Electronic Supplementary Information

A cationic BODIPY photosensitizer decorated with quaternary ammonium for high-efficiency photodynamic inhibition of bacteria growth

Hongyu Wang,[ab] Chaonan Li,[ab] Qihang Wu,[ab] Hui Wen,[ab] Tingting Sun,*[a] and Zhigang Xie[ab]

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China

[b] School of Applied Chemistry and Engineering University of Science and Technology of China, Hefei 230026, P. R. China

***Corresponding author:**

E-mail: suntt@ciac.ac.cn

Experimental section

Materials and characterizations

1,6-Dibromohexane was purchased from Shanghai Youshi Chemical Co., Ltd.. Triethylamine was purchased from Shanghai Chemical Reagent Co., Ltd.. 2,4-Dimethyl pyrrole was obtained from Tokyo Chemical Industry Co., Ltd.. DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) and N-bromosuccinimide (NBS) was purchased from Tianjin Heowns Biochemical Technology Co., Ltd.. Trimethylamine was purchased from TCI Development Co., Ltd.. Living cell nucleic acid dyes (SYTO green) was purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide) and Live-Dead Cell Staining Kit were provided from NanjingKeyGen Biotech Co., Ltd.. The other chemicals were used as obtained commercially. ^{1}H NMR spectra were measured in $CDCl_3$ at room temperature by an AV-400 NMR spectrometer from Bruker. Analytical balance (XS105DU) and Rainin Pipettes from METTLER TOLEDO were used to quantify solid and liquid respectively. The absorption spectra were obtained from a TU-1901 UV-vis spectrophotometer (Persee). TEM and DLS results of NPs were determined by JEOL JEM-1011 electron microscope (acceleration voltage of 100 kV) and Malvern Zeta-sizer Nano. CLSM images were obtained from a Zeiss LSM 700 (Zurich, Switzerland).

Synthesis of 4-(6-bromohexoxy) benzaldehyde (1)

4-(6-Bromohexoxy)benzaldehyde was synthesized according to a reported method.[1] 1,6-Dibromohexane (4 g, 16 mmol), p-hydroxybenzaldehyde (2 g, 16 mmol) and K_2CO_3 (4 g, 29 mmol) were added to MeCN (50 mL), and the mixture was heated to reflux at 85 ^oC for 24 h at N_2 atmosphere. After the reaction mixture was cooled down to room temperature, the solution was drained under vacuum and water was added to dissolve it. Then, the mixture was extracted with ethyl acetate (50 mL) for 3 times. The organic phases were combined, washed with 20 mL of saturated salt water, dried with $MgSO_4$, filtered and spun dry on a rotary evaporator to obtain a white solid crude product, which was purified on a silica gel column (CH_2Cl_2 : n-hexane = 1 : 2).

Synthesis of 2

1 (2.84 g, 10 mmol) and 2,4-dimethyl pyrrole (2.26 mL, 22 mmol) were added in CH_2Cl_2（400 mL), followed by the addition of 5 drops of trifluoroacetic acid. The above mixture was stirred overnight at room temperature with protection from light in N_2 atmosphere. Then, DDQ (2.27 g, 10 mmol) was slowly added. After reacting for 4 h, triethylamine (10 mL, 73 mmol) was added. 30 min later, boron trifluoride ethyl ether (12 mL, 95 mmol) was slowly added. After 2 h, the reaction was stopped. The mixture was washed with distilled water (50 mL) for 3 times, dried with $MgSO_4$, and the reaction mixture was concentrated after filtration. The brown oily residue was purified through a silica gel column (CH_2Cl_2 : n-hexane = 1 : 1), and the product

was subsequently collected and concentrated to give a reddish brown solid.

Synthesis of BODIPY-Br

2 (100 mg, 0.2 mmol) and N-bromosuccinimide (NBS) (178.86 mg, 0.8 mmol) were added in CH_2Cl_2 (10 mL). After reacting for 4 h, the crude product was purified on a silica gel column (CH_2Cl_2 : n-hexane = 1 : 2) to obtain a dark red solid product.

