

Manganese Phthalocyanine Nanoassemblies Showing Stronger Peroxidase-like Activity than Porphyrins for Anti-tumor Therapy

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ABSTRACT:

Similar to porphyrins, phthalocyanine is a macrocyclic conjugated metal ligand, which is expected to achieve a highly efficient peroxidase-like (POD-like) catalytic effect, generating free radicals and inhibiting the proliferation of cancer cells. Herein, using a rapid self-assembly method, manganese phthalocyanine (MnPc) possessing a metal-N-C active center assembled into various nanostructures, depending on the added emulsifier. During the shrinkage of the microemulsion droplets, the hydrophobic chain of the emulsifier was inserted between the MnPc molecules to expose more active sites, showing stronger peroxidase-like catalytic activity than Mn porphyrin assemblies. The assembly had a highly efficient microenvironmental response to the catalytic activity and has a water-dispersed nanostructure, which is conducive to accumulation at the tumor site. Their higher catalytic activity only emerged under the acidic tumor-like microenvironment, which causes less damage to normal tissues in biomedical applications. In vivo and in vitro catalytic therapy tests showed excellent anti-tumor effects. This work explored a new way for the application of metal-organic macromolecules such as MnPc as nanozymes for catalytic tumor therapy.

KEYWORDS:

Self-assembly, Manganese Phthalocyanine, Nanozymes, Peroxidase, Catalytic Therapy

As engineered enzyme mimics, nanozymes have excellent catalytic activities under physiological conditions.¹⁻⁵ In addition, nanozymes have higher stability and lower cost, and have been extensively used in biomedical fields such as biosensors, theranostics and antibacterial applications.⁶⁻¹⁰ Among them, nanozymes are particularly attractive for disease treatment such as cancer therapy.¹¹⁻¹⁵

Tumor tissues have a special microenvironment that is different from normal tissues, including lower pH and an excessive H₂O₂ content.^{16, 17} Therefore, nanozyme-based tumor therapy in response to tumor microenvironment (TME) might exhibit high specificity for tumors and low toxicity to normal tissues. Some nanozymes can specifically generate reactive oxygen species (ROS) at tumor sites, which can induce tumor cell apoptosis or necrosis. Previous work indicated that iron oxide nanoparticles (IONPs) have peroxidase-like (POD-like) catalytic activity in the tumor microenvironment.¹⁸⁻²¹ Fe atoms can effectively trigger the tumor-specific Fenton reaction in the acidic tumor microenvironment, which produces a large amount of toxic hydroxyl radicals ($\bullet$ OH), thereby effectively inhibiting tumors and avoiding damages to normal tissues.^{22, 23} In addition, manganese (Mn)-based materials have also been confirmed to exhibit POD-like activities.^{24, 25} Furthermore, Mn is a key cofactor for a variety of metallo-enzymes, playing important regulatory roles to affect metabolism and biological redox reactions.²⁶⁻²⁸

Apart from inorganic nanomaterials, some carbon-based nanomaterials with better biocompatibility also possess excellent POD-like activities.²⁹⁻³¹ Gao and co-workers used density functional theory (DFT) to reveal that large aromatic domains played a significant role for carbon-based nanomaterials as peroxidase mimics.³² Porphyrins are macrocyclic compounds containing C and N.^{33, 34} The porphyrin center can chelate different metal ions to form metalloporphyrins, and in particular, Fe porphyrins have been used to mimic enzyme catalysis.³⁵⁻³⁷

Similar to porphyrin, phthalocyanine (Pc) is a synthetic macromolecule with an 18 π -electron conjugated structure. It has good biocompatibility and has been widely used in bio-phototherapy.³⁸⁻⁴¹ Pcs are thermally stable with rich redox properties, which can be easily adjusted by changing the metal center. In addition, for variable valence metal ions, they usually exhibit the lower valence state when chelated in phthalocyanines, and the low metal

valence state is more conducive to the decomposition of H_2O_2 to generate $\cdot\text{OH}$ in acidic systems.^{42, 43} However, metal phthalocyanines are difficult to be directly used in biological environment because of their poor water solubility. This solubility problem might be solved by assembly of Pcs to form nanomaterials, similar to what has done for porphyrins and their derivatives.⁴⁴⁻⁴⁶ However, highly ordered assembly can also mask more active sites while enhancing electron transport between molecules. Thus, shorter assembly cycles and more disorganized assemblies are expected to have better catalytic activities (**Figure S1a**).

Herein, we report a MnPc assembly with good water dispersibility via supramolecular self-assembly (**Figure 1a**). Mn phthalocyanine molecules (MnPcs, **Figure S1b**) were dissolved in an oil-soluble reagent such as chloroform (CHCl_3), and mixed with an aqueous solution of emulsifier using high-intensity ultrasound to form an emulsion. At a high temperature (63°C), the chloroform was quickly volatilized from the microemulsion droplets, thereby obtaining MnPc nanomaterials coated with emulsifiers on the surface. The hydrophobic chains of emulsifiers were inserted between the MnPc molecules to increase the molecular distance, thereby exposing more active sites.

