Electronic Supplementary Information (ESI)

Constructing Reduction-Sensitive PEGylated NIRF Mesoporous Silica Nanoparticles via One-Pot Passerini Reaction for Photothermal / Chemo Therapy

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Experimental

Materials

The chemicals used for the Dox@MSN-BDP-PEG synthesis were: 2,4-dimethyl-pyrrole, 4-methoxybezaldehyde and 4-Pyridinecarboxald-ehyde (TCI, Aladdin and Innochem reagents 98%), Tetraethylorthosilicate (TEOS, Aladdin), N-cetyltrimethylammonium bromide (CTAB, YiLi fine chemicals Co. Ltd. Beijing), 3-mercaptopropyltrimethoxysilane (MPTMS, Aladdin), 3-mercaptopropionic acid (Aldrich), 2,2'-dithiodipyridine(aldrithiol-2, Zhejiang Shou&Fu chemical Co. Ltd), reduced glutathione (GSH, Aladdin) were used as received. Doxorubicin hydrochloride (Dox·HCl) was purchased from Zhejiang Hisun Pharmaceutical Co.Ltd. 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) and 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. Other reagents were commercially available and used as received.

Characterizations

Transmission electron microscopy (TEM) studies were performed on a JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. Fourier transform infrared spectrophotometer (FT-IR) spectra were recorded on a Bruker Vertex70 Win-IR instrument. Thermogravimetric analyses were carried out on a Perkin Elmer Pyris Diamond TG/DTA analyzer, using an oxidant atmosphere (Air) with a heating program consisting of a dynamic segment (10 °C min^{-1}) from 373 to 1073 K. ^{1}H-NMR spectra were recorded on a Bruker NMR 400 DRXS pectrometer using $CDCl_3$ as solvent. UV-Vis absorption and emission measurement were carried on by using a Shimadzu UV2450 PC UV-Vis Spectrophotometer and a PerkinElmer LS-55 Fluorescence Spectrophotometer, respectively. Confocal laser scanning microscopy (CLSM) images were taken using a Zeiss LSM 700 (Zurich, Switzerland). Statistical

significance analysis was assessed by SPSS via one-way ANOVA test. *P < 0.05 was considered statistically highly significant.

Synthesis of MSN-SS-COOH

Firstly, we synthesized MSN-SH, and it was prepared according to the published method.[1] Then under an argon atmosphere, MSN-SH (1.00 g) and 2,2'-dithiodipyridine (0.44 g, 2 mmol) were first dispersed in 200 mL of deionized water. The pH of the solution was adjusted to 2.0 using 1.0 M of HCl to render 2,2'-dithiodipyridinesoluble in water. After that, the reaction was kept at room temperature for 24 h. The resulting MSN-SS-Py particles were collected by centrifugation and washed with water and methanol to remove excess 2,2'-dithiodipyridine.

To obtain MSN-SS-COOH, MSN-SS-Py was added to a DMF solution of 3-mercaptopropionic acid (0.11 g, 1 mmol) containing acetic acid (4 mL). The reaction was allowed to proceed under an argon atmosphere at 40 °C for 24 h. The particles were isolated by centrifugation, extensively washed with methanol and dried under vacuum.[2]

Synthesis of BODIPY 5

The BODIPY 1, 2 and 3 were synthesized by following the literature procedure.[3] BODIPY 4 and 5 was prepared as follows: BODIPY 3 (200 mg, 0.35 mmol) dissolved in ethyl formate under argon, then the reaction mixture was stirred for 4 days at 70 ºC. The solvent was removed and extracted with CH_2Cl_2 (15 mL), then washed three times with NH_4Cl solution and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuum, and the residue was purified by column chromatography (silica gel, dichloromethane: EtOAc = 5:1, v/v) to afford dark blue powder (147 mg, 73.5 %).

BODIPY 4 (120 mg,0.2 mmol)was dissolved in dry dichloromethane, and triethylamine and phosphorous oxychloride were added in the mixture, then the reaction mixture was stirred for 2 hours at 0 ºC, at the end added $NaHCO_3$(2 mol^{-1} L,10 mL) and stired for 20 min at 25 ºC. The mixture was extracted with CH_2Cl_2 and purified by column chromatography (silica gel, dichloromethane: triethylamine = 50:

1, v/v) to get BODIPY5 (100 mg, 83.3 %).

Passerini Three-Component Reaction on MSNs

To a magnetically stirred solution of MSN-SS-COOH (20 mg), BODIPY 5(33 mg, 0.06 mmol) and PEG-CHO (M_n = 750, 37.5 mg, 0.05 mmol) was added a solution of dry dichloromethane (0.5 mL) at 25 °C. The mixture was stirred for 72 h and then the particles were isolated by centrifugation, extensively washed with CH_2Cl_2 for 5 times and dried under vacuum.

Preparation of Dox loaded nanoparticles and In Vitro Drug Release

Typically, Dox (4.00 mg) and 3-time excess of triethylamine (TEA) in DMSO (1.00 mL) were added to THF (2 mL) solution containing MSN-BDP-PEG (15 mg). The mixture was stirred for 2 h. Then, deionized water (5 mL) was added to this solution dropwise and stirred for an additional 2 h. After that, THF was removed by a rotating evaporator, and the mixture was dialyzed against deionized water for 48 h (MWCO = 3500) and freeze-dried.[2] The drug-loaded MSNs were named as Dox@MSN-BDP-PEG.

In vitro drug release profiles of drug-loaded nanoparticles were investigated in PBS (at pH 5.0 and 7.4). The steps are as follows: Dox@MSN-BDP-PEG were dissolved in phosphate buffer (pH =7.4) or acetate buffer (pH= 5.0) solutions (1 mg /mL, 5mL) at different GSH concentrations (0 or 10 m M). Then the mixture were transferred to a dialysis bag (MWCO = 3500) and immersed in 20 mL of corresponding PBS solution at 37 ^oC under oscillation (90 r min^{-1}). At predetermined time intervals, 2 mL of buffer outside the dialysis bag was taken out for UV-Vis measurement and an equal volume of fresh buffer was added. The release experiments were conducted in triplicate. The amount of released Dox was examined from the absorbance at 480 nm with the help of a calibration curve of Dox in the same buffer.

Cellular uptake and Intracellular release behaviors

The cellular uptake and intracellular release behaviors of Dox@MSN-BDP-PEG and MSN-BDP-PEG were assessed by confocal laser scanning microscopy (CLSM). For a CLSM study, HeLa cells were seeded in 6-well plates at a density of 105 cells per well in 2 mL of complete Dulbecco's modified Eagle's medium (DMEM)

containing 10 % fetal bovine serum, supplemented with 50 IU mL^{-1}penicillin and 50 IU mL^{-1} streptomycin. After incubation for 24 h, the culture media were withdrawn and culture media containing Dox@MSN-BDP-PEG or MSN-BDP-PEG were supplemented (final BODIPY concentration: 5μg mL^{-1}). The cells were incubated for another 2 h, 15 h and 24 h at 37 ^{o}C. After washing with PBS five times, the cells were then fixed in 4% paraformaldehyde for 20 min and washed with PBS five times. For staining the nuclei, the cells were incubated with 4,6-diamidino-2-phenylindole (DAPI, blue) for 2 min. The images of cells were observed using a laser scanning confocal microscope (Olympus FluoView 1000).

