

# Simultaneously Self-supply of H<sub>2</sub>O<sub>2</sub> and GSH-depleted Intracellular Oxidative Stress for Enhanced Photodynamic /Photothermal /Chemodynamic Therapy

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## **S1. Materials**

Hexadecyltrimethylammonium bromide (CTAB), sodium hydroxide (NaOH), tetraethyl orthosilicate (TEOS, 95%), hydrochloric acid (HCl, 37%), (3-Mercaptopropyl)trimethoxysilane (MPTMS, 97%), methanol, indocyanine green (ICG, 75%), dopamine hydrochloride (99.0%), methylene blue (MB, 98%), Tris(hydroxymethyl)aminomethane (Tris, 99.9%), sodium bicarbonate (NaHCO<sub>3</sub>, 99.7%), 1,3-Diphenylisobenzofuran (DPBF, 97%), glutathione (GSH, 98%), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%), 4-(N-Maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester sodium salt (Sulfo-SMCC, 98%), potassium permanganate (KMnO<sub>4</sub>, 99%), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, AR), calcium chloride dehydrate (CaCl<sub>2</sub>·2H<sub>2</sub>O, AR), 5,5'-Dithio bis-(2-nitrobenzoic acid) (DTNB, 98%), hydroxyphenyl fluorescein (HPF, 98%), thiazolyl blue tetrazolium bromide (MTT, 97.5%). Reactive Oxygen Species Assay Kit were purchased from Beyotime, AnnexinV-FITC Apoptosis detection kit were purchased from BD Bioscience. UV-2700i UV-visible (UV-Vis) spectrophotometer (Hitachi, JAPAN). SpectraMax i3x multifunctional microplate reader (Molecular Devices, USA). FLIR-A600-Series infrared thermal camera (Teledyne FLIR, USA). Axio Vert. A1 fluorescence microscope (ZEISS, GERMANY). CytoFLex(A00-1-1102) flow cytometry (Beckman Coulter, USA). All other chemicals were commercially available and used. All chemicals were used as received unless otherwise noted.

## **S2. Synthesis of Sulfhydryl modified mesoporous silica nanoparticles**

Silica nanoparticles with CTAB as the skeleton were synthesized according to reported method<sup>[1]</sup>. CTAB (0.5 g) was dissolved in deionized water (96 mL) under stirring. Then, NaOH (2 M, 0.7 mL) was added and the reaction system was heated to 85°C. Then TEOS (0.25 mL) was slowly added under stirring, MPTMS (0.2 mL) and TEOS (0.25 mL) was slowly added after 5 min. The solution gradually became turbid. After reaction for 3 h, the solution was centrifuged while hot, washed with deionized water and methanol. The white precipitate was ultrasonically dispersed in methanol solution, then heated and refluxed with methanol (16 mL) and hydrochloric acid (0.9 mL) for 24 h. and washed with methanol and deionized water three times.

### **S3. Load of GOD**

GOD (45 mg) was dissolved in deionized water (2 mL). Sulfo-SMCC (10 mg/mL) was dissolved in DMF/water (v/v = 1:1) solution (2 mL) and then was added to the GOD solution. The mixture was stirred for 1 h at room temperature. Afterwards, sul-Silica NPs (10 mg/mL, 10 mL) were added. The mixture was reacted for 2 h while stirring. The GOD loaded silica nanoparticles (GOD@SiO<sub>2</sub> NPs) were obtained by centrifugation and washed three times with deionized water.

### **S4. Synthesis of SM NPs.**

The GOD@SiO<sub>2</sub> NPs solution (1 mg/mL, 50 mL) was added dropwise to KMnO<sub>4</sub> solution (1 mg/mL, 50 mL). The mixture was stirred for 10 min and then purified by repetitive centrifugation (three times for 5 min, 12000 rpm). After that, SM NPs were obtained and redispersed in deionized water.