According to our previous work, the choice of different emulsifiers can impact the morphology of the final assemblies. For our current experiment, spherical nanoparticle assemblies (MnPcNPs) were obtained when sodium dodecyl sulfate (SDS) was used, whereas linear assemblies (MnPcNWs) were obtained by using sodium dodecyl benzene sulfonate (SDBS) under the same experimental conditions. As shown in scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images (**Figure 1b, c**), the MnPcNPs were well dispersed with a relatively regular morphology and a diameter of ~ 62 nm (**Figure 1c inset**). Meanwhile, the linear MnPcNWs were generally more than $1\ \mu\text{m}$ in length (**Figure 1d, e**). As observed by ultraviolet-visible spectroscopy (UV-vis, **Figure S2a**), the absorption of the assemblies has undergone different degrees of broadening and red shift compared with single molecules. The crystal structures of the MnPc powder and MnPc assemblies were further characterized by X-ray diffraction (XRD, **Figure S2b**), and the assembled MnPcNWs and MnPcNPs show different crystal structures. The assembly maintains the large conjugated structure of the assembled element, and at the same time increases the weak bond interaction between molecules, further improves the electron transfer ability, and is expected to achieve

an efficient catalytic effect.

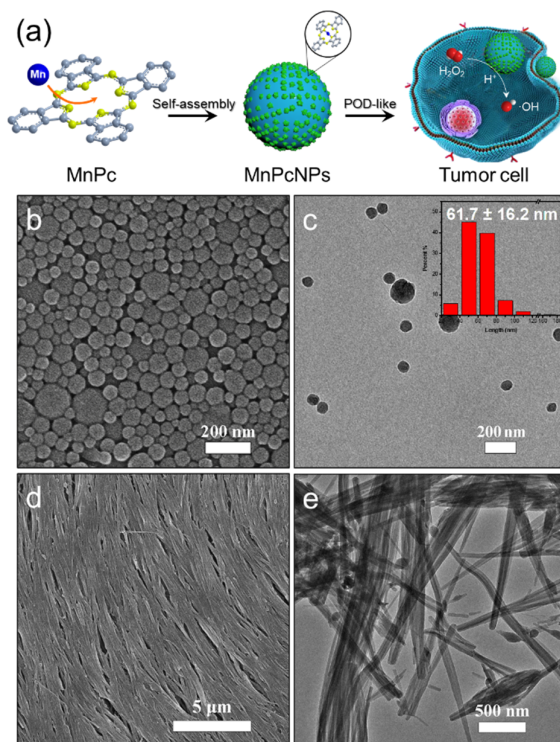


Figure 1. Schematic diagram of the synthesis of MnPc assemblies and the corresponding catalytic treatment of tumor microenvironment (a) and morphology characterization of MnPc assemblies. (b) (d) SEM and (c) (e)TEM images of MnPcNPs and MnPcNWs, and the inset in (c) is a particle size histogram of the MnPcNPs.

It was reported that nanozymes with peroxidase-like (POD-like) activity can induce the decomposition of H_2O_2 to generate $\cdot\text{OH}$, thereby catalyzing the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) to oxidized TMB (oxTMB). The blue oxTMB has an absorption peak at 652 nm ($\epsilon=3.9\times10^4 \text{ M}^{-1}\text{cm}^{-1}$), allowing UV-vis based characterization (**Figure 2a**).^{43, 47} The valence state of the metal ion as the catalytic center was tested by X-ray photoelectron spectroscopy (XPS, **Figure 2b**). As determined by the results, both of the MnPc assemblies showed four characteristic peaks: C 1s (285 eV), O 1s (530 eV), N 1s (397 eV), and Mn 2p (641 eV), which were consistent with the MnPc powder before the self-assembly. In addition, the characteristic Mn 2p peaks of the MnPc assemblies (**Figure 2c**) were also consistent with those of the MnPc powder, suggesting that the oxidation state of Mn remained +2 during the

assembly process, and low-valence metals valence state be beneficial to the decomposition of H_2O_2 to generate $\cdot\text{OH}$ under acidic conditions. Therefore, the assembly can solve the water dispersion of the MnPc monomers while maintaining a low-valence central metal.

During the test, the catalytic activity was closely related to the pH and temperature of the reaction system (**Figure S3**), where the maximal activity was reached under weakly acidic conditions (pH 5.2~6.0) at 35°C , similar to the TME. In addition, in neutral and alkaline environments, the MnPc assemblies hardly had any POD-like activity, which would have less damages to normal tissues. The kinetics of the reactions were studied with different concentrations of TMB and a fixed concentration of H_2O_2 (0.53 M). Under the most optimal catalytic conditions, the absorbance value of the reaction system at 652 nm increased with the increase and finally reached a plateau (**Figure 2d**). Interestingly, the catalytic activity of the MnPcNPs was significantly higher than that of the MnPcNWs at the same concentration of the assemblies (**Figure S4**).

