

## Supporting Information

# “Dual-Key-and-Lock” Dual Drug Carrier for Dual Mode Imaging Guided Chemo-Photothermal Therapy

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## **Materials and Characterization Methods**

**Materials.** Potassium ferricyanide ( $K_3[Fe(CN)_6]$ ), concentrated hydrochloric acid (HCl, 36.0-38.0%), poly(vinylpyrrolidone) (PVP, K-30), ammonium hydroxide ( $NH_3 \cdot H_2O$ , 25.0-28.0%), tetraethyl orthosilicate (TEOS), sodium hydroxide, (3-Aminopropyl)triethoxysilane (APTES), thioglycolic acid, ibuprofen (IBU), doxorubicin (DOX), sodium hydroxide, ethanol absolute, diethyl ether, acetone, cyclohexane, methanol, glutathione (Reduced) were purchased from the Aladdin Chemistry, Co. (Shanghai) and used as received. PEG-SH was purchased from DingGuo Chang Sheng Biotech. Co., Ltd. Aqueous solutions were prepared with deionized water (18.2 MU cm) produced from a Milli-Q water purification system. All other chemicals used in this work were of analytical grade, obtained from commercial suppliers, and used without further purification unless otherwise noted.

**Characterization Methods.** Powder X-ray diffraction (XRD) patterns were collected on a Japan Rigaku D/MAX — IIIC X-ray diffractometer equipped with Cu K $\alpha$  radiation over the  $2\theta$  range of 5-80. Transmission electron microscopy (TEM) images were obtained on a Tecnai G2 F30/F20 JEOL-2100F transmission electron microscope. Field emission scanning electron microscopy (FE-SEM) images were performed on a JEOL JSM7100 F scanning electron microscope. ICP-MS measurements were carried out on an Optima 8000 inductively coupled plasma-atomic emission spectrometer (ICP-AES). The elements distribution was characterized using a scanning electron microscopy (SEM, JSM6510LV) with energy dispersive X-ray (EDX). Specific surface areas were calculated from the results of  $N_2$

physisorption at 77 K (Belsorp-max) by using the BET (Brunauer Emmete Teller). The pore volume and pore size were calculated according to BJH (Barrette Joynere Halenda) formula applied to the adsorption branch. FTIR spectrum was determined using a Magna-IR 750 spectrometer in the range of 500-4000  $\text{cm}^{-1}$  with a resolution of 0.5  $\text{cm}^{-1}$ . Ultraviolet-Visible (UV-Vis) Absorption Spectra were measured on a Lambda 35 ultraviolet visible absorption spectrometer. Temperature was measured using a digital non-contact Infrared Thermometer with a resolution of 0.1  $^{\circ}\text{C}$ . The size of the nanospheres in distilled water was measured by a commercial dynamiclight scattering (DLS) spectrometer (Zetasizer Nano ZSP).

### **Synthesis of p-PB@d-SiO<sub>2</sub>-SS-PEG**

**Synthesis of PB nanoparticles.** PB was synthesized according to the report by Yamauchi group.<sup>1</sup> Typically, 3 g PVP and 131.7 mg K<sub>3</sub>[Fe(CN)<sub>6</sub>] were dissolved into HCl solution (0.01 M, 40 mL) under vigorous magnetic stirring. After stirring for 30 min, the beaker was placed in an electric oven and heated at 80  $^{\circ}\text{C}$  for 20 hours. (The rate of warming and cooling was controlled to change the particle size of the product PB.) After cooling, the precipitate was collected by centrifugation and washed several times with distilled water and ethanol. The product was obtained after drying at 60  $^{\circ}\text{C}$  for 12 hours.

