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Supporting Information

Rodlike nanomaterials from organic diradicaloid with high photothermal conversion capability for tumor treatment

Tingting Sun, Jiaxiang Guo, Hui Wen, Qing Pei, Qihang Wu, Dengyuan Hao, Chuandong Dou,* and Zhigang Xie*

Dr. T. Sun, H. Wen, Q. Pei, Q. Wu, D. Hao, Prof. Z. Xie
State Key Laboratory of Polymer Physics and Chemistry
Changchun Institute of Applied Chemistry
Chinese Academy of Sciences
Changchun, Jilin 130022, China
E-mail: xiez@ciac.ac.cn

J. Guo, Prof. C. Dou
State Key Laboratory of Supramolecular Structure and Materials
College of Chemistry
Jilin University
Changchun, Jilin 130012, China
E-mail: chuandong@jlu.edu.cn

Materials and Characterizations

1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol)$_{2K}$ (DSPE- PEG) was purchased from Ponsure Biotechnology Co., Ltd.. Cell Counting Kit-8 (CCK-8) was purchased from Biosharp (Hefei, China). 0.25% Trypsin-EDTA, Phenol Red(modified) was purchased from Dalian Meilun Biotechnology Co., Ltd.. Calcein/PI Cell Viability/Cytotoxicity Assay Kit was purchased from Shanghai Beyotime Biotechnology Co., Ltd.. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide) was purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. The other chemicals were used as obtained commercially. Cell culture dishes and centrifuge tubes were obtained from NEST Biotechnology Co., Ltd.. Six-well and 96-well plates were obtained from Guangzhou Jet Bio-Filtration Co., Ltd.. Rainin Pipettes and Analytical balance (XS105DU) from METTLER TOLEDO were utilized for quantification of liquid and solid. JEOL JEM-1011 electron microscope was used to obtain TEM image. The absorption spectra were measured by a TU-1901 UV-Vis spectrophotometer (Persee).

Preparation of BOD NPs

The compound BOD was synthesized via a three-step reaction in our previous work.[1] 2 mg of BOD and 1 mg of DSPE-PEG were dissolved in 8 mL of THF. The solution was added dropwise to deionized water (20 mL) under stirring. After THF was fully evaporated, the mixture was dialyzed for 24 h followed by concentration with ultrafiltration tube. For the preparation of IR780 loaded BOD NPs, IR780 at a mass ratio of 50% to DSPE-PEG was dissolved in acetone, and then mixed with the THF solution of BOD and DSPE-PEG. The subsequent steps are similar with the preparation method of BOD NPs.

Loading Efficiency and Loading Content of BOD in BOD NPs

To determine the loading efficiency and loading content of BOD in BOD NPs, the freshly prepared BOD NPs was lyophilized and weighed. The weight of BOD in the lyophilized

powder (BOD NPs) was determined *via* a standard curve obtained from UV-vis-NIR spectrophotometer. The loading efficiency and loading content were calculated by the following equations:

loading efficiency (wt%)

= total weight of BOD in freshly prepared BOD NPs / feeding weight of BOD × 100%

loading content (wt%)

= weight of BOD in BOD NPs / weight of BOD NPs × 100%

Photothermal Effect of BOD NPs

BOD NPs at various concentrations (0-20 μg mL^{-1}) were subjected to 730 nm laser irradiation (0.6 W cm^{-2}) for 10 min respectively. For BOD NPs at a fixed concentration of 20 μg mL^{-1}, the temperature of BOD NPs under 730 nm laser irradiation of different power densities (0.4-1.0 W cm^{-2}) was also recorded. The photothermal conversion efficiency of BOD NPs was measured according to a previous method[2] by recording the photothermal response of BOD NPs under 730 nm laser irradiation (0.6 W cm^{-2}) and the temperature during the cooling period. The photostability of BOD NPs upon 730 nm laser irradiation was investigated by measuring the temperature variations of BOD NPs in water over 5 cycles of heating and natural cooling.