Synthesis of BODIPY-TMA

BODIPY-Br (75 mg, 0.1 mmol) was added into a round bottom flask, then 15 mL of ethanol was added to dissolve BODIPY-Br, and $N(CH_3)_3$ (0.6 mL, 6 mmol) was added under stirring at room temperature. After heating and refluxing for 24 h, the solvent was removed under reduced pressure and the product was obtained by precipitation with ether.

Preparation of nanoparticles

BODIPY-TMA (1 mg) was fully dissolved in 2 mL of acetone, and the solution was added dropwise to 5 mL of water under stirring. After stirring at room temperature for 8 h to remove the organic solvent, BODIPY-TMA NPs were obtained. The standard curve of BODIPY-TMA was developed by UV-vis spectrophotometer to determine the concentration of BODIPY-TMA NPs. BODIPY-Br NPs were obtained with the same method as BODIPY-TMA NPs

Photodynamic effect

1 mg of DPBF was dissolved in 1 mL of DMF (1 mg mL^{-1}). BODIPY-Br NPs and BODIPY-TMA NPs were diluted to the same concentration. 90 μL of DPBF solution was added to 3 mL of deionized water, BODIPY-Br NPs and BODIPY-TMA NPs respectively, and their absorption spectra were measured after green light irradiation (green light, 12 mW cm^{-2}) for different times. The detection interval was 30 s.

Singlet oxygen quantum yields

The Φ_Δ value of BODIPY-TMA NPs and BODIPY-Br NPs was determined by using DPBF as the singlet oxygen sensor. The value was calculated from the equation:[2]

$$\phi_\Delta = \phi_{\Delta(std)} \frac{RF^{std}}{FR^{std}}$$

where, $\Phi_{\Delta(std)}$ is the standard singlet oxygen quantum yield of rose bengal (RB, $\Phi_{\Delta(std)}$ = 0.75 in water).[3] R and R_{std} are the slope of the absorbance over time of DPBF in the presence of BODIPY-TMA NPs / BODIPY-Br NPs and RB, respectively. F and F_{std} are the absorption correction factors for BODIPY-TMA NPs / BODIPY-Br NPs, which were calculated by $F = 1 - 10^{-OD}$ (OD at the irradiation wavelength).

Antibacterial assays

S. aureus or *E. coli* (10^5 CFU mL^{-1}) were mixed with different concentrations of BODIPY-Br NPs or BODIPY-TMA NPs and inoculated on 96-well plates, where the bacteria in the Light groups were irradiated with a green light (12 mW cm^{-2}) for 10

min and placed in a 37 ^{o}C incubator, while those in the Dark groups were incubated under the same conditions. After incubation for 24 h, the viabilities of the bacteria were determined via the absorbance detected by a microplate reader. In addition, a mixture of different concentrations of NPs and bacteria was applied to the solid media. The bacteria in the Light groups were irradiated with green light (12 mW cm^{-2}) for 10 min. 100 μL of the bacteria suffered from different treatments were taken out, and then spread on agar plates. All plates were placed in a 37 ^{o}C incubator and cultivated for 24 h.

Crystalline violet staining assays

A mixture of 1mL of *S. aureus*/*E. coli* (10^5 CFU mL^{-1}) and nanoparticles was added to a 24-well plate, and the bacteria in the Light groups (green light, 12 mW cm^{-2}, 10 min) and the Dark groups were incubated under the same conditions after different treatments. After incubation for 24 h, 1 mL of 0.01% crystalline violet was added to each well and stained for 30 min. After the liquid in each well was carefully removed, the plate was washed with 1 mL of PBS for 3 times, and then 1mL of ethanol was added. Finally, the absorbance of each well was detected by a microplate reader.

Live and dead bacterial staining assays

500 μL of *S. aureus* solution (10^7 CFU mL^{-1}) was added to the 24-well plate, which was then placed in a 37 ^{o}C incubator. After incubation for 24 h, each well was

washed with PBS solution for 3 times. BODIPY-Br NPs and BODIPY-TMA NPs were added respectively. The bacteria in the Light groups were irradiated with green light (12 mW cm^{-2}) for 10 min, and then incubated in the incubator together with those in the Dark groups. After incubation for 4 h, they were stained with PI and SYTO for 30 min. Subsequently, the dye solutions were removed. And the *S. aureus* were imaged by confocal laser scanning microscopy.

