption and desorption isotherms (a, c) and pore size distribution (imitated using QSDFT; b, d) of N-C (a, b), all the Fe-N-C (a, b) and CB (c, d) nanozymes.
**Figure S5.** TEM (a, c, e) and high-resolution TEM (b, d, f) images of $\text{Co}_0.5$-N-C (a, b), $\text{Co}_1.5$-N-C (c, d) and $\text{Co}_4.5$-N-C (e, f) nanozymes, respectively.

**Figure S6.** $\text{N}_2$ adsorption and desorption isotherms (a) and pore size distribution (imitated using QSDFT; b) of Co-N-C nanozymes.
**Figure S7.** X-ray powder diffraction spectra of N-C and all the Fe-N-C nanozymes.

| Fe<sub>4.5</sub>-N-C | Fe<sub>1.5</sub>-N-C | Fe<sub>0.5</sub>-N-C | Fe<sub>0.15</sub>-N-C |
|---------------------|---------------------|---------------------|---------------------|
| N-C                 | N-C                 | N-C                 | N-C                 |

**Figure S8.** X-ray powder diffraction spectra of M<sub>0.5</sub>-N-C (M=Mn, Ni and Cu; a) and all the Co-N-C (b) nanozymes.

| Co<sub>4.5</sub>-N-C | Co<sub>1.5</sub>-N-C | Co<sub>0.5</sub>-N-C |
|---------------------|---------------------|---------------------|
| N-C                 | N-C                 | N-C                 |

**Figure S9.** Raman spectra of N-C (a), all the Fe-N-C (a), all the Co-N-C (b) and M<sub>0.5</sub>-N-C (M=Mn, Ni and Cu; c) nanozymes.

Raman spectrum of Fe<sub>0.5</sub>-N-C typically exhibited D band (~1350 cm<sup>-1</sup>) and G band (~1580 cm<sup>-1</sup>), which indicating the carbon disorder and crystallization, respectively. The high intensity ratio of D-to-G band (I<sub>D</sub>/I<sub>G</sub>) confirmed again the disorder in graphene basal planes, and it is negligibly influenced by the iron concentration. The other M-N-C was also composed of amorphous carbon.
Figure S10. Deconvoluted high-resolution N 1s spectra of N-C (a), Fe$_{0.15}$-N-C (b), Fe$_{1.5}$-N-C (c) and Fe$_{4.5}$-N-C (d) nanozymes.

Figure S11. Deconvoluted high-resolution N 1s spectra of Co$_{1.5}$-N-C (a) and Co$_{4.5}$-N-C (b) nanozymes.
Figure S12. Deconvoluted high-resolution N 1s spectra of Mn₀.₅-N-C (a), Ni₀.₅-N-C (b) and Cu₀.₅-N-C (c) nanozymes.
Figure S13. High-resolution Fe 2p spectra of all the Fe-N-C nanozymes.

Figure S14. High-resolution Co 2p spectra of all the Co-N-C nanozymes.
**Figure S15.** High-resolution Mn (a), Ni (b) and Cu (c) 2p spectra of M-N-C (M=Mn, Ni and Cu) nanozymes.

**Figure S16.** The typical Michaelis-Menten curves for 20 µg/mL Fe₉₅-N-C with TMB (a; [H₂O₂]: 0.1 M) and H₂O₂ (b; [TMB]: 1 mM) as substrate in 0.1 M HAc-NaAc (pH 3.5), respectively.
**Figure S17.** The initial velocity of catalytic oxidation of 1 mM TMB with 100 mM H$_2$O$_2$ in the presence of 20 μg/mL of Fe-N-C nanozymes in 0.1 M HAc-NaAc (pH 3.5).

The catalytic activity of Fe-N-C can be regulated by the iron content in the precursor. With increasing the iron content, the activity of Fe-N-C increases and reached a plateau, which presumably due to the existence of a maximum M-N$_x$ doping.