### **S5. Synthesis of SMP NPs.**

The SM NPs were dispersed in 10 mL Tris buffer (pH8.5,  $10 \times 10^{-3}$  M) containing dopamine hydrochloride (10 mg). Then the mixture was stirred for 6 h in the dark at room temperature. The SMP NPs were purified by centrifugation three times and redispersed in deionized water.

### **S6. Synthesis of SMP@I NPs.**

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (10 mg/mL, 1 mL) and ICG (2 mg/mL, 2 mL) were added to SMP NPs dispersion (10 mg/mL, 10 mL). The mixture was stirred for 12 h. Then, the mixture was centrifuged and redispersed in 50 mL deionized water. After that,  $\text{Na}_2\text{CO}_3$  (10 mg/mL, 0.2 mL) and ICG (2 mg/mL, 2 mL) were added into the mixture and reacted for 6 h. The mixture was further centrifuged and dialyzed against deionized water for 24 h. SMP@I NPs were obtained by freeze drying.

### **S7. Characterization of NPs**

The surface morphology and particle size of nanoparticles were measured by transmission electron microscopy (TEM). The elemental composition of SMP@I NPs was analyzed by energy dispersive X-ray spectroscopy (EDS). The absorption spectra of the NPs were obtained by UV-vis-NIR spectrophotometer. The infrared spectra of NPs was obtained by infrared spectroscopy. The surface properties of NPs were analyzed by X-ray photoelectron spectroscopy (XPS).

### **S8. Degradation of SMP@I NPs**

SMP@I solution (1 mg/mL, 1 mL) was mixed with GSH solution (10 mM) at pH 5.5. Then the mixture was irradiated with 808 nm laser ( $2 \text{ W/cm}^2$ ) for 5 min. The

morphology of SMP@I NPs was observed via TEM after 1 h or 12 h incubation. SMP@I solution (1 mg/mL, 1 mL) was mixed with GSH solution (10 mM) at pH 7.4 and irradiated with 808 nm laser (2 W/cm<sup>2</sup>) for 5 min or mixed with GSH solution (10 mM) at pH 5.5. The morphology of SMP@I NPs was observed via TEM after 12 h incubation.

### **S9. In vitro photothermal performance**

The in vitro photothermal property of SMP@I NPs was measured by infrared thermal camera (FLIR-A600-Series). In order to study the effect of PDA shell on the photothermal properties of NPs, four kinds of NPs solution (SiO<sub>2</sub>, SM, SMP and SMP@I) were irradiated with a 808 nm laser (2 W/cm<sup>2</sup>, 10 min). Deionized water was used as control. To examine the concentration-dependency of the photothermal effect, different concentrations of SMP@I solution (0, 200, 400, 600, 800 and 1000 µg/mL) were irradiated for 15 min. In order to study the optical power dependence of the photothermal effect, SMP@I solution (1000 µg/mL) was irradiated with different laser intensity (0.5, 1, 1.5 and 2 W/cm<sup>2</sup>) for 5 min. Deionized water was used as control. In order to study the photothermal stability, SMP@I solution (1000 µg/mL) was repetitively irradiated for 4 cycles with 15 min ON and 15 min OFF. The temperature change during irradiation was monitored by infrared thermal camera.

### **S10. Detection of reactive oxygen species (ROS) in vitro**

DPBF was used to detect <sup>1</sup>O<sub>2</sub>. SMP@I solution (1 mg/mL) and DPBF (20 µM) were mixed and further irradiated with 808 nm laser (2 W/cm<sup>2</sup>) in the presence of

GSH (10 mM) and hydrogen peroxide (8 mM) at pH 5.5. The generated  $^1\text{O}_2$  was monitored by measuring the absorbance change of DPBF at 426 nm at different time points. MB was used to detect  $\bullet\text{OH}$ . SMP@I solution (1 mg/mL) and MB (10  $\mu\text{g}/\text{mL}$ ) were mixed and irradiated with 808 nm laser (2  $\text{W}/\text{cm}^2$ ) in the presence of GSH (10mM) and hydrogen peroxide (8 mM) at pH 5.5. The absorbance value of MB in the reaction solution at 665 nm was detected by UV-vis spectrophotometer to monitor  $\bullet\text{OH}$  generation.