Cytotoxicity assays

NIH 3T3 cells were inoculated in a 96-well plate, which was placed in a 37 ^{o}C incubator. After incubation for 24 h, different concentrations of BODIPY-TMA NPs were added in the well plate with cells. After incubation for another 24 h, 20 μL of MTT solution (5 mg mL^{-1}) was added to each well. After 4 h, the supernatant was aspirated and 150 μL of DMSO was added. The well plate was placed in a microplate reader to detect their OD values at 490 nm.

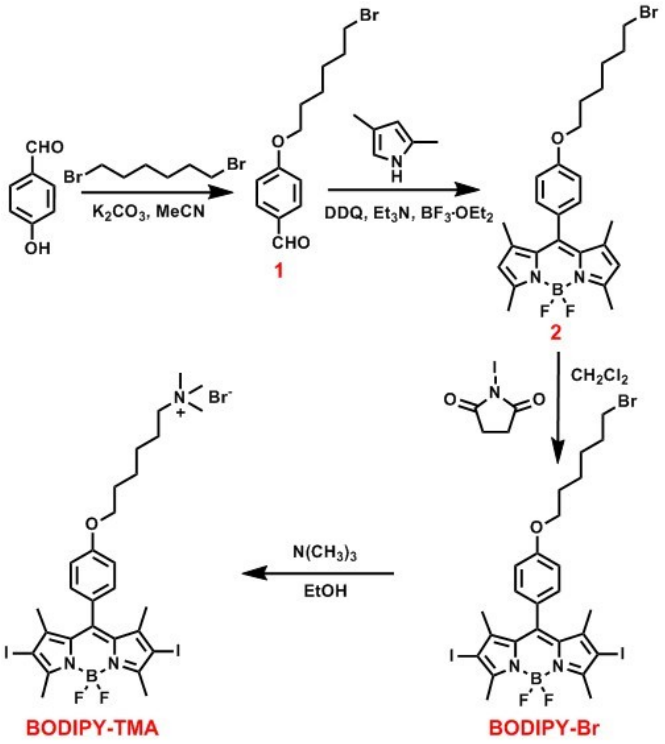

Fig. S1 The synthetic route of BODIPY-TMA.