In order to quantitatively demonstrate the endocytosis of the cells, we performed flow cytometry analysis (FCS). HeLa cells were placed into 6-well plates (2×10^5 cells/well) and cultured in 2.0 mL of complete DMEM for 24 h. The culture media were then withdrawn and culture media with MSN-BDP-PEG, MSN-BDP-PEG + 10 mM GSH, Dox@MSN-BDP-PEG and Dox@MSN-BDP-PEG +10 mM GSH were supplemented at a final BODIPY 5 concentration of 2.5 μg /mL. The cells were incubated for additional 2 h, 5 h and 24 h, followed by washing with PBS three times and trypsinized. Then, 1.0 mL of PBS was added, and the solutions were centrifuged for 5 min at 3000 rpm and the cells were resuspended in 0.4 mL of PBS. The analysis was performed by flow cytometer (Beckman, California, U.S.A.) for 5×10^3 cells.

Cell viability assays

To study the dark toxicity and phototoxicity of MSN-BDP-PEG and Dox@MSN-BDP-PEG to HeLa cells, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was carried out in the presence or absence of the 638 nm light source. HeLa cells were inoculated at the density of $5*10^3$ cells/well in 96-well plates and incubated in 100 mL of medium overnight. Then add 100mL solution of the MSN-BDP-PEG and Dox@MSN-BDP-PEG concentrations of 0, 10, 25, 50 and 100 ugmL^{-1} to 96-well plates respectively and culture for 6 hours. The light group was taken out of the incubator and each well was illuminated with a 638 nm light source for 5 min. The formulations were replaced by 5 mg/mL MTT-

containing DMEM after 24h. After incubation of the cells for 4 h, MTT was aspirated off and formazan crystals were dissolved by adding DMSO. The absorbance was detected by a microplate reader (Bio-Rad Model 680, Watford, UK) at 490 nm, and calibrated to zero by using untreated cells with 100% viability as control and the cells without MTT addition as blank.

We conducted a control experiment about the combination of Dox with BODIPY (BODIPY-Dox). The MTT assay was carried out in the presence or absence of the 638 nm light source to study the dark toxicity and phototoxicity of BODIPY-Dox to HeLa cells. HeLa cells were inoculated at the density of 5×10^3 cells/well in 96-well plates and incubated in 100 μL of medium overnight. The BODIPY-Dox solution was obtained by dissolving 1.2 mg of BODIPY 5 with DMSO and then adding 1.5 mg of Dox. Then added 100 μL solution of the BODIPY 5 concentrations of 0, 0.44, 1.1, 2.2 and 3.5 μg mL^{-1} to 96-well plates respectively and cultured for 6 hours. The light group was taken out of the incubator and each well was illuminated with a 638-nm light for 5 min. The subsequent steps were as described above.

In vivo antitumor efficacy of MSN-BDP-PEG and Dox@MSN-BDP-PEG

All animal experiments were performed in compliance with the guidelines of the Chinese Academy of Science Committee for Animal Use and Care. Chinese Academy of Science Committee approved the experiments in this work. The xenograft tumors were established by subcutaneously injecting U14 cells into the armpit of the female Kunming mice use for treatment and bio-imaging. Treatment was initiated after tumors of 7-8 mm in diameter were palpable. The tumor-bearing mice were divided into 6 groups: Control, Control+Light, MSN-BDP-PEG, MSN-BDP-PEG+Light, Dox@MSN-BDP-PEG, Dox@MSN-BDP-PEG+Light. And 4 mice were used in each group. Each group of mice were intratumorally injected with the corresponding drugs or saline and take the mice that need light out, followed by 10 min of irradiation by the 638 nm laser at a power density of 1 W cm^{-2}. Upon laser irradiation (638 nm, 1W cm^{-2}), the temperature change in tumors under laser irradiation is monitored by infrared thermal camera. Elevation of temperature could be seen directly, indicating

the photothermal activity is kept in vivo. For tumor-bearing mice injected with MSN-BDP-PEG and Dox@MSN-BDP-PEG, the local tumor temperature is adjusted between 42 to 45 °C for 10 min, which is high enough to kill the tumor in vivo. As a control, the tumor temperature is slightly altered under the same laser conditions toward the mice treated with saline. The tumor size and body weight of each mouse were measured every two days. After 16 days of treatment, the antitumor activity was evaluated by measuring the terminal tumor volume. Tumors and major organs (heart, liver, spleen, lung and kidney) were excised and weigh tumor weight.

References:

1. B. G. T. Cheng-Yu Lai, Dusan M. Jeftinija, Ksenija Jeftinija, Shu Xu, Srdija Jeftinija, and Victor S.-Y. Lin, *J. Am. Chem. Soc.* 2003**, 15**, 4451-4459.

2. H. He, H. kuang, L. Yan, F. Meng, Z. Xie, X. Jing and Y. Huang, *Phys. Chem. Chem. Phys.* 2013**, 15**, 14210-14218.

3. (a) M. H. Y. Cheng, H. Savoie, F. Bryden and R. W. Boyle, *Photochem. Photobiol. Sci.* 2017**, 16**, 1260-1267. (b) M. Cordaro, P. Mineo, F. Nastasi and G. Magazzù, *RSC Adv.* 2014**, 4**, 43931-43933. (c) X. Guan, X. Hu, S. Liu, X. Sun and X. Gai, *RSC Adv.* 2016**, 6**, 1151-1157. (d) X.-D. Jiang, X. Liu, T. Fang and C. Sun, *Dyes Pigm.* 2017**, 146**, 438-444.

Table S1. Textural and structural parameters

Material	Surface area (m^2g^{-1})	Pore size (nm)
MSN-SH	946.909	3.537
MSN-SS-COOH	877.918	3.415
MSN-BDP-PEG	211.068	3.056

Table S2. IC_{50} of BDP –loaded nanoparticles.

Simple	MSN-BDP-PEG	Dox@MSN-BDP-PEG	MSN-BDP-PEG+Light	Dox@MSN-BDP-PEG+Light
IC_{50} (μg/mL)	317.7	57.8	68.9	8.9

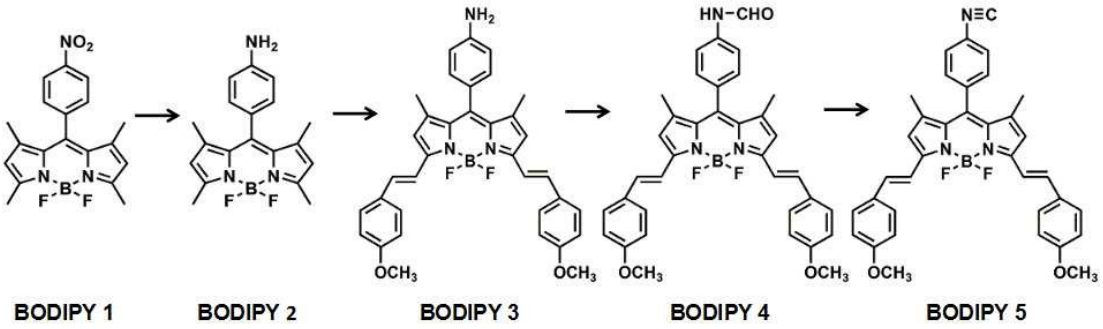

Fig. S1 Synthesis procedure of BODIPY 5.