**Synthesis of p-PB nanoparticles.** Porous Prussian blue nanoparticles (p-PB) were prepared by improving the reported methods.<sup>2</sup> Typically, solid PB nanoparticles (20 mg) and PVP (55 mg) were added to HCl solution (1.0 M, 20 mL) in a Teflon vessel under magnetic stirring. After 2 h, the vessel was transferred into a stainless autoclave

and heated at 140 °C for 4 h (heating and cooling rate are 10 °C/h and 20 °C/h respectively) in an electric oven. After aging, the precipitates were collected by centrifugation and washed in distilled water and ethanol several times. After drying at 60 °C for 12 h, p-PB mesocrystals were obtained.

**Synthesis of p-PB@d-SiO<sub>2</sub> nanoparticles.** p-PB was coated by dendrimer-like amino-functionalized silica according to the reported method.<sup>3</sup> Typically, The p-PB nanoparticles (109 mg) and CTAB (500 mg) were added into mixed solution of deionized water (70 mL), NH<sub>3</sub>·H<sub>2</sub>O (0.8 mL), diethyl ether (15 mL) and ethanol absolute (15 mL). After continuous stirring for 30 min, TEOS (2.5 mL) and APTES (0.1 mL) were rapidly dropped and stirred for 4 h at 10 °C. 1 mL of HCl (36.0%-38.0%) was dropped into the mixed solution to after the reaction was completed. The product was collected by centrifugation and washed several times with ethanol and acetone. After drying for 24 hours at 40 °C, the product was obtained.

**Synthesis of p-PB@d-SiO<sub>2</sub>-SS-PEG.** 0.1 g p-PB@SiO<sub>2</sub> was added to a solution of ethanol (25 mL) containing 10 µL of thioglycolic acid, and stirred at 40 °C for 24 hours. The product was collected by centrifugation and washed several times with ethanol and deionized water. After drying at 40 °C for 24 hours the p-PB@d-SiO<sub>2</sub>-SH was obtained. 0.1 g p-PB@d-SiO<sub>2</sub>-SH was mixed with PEG-SH (2.5 mg) into methanol (20 mL), then 0.1 M NaOH solution was used to adjust the pH so that the pH of the mixture was around 9. The mixed solution was stirred for 24 hours in 50 °C. After the reaction is complete, the solution is cooled. The product was collected by

centrifugation and washed several times with ethanol and acetone. After drying at 40 °C for 24 hours the product was obtained.

**Synthesis of dual drugs loaded p-PB@d-SiO<sub>2</sub>-SS-PEG.** 0.1 g of p-PB@d-SiO<sub>2</sub>-SH was dispersed into 25 mL of IBU solution (0.5 g/L) for 24h, sealing the vials to prevent the evaporation of cyclohexane, then the supernatant was collected and washed by water to clean the IBU of the surface of d-SiO<sub>2</sub>. After drying at 40 °C for 24h, the IBU loaded p-PB@d-SiO<sub>2</sub>-SH was dispersed into 25 mL of DOX solution (0.5 g/L) for 24h, then washed by water and dried at 40 °C for 24h, the dual drugs loaded p-PB@d-SiO<sub>2</sub>-SS-PEG was obtained. Using the same method as the above-mentioned synthesis of p-PB@d-SiO<sub>2</sub>-SS-PEG, dual drug loaded p-PB@d-SiO<sub>2</sub>-SS-PEG was obtained.