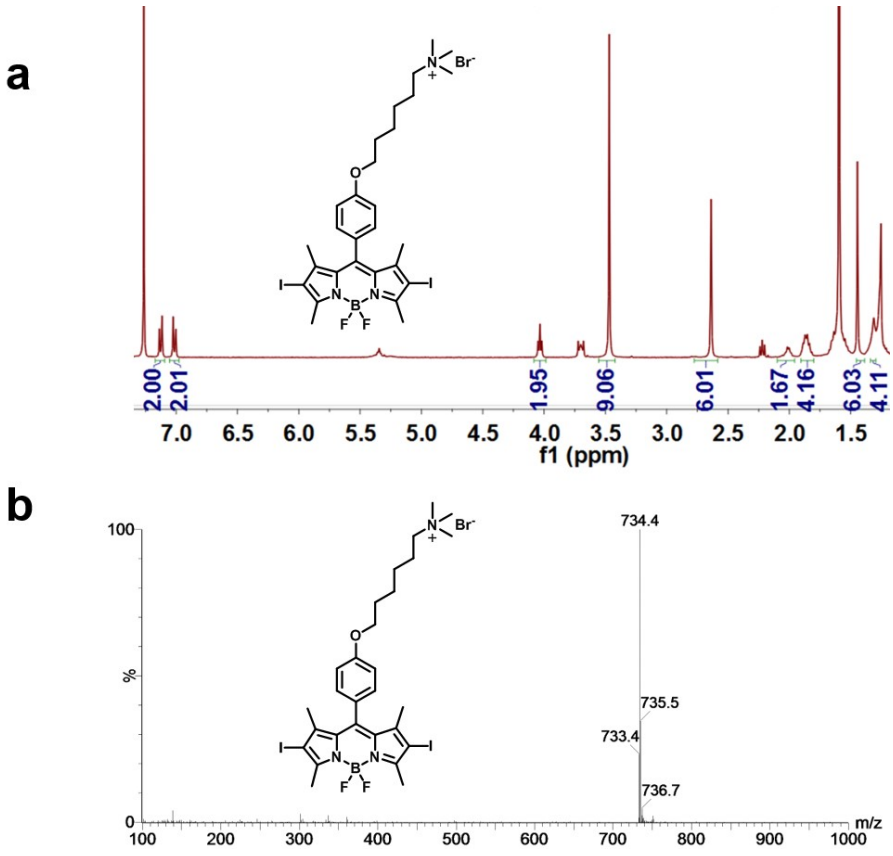

Fig. S2 (a) ^{1}H NMR and (b) ESI-MS spectra of BODIPY-TMA.

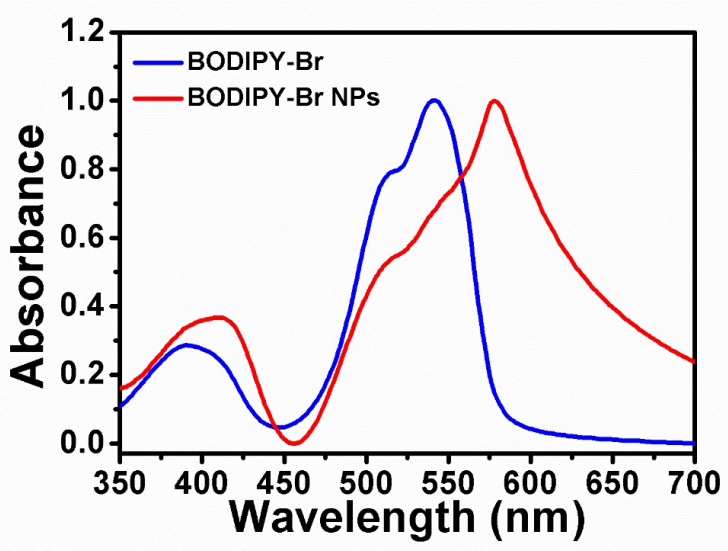

Fig. S3 Absorption spectra of BODIPY-Br in acetone and BODIPY-Br NPs in water.

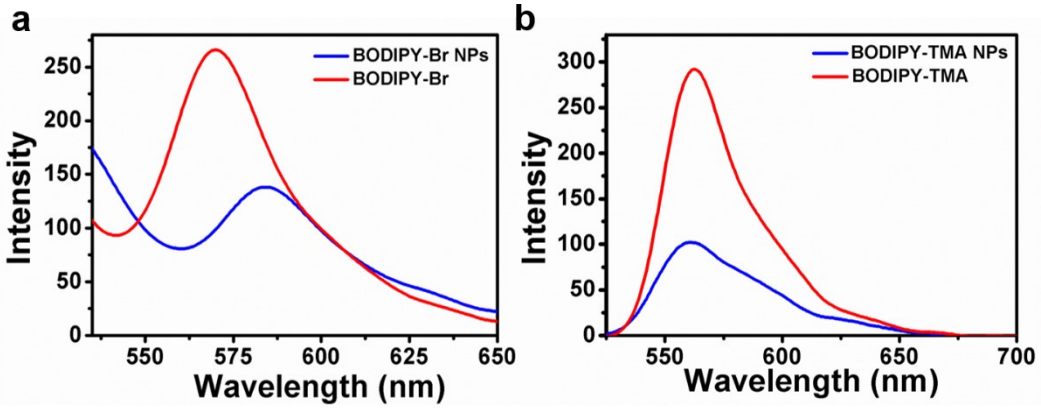

Fig. S4 Fluorescence spectra of (a) BODIPY-Br and BODIPY-Br NPs (20 μg mL^{-1}), as well as (b) BODIPY-TMA and BODIPY-TMA NPs (20 μg mL^{-1}).