### **S11. Detection of GSH in vitro**

The in vitro consumption of GSH was detected by DTNB. Different concentration of SMP@I solution (200, 400, 600, 800 and 1000  $\mu\text{g}/\text{mL}$ ) was mixed with GSH solution (5 mM). After laser irradiation (808 nm, 2  $\text{W}/\text{cm}^2$ , 5 min), DTNB (20  $\mu\text{M}$ ) was added for 5 min. By measuring the absorbance of DTNB at 412 nm after reaction, the concentration of residual GSH in the solution was detected to prove the consumption of GSH by SMP@I.

### **S12. Cell viability and live/dead assay**

The cytotoxicity of SMP@I was determined by examining the viabilities of ACC-83 and 4T1 cells after SMP@I treatment using the MTT assay.  $7 \times 10^3$  4T1 cells or ACC-83 cells per well were seeded onto 96-well plates. Cells were incubated at 37°C for 12 hours. Then, medium containing SMP@I (50, 25, 12.5, 6.25 and 3.125  $\mu\text{g}/\text{mL}$ ) was used to replace the original medium. After 6 hour, the medium containing SMP@I was replaced with fresh medium, and the cells were exposed to NIR laser (808 nm, 2  $\text{W}/\text{cm}^2$ , 5 min) and then cultured for 12 h or cultured directly

for 12 h without light. MTT working solution was added as 20  $\mu\text{L}$  per well. Cell viability was assessed by the measurement of the absorbance at the wavelength of 490 nm and 630 nm on the microplate reader. The activity and toxicity of 4T1 or ACC-83 cells were detected by Calcein AM/PI staining. The cells were incubated with SMP@I solution (25 and 50  $\mu\text{g}/\text{mL}$ ) for 6 h and exposed to NIR laser (808 nm, 2  $\text{W}/\text{cm}^2$ , 5 min). Then 100  $\mu\text{L}$  Calcein AM/PI working solution was added and incubated at 37°C in the dark for 30 min. The live/dead cells images were observed using fluorescence microscope (Axio Vert. A1).

### **S13. Detection of intracellular ROS**

DCFH-DA was used to detect the generated ROS in the intracellular environment. Typically,  $3 \times 10^5$  4T1 cells were seeded into 6-well plates and incubated overnight at 37°C. Then, SMP@I NPs (25  $\mu\text{g}/\text{mL}$ ) were added and cultured for 6 hours, followed by incubation with DCFH-DA (10  $\mu\text{M}$ ) for additional 20 min. Cells were washed with serum-free medium three times and then exposed to NIR laser (808 nm, 2  $\text{W}/\text{cm}^2$ ) for 5 min. The generated ROS was detected using fluorescence inverted microscope.

HPF was used to detect the generated  $\bullet\text{OH}$  in the intracellular environment.  $3 \times 10^5$  4T1 cells were seeded into 6-well plates and incubated overnight at 37°C. Then, SMP@I NPs (12.5 or 25  $\mu\text{g}/\text{mL}$ ) were added and cultured for 6 hours, followed by incubation with HPF (10  $\mu\text{M}$ ) for additional 60 min. Cells were washed with serum-free medium three times and then exposed to NIR laser (808 nm, 2  $\text{W}/\text{cm}^2$ ) for 5 min. The generated  $\bullet\text{OH}$  was detected using fluorescence inverted microscope.