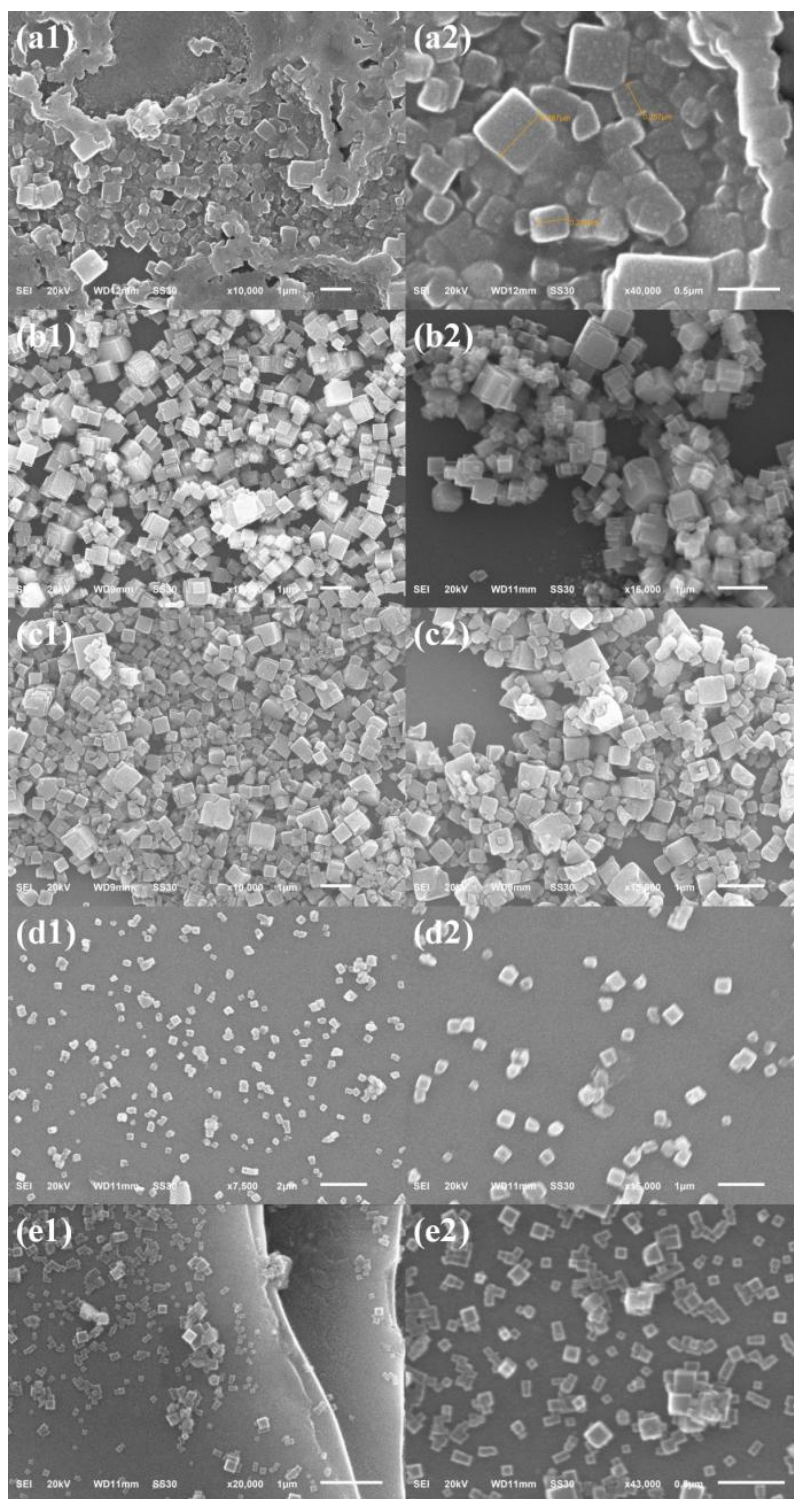
### **Cellular toxicity , Photothermal effect and MRI**

**Cell toxicity.** Mouse breast cancer (4T1) cells were cultured in standard cell media into 96-well plates and incubated in 5% CO<sub>2</sub> at 37 °C for 24 h. Then different concentrations of particles were added into the 96-well plates and continued to be cultured for 24 h. CKK-8 assay was used to analyze cell viability. Typically, the cells were incubated for 24 h with 100 mL free IBU and DOX, p-PB@d-SiO<sub>2</sub>-SS-PEG, IBU loaded p-PB@d-SiO<sub>2</sub>-SS-PEG and DOX loaded p-PB@d-SiO<sub>2</sub>@DOX-SS-PEG in PBS buffer (25, 50, 100, 200, and 500 µg/mL). Cells were rinsed with PBS for three times and then each well was added to 100 µL of 0.1 mg/mL CKK-8 solution and incubated for 30 min at 37 °C. Finally, an ELISA readers was also used to measure the absorbance at 480 nm of each well. Results were

expressed as the percentage of cell viability. Five independent experiments were carried out to minimize the deviations.