Cytotoxicity of BOD NPs

The cytotoxicity of BOD NPs toward CT26 cells with or without laser irradiation was measured via Cell Counting Kit-8 (CCK-8) assay. CT26 cells in a logarithmic growth phase were seeded in 96-well plates and incubated in DMEM for 24 h. Thereafter, cells were incubated with DMEM or BOD NPs at various concentrations (0-20 μg mL^{-1}) at 37 °C for 4 h. Then, 730 nm laser irradiation (0.6 W cm^{-2}, 10 min) was performed on cells in BOD NPs + Laser groups. After that, the cells were incubated for another 20 h. The culture media were

replaced with 100 μL of fresh media, followed by the addition of CCK-8 solution (10 μL). Finally, the absorbance at 450 nm was measured via a microplate reader. For HeLa cells, after the cells were incubated for another 20 h, MTT solution (5 mg mL^{-1} in PBS, 20 μL) was added, and they were further incubated at 37 °C for 4 h. Then, the culture medium containing MTT was removed, and 150 μL of dimethyl sulfoxide was added to each well. Finally, the absorbance of formazan product at 490 nm was measured via a microplate reader.

To visualize the PTT effect of BOD NPs, CT26 and HeLa cells were incubated with DMEM or BOD NPs (20 μg mL^{-1}) at 37 °C for 4 h. Then 730 nm laser irradiation (0.6 W cm^{-2}, 10 min) was performed on Laser and BOD NPs + Laser groups, respectively. After that, the cells were incubated for another 20 h, followed by the addition of calcein-AM and PI to stain the cells at room temperature for 30 min. Finally, the stained cells were observed via a fluorescence microscope.

In Vivo Fluorescence Imaging of BOD NPs

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences and approved by the Animal Ethics Committee of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences (Approved No. 20220005). To explore the distribution of BOD NPs in tumors, BOD NPs were labeled with IR780, and in vivo imaging was performed toward a CT26 tumor-bearing BALB/c mouse. The tumor-bearing mouse was intravenously injected with IR780 labeled BOD NPs, and fluorescence imaging of the mouse was conducted 1, 4, 8, 12, 24 and 48 h post injection. After administration for 48 h, the mouse was sacrificed followed by the excision of the tumor and the main organs, and ex vivo fluorescence image were obtained.

In Vivo PTT Effect of BOD NPs

To evaluate the antitumor effects, the mice bearing CT26 tumors were randomly divided into

4 groups, namely, Control, Laser, BOD NPs and BOD NPs + Laser. After the mice in BOD NPs and BOD NPs + Laser groups were intravenously injected with BOD NPs (6 mg kg^{-1}) for 12 h, the mice in Laser and BOD NPs + Laser groups were irradiated with 730 nm laser (0.6 W cm^{-2}) for 10 min. The temperature changes of tumors during irradiation were recorded with an IR thermal imager every two minutes. The tumor dimensions (length and width) and body weights of mice were measured every other day. And the tumor volumes were calculated with the formula: $width^2 \times length/2$ (mm^3). 14 days later, the mice in 4 groups were all sacrificed followed by dissection of tumors and main organs.

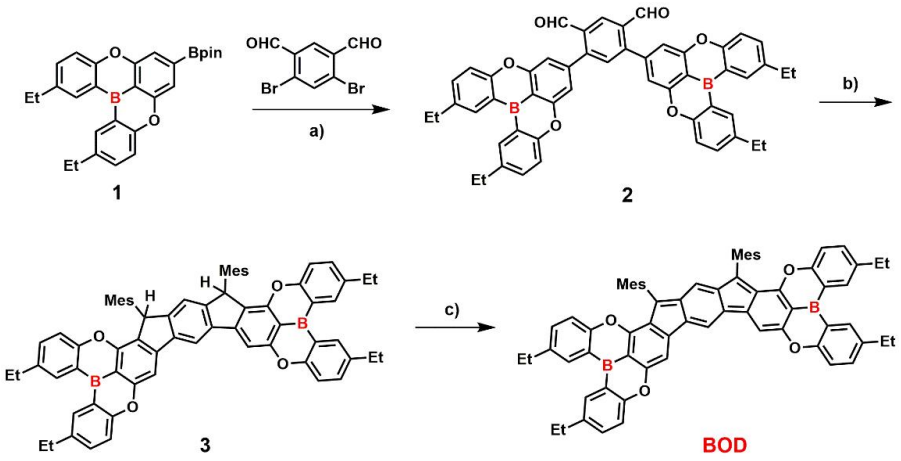

Figure S1. Synthesis of BOD.[1] (a) $Pd(PPh_3)_4$, K_2CO_3, toluene:EtOH:H_2O (2:1:1), 80 °C; (b) (i) mesitylmagnesium bromide, THF, 25 °C; (ii) $BF_3 \cdot Et_2O$, DCM, 25 °C; (c) DDQ, toluene, 80 °C.