**Figure S18.** The pH (a) and temperature (b) dependent activity of the catalytic oxidation of 1 mM TMB with 100 mM H$_2$O$_2$ in the presence of 20 μg/mL Fe-N-C nanozyme.
**Figure S19.** The specific activity of Fe$_{0.5}$-N-C calculated using the nanozyme activity standardization method. [TMB]: 1 mM; [H$_2$O$_2$]: 100 mM.

**Figure S20.** The time evolution of chemiluminescence intensity at 425 nm for monitoring the catalytic oxidation of 2.5 mM luminol with 250 mM H$_2$O$_2$ in the presence of 50 µg mL$^{-1}$ Co-N-C nanozymes in 0.01 M NaOH.

**Figure S21.** The time evolution of chemiluminescence intensity at 425 nm for monitoring the catalytic oxidation of 2.5 mM luminol with 250 mM H$_2$O$_2$ in the presence of 50 µg mL$^{-1}$ Co$_{0.5}$-N-C nanozyme in 0.01 M NaOH with the different mixing orders of Co$_{0.5}$-N-C, H$_2$O$_2$ and luminol (S1:
Co$_{0.5}$-N-C, H$_2$O$_2$ and luminol in sequence; S2: luminol, Co$_{0.5}$-N-C and H$_2$O$_2$ in sequence; S3: luminol, H$_2$O$_2$ and Co$_{0.5}$-N-C in sequence).

To further investigate the catalytic behavior of Co$_{0.5}$-N-C, the different mixing orders of Co$_{0.5}$-N-C, H$_2$O$_2$ and luminol were operated. A slightly weakened light signal accompanied with weak decay was observed when injecting H$_2$O$_2$ into the mixture of Co$_{0.5}$-N-C and luminol, while the sharply enhanced emission intensity with accelerated damping was occurred when injecting Co$_{0.5}$-N-C into the mixture of H$_2$O$_2$ and luminol.

![Figure S22](image)

**Figure S22.** The influences of TMB and Luminol on the decomposition of H$_2$O$_2$ catalyzed by M$_{0.5}$-N-C (M = Fe, Co) in 0.1 M HAc-NaAc (a) and 0.01 M NaOH (b), respectively.

![Figure S23](image)

**Figure S23.** Top (a) and side (b) view of H$_2$O$_2$ adsorption on potential M-N-C nanozyme. The red, white, purple, blue and grey spheres represent oxygen, hydrogen, metal, nitrogen and carbon atoms, respectively.
The catalytic mechanism of Fe-N-C Catalase-like mechanism: 1) H$_2$O$_2$ binds to N coordinated Fe$^{III}$ of Fe-N-C to form Fe$^{III}$-superoxo species; 2) O-O cleavage of H$_2$O$_2$ to release a H$_2$O molecule with the oxidation of Fe$^{III}$ to Fe$^{V}$=O; 3) the second H$_2$O$_2$ is oxidized by Fe$^{V}$=O through two-step proton coupled electron transfer to form O$_2$ and H$_2$O molecular. Peroxidase-like mechanism: 1) H$_2$O$_2$ binds to N coordinated Fe$^{III}$ of Fe-N-C to form Fe$^{III}$-superoxo species; 2) O-O cleavage of H$_2$O$_2$ to release a H$_2$O molecule with the oxidation of Fe$^{III}$ to Fe$^{V}$=O; 3) two TMB are oxidized successively by Fe$^{V}$=O and Fe$^{IV}$=O through one-step proton coupled electron transfer to form oxidized TMB with the reduction of Fe$^{IV}$=O to Fe$^{III}$.

The catalytic mechanism of Co-N-C Catalase-like mechanism: 1) HO$_2^-$ binds to N coordinated Co$^{II}$ of Co-N-C to form Co$^{II}$-superoxo species; 2) O-O cleavage of HO$_2^-$ to release a OH$^-$ with the oxidation of Co$^{II}$ to Co$^{IV}$=O; 3) the second HO$_2^-$ is oxidized by Co$^{IV}$=O through two-step proton coupled electron transfer to form O$_2$ and OH$^-$. Peroxidase-like mechanism: 1) HO$_2^-$ binds to N coordinated Co$^{II}$ of Co-N-C to form Co$^{II}$-superoxo species; 2) O-O cleavage of HO$_2^-$ to release a OH$^-$ with the oxidation of Co$^{II}$ to Co$^{IV}$=O; 3) luminol binds to Co$^{IV}$=O to form luminol radical and Co$^{III}$-O; 4) the second HO$_2^-$ is oxidized by Co$^{III}$-O through one-step proton coupled electron transfer to form superoxide radical and OH$^-$ with the reduction of Co$^{III}$-O to Co$^{II}$. 5) the desorbed superoxide radical react with luminol radical.
Table S1. Specific surface area of N-C, all the Fe-N-C and Co-N-C nanozymes.