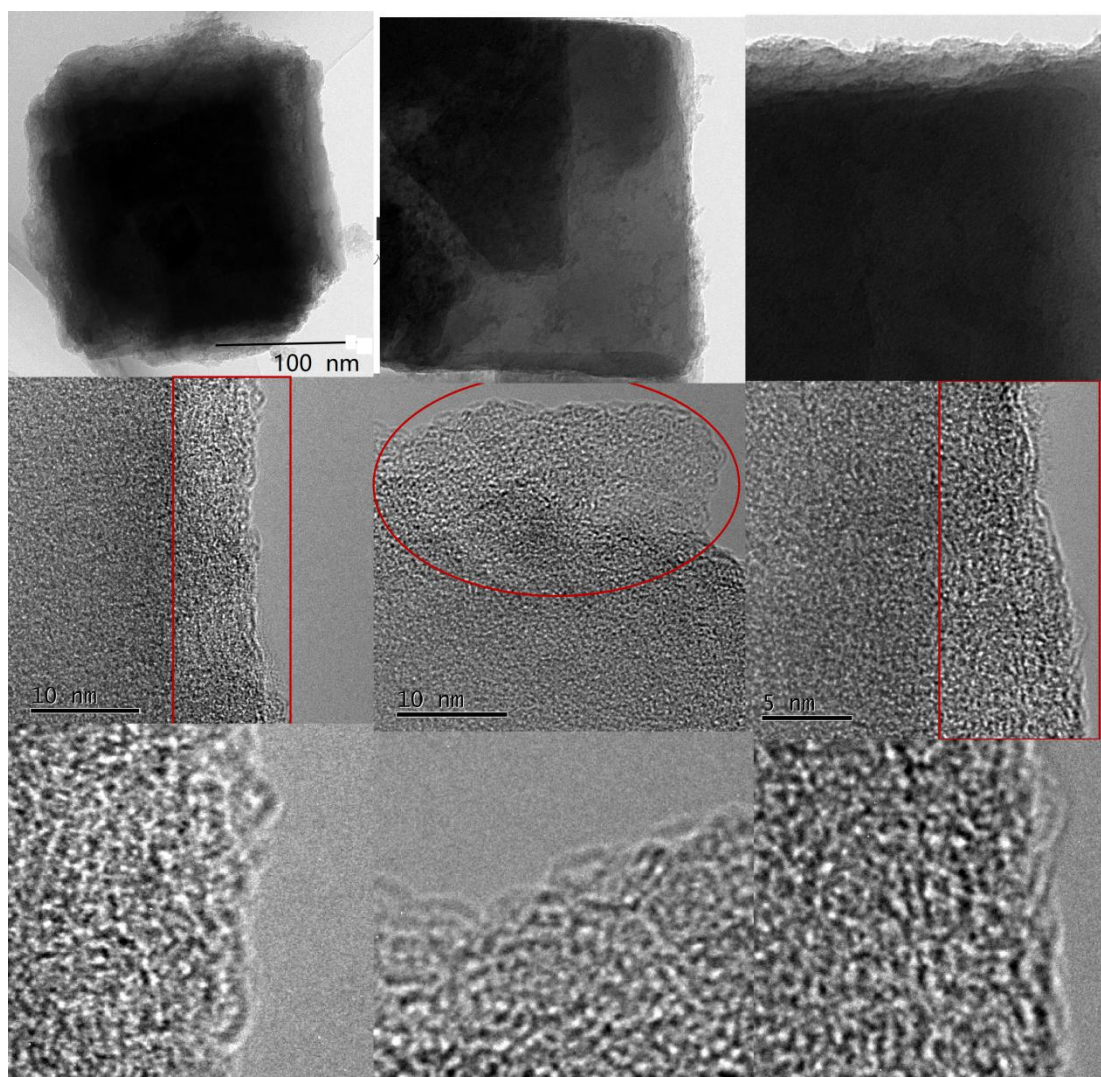
**Photothermal effect.** A quantity of 1.0 mL of aqueous solution containing the p-PB@d-SiO<sub>2</sub>-SS-PEG (0.02, 0.05, 0.2 mg/mL) was transferred to a cuvette and irradiated with the NIR laser (808 nm, 2 W/cm<sup>2</sup>). At selected time intervals (30 s), temperature of the aqueous solution was monitored with a digital non-contact infrared thermometer. The control experiment of negative sample (H<sub>2</sub>O) was also measured under the same conditions.