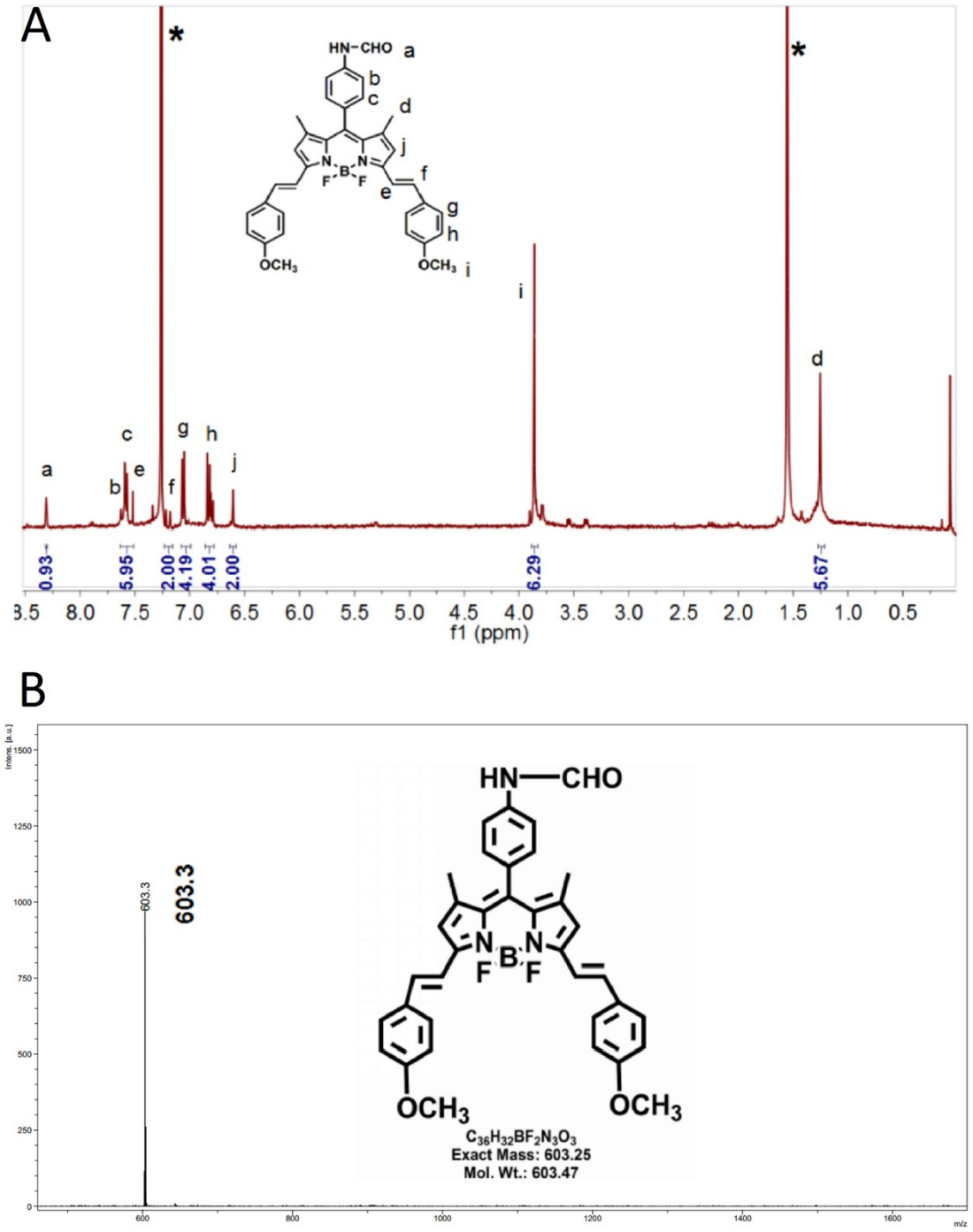

Fig. S2 (A) ^{1}H-NMR and (B) MALDI - TOF - MS of BODIPY 4.

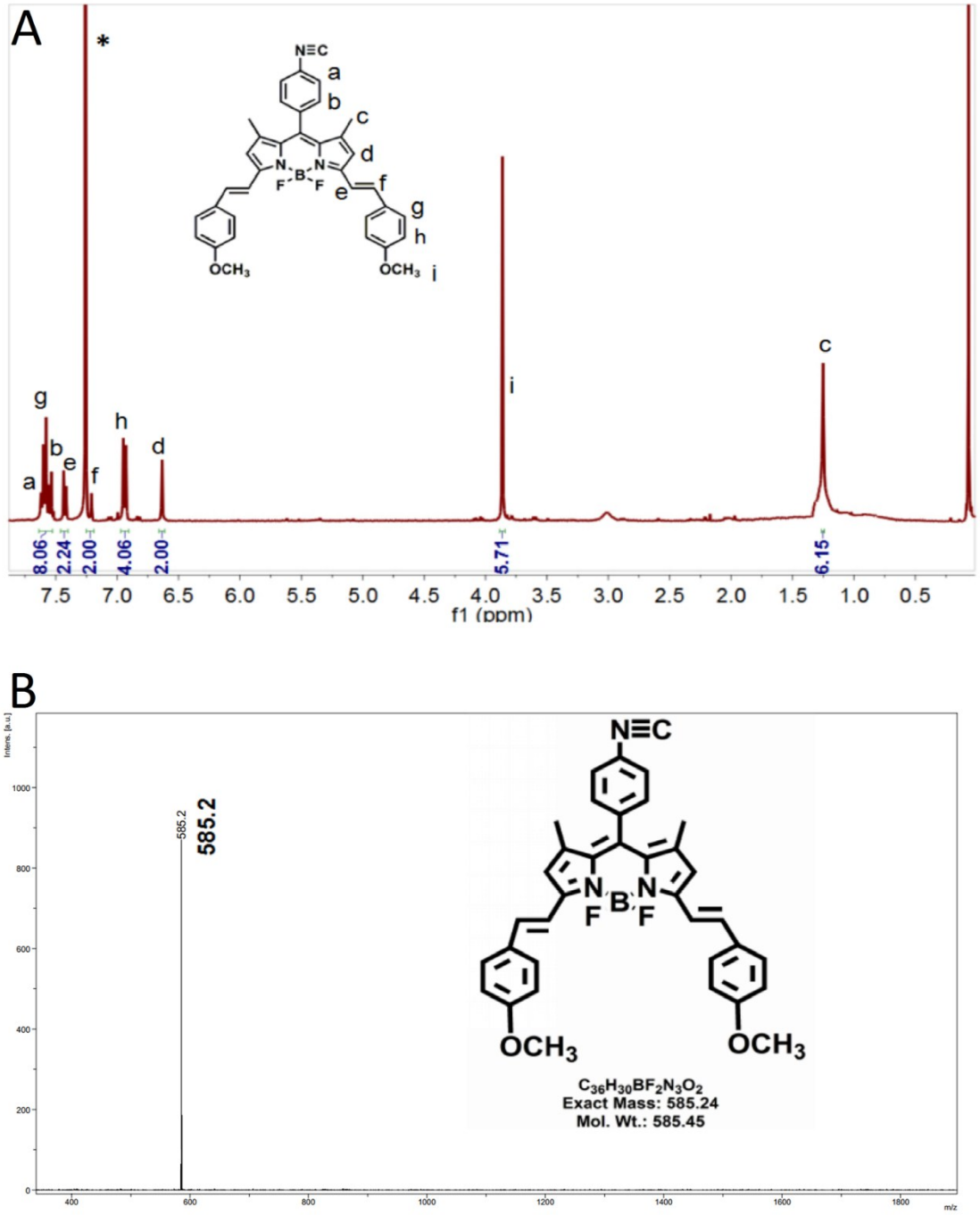

Fig. S3 (A) ^{1}H-NMR and (B) MALDI - TOF - MS of BODIPY 5.