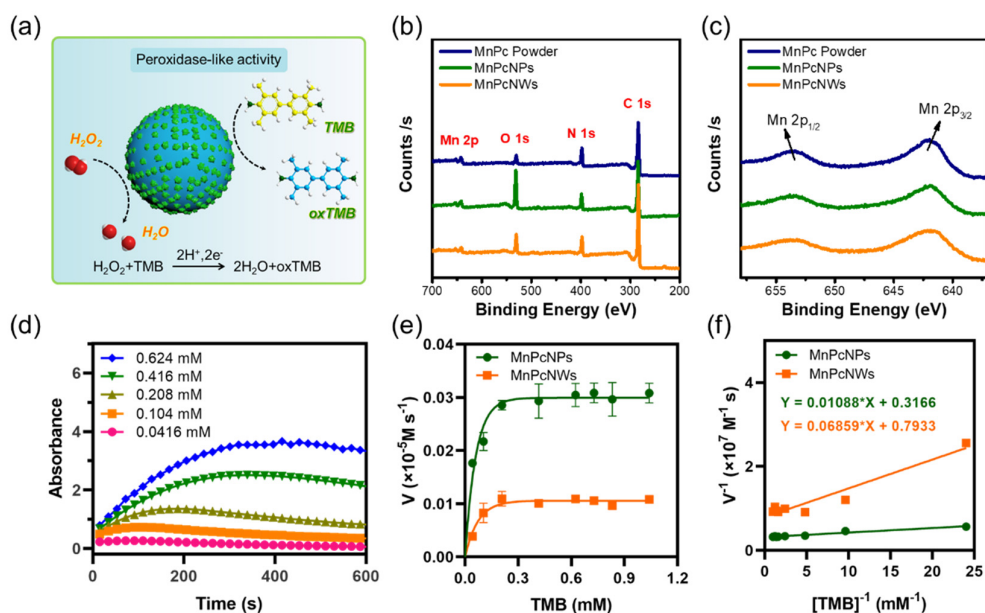


Figure 2. Characterizations of enzyme-like activities of MnPc assemblies. (a) Schematic presentation of peroxidase-like (POD-like) activity of MnPc assemblies. (b) XPS survey spectra and (c) Mn 2p_{3/2} core-level XPS spectra of Mn-Pc monomers, MnPcNPs and MnPcNWs. (d) Time-course absorbance@652nm of MnPcNPs upon the addition of varied concentrations of TMB as a reaction substrate. (e) Michaelis-Menten kinetics and (f) Lineweaver-Burk plotting of Kinetics for POD-like activity of MnPc assemblies using

different concentrations of TMB. The initial velocities were calculated in six periods (15 s per period) in one experiment.

We then studied the Michaelis-Menten steady-state kinetics with H₂O₂ and TMB substrates in order to systematically evaluate the catalytic performance of the system.¹² Specifically, the corresponding average initial velocities were calculated through the time-dependent absorbance change curves, and all the average initial velocity of absorbance changes were converted to the initial velocity of generating the cationic radical product (v_0) through Beer-Lambert law (Eq. 1 in **Figure S5**), which was plotted with the corresponding substrate concentration to fit with the Michaelis-Menten equation (Eq. 2 in **Figure S5**) as shown in **Figure 2e and S6a**. Then, a double-reciprocal plot in **Figure 2f and S6b** was obtained (Eq. 3, **Figure S5**) to determine the Michaelis-Menten constant (K_M) and maximum velocity (V_{max}). The K_M and V_{max} were calculated to be 0.034 mM and $3.16 \times 10^{-7} \text{ M s}^{-1}$ for MnPcNPs. Its V_{max} for TMB and H₂O₂ were 2.5 and 1.7-fold of that of the MnPcNWs, respectively (**Supplementary Table 1**).

To explore the source of the catalytic differences between different assembled structures, the surface elemental compositions of the MnPc assemblies were compared (**Supplementary Table 2**), which was different for the MnPcNPs, MnPcNWs and MnPc powders, suggesting that the type and amount of emulsifier covered on the surface of the assembled nanomaterials were different. The surface S element came from the emulsifier. The surface of MnPcNPs has the largest proportion of S element (6.89%), which means that more emulsifiers were wrapped on the surface, but its surface C element was the least of the three. Therefore, we speculated that in the process of forming MnPcNPs, the hydrophobic alkyl chain in the emulsifier was between the MnPc molecules (**Figure S7**), which increased the distance between MnPc molecules and also increased the contact area. It is worth noting that the proportion of Mn on the surface of MnPcNPs was close to that of unassembled MnPc molecules, indicating that more active sites were exposed.

It was reported that for enzyme or chemical catalysis, the POD-like catalytic reactions are similar to the oxygen reduction in ORR (oxygen reduction reaction).^{31, 48} For nitrogen-doped carbon materials (N-CNMs) such as phthalocyanine, the metal-N-C structure can

significantly improve the catalytic performance of ORR.⁴⁹ Related research also pointed out that the metal-N active site in the metal-N-C structure plays a key role in the ORR of acidic systems, and more single-atom metal sites can improve the catalytic performance.⁵⁰ Compared with N-doped nanocarbon or graphene oxide with the POD-like catalytic performance, the MnPcNPs had better substrate affinity and a greater reaction rate as shown in **supplementary Table 3**.^{31, 51}

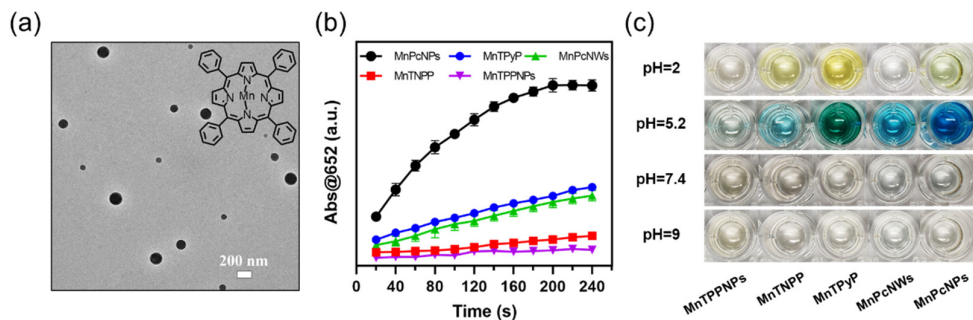


Figure 3. (a) Morphology TEM characterization of MnTPPNPs. (b) The time-dependent absorbance change curves of MnPc assemblies, MnTPP assemblies, MnTPPy and MnTNPP monomers. (c) Photographs of TMB peroxidation catalyzed by different materials in 0.025 mg/mL with pH of 2 to 9.