The generated intracellular  $^1\text{O}_2$  was detected by  $^1\text{O}_2$  detection kit. Phenylanthracene fluorescent probe can freely pass through the cell membrane and react with  $^1\text{O}_2$  to be oxidized to green fluorescent substances.  $7 \times 10^3$  4T1 cells were seeded into 96-well plates. Then the cells were incubated with SMP@I (12.5 or 25  $\mu\text{g}/\text{mL}$ ) for 6 hours and exposed to 808 nm laser (5 min, 2  $\text{W}/\text{cm}^2$ ). The cells were incubated with phenylanthracene probe (10  $\mu\text{M}$ ) at 37  $^\circ\text{C}$  for 1 hours. After, cells were washed with PBS for three times, and the generated  $^1\text{O}_2$  was observed via fluorescence inverted microscope.

Superoxide anion ( $\text{O}_2\cdot^-$ ) production in cells was detected by superoxide assay kit. WST-1 is a compound similar to MTT that can be reduced by superoxide to form orange Formazan.  $7 \times 10^3$  4T1 cells were inoculated into 96-well plates. Then cells were incubated with SMP@I NPs (12.5 or 25  $\mu\text{g}/\text{mL}$ ) for 6 hours. 200  $\mu\text{L}$  superoxide anion working solution was added into each well, incubated at 37  $^\circ\text{C}$  for 3 min, followed by exposure to 808 nm laser (5 min, 2  $\text{W}/\text{cm}^2$ ). The 450 nm absorbance was detected by microplate reader.

#### **S14. Cell apoptosis in vitro**

The apoptosis induced by SMP@I was further analyzed by flow cytometry. 4T1 cells ( $5 \times 10^5$  cells per well) were seeded into 6 well plates and cultured for 12 h. Then the 4T1 cells were incubated with SMP@I (12.5, 25 and 50  $\mu\text{g}/\text{mL}$ ) for 6 h and exposure to NIR laser (808 nm, 2  $\text{W}/\text{cm}^2$ ) for 5 min. After 24 h incubation, the cells were treated according to the instructions of the Apoptosis Kit and detected by flow cytometry (CytoFLex (A00-1-1102)).

### **S15. Establishment of tumor model in vivo**

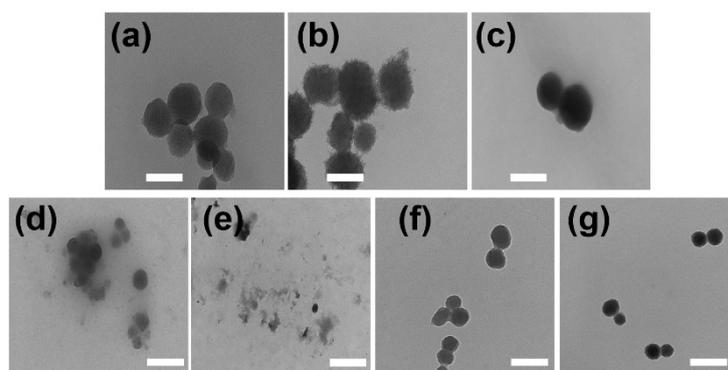
BALB/c mice (female, 4~6 weeks, 15~18g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. 4T1 cells ( $2 \times 10^6$ ) were dispersed in 100  $\mu$ L fresh serum-free 1640 medium and injected subcutaneously in the left armpit of each mouse. Then, all mice were maintained under specific pathogen-free conditions and had free access to food and water for 10 days. The tumor volume was monitored every day until the tumor size reached 100-150 mm<sup>3</sup>. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th edition, 2011). The animal protocol was approved by the local research ethics review board of the Animal Ethics Committee of Weifang Medical University (certificate number is 2021SDL328).