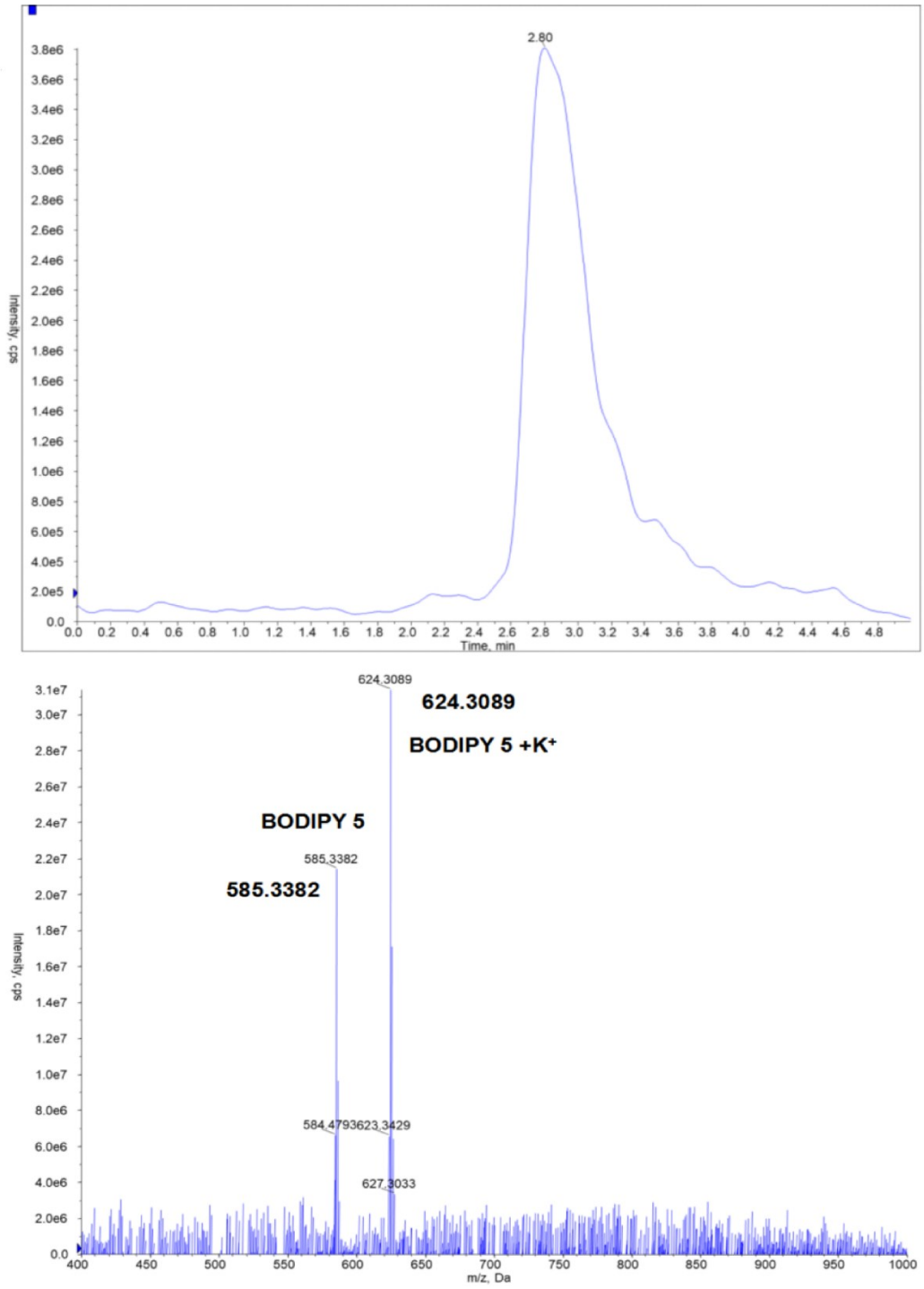

Fig. S4 HPLC-ESI-MS/MS of BODIPY 5

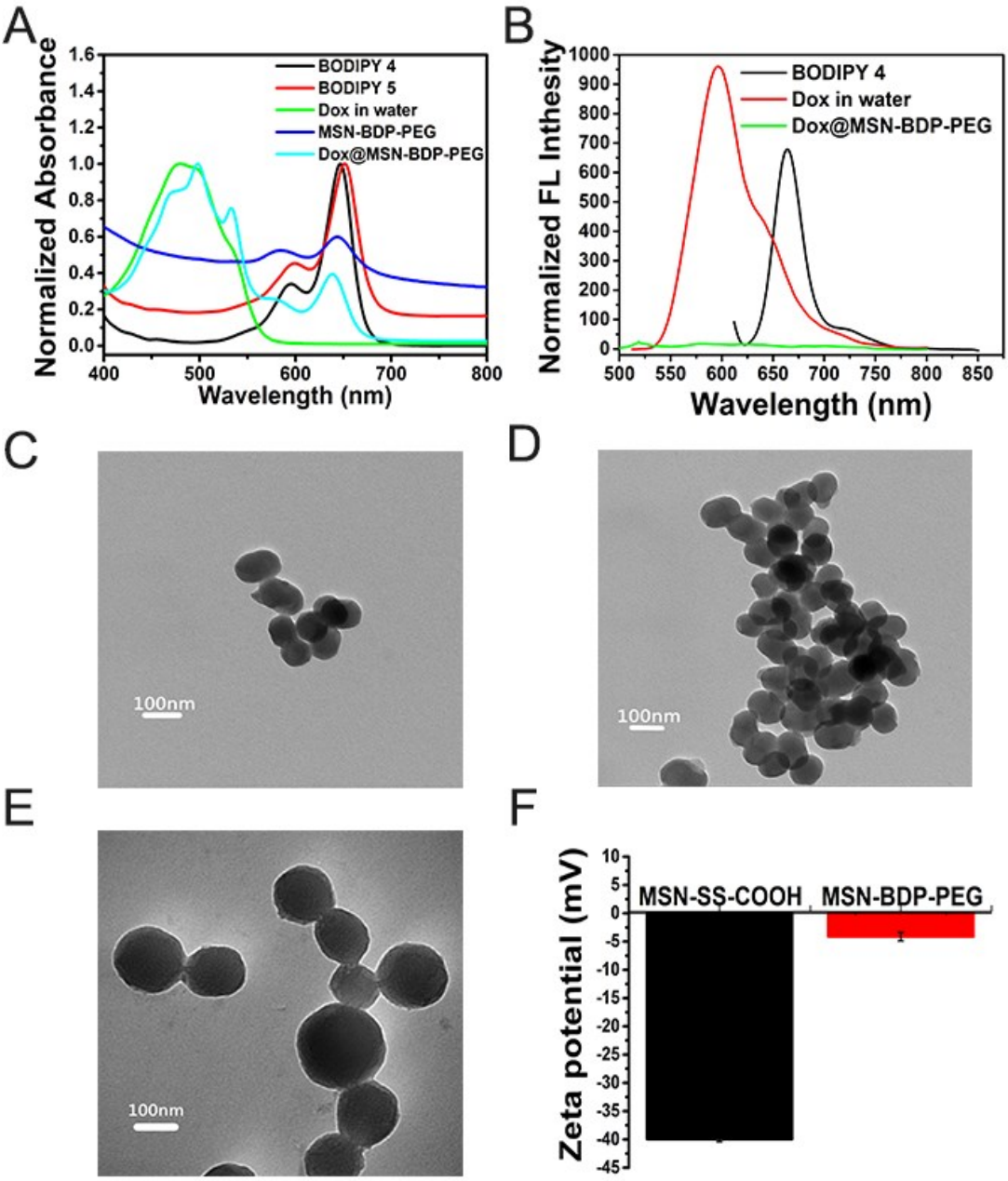

Fig. S5 (A) UV absorption spectra of BODIPY 4, BODIPY 5, Dox in water, MSN-BDP-PEG and Dox@MSN-BDP-PEG. (B) Fluorescence spectra of BODIPY 4, Dox in water and Dox@MSN-BDP-PEG. TEM images of (C) MSN-SS-COOH, (D) MSN-BDP-PEG and (E) Dox@MSN-BDP-PEG. Scale bar, 100 nm. (F) Zeta potentials of MSN-SS-COOH and MSN-BDP-PEG.

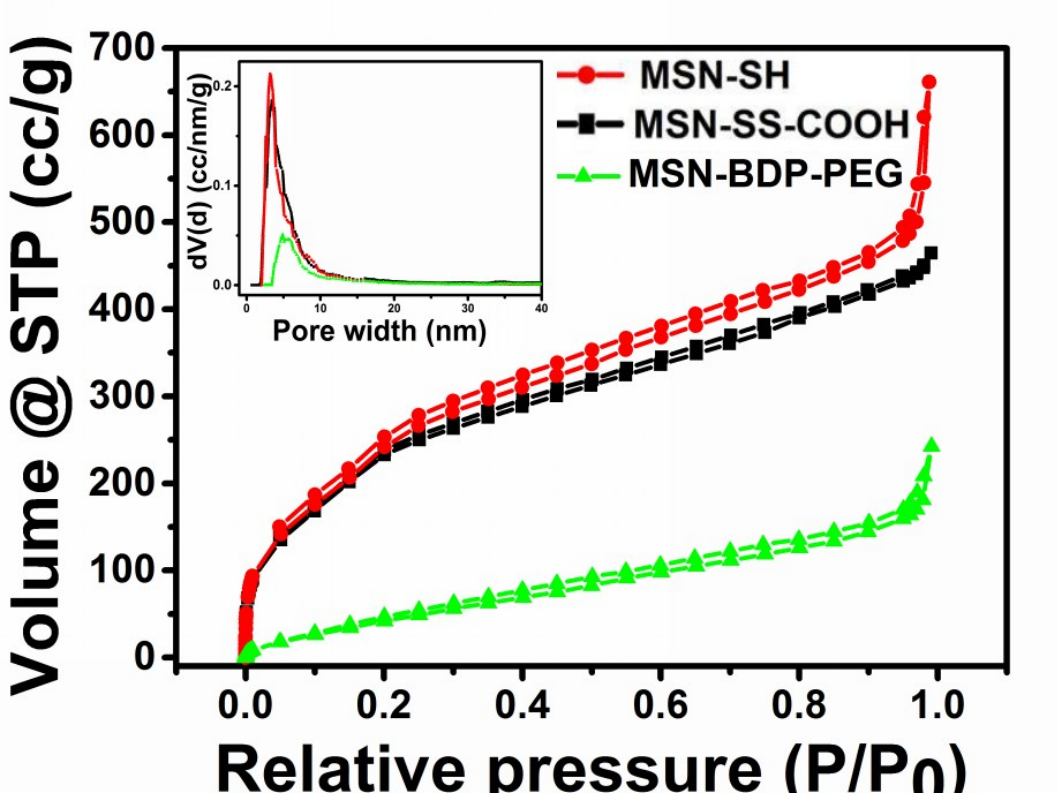