Furthermore, we compared the catalytic activity of the MnPc and a few Mn porphyrins. We first prepared the MnTPP (molecular structure shown in **Figure S8a**) assemblies (MnTPPNPs) by the microemulsion-assisted method, whose morphology (**Figure 3a**) was similar to the MnPcNPs. In addition, we selected two water-soluble porphyrins (MnTNPP and MnTPPy, **Figure S8b, c**) as controls to further analyze the effect of porphyrin substituents on the catalytic activity. As observed by the reaction kinetic curves, the MnPcNPs had the fastest reaction rate (**Figure 3b**). Interestingly, the MnTPPNPs had almost no activity at all. In some reports, it was only pointed out that manganese porphyrin has the catalytic activity of superoxide dismutase (SOD), which could not generate ROS.^{52, 53}

The catalytic performance at different pH was more intuitively reflected by the change of color (**Figure 3c**). The color difference trend presented at pH 5.2 was consistent with **Figure 3b**. These results suggest that the catalytic activity of the MnPc assemblies was higher than that of the Mn porphyrin assemblies, which might be related to the larger N doping ratio, the

larger conjugated structure and different oxidation states of the metal. Furthermore, the electron-withdrawing substituent (MnTPyP) can induce stronger catalytic activity than the electron-donating substituent for the water-dispersed Mn porphyrin monomer.⁵⁴⁻⁵⁶

After finding the high catalytic activity of the MnPcNPs, in vitro experiments on the tumor cells survival were carried out by a standard Cell Counting Kit-8 (CCK-8) assay. The proliferation activity of HeLa cells was less affected by changes in the incubation environment or treated with MnPcNPs without adding H₂O₂ (**Figure S9**). With 100 μ M H₂O₂ to simulate the H₂O₂ level in the tumor microenvironment, the survival rate of HeLa cells was significantly reduced when the concentration of the MnPcNPs was 100 μ g/mL in the acidic medium (pH 6.0). In the neutral incubation environment, the survival rate of HeLa cells was improved (**Figure 4a and 4b**).

The intracellular $\bullet$ OH due to the MnPcNPs could be further observe through confocal laser scanning microscopy (CLSM) by using DCFH-DA as a fluorescent probe. DCFH-DA can be immediately oxidized to generate DCF when $\bullet$ OH is present, and green fluorescent signal can be detected. The fluorescence in the HeLa cells treated with MnPcNPs+H₂O₂ was greatly enhanced compared with that after treatment with MnPcNPs or H₂O₂ (**Figure 4c**). In addition, the treated HeLa cells were stained by calcein-AM (living cells, green fluorescence) and PI (dead cells, red fluorescence). The HeLa cells treated with MnPcNPs+H₂O₂ mainly showed red fluorescence under the slightly acidic environment (**Figure 4d**), which confirmed that the production of $\bullet$ OH caused apoptosis. In addition, the cytotoxicity induced by MnPcNPs on cancer cells was alleviated by adding an antioxidant ascorbic acid (AA, vitamin C), which further indicated that the cytotoxicity of the cancer cells stems from the oxidative damage of active free radicals generated by the MnPcNPs (**Figure S10**).

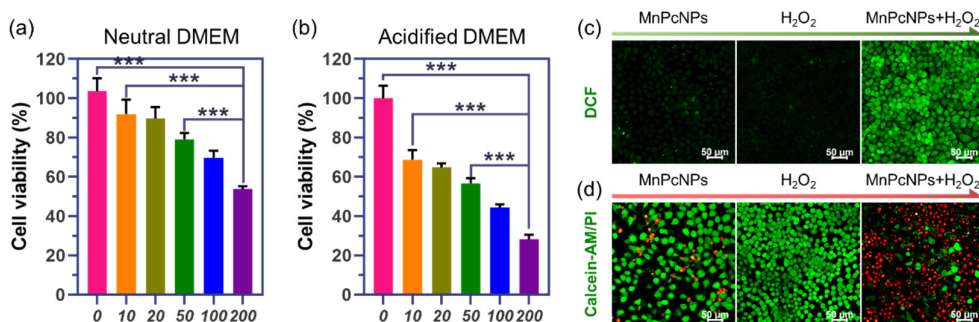


Figure 4. In vitro catalytic therapy of MnPc assemblies and its effect on cell viability. (a)

HeLa tumor cells viability assays of MnPcNPs + H₂O₂ under neutral culture media (pH = 7.4) and (b) acidified (pH = 6.0). Values are presented as means $\pm$ s.d. (six replicates). *P < 0.05, **P < 0.01, ***P < 0.001. Confocal microscopic images (CLSM) for cells treated with MnPcNPs, H₂O₂ and MnPcNPs + H₂O₂ to (c) DCFH-DA cell-staining or (d) Calcein-AM/PI cell-staining, respectively. Conditions: MnPcNPs: 100 μ g/mL, H₂O₂: 100 μ M, pH 6.0.

The effects of MnPc assemblies with different morphologies on cell internalization and generation of active free radicals in a slightly acidic environment containing H₂O₂ (100 μ M) were also compared. Using CLSM (**Figure S11**), Pc can produce a red fluorescence emission peak similar to porphyrin, which can be used for biological imaging. Based on the red fluorescence, the MnPcNPs appeared to be internalized more than MnPcNWs, which could be due to smaller size of the MnPcNPs.⁵⁷ There was also a difference between the two MnPc assemblies from the green fluorescence intensity of the detected DCF probe, which was attributed to the difference between the catalyzed production of •OH. According to these results, we confirmed that MnPcNPs can be more easily internalized by HeLa cells and cause PDT-like effect to produce •OH in the cells.

