Supporting Information

The photodynamic therapy for hypoxic solid tumor *via* Mn-MOF as photosensitizer

Jun Lu, Li Yang, Wei Zhang*, Ping Li, Xiaonan Gao, Wen Zhang, Hui Wang, and Bo Tang*

College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Institute of Biomedical Sciences, Shandong Normal University. Jinan 250014 (P. R. China)

* E-mail: tangb@sdnu.edu.cn; zhangwei@sdnu.edu.cn

Table of Contents

EXPERIMENTAL SECTION

Materials and Instruments.

Meterials.

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from Beyotime (Shanghai, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Disodium of 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABMD) and 4-formylbenzoic acid were purchased from Sigma-Aldrich (Shanghai, China). Trypsin, Dulbecco's modified Eagle's Medium (DMEM) and fetal bovine serums were acquired from Sangon. 4T1 cell line (mouse mammary carcinoma cells) was purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Instruments.

Transmission electron microscopy (TEM) was carried out on a HITACHI HT7700 electron microscope. Absorbance was measured in a microplate reader (RT-6000, Rayto, USA) in the MTT assay. Confocal fluorescence imaging studies were performed with a TCS SP8 confocal laser scanning microscope (Leica, Germany).

Synthesis of Mn-MOF.

The organic ligand meso-tetrakis (4-carboxylphenyl) porphyrin (H_6L) and the Nano-MOF were synthesized according to the literature.[1] First, 4-formylbenzoic acid (3.0 g, 0.02 mol) was added to a solution of propionic acid (200 mL) with pyrrole (1.39 mL, 0.02 mol). Next, the mixture was heated to reflux for 1 h. After cooling, a brown-purple precipitate formed. The precipitate was isolated by filtration, washed with CH_2Cl_2, and then dried under vacuum for 6 h.

A mixture of $AlCl_3{\cdot}6H_2O$ (0.125 mmol, 0.030 g), H_6L (0.063 mmol, 0.050 g), and cetyltrimethylammonium bromide (CTAB) (0.1 mmol, 0.036 g) in water (5 mL) was stirred for 10 min at room temperature and then heated in a 20 mL Teflon-lined autoclave at 180 °C for 16 h. After being cooled to room temperature slowly, the purple nanoscale crystals (referred as Al-MOF) were collected by centrifugal separation and washed with DMF, H_2O, and acetone in turn for several times. Afterwards, a mixture of the activated Al-MOF (170 °C under vacuum, overnight) (10 mg) and $Mn(Ac)_2{\cdot}4H_2O$ (0.08 mmol, 0.0196 g) in DMF (2 mL) was stirred for 10 min at room temperature and then heated in a 20 mL Teflon-lined autoclave at 100 °C for 24 h. After cooling slowly to room temperature, the sample was collected by centrifugal separation and washed with DMF, H_2O, and acetone in turn for several times (referred as Mn-MOF).

Cell culture.

4T1 cells were cultured in high glucose DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin, 1% streptomycin. Normal cells were maintained in a humidified incubator in a 5% CO_2, 95% air at 37 °C. Hypoxic cells were maintained in a humidified incubator in 1% O_2, 5% CO_2 and 94% N_2 at 37 °C for another 3 h.

O_2 production quantification from H_2O_2 catalyzed by Mn-MOF.

We investigated the performance of Mn-MOF in the chemical system for the production of O_2 from H_2O_2. The oxygen measurement experiments were divided into two groups, in which the control group was only added with 10 mM H_2O_2, and the experimental group was added with 10 mM H_2O_2 and 0.25 mM Mn-MOF. The oxygen meter was used to measure the oxygen production of the two groups over time.

ROS produced under Mn-MOF illumination.

We used ROS fluorescent probes 2',7'-dichlorodihydrofluorescein (DCFH)[2] to detect the amount of ROS produced by Mn-MOF in the chemical system. The ROS assay was divided into two groups: one group was added with Mn-MOF and ROS fluorescent probes. After incubation for 20 min and centrifugation, the supernatant liquid was taken to measure the fluorescence intensity. The other group was added with Mn-MOF and ROS fluorescent probes, incubated for 10 min, 650 nm laser irradiation for 10 min, centrifuged, and the upper layer of the solution was taken to quantify the fluorescence intensity.