| Sample  | SSA  (m² g⁻¹) | SSAₘ (m² g⁻¹) | SSAₑ (m² g⁻¹) |
|---------|---------------|---------------|---------------|
| CB      | 1199.328      | 1188.060      | 11.268        |
| N-C     | 653.714       | 361.153       | 292.560       |
| Fe₀.₁₅-N-C | 551.837    | 308.141       | 243.696       |
| Fe₀.₅-N-C  | 490.074     | 260.118       | 229.956       |
| Fe₁.₅-N-C  | 390.629     | 87.177        | 303.451       |
| Fe₄.₅-N-C  | 378.542     | 117.676       | 260.866       |
| Co₀.₅-N-C  | 440.893     | 77.916        | 362.977       |
| Co₁.₅-N-C  | 327.500     | 77.398        | 250.102       |
| Co₄.₅-N-C  | 448.549     | 121.246       | 327.303       |

SSA is the Brunauer-Emmet-Teller (BET) specific surface area; SSAₘ is the micropore specific surface area and SSAₑ is the external specific surface area based on the t-plot method.

Table S2. The percentage of nitrogen species of N-C and M-N-C (M= Fe, Co, Mn, Ni and Cu) nanozymes.

| Nₓ-Metal and (or) xxx (%) | Pyridinic-N (%) | Pyrrolic-N (%) | Graphitic-N (%) | Oxidized-N (%) |
|---------------------------|-----------------|----------------|-----------------|---------------|
| N-C                       | 14.45           | 34.19          | 37.33           | 8.53          | 5.51          |
| Fe₀.₁₅-N-C                | 18.28           | 34.95          | 35.12           | 7.23          | 4.43          |
| Fe₀.₅-N-C                 | 18.49           | 32.98          | 35.91           | 7.45          | 5.17          |
| Fe₁.₅-N-C                 | 18.88           | 31.01          | 36.41           | 8.16          | 5.43          |
| Fe₄.₅-N-C                 | 18.99           | 32.04          | 35.95           | 8.63          | 4.89          |
| Co₀.₅-N-C                 | 18.04           | 33.74          | 34.69           | 8.32          | 5.22          |
| Co₁.₅-N-C                 | 19.58           | 35.53          | 33.81           | 6.68          | 4.41          |
| Co₄.₅-N-C                 | 19.72           | 29.15          | 36.00           | 9.13          | 6.00          |
| Mn₀.₅-N-C                 | 20.03           | 34.55          | 32.90           | 7.84          | 4.68          |
| Ni₀.₅-N-C                 | 20.01           | 34.90          | 32.74           | 7.30          | 5.04          |
| Cu₀.₅-N-C                 | 16.76           | 36.43          | 34.47           | 7.16          | 5.18          |
Table S3. The kinetic constants and specific activity of Fe_{0.5}-N-C.

|                | [E] (μg mL\(^{-1}\)) | \(K_m\) (mM) | \(v_{\text{max}}\) (μM s\(^{-1}\)) | SA (U mg\(^{-1}\)) |
|----------------|----------------------|---------------|-------------------------------|-------------------|
| Fe_{0.5}-N-C   | 20                   | TMB 0.8922    | 11.0108                       | 16.2679           |
|                |                      | H\(_2\)O\(_2\) 25.2612 | 6.3837                       |                   |

[E] is the nanozyme concentration, \(K_m\) is the Michaelis constant, \(v_{\text{max}}\) is the maximal reaction velocity and SA is the specific activity expressed in units per milligram.