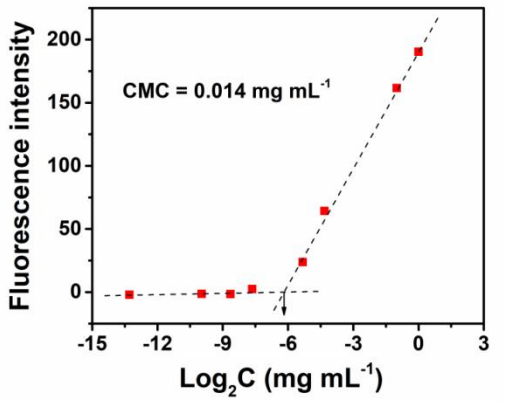

Figure S2. CMC determination of DSPE-PEG with nile red as the fluorescent probe.

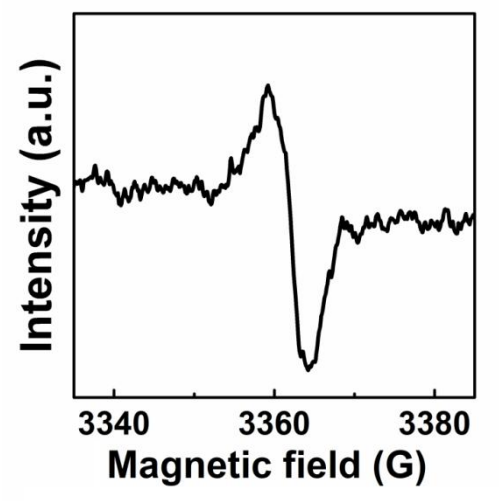

Figure S3. EPR spectrum of BOD NPs.

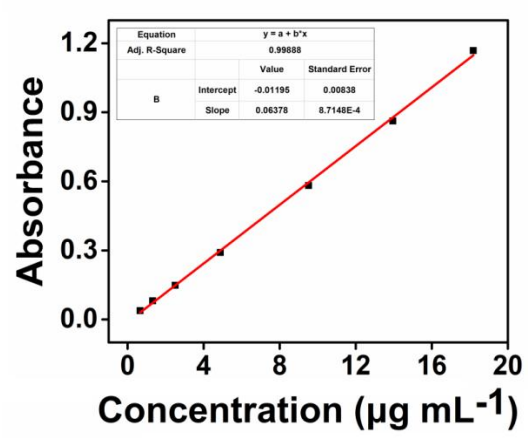

Figure S4. The standard curve of BOD in THF:H_2O=9:1.

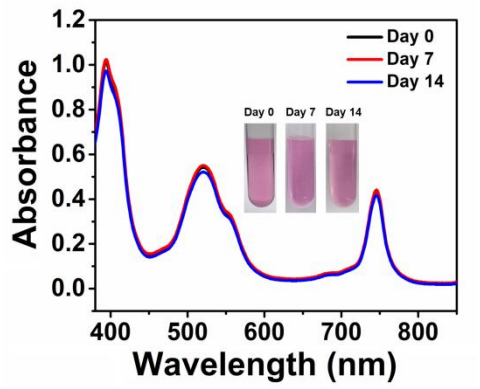

Figure S5. The absorption spectra and the photos of BOD NPs after storage in water for 0, 7, and 14 days.

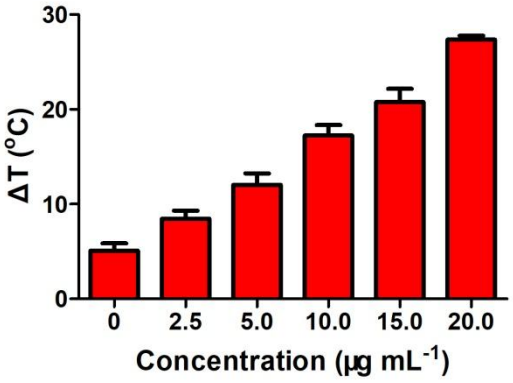

Figure S6. Temperature variations of various concentrations of BOD NPs under laser irradiation.

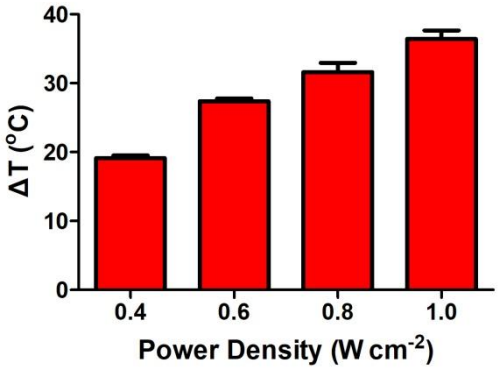

Figure S7. Temperature variations of 20 μg mL^{-1} of BOD NPs under laser irradiation of diverse power densities.