### **S16. Animal experiment**

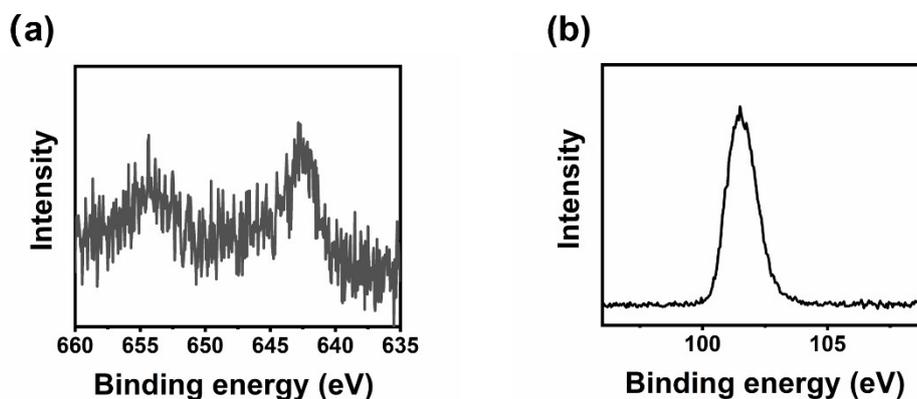
The anticancer efficacy was evaluated by monitoring the tumor volumes using tumor-bearing mice. When tumor nodules reached approximately 100 mm<sup>3</sup>, mice were randomly divided into 7 groups (n=5/group): (1) a group of tumor-bearing mice was injected with PBS (PBS group); (2) a group of tumor-bearing mice was injected with PBS, followed by 808 nm laser irradiation (PBS+808 nm group); (3) a group of tumor-bearing mice was injected with 1 mg/kg GOD (GOD group); (4) a group of tumor-bearing mice was injected with 2 mg/kg ICG (ICG group); (5) a group of tumor-bearing mice was injected with 2 mg/kg ICG, followed by 808 nm laser irradiation (ICG+808 nm group); (6) a group of tumor-bearing mice was

injected with 10 mg/kg SMP@I (SMP@I group); (7) a group of tumor-bearing mice was injected with 10 mg/kg SMP@I, followed by 808 nm laser irradiation (SMP@I+808 nm group). Each injection was performed via intratumoral injection. Tumor site was irradiated for 5 min with an intensity of 2 W/cm<sup>2</sup>. Temperature increase at tumor sites was recorded using a digital infrared thermal imaging camera. The size of tumor and the weight of mice were recorded continuously for 2 weeks. At the end of tumor inhibition experiments, the mice from each group were sacrificed and tumor were collected. Main organs including heart, liver, lung, kidney and spleen were also collected for H&E staining.