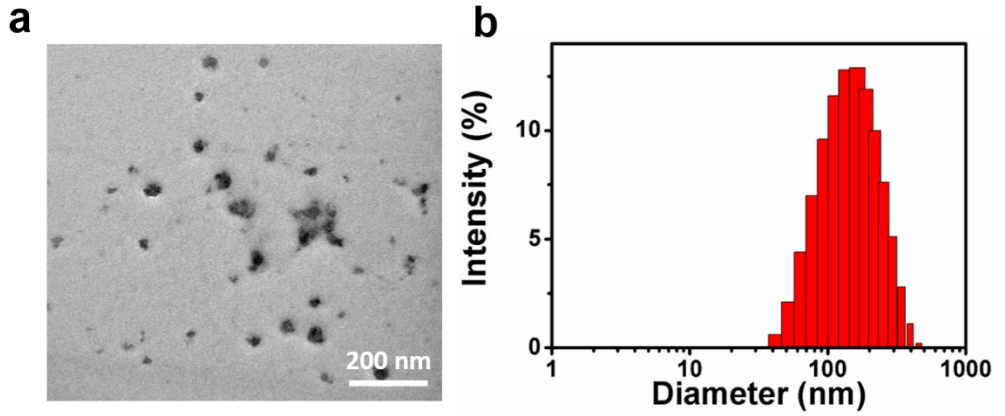

Fig. S5 (a) TEM image and (b) the size distribution of BODIPY-Br NPs.

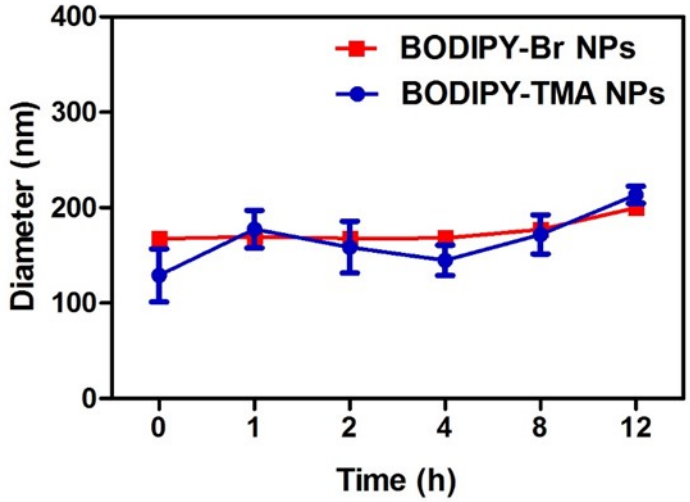

Fig. S6 The size changes of BODIPY-Br NPs and BODIPY-TMA NPs in PBS solution containing 10% fetal bovine serum within 12 h.

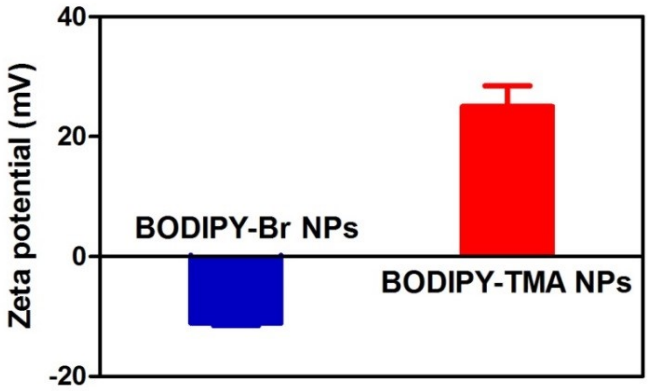

Fig. S7 The zeta potential of BODIPY-Br NPs and BODIPY-TMA NPs.