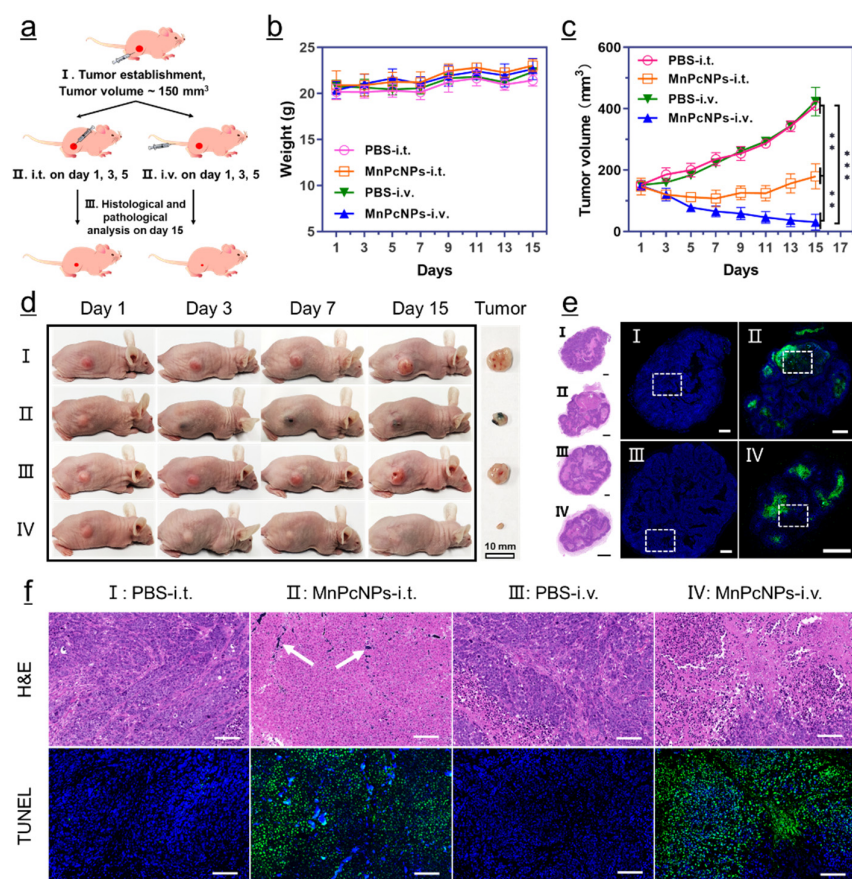


Figure 5. In vivo nanozyme-based tumor treatment of MnPcNPs against HeLa tumor xenografts. (a) Schematic diagram of HeLa tumor xenograft establishment and treatment evaluation. (b) Body weight and (c) tumor volume changes of HeLa tumor-bearing mice after different treatment time. Values are presented as means $\pm$ s.d. (n = 5 per group), *P < 0.05, **P < 0.01, ***P < 0.001). (d) Photographs of the mice during the therapy process. (e) Panoramic H&E and TUNEL staining images of the entire tumor area. Scale bars = 1000 μm. (I, PBS-i.t.; II, MnPcNPs-i.t.; III, PBS-i.v.; IV, MnPcNPs-i.v.). (f) Enlarged view of the area in Figure 5e, and the white arrow indicates the position of MnPcNPs remaining in the tumor area. Scale bars = 100 μm.

Finally, the in vivo catalytic anti-tumor therapeutic properties of MnPcNPs were evaluated after 15 days of treatment by intratumor (i.t.) or intravenous (i.v.) injection (**Figure 5a**). Throughout the treatment cycle, the weight of the mice in each group remained stable and in satisfactory conditions (**Figure 5b**). The tumors of PBS-treated mice continued to grow

rapidly, while MnPcNPs with i.t. and i.v. could significantly inhibit tumor growth (**Figure 5c**). The treatment results can also be observed intuitively from the photographs of each mouse group at different treatment times and the dissected tumors (**Figure 5d**).

To further assess the *in vivo* catalytic therapeutic effect, histological analysis of tumors by hematoxylineosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick end labeling assay (TUNEL) were carried out. The tumors in the control group had no obvious apoptosis, while the tumor volume of the mice receiving drug treatment was significantly reduced. In addition, and the internal dead area increased compared with the control group (green fluorescence in **Figure 5e**). A large number of tumor cells apoptosis in the area where the drug remains in the i.t. group was observed through the enlarged staining image. Owing to the enhanced permeability and retention effect (EPR) of solid tumors in the i.v. group, the drugs can be evenly distributed in the tumors, resulting in more apoptosis (**Figure 5f**). No obvious lesions occurred in the main organs of the mice though the H&E staining analysis after 15 days of treatment (**Figure S12**). Blood routine and blood biochemical analysis also showed excellent liver and kidney safety (**Figure S13**), which confirmed *in vivo* biocompatibility of the MnPcNPs.

In summary, we accelerated the assembly process to insert the hydrophobic alkyl chain of the emulsifier between the metal phthalocyanine molecules to expand the molecular distance while exposing more active sites. The MnPcNPs showed a high POD-like catalytic performance, better than the tested Mn porphyrin assemblies. Moreover, the catalysis of MnPcNPs can be achieved under conditions similar to the tumor microenvironment, which reduces damage to normal tissues. It shows excellent effects in anti-tumor catalytic therapy *in vivo* and *in vitro*. These results provide a promising strategy for promoting tumor ablation and greatly reduce the side effects on normal tissues/organs.