Fig. S6 Nitrogen adsorption – desorption isotherms of MSN-SH, MSN-SS-COOH and MSN-BDP-PEG; the inset demonstrates the BJH pore size distribution of the corresponding samples.

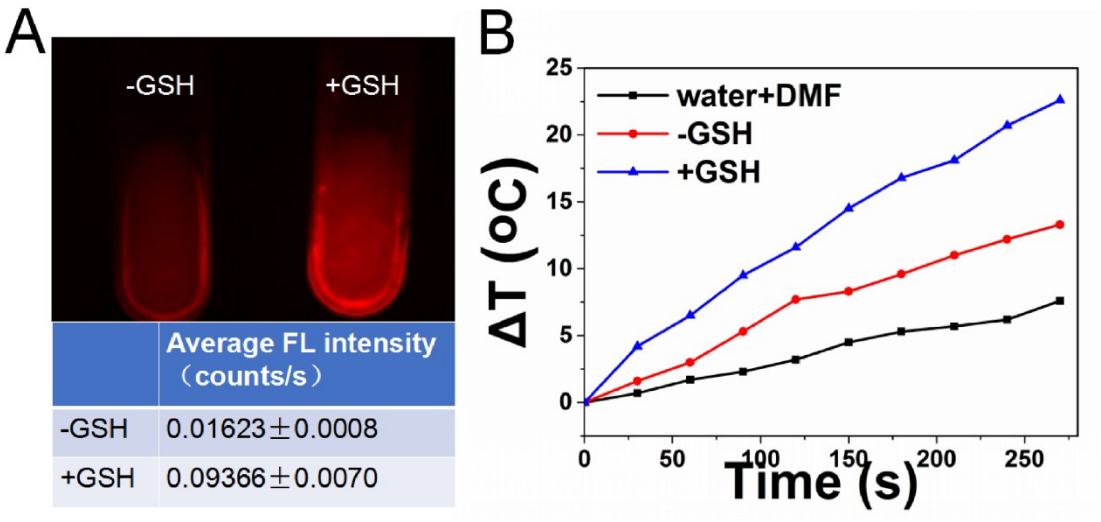

Fig. S7 (A) Fluorescence imaging of MSN-BDP-PEG after adding GSH. (B) Photothermal heating curves of water +DMF (v/v, 1:1), MSN-BDP-PEG in water +DMF (v/v, 1:1) after adding GSH. -GSH, without GSH. +GSH, adding GSH (10 mM) for 0.5 h.