**MRI.** MRI experiments were acquired with a clinical magnetic resonance scanner (Siemens Magnetom Trio 3.0 T) to test the relaxation properties of the as-prepared samples in deionized water. The concentration of Fe element is calculated based from ICP tests. T<sub>1</sub> and T<sub>2</sub> estimation were performed using a multiple contrast spin echo sequence with the following parameters: number of slices = 1, slice thickness = 2 mm, field-of-view = 58 × 58 mm, acquisition matrix = 256 × 256, TR = 5000 ms, TE times = 30 to 300 ms with 30 ms increments.

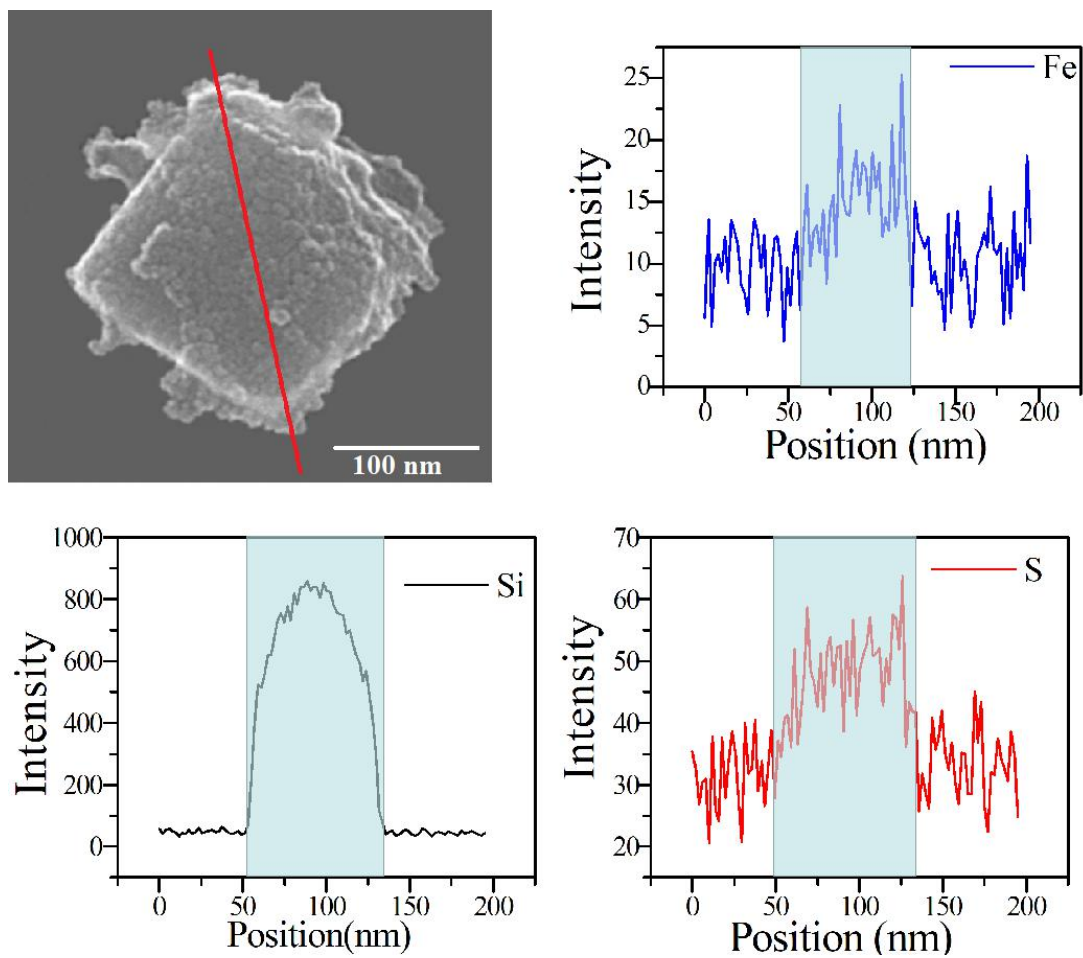


**Figure S1.** (a1-a2)-(e1-e2) The SEM images of PB synthesized that heating and cooling rates are 80, 60, 40, 20, and 10 °C/h, respectively.

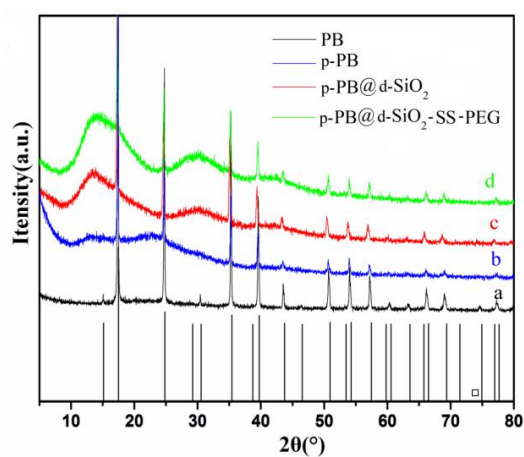




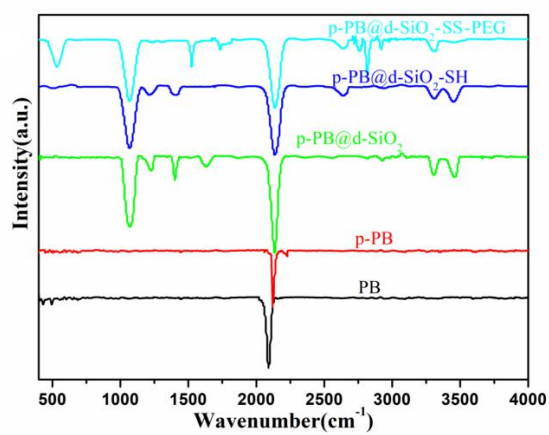
**Figure S2** High Resolution TEM images of p-PB@d-SiO<sub>2</sub>



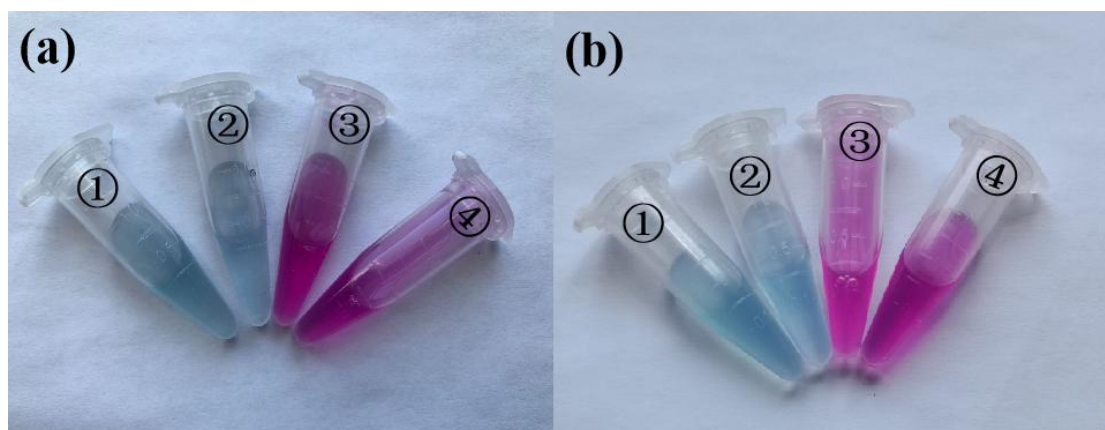
**Figure S3.** Image and cross-sectional compositional line profiles of p-PB@d-SiO<sub>2</sub>-SS-PEG.