$^1O_2$ Produced under Mn-MOF illumination.

The generation of $^1O_2$ by Mn-MOF under illumination was evaluated by Disodium of 9,10-anthracenediylbis (methylene) dimalonic acid (ABMD) [3]. ABMD molecule can react with $^1O_2$ to produce endoperoxide, which caused an absorption intensity decrease for itself [4]. The experiment was divided into two groups, one group was added Mn-MOF and ABMD for 20 min incubation, but the other group was added with Mn-MOF and ABMD, incubated for 10 min followed by 650 nm laser irradiation for 10 min; both groups were centrifuged, and the supernatant were subtracted to acquire the UV-Vis absorbance in the wavelength range of 300-500 nm.

ROS generation ability in normal and hypoxic cells.

To explore the ROS generation ability of Al-MOF and in normal and hypoxic cells, four different treatment groups were set. Two groups were 4T1 cells cultured under normal conditions, while the rest two groups were 4T1 cells cultured under hypoxic conditions for another 3 h, and then incubated with 100 μM Al-MOF or Mn-MOF respectively for 1 h. Afterwards, each group was incubated with DCFH-DA for 15 min, and 650 nm laser illumination was applied for 5 min. Finally, the excess material and probe were removed, and the cells were washed three times with PBS buffer solution, and the fluorescence of the cells was observed under a confocal microscope. The excitation wavelength was 488 nm and the emission was collected between 500-550 nm.

Mn-MOF induced apoptosis in hypoxic cancer cells.

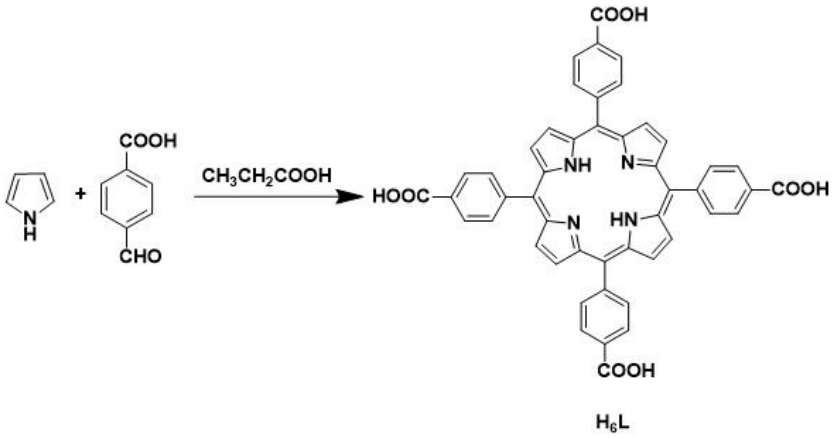
To compare the apoptosis induced by Al-MOF and Mn-MOF under hypoxic conditions, we investigated the cytotoxicity of the two materials for hypoxic 4T1 cells, and the survival rate of hypoxic 4T1 cells was assessed using standard MTT assays. Hypoxic 4T1 cells were seeded in 96-well plates at 5×10^4 cells/well, then cultured in high glucose DMEM containing 10% fetal bovine serum and 1% antibiotics in a 37 °C, 5% CO_2 incubator for 24 h, followed by another 3 h incubation under hypoxia. The cells were incubated with PBS, Al-MOF, Mn-MOF respectively in hypoxic condition for 24 h. All the three

groups, PBS+Laser, Al-MOF+Laser and Mn-MOF+Laser were irradiated with a 650 nm (50 mW/cm^2) laser for 15 min. The medium containing the above reagent was removed, and 20 μL of MTT solution (5 mg/mL) was added to each well. After 4 h, the MTT solution was removed, 150 μL of dimethyl sulfoxide (DMSO) was added to each well, and after 10 min of shaking in the dark, the absorbance at 490 nm was measured with a microplate reader, and cell survival rate was calculated using the cell survival formula (cell viability = (experimental group / control group)×100%).