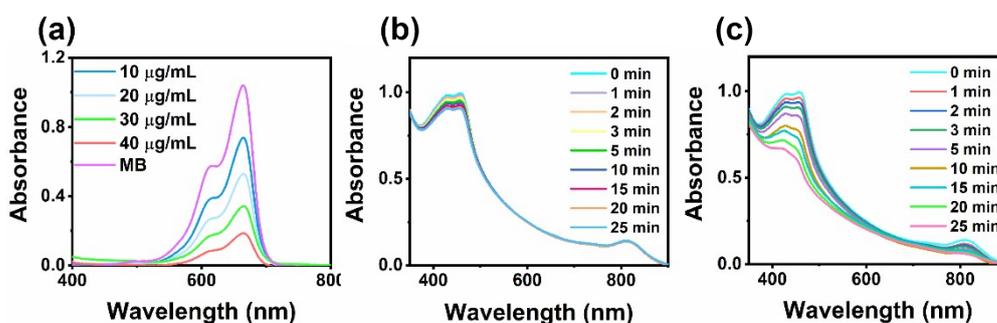
### S17. Supplementary Figures



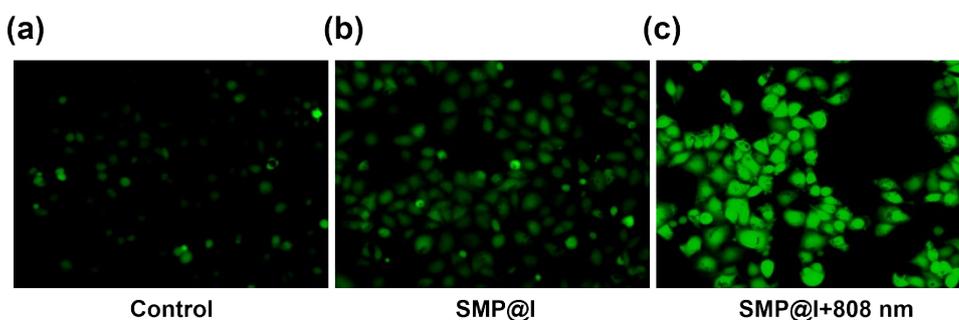
**Figure S1.** TEM images of (a) SiO<sub>2</sub>, (b) SM, (c) SMP, (d) SMP@I after 1 h reaction under pH5.5 and irradiation(808nm, 2W/cm<sup>2</sup>, 5min) and (e) SMP@I after 12 h reaction under pH5.5 irradiation (808nm, 2W/cm<sup>2</sup>, 5min) , (f) SMP@I after 12 h reaction under physiological pH irradiation (808nm, 2W/cm<sup>2</sup>, 5min), (g) SMP@I after 12 h reaction under pH5.5. (a, b, c -Scale bars=100 nm; d, f, g -Scale bars=200 nm; e -Scale bars=500 nm)



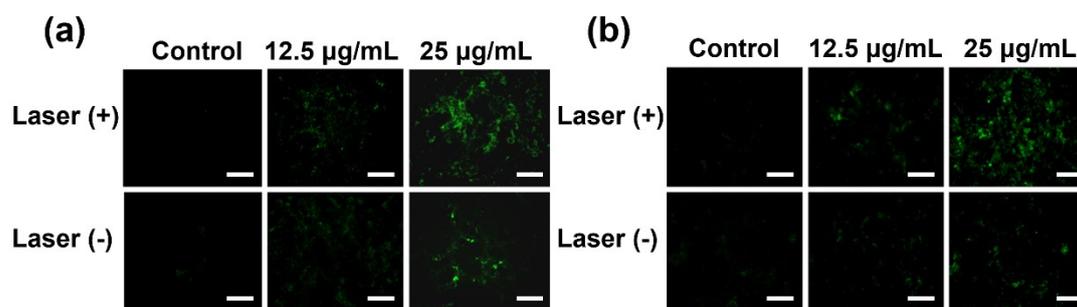
**Figure S2.** (a) high resolution Mn 2p XPS spectra of SMP@I (b) high resolution Si 1s XPS spectra of SMP@I.



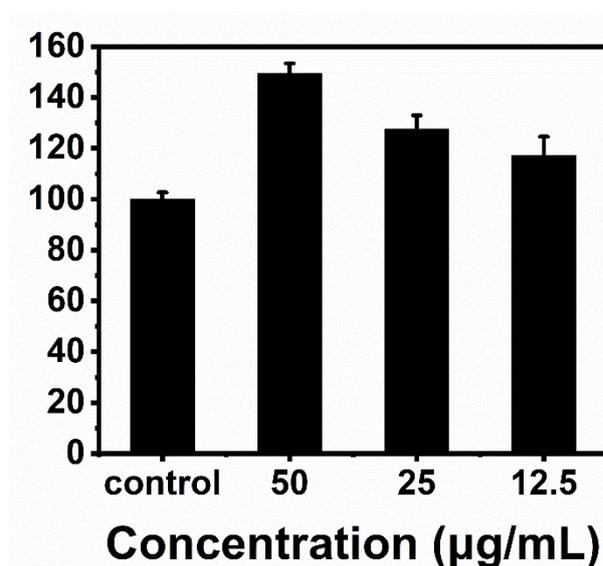
**Figure S3.** (a) UV-vis spectra of the remaining MB after treatment with different concentrations of SMP@I solution (b) UV-vis spectra of the DPBF solution treated by SMP@I for different times (c) UV-vis spectra of the DPBF solution treated by SMP@I and 808 nm laser irradiation for different times