Supporting Information

Supporting Information Available:

AUTHOR INFORMATION

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

1. Gao, L.; Zhuang, J.; Nie, L.; Zhang, J.; Zhang, Y.; Gu, N.; Wang, T.; Feng, J.; Yang, D.; Perrett, S.; Yan, X., Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat Nanotechnol* 2007, 2 (9), 577-83.
2. Gao, L.; Yan, X., Nanozymes: an emerging field bridging nanotechnology and biology. *Sci China Life Sci* **2016**, 59 (4), 400-2.
3. He, W.; Wamer, W.; Xia, Q.; Yin, J. J.; Fu, P. P., Enzyme-like activity of nanomaterials. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* **2014**, 32 (2), 186-211.
4. Wei, H.; Wang, E., Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. *Chem. Soc. Rev.* **2013**, 42 (14), 6060-93.
5. Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H., Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). *Chem. Soc. Rev.* **2019**, 48 (4), 1004-1076.
6. Wang, X.; Hu, Y.; Wei, H., Nanozymes in bionanotechnology: from sensing to therapeutics and beyond. *Inorganic Chemistry Frontiers* **2016**, 3 (1), 41-60.
7. Huang, Y.; Ren, J.; Qu, X., Nanozymes: Classification, Catalytic Mechanisms, Activity Regulation, and Applications. *Chem Rev* **2019**, 119 (6), 4357-4412.
8. Li, Y.; Liu, J., Nanozyme's catching up: activity, specificity, reaction conditions and reaction types. *Materials Horizons* **2021**, 8 (2), 336-350.
9. Chang, Y.; Gao, S.; Liu, M.; Liu, J., Designing signal-on sensors by regulating nanozyme activity. *Anal Methods* **2020**, 12 (39), 4708-4723.
10. Chen, Z. J.; Huang, Z.; Sun, Y. M.; Xu, Z. L.; Liu, J., The Most Active Oxidase-Mimicking Mn₂O₃ Nanozyme for Biosensor Signal Generation. *Chemistry* **2021**, 27 (37), 9597-9604.
11. Zhu, P.; Chen, Y.; Shi, J., Nanoenzyme-Augmented Cancer Sonodynamic Therapy by Catalytic Tumor Oxygenation. *ACS Nano* **2018**, 12 (4), 3780-3795.

12. Huo, M.; Wang, L.; Chen, Y.; Shi, J., Tumor-selective catalytic nanomedicine by nanocatalyst delivery. *Nat Commun* **2017**, *8* (1), 357.
13. Wang, L.; Huo, M.; Chen, Y.; Shi, J., Iron-engineered mesoporous silica nanocatalyst with biodegradable and catalytic framework for tumor-specific therapy. *Biomaterials* **2018**, *163*, 1-13.
14. Liang, Q.; Xi, J.; Gao, X. J.; Zhang, R.; Yang, Y.; Gao, X.; Yan, X.; Gao, L.; Fan, K., A metal-free nanozyme-activated prodrug strategy for targeted tumor catalytic therapy. *Nano Today* **2020**, *35*.
15. Chen, Q.; Liu, Y.; Liu, J.; Liu, J., Liposome-Boosted Peroxidase-Mimicking Nanozymes Breaking the pH Limit. *Chemistry* **2020**, *26* (70), 16659-16665.
16. Webb, B. A.; Chimenti, M.; Jacobson, M. P.; Barber, D. L., Dysregulated pH: a perfect storm for cancer progression. *Nat. Rev. Cancer* **2011**, *11* (9), 671-7.
17. Bai, J.; Jia, X.; Zhen, W.; Cheng, W.; Jiang, X., A Facile Ion-Doping Strategy To Regulate Tumor Microenvironments for Enhanced Multimodal Tumor Theranostics. *J. Am. Chem. Soc.* **2018**, *140* (1), 106-109.
18. Jana, D.; Wang, D.; Bindra, A. K.; Guo, Y.; Liu, J.; Zhao, Y., Ultrasmall Alloy Nanozyme for Ultrasound- and Near-Infrared Light-Promoted Tumor Ablation. *ACS Nano* **2021**, *15* (4), 7774-7782.
19. Cheng, G.; Zong, W.; Guo, H.; Li, F.; Zhang, X.; Yu, P.; Ren, F.; Zhang, X.; Shi, X.; Gao, F.; Chang, J.; Wang, S., Programmed Size-Changeable Nanotheranostic Agents for Enhanced Imaging-Guided Chemo/Photodynamic Combination Therapy and Fast Elimination. *Adv. Mater.* **2021**, *33* (21), e2100398.
20. Ma, P.; Xiao, H.; Yu, C.; Liu, J.; Cheng, Z.; Song, H.; Zhang, X.; Li, C.; Wang, J.; Gu, Z.; Lin, J., Enhanced Cisplatin Chemotherapy by Iron Oxide Nanocarrier-Mediated Generation of Highly Toxic Reactive Oxygen Species. *Nano Lett.* **2017**, *17* (2), 928-937.
21. Liu, Y.; Zhen, W.; Jin, L.; Zhang, S.; Sun, G.; Zhang, T.; Xu, X.; Song, S.; Wang, Y.; Liu, J.; Zhang, H., All-in-One Theranostic Nanoagent with Enhanced Reactive Oxygen Species Generation and Modulating Tumor Microenvironment Ability for Effective Tumor Eradication. *ACS Nano* **2018**, *12* (5), 4886-4893.
22. Ranji-Burachaloo, H.; Gurr, P. A.; Dunstan, D. E.; Qiao, G. G., Cancer Treatment through Nanoparticle-Facilitated Fenton Reaction. *ACS Nano* **2018**, *12* (12), 11819-11837.
23. Huo, M.; Wang, L.; Wang, Y.; Chen, Y.; Shi, J., Nanocatalytic Tumor Therapy by Single-Atom Catalysts. *ACS Nano* **2019**, *13* (2), 2643-2653.
24. Zhu, Y.; Wang, W.; Cheng, J.; Qu, Y.; Dai, Y.; Liu, M.; Yu, J.; Wang, C.; Wang, H.; Wang, S.; Zhao, C.; Wu, Y.; Liu, Y., Stimuli-Responsive Manganese Single-Atom Nanozyme for Tumor Therapy via Integrated Cascade Reactions. *Angew. Chem. Int. Ed. Engl.* **2021**, *60* (17), 9480-9488.
25. Lu, W.; Chen, J.; Kong, L.; Zhu, F.; Feng, Z.; Zhan, J., Oxygen vacancies modulation Mn₃O₄ nanozyme with enhanced oxidase-mimicking performance for l-cysteine detection. *Sensors Actuators B: Chem.* **2021**, *333*.
26. Lv, M.; Chen, M.; Zhang, R.; Zhang, W.; Wang, C.; Zhang, Y.; Wei, X.; Guan, Y.; Liu, J.; Feng, K.; Jing, M.; Wang, X.; Liu, Y. C.; Mei, Q.; Han, W.; Jiang, Z., Manganese is critical for antitumor immune responses via cGAS-STING and improves the efficacy of clinical immunotherapy. *Cell Res.* **2020**, *30* (11), 966-979.