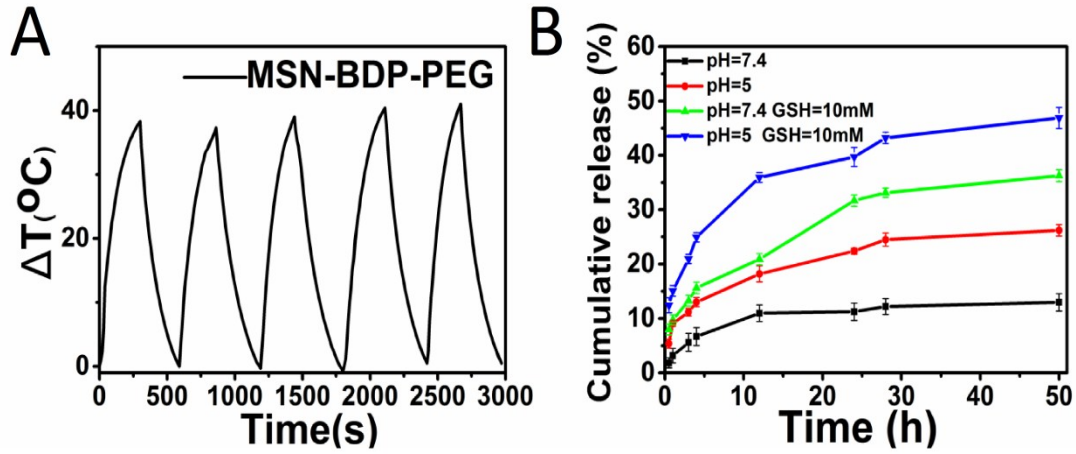

Fig. S8 (A) The heating curve of the MSN-BDP-PEG in water for five laser on/off cycles. (B) Dox release from Dox@MSN-BDP-PEG at different GSH concentrations in PBS (pH 7.4 and 5.0), 37 ^{o}C in vitro.

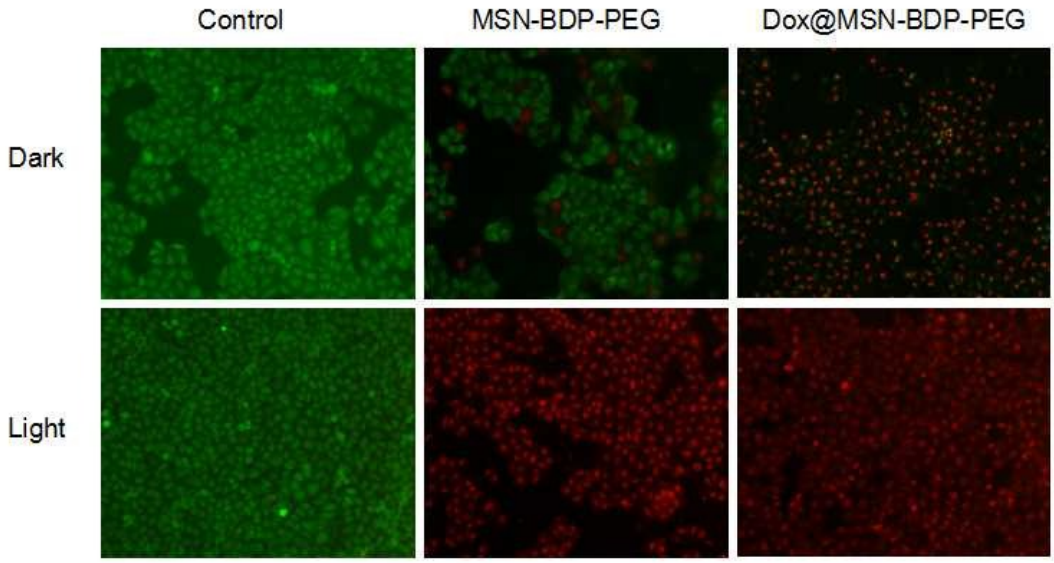

Fig. S9 Fluorescence imaging of Hela cells cultured in different ways (Control, Control + Light, MSN-BDP-PEG，MSN-BDP-PEG + Light，Dox@MSN-BDP-PEG, Dox@MSN-BDP-PEG+Light). Cells were treated with live/dead staining. Live cells were stained with calcein-AM to emit green fluorescence and dead/late apoptotic cells were stained with propidium iodide (PI) to emit red fluorescence.

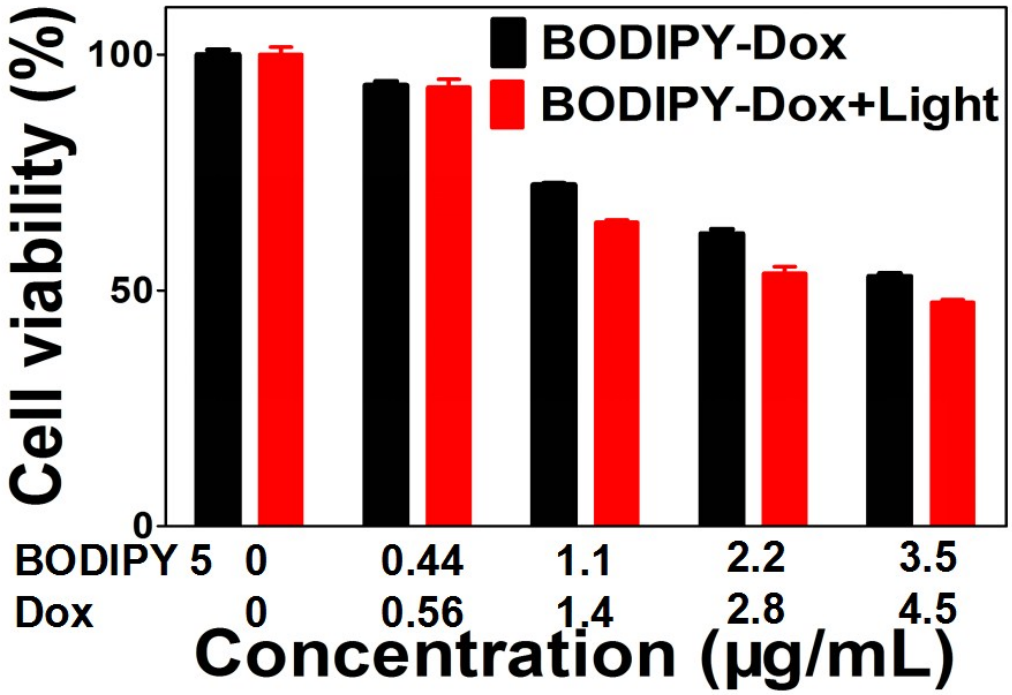

Fig. S10 Cell viability of BODIPY-Dox towards HeLa cells at different concentrations after 24 h of incubation.