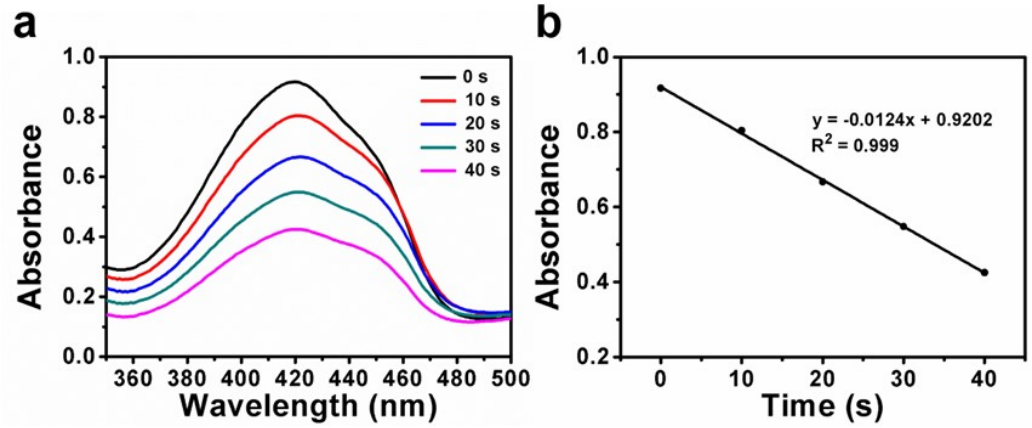

Fig. S8 (a) The spectra of DPBF with RB in water for the determination of Φ_{Δ}. (b) Plot of the absorbance changes of DPBF versus time.

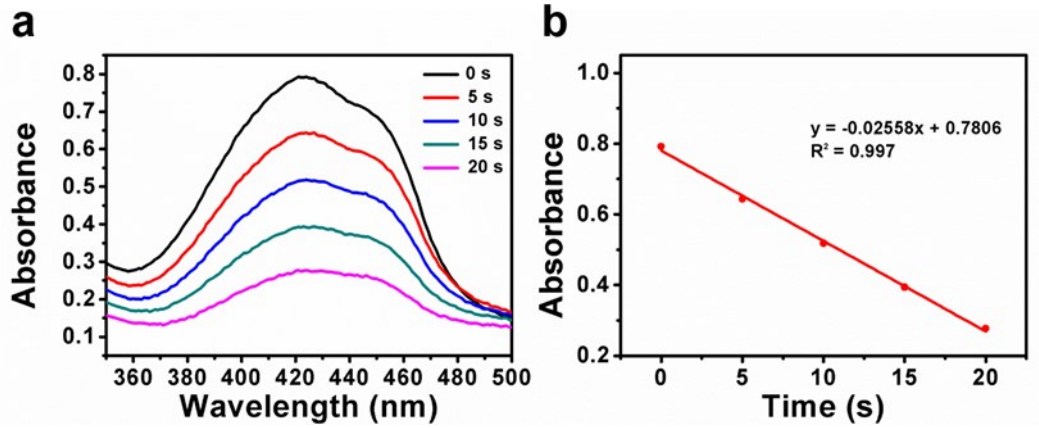

Fig. S9 The spectra of DPBF with BODIPY-TMA NPs in water for the determination of Φ_{Δ}. (b) Plot of the absorbance changes of DPBF versus time.