27. Waldron, K. J.; Rutherford, J. C.; Ford, D.; Robinson, N. J., Metalloproteins and metal sensing. *Nature* **2009**, *460* (7257), 823-30.
28. Signorella, S.; Palopoli, C.; Ledesma, G., Rationally designed mimics of antioxidant manganoenzymes: Role of structural features in the quest for catalysts with catalase and superoxide dismutase activity. *Coord. Chem. Rev.* **2018**, *365*, 75-102.
29. Zhang, J.; Lu, X.; Tang, D.; Wu, S.; Hou, X.; Liu, J.; Wu, P., Phosphorescent Carbon Dots for Highly Efficient Oxygen Photosensitization and as Photo-oxidative Nanozymes. *ACS Appl Mater Interfaces* **2018**, *10* (47), 40808-40814.
30. Ding, H.; Hu, B.; Zhang, B.; Zhang, H.; Yan, X.; Nie, G.; Liang, M., Carbon-based nanozymes for biomedical applications. *Nano Research* **2020**, *14* (3), 570-583.
31. Fan, K.; Xi, J.; Fan, L.; Wang, P.; Zhu, C.; Tang, Y.; Xu, X.; Liang, M.; Jiang, B.; Yan, X.; Gao, L., In vivo guiding nitrogen-doped carbon nanozyme for tumor catalytic therapy. *Nat Commun* **2018**, *9* (1), 1440.
32. Zhao, R.; Zhao, X.; Gao, X., Molecular-level insights into intrinsic peroxidase-like activity of nanocarbon oxides. *Chemistry* **2015**, *21* (3), 960-4.
33. Hiroto, S.; Miyake, Y.; Shinokubo, H., Synthesis and Functionalization of Porphyrins through Organometallic Methodologies. *Chem Rev* **2017**, *117* (4), 2910-3043.
34. Park, J. M.; Hong, K. I.; Lee, H.; Jang, W. D., Bioinspired Applications of Porphyrin Derivatives. *Acc. Chem. Res.* **2021**, *54* (9), 2249-2260.
35. Gao, Z.; Li, Y.; Zhang, Y.; Cheng, K.; An, P.; Chen, F.; Chen, J.; You, C.; Zhu, Q.; Sun, B., Biomimetic Platinum Nanozyme Immobilized on 2D Metal-Organic Frameworks for Mitochondrion-Targeting and Oxygen Self-Supply Photodynamic Therapy. *ACS Appl Mater Interfaces* **2020**, *12* (2), 1963-1972.
36. Ling, P.; Cheng, S.; Chen, N.; Qian, C.; Gao, F., Nanozyme-Modified Metal-Organic Frameworks with Multienzymes Activity as Biomimetic Catalysts and Electrocatalytic Interfaces. *ACS Appl Mater Interfaces* **2020**, *12* (15), 17185-17192.
37. Cao-Milán, R.; Gopalakrishnan, S.; He, L. D.; Huang, R.; Wang, L.-S.; Castellanos, L.; Luther, D. C.; Landis, R. F.; Makabenta, J. M. V.; Li, C.-H.; Zhang, X.; Scaletti, F.; Vachet, R. W.; Rotello, V. M., Thermally Gated Bio-orthogonal Nanozymes with Supramolecularly Confined Porphyrin Catalysts for Antimicrobial Uses. *Chem* **2020**, *6* (5), 1113-1124.
38. Zheng, B.-D.; He, Q.-X.; Li, X.; Yoon, J.; Huang, J.-D., Phthalocyanines as contrast agents for photothermal therapy. *Coord. Chem. Rev.* **2021**, 426.
39. Lo, P. C.; Rodriguez-Morgade, M. S.; Pandey, R. K.; Ng, D. K. P.; Torres, T.; Dumoulin, F., The unique features and promises of phthalocyanines as advanced photosensitisers for photodynamic therapy of cancer. *Chem. Soc. Rev.* **2020**, *49* (4), 1041-1056.
40. Chow, S. Y. S.; Wong, R. C. H.; Zhao, S.; Lo, P. C.; Ng, D. K. P., Disulfide-Linked Dendritic Oligomeric Phthalocyanines as Glutathione-Responsive Photosensitizers for Photodynamic Therapy. *Chemistry* **2018**, *24* (22), 5779-5789.
41. Shi, W. J.; Ng, D. K. P.; Zhao, S.; Lo, P. C., A Phthalocyanine - Based Glutathione - Activated Photosensitizer with a Ferrocenyl Boron Dipyrromethene Dark Quencher for Photodynamic Therapy. *ChemPhotoChem* **2019**, *3* (10), 1004-1013.
42. Ma, B.; Wang, S.; Liu, F.; Zhang, S.; Duan, J.; Li, Z.; Kong, Y.; Sang, Y.; Liu, H.; Bu, W.; Li, L., Self-Assembled Copper-Amino Acid Nanoparticles for in Situ Glutathione "AND" H₂O₂ Sequentially Triggered Chemodynamic Therapy. *J. Am. Chem. Soc.* **2019**, *141* (2),

849-857.