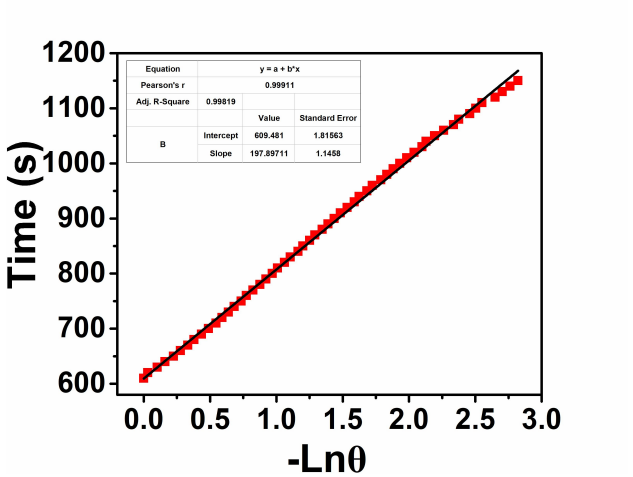

Figure S8. Linear plot of the cooling time versus -Lnθ calculated from the cooling stage.

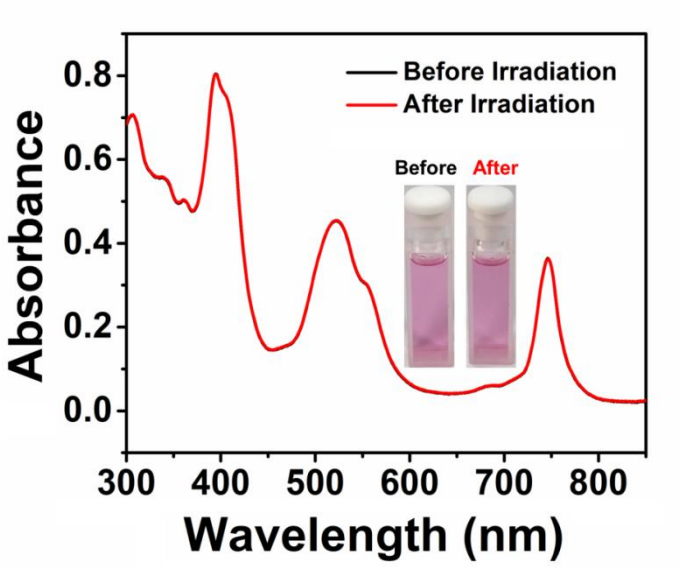

Figure S9. Absorption spectra of BOD NPs before and after 730 nm laser irradiation.

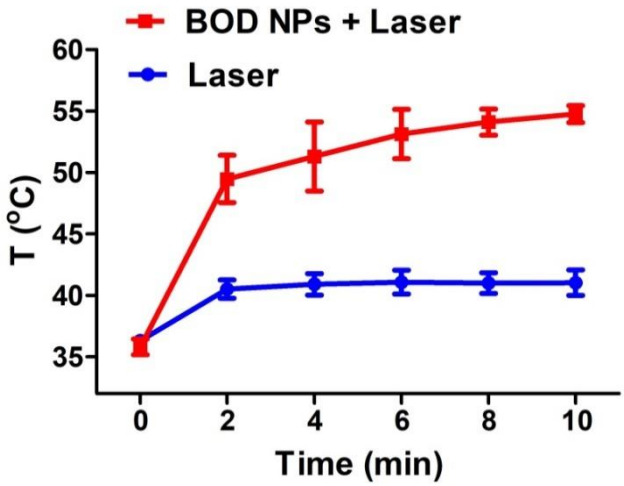

Figure S10. Temperature changes of the tumors treated with (BOD NPs + Laser) or without (Laser) BOD NPs (6 mg kg^{-1}) under 730 nm laser irradiation (0.6 W cm^{-2}) for 10 min.

Reference

1. J. Guo, Y. Yang, C. Dou, Y. Wang, *J. Am. Chem. Soc.* **2021**, *143*, 18272.

2. Y. Liu, K. Ai, J. Liu, M. Deng, Y. He, L. Lu, *Adv. Mater.* **2013**, *25*, 1353.