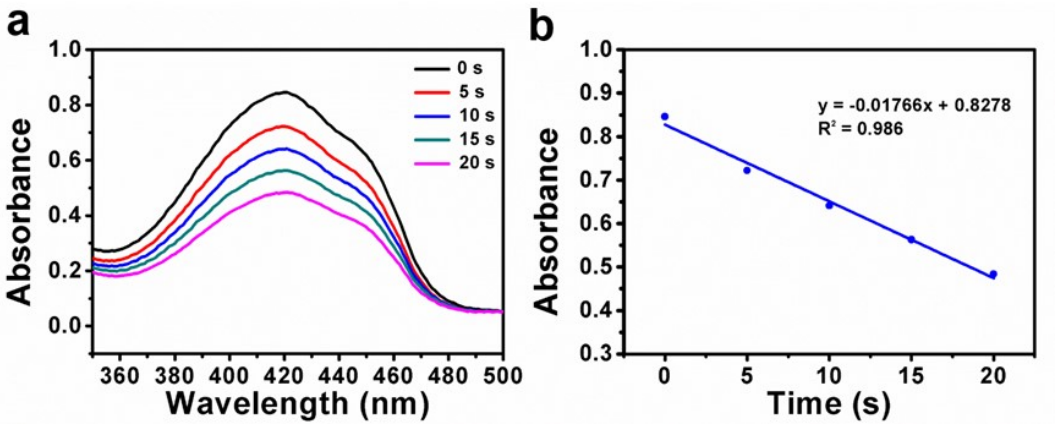

Fig. S10 The spectra of DPBF with BODIPY-Br NPs in water for the determination of Φ_{Δ}. (b) Plot of the absorbance changes of DPBF versus time.

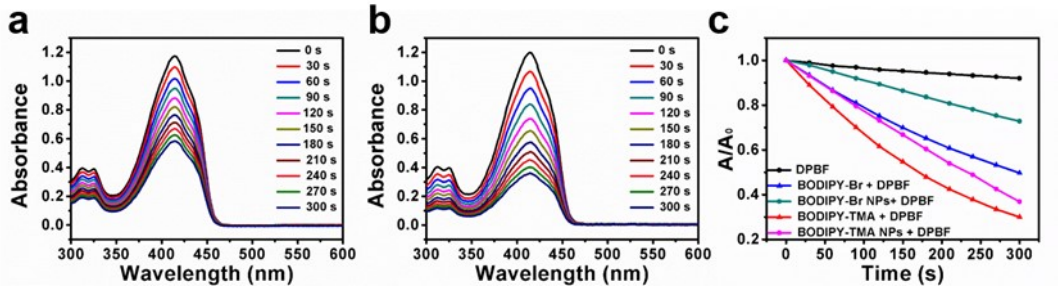

Fig. S11 Changes in the absorption spectra of (a) BODIPY-Br + DPBF and (b) BODIPY-TMA + DPBF under green light irradiation. (c) The relative changes of the absorbance of DPBF, BODIPY-Br + DPBF, BODIPY-Br NPs + DPBF, BODIPY-TMA + DPBF and BODIPY-TMA NPs + DPBF under green light irradiation. A_0 is the initial absorbance of DPBF, BODIPY-Br + DPBF, BODIPY-Br NPs + DPBF, BODIPY-TMA + DPBF and BODIPY-TMA NPs + DPBF without irradiation, and A is the corresponding absorbance of them after irradiation for different times.

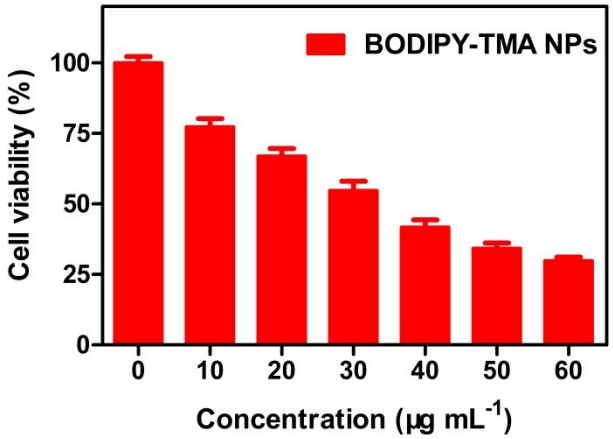

Fig. S12 Cytotoxicity of BODIPY-TMA NPs at different concentrations toward NIH 3T3 cells.

Reference

1 F. Bahrami, F. Panahi, F. Daneshgar, R. Yousefi, M. B. Shahsavani and A. Khalafi-Nezhad, *RSC Adv.*, 2016, **6**, 5915-5924.

2 L. Yin, Z. Wang, Q. Wu, L. Liu, N. Zhang, Z. Xie and G. Zhu, *ACS Nano*, 2022, **16**, 6197-6205.

3 Y. Gao, X. Wang, X. He, Z. He, X. Yang, S. Tian, F. Meng, D. Ding, L. Luo and B. Z. Tang, *Adv. Funct. Mater.*, 2019, **29**, 1902673.