43. Xu, B.; Cui, Y.; Wang, W.; Li, S.; Lyu, C.; Wang, S.; Bao, W.; Wang, H.; Qin, M.; Liu, Z.; Wei, W.; Liu, H., Immunomodulation-Enhanced Nanozyme-Based Tumor Catalytic Therapy. *Adv. Mater.* **2020**, *32* (33), e2003563.
44. Wang, J.; Zhong, Y.; Wang, X.; Yang, W.; Bai, F.; Zhang, B.; Alarid, L.; Bian, K.; Fan, H., pH-Dependent Assembly of Porphyrin-Silica Nanocomposites and Their Application in Targeted Photodynamic Therapy. *Nano Lett.* **2017**, *17* (11), 6916-6921.
45. Wang, D.; Niu, L.; Qiao, Z. Y.; Cheng, D. B.; Wang, J.; Zhong, Y.; Bai, F.; Wang, H.; Fan, H., Synthesis of Self-Assembled Porphyrin Nanoparticle Photosensitizers. *ACS Nano* **2018**, *12* (4), 3796-3803.
46. Wang, X.; Wang, J.; Wang, J.; Zhong, Y.; Han, L.; Yan, J.; Duan, P.; Shi, B.; Bai, F., Noncovalent Self-Assembled Smart Gold(III) Porphyrin Nanodrug for Synergistic Chemo-Photothermal Therapy. *Nano Lett.* **2021**, *21* (8), 3418-3425.
47. Li, S.; Shang, L.; Xu, B.; Wang, S.; Gu, K.; Wu, Q.; Sun, Y.; Zhang, Q.; Yang, H.; Zhang, F.; Gu, L.; Zhang, T.; Liu, H., A Nanozyme with Photo-Enhanced Dual Enzyme-Like Activities for Deep Pancreatic Cancer Therapy. *Angew. Chem. Int. Ed. Engl.* **2019**, *58* (36), 12624-12631.
48. Liang, H. W.; Zhuang, X.; Bruller, S.; Feng, X.; Mullen, K., Hierarchically porous carbons with optimized nitrogen doping as highly active electrocatalysts for oxygen reduction. *Nat Commun* **2014**, *5*, 4973.
49. He, Y.; Liu, S.; Priest, C.; Shi, Q.; Wu, G., Atomically dispersed metal-nitrogen-carbon catalysts for fuel cells: advances in catalyst design, electrode performance, and durability improvement. *Chem. Soc. Rev.* **2020**, *49* (11), 3484-3524.
50. Chen, M. X.; Zhu, M.; Zuo, M.; Chu, S. Q.; Zhang, J.; Wu, Y.; Liang, H. W.; Feng, X., Identification of Catalytic Sites for Oxygen Reduction in Metal/Nitrogen-Doped Carbons with Encapsulated Metal Nanoparticles. *Angew. Chem. Int. Ed. Engl.* **2020**, *59* (4), 1627-1633.
51. Song, Y.; Qu, K.; Zhao, C.; Ren, J.; Qu, X., Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection. *Adv. Mater.* **2010**, *22* (19), 2206-10.
52. Tovmasyan, A.; Carballal, S.; Ghazaryan, R.; Melikyan, L.; Weitner, T.; Maia, C. G.; Reboucas, J. S.; Radi, R.; Spasojevic, I.; Benov, L.; Batinic-Haberle, I., Rational design of superoxide dismutase (SOD) mimics: the evaluation of the therapeutic potential of new cationic Mn porphyrins with linear and cyclic substituents. *Inorg. Chem.* **2014**, *53* (21), 11467-83.
53. Miriyala, S.; Spasojevic, I.; Tovmasyan, A.; Salvemini, D.; Vujaskovic, Z.; St Clair, D.; Batinic-Haberle, I., Manganese superoxide dismutase, MnSOD and its mimics. *Biochim Biophys Acta* **2012**, *1822* (5), 794-814.
54. Tabor, E.; Połtowicz, J.; Pamin, K.; Basąg, S.; Kubiak, W., Influence of substituents in meso-aryl groups of iron μ -oxo porphyrins on their catalytic activity in the oxidation of cycloalkanes. *Polyhedron* **2016**, *119*, 342-349.
55. Xie, J.; Ma, G.; Ouyang, X.; Zhao, L.; Qiu, X., Metalloporphyrin as a Biomimetic Catalyst for the Catalytic Oxidative Degradation of Lignin to Produce Aromatic Monomers. *Waste and Biomass Valorization* **2019**, *11* (8), 4481-4489.
56. Antonangelo, A. R.; Westrup, K. C. M.; Burt, L. A.; Bezzu, C. G.; Malewschik, T.; Machado, G. S.; Nunes, F. S.; McKeown, N. B.; Nakagaki, S., Synthesis, crystallographic

characterization and homogeneous catalytic activity of novel unsymmetric porphyrins. *RSC Adv.* **2017**, 7 (80), 50610-50618.

57. Wang, X.; Li, L.; Song, F., Interplay of Nanoparticle Properties during Endocytosis. *Crystals* **2021**, 11 (7).