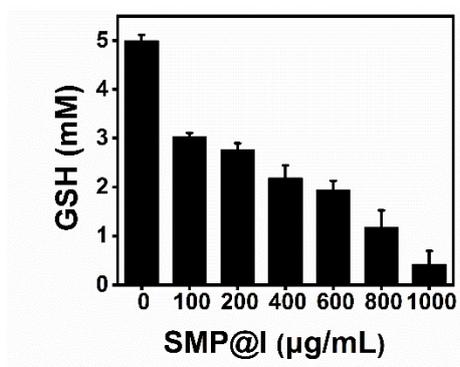
**Figure S4.** DCF fluorescence of ACC-83 cells treated with SMP@I under different light conditions (Scale bars=100  $\mu\text{m}$ )



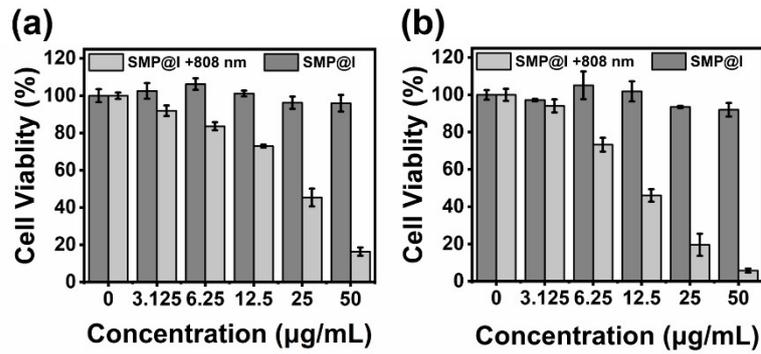
**Figure S5.** (a) HFP fluorescence of 4T1 cells after different treatment (Scale bars=100  $\mu\text{m}$ ) (b)  $^1\text{O}_2$  sensitive probe fluorescence of 4T1 cells after different treatment.



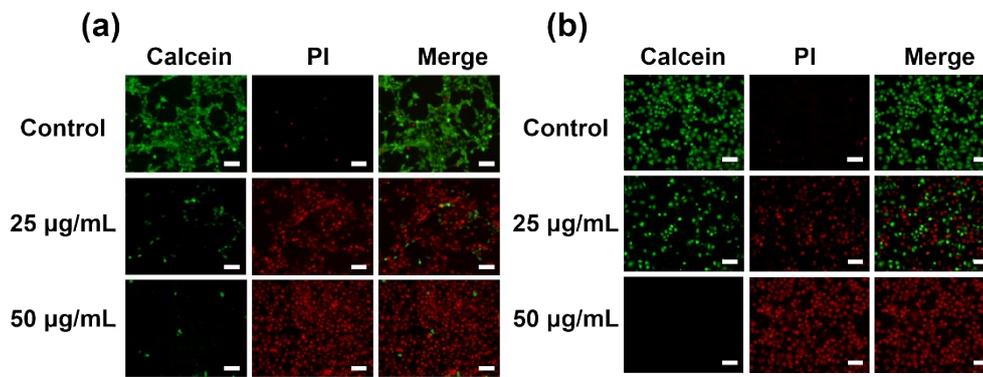
**Figure S6.** Superoxide anion level of 4T1 cells after the incubation with SMP@I (12.5, 25 and 50  $\mu\text{g/mL}$ ) and 808 nm laser irradiation (2  $\text{W/cm}^2$ )