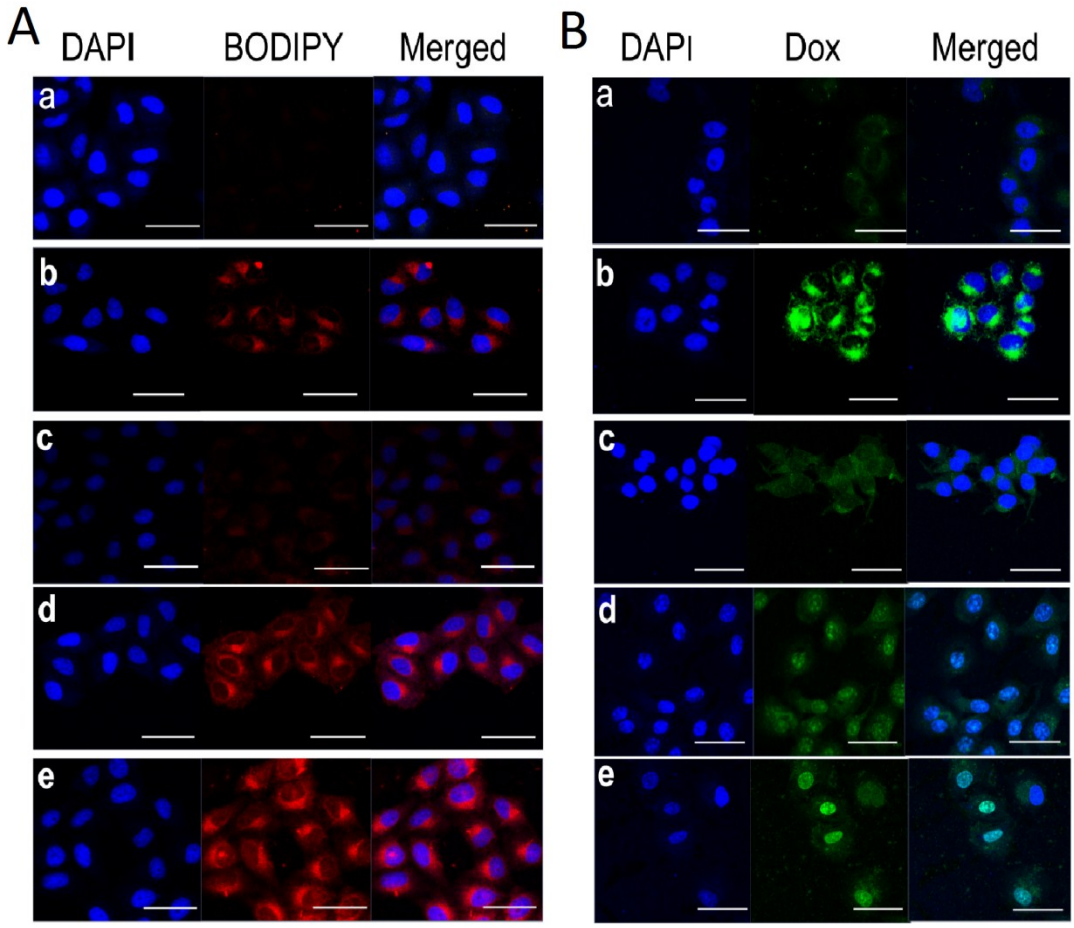

Fig. S11 CLSM images of HeLa cells incubated with (A) MSN-BDP-PEG and (B) Dox@MSN-BDP-PEG. In (A) cell nuclei stained by DAPI showed blue fluorescence and BODIPY showed red fluorescence in cells.The overlays of both images were shown in the rightmost. From top to bottom, it represents the HeLa cells incubated with MSN-BDP-PEG for (a)2 h, (c) 5 h, (d) 24 h and MSN-BDP-PEG + 10 mM GSH for (b) 2 h and (e) 24h. In (B) Dox showed green fluorescence in cells. The overlays of both images were shown in the rightmost. From top to bottom, it represents the HeLa cells incubated with Dox@MSN-BDP-PEG for (a)2 h, (c) 5 h, (d) 24 h and Dox@MSN-BDP-PEG + 10 mM GSH for (b) 2 h and (e) 24 h. Scale bar, 50 μm.

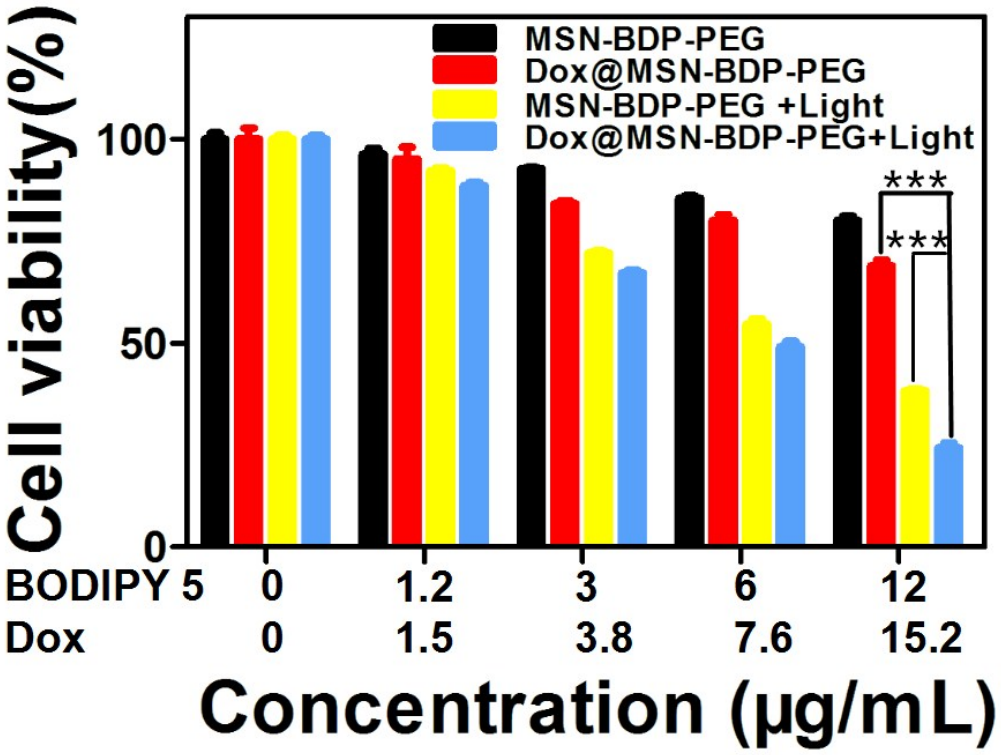

Fig. S12 Cell viability of U14 cells after 24 h incubation at 37 °C.