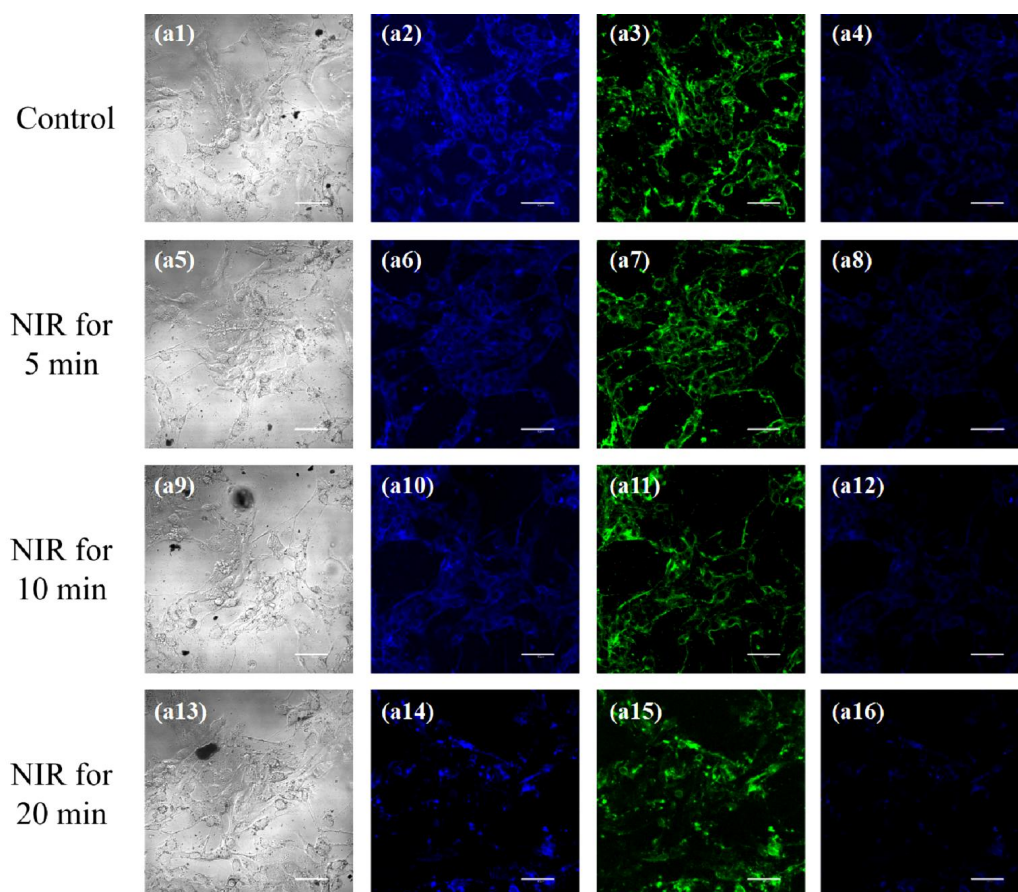
**Figure S4** XRD pattern of PB, p-PB, p-PB@d-SiO<sub>2</sub> and p-PB@d-SiO<sub>2</sub>-SS-PEG.



**Figure S5** FTIR spectra of PB, p-PB, p-PB@d-SiO<sub>2</sub>, p-PB@d-SiO<sub>2</sub>-SH and p-PB@d-SiO<sub>2</sub>-SS-PEG.