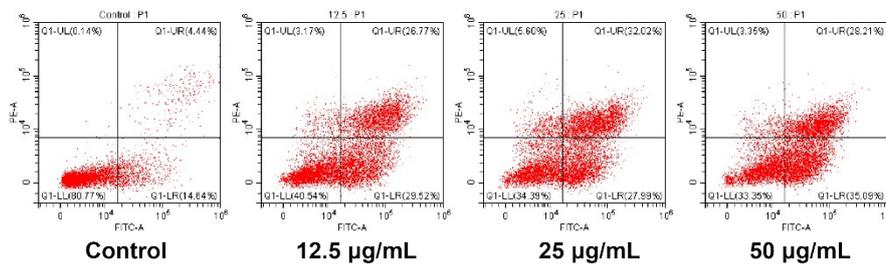
**Figure S7.** GSH levels after treatment with SMP@I (100, 200, 400, 600, 800 and 1000  $\mu\text{g/mL}$ ) and 808 nm laser irradiation (2  $\text{W/cm}^2$ )



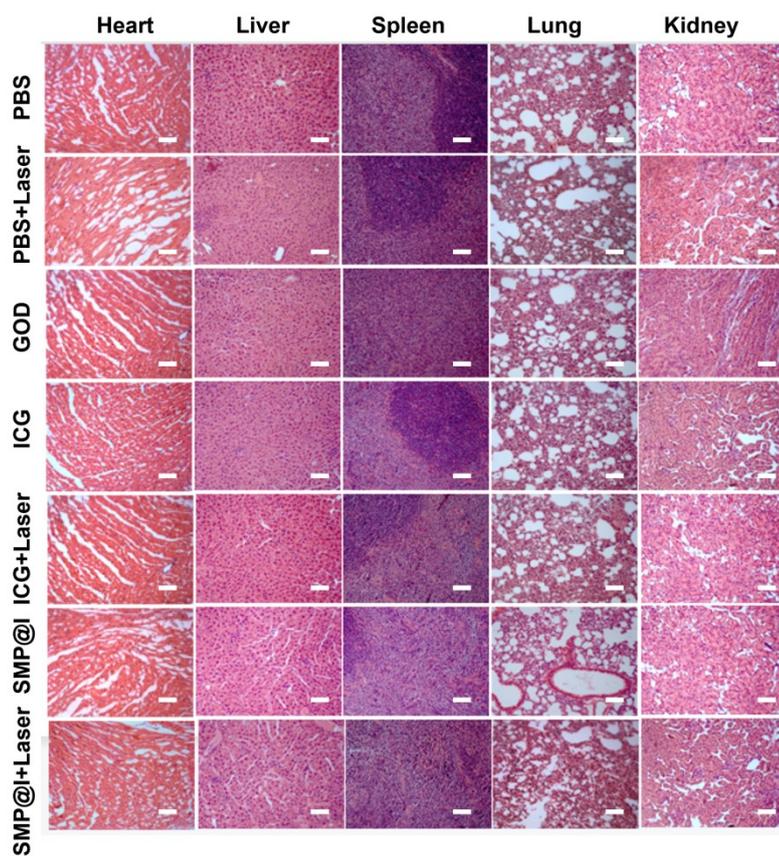
**Figure S8.** (a) Viability of ACC-83 cells after 24 h of incubation with different conditions (b) Viability of 4T1 cells after 24 h of incubation with different conditions.



**Figure S9.** (a) Live/Dead assays on the 4T1 cells treated with SMP@I (25 and 50 µg/mL) under 808 nm laser irradiation (b) Live/Dead assays on the ACC-83 cells treated with SMP@I (25 and 50 µg/mL) under 808 nm laser irradiation (Scale bars=100 µm)



**S10.** Apoptosis assays on the 4T1 cells treated with SMP@I (12.5, 25 and 50 µg/mL) under 808 nm laser irradiation



**Figure S11.** H&E staining images of major organs dissected from various groups receiving different treatments (Scale bars=200  $\mu\text{m}$ ).

### S18. Reference

- [1]. C. Lai, B. G. Trewyn, D. M. Jeftinija, K. Jeftinija, S. Xu, S. Jeftinija and V. S.-Y. Lin, *J. Am. Chem. Soc.*, 2003, 125, 4451-4459.