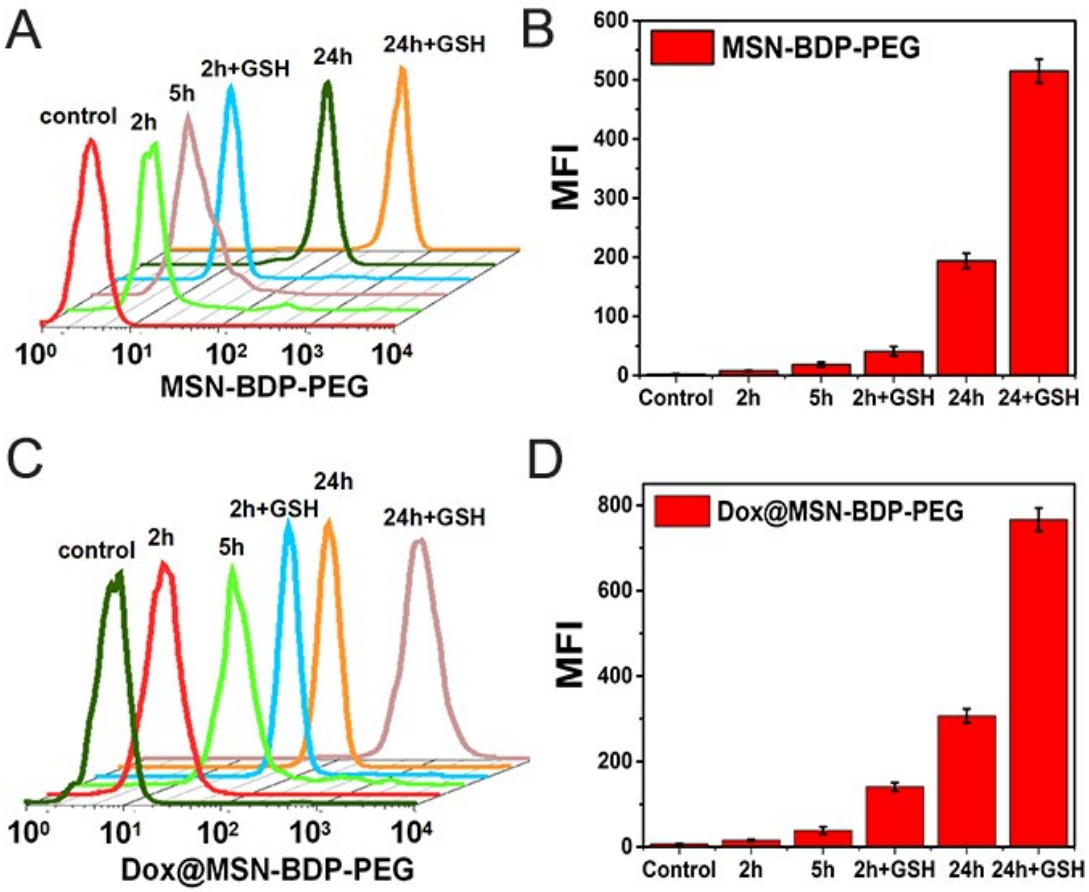

Fig. S13 (A) FCS of HeLa cells incubated with MSN-BDP-PEG at 37 °C for 2h, 2h+GSH, 5h, 24h and 24h+GSH. (B) Mean fluorescence intensity (MFI) quantification analyses against cellular uptake of MSN-BDP-PEG. (C) FCS of HeLa cells incubated with Dox@MSN-BDP-PEG at 37 °C for 2h, 2h+GSH, 5h, 24h and 24h+GSH. (D) MFI quantification analyses against cellular uptake of Dox@MSN-BDP-PEG.

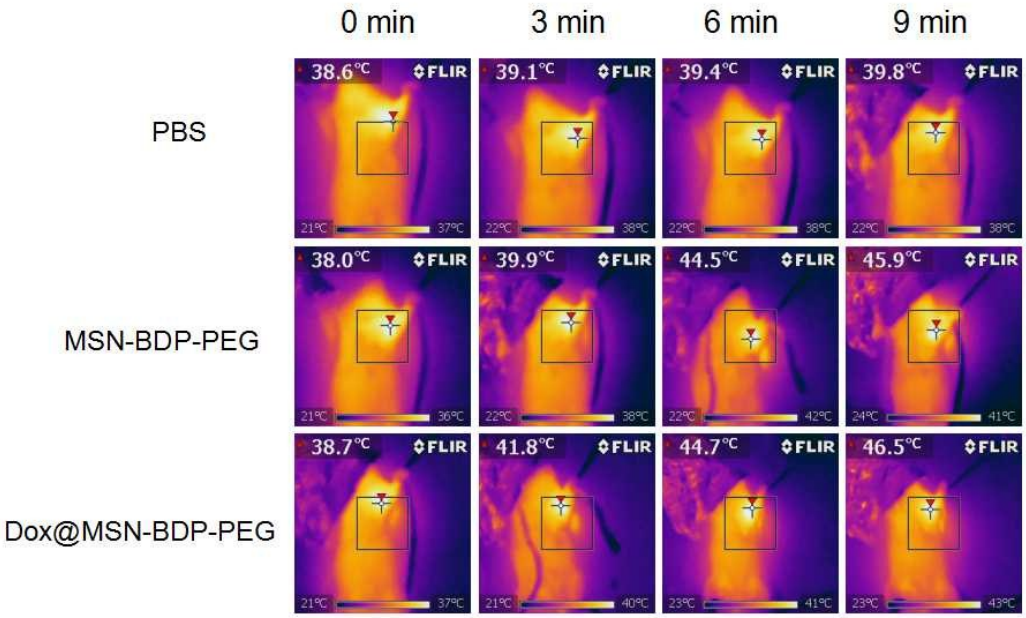

Fig. S14 The whole-body photothermal images of mouse after intratumoral injection of PBS, MSN-BDP-PEG and Dox@MSN-BDP-PEG (0.2 mL, 1 mg mL^{-1}) under 638 nm laser (1 W cm^{-2}) at different time point.

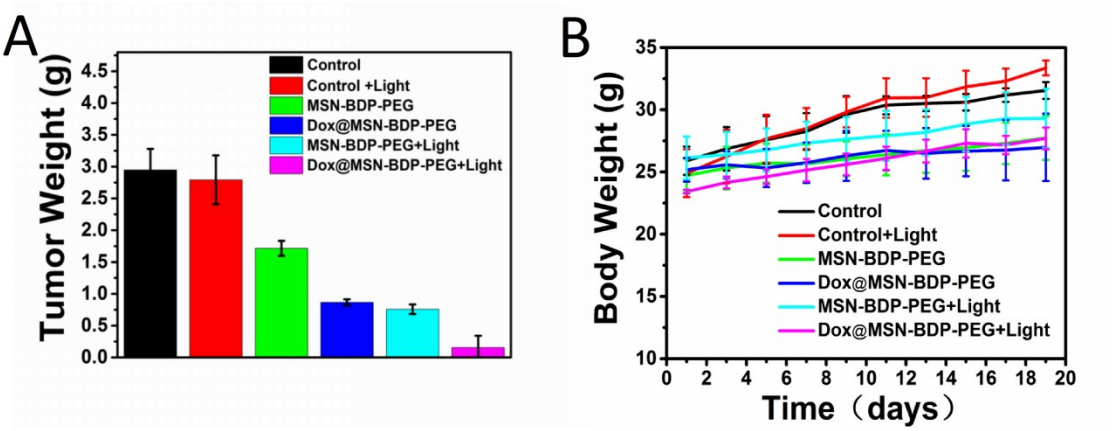

Fig. S15 (A) Tumor weights of each group. (B) Body weights of mice with various treatments.

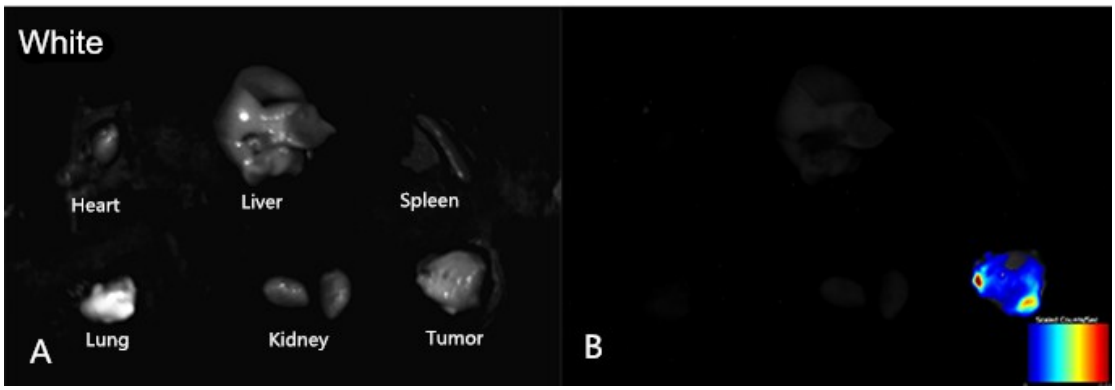

Fig. S16 Ex vivo (A) white light image and (B) fluorescence image of heart, liver, spleen, lung, kidney and tumor.