Breast cancer mouse treatment experiment.

This experiment used 4-6 weeks of wild-type BalB/C mice (female). The mouse was purchased from the Experimental Animal Center of Shandong University. 4T1 tumor model was established by subcutaneous injection of 4T1 cells (10^6 per mouse) into the right flank of mice. When the tumor volume (V) reached 100-150 mm^3, the mice were treated by intratumoral injection. In addition, the tumor volume (V) is calculated by measuring the length (L), and the width (W), and calculated as $V = (W^2 \times L) / 2$. The relative tumor volume of each mouse was calculated as V / V_0 (the initial tumor volume V_0 was set when treatment initiated). All animal experiments were conducted in accordance with the relevant laws and guidelines promulgated by the Ethics Committee of Shandong University. Tumor-bearing mice were randomly divided into 6 groups of 3 each (the mice grew in the same environment except for special operations). In the treatment experiment, the light group was required to light once a day, injected medicine(PBS/Al-MOF/Mn-MOF) once every other day, and the tumor volume and body weight of the mice were recorded every other day. The tumor comparison images of mice in different treatment groups were obtained after 14 days of treatment.

SUPPLEMENTAL FIGURES



$$H_6L + AlCl_3 \cdot 6H_2O \xrightarrow[180°C\ \ 16\ h]{H_2O\ \ CTAB} \text{Al-MOF}$$

$$\text{Al-MOF} + Mn(Ac)_2 \cdot 4H_2O \xrightarrow[100°C\ \ 24\ h]{DMF} \text{Mn-MOF}$$

Fig. S1 Schematic illustration for the synthesis of the Mn-MOF.

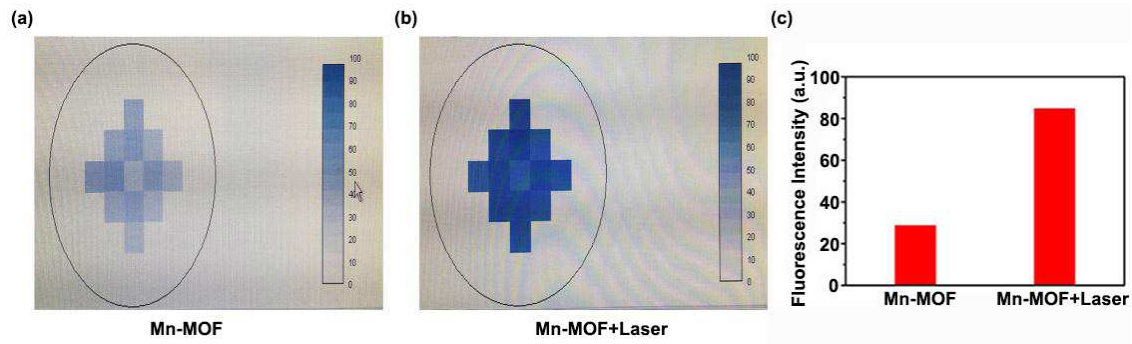


Fig. S2 Produced ROS of Mn-MOF in chemical systems using ROS fluorescent probe DCFH. The fluorescence intensities of ROS produced by (a) the Mn-MOF and (b) Mn-MOF+Laser. (c) The data output of the fluorescence intensities under the Mn-MOF and Mn-MOF+Laser (light irradiation 650 nm), respectively.

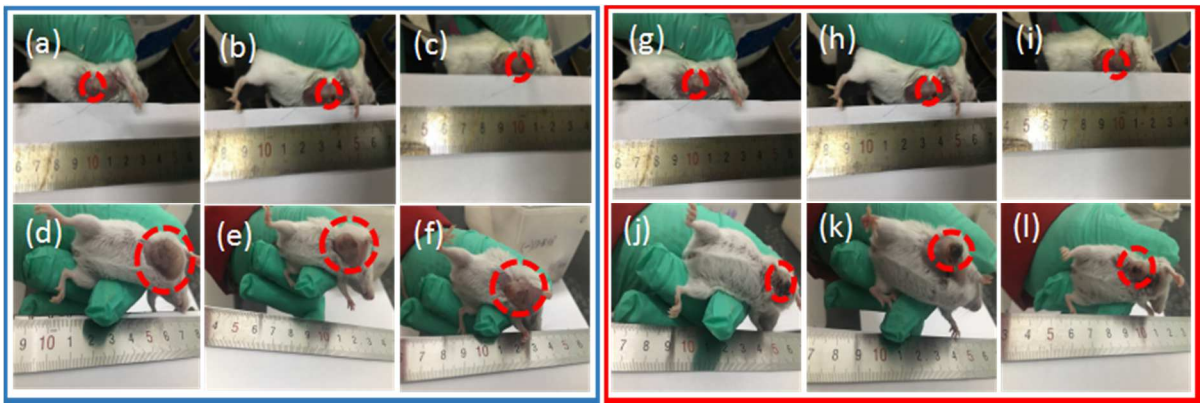


Fig. S3 *In vivo* antitumor efficacy of the Mn-MOF. The mice pictures of the control group with PBS buffer only: (a-c) three parallel groups of mice implanted with breast cancer of tumors growing to about 100-150 mm^3; (d-f) corresponding mice with treatment of PBS after 14 days treatment. The picture of mice treated with Mn-MOF: (g-i) three parallel groups of mice implanted with breast cancer of tumors growing to about 100-150 mm^3; (j-l) corresponding mice with treatment of Mn-MOF after 14 days. Light irradiation is under 50 mW/cm^2 650 nm light irradiation 15 min every day for 14 days.

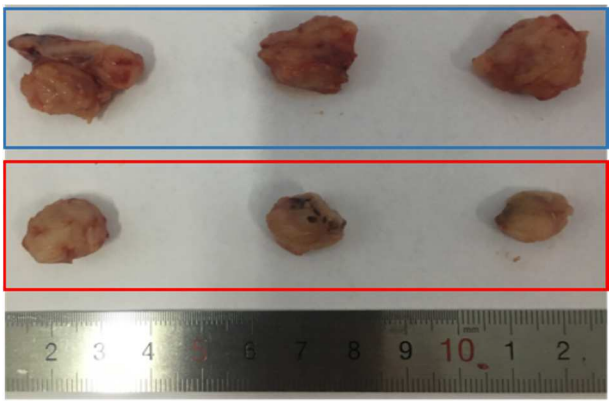


Fig. S4 Therapeutic effects of the Mn-MOF regard from tumor sizes. The picture for tumors of the control group with only PBS buffer injected (Top blue box, three times in parallel), and tumors after treatment with Mn-MOF (Bottom Red box, three times in parallel).

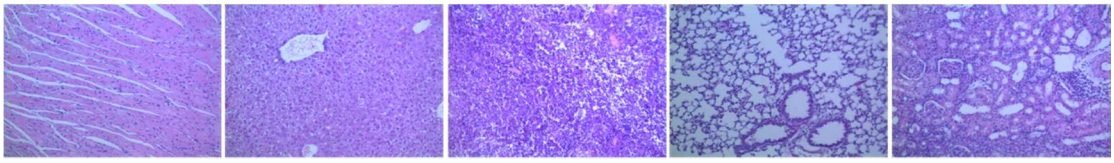


Fig. S5 Experiments of tissue slides (heart, liver, spleen, lung and kidney, respectively). The Mn-MOF was injected directly into the tumor section.

References

1. Y. Ma, H. Su, X. Kuang, X. Li, T. Zhang and B. Tang, *Anal. Chem. ,* 2014, **86**, 11459-11463.
2. E. Ju, K. Dong, Z. Chen, Z. Liu, C. Liu, Y. Huang, Z. Wang, F. Pu, J. Ren and X. Qu, *Angew. Chem. Int. Ed. ,* 2016, **55**, 11467-11471.
3. Z. Yu, W. Pan, N. Li and B. Tang, *Chem. Sci.,* 2016, **7**, 4237-4244.
4. Y. Ma, X. Li, A. Li, P. Yang, C. Zhang and B. Tang. *Angew. Chem. Int. Ed.,* 2017, **56**, 13752-13756.