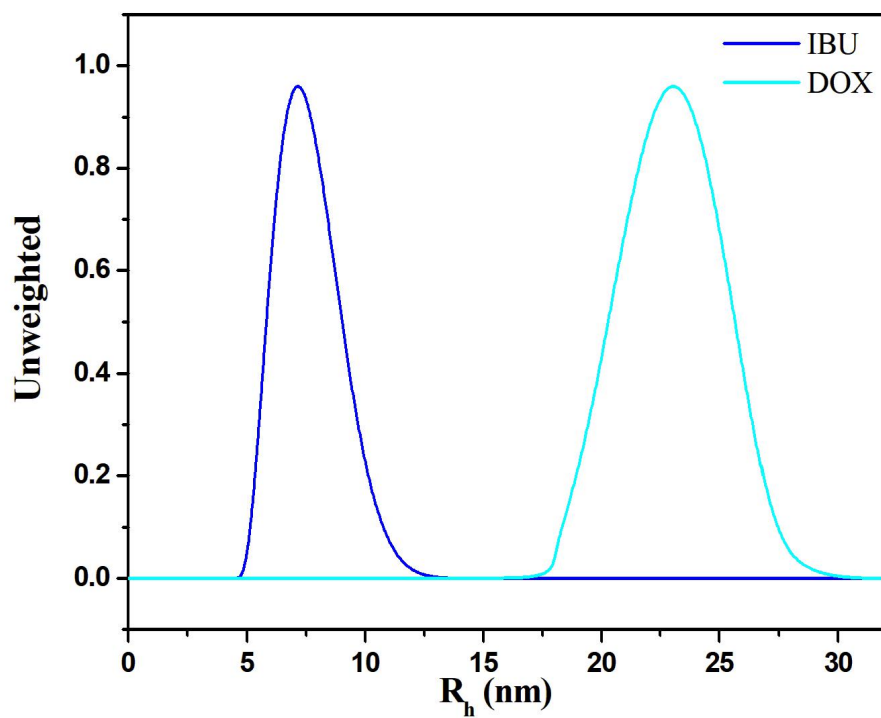


**Figure S6** Photographs (from ① to ④) of p-PB@d-SiO<sub>2</sub>-SS-PEG dispersed in DI water; PBS (pH:7.4); DMEM (without FBS); DMEM (with FBS). (a) 10 mins. (b) 24 h.

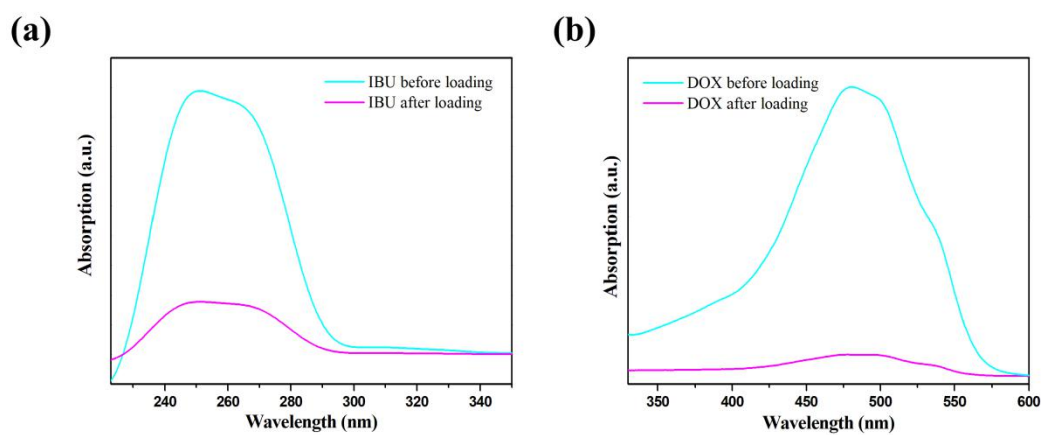


**Figure S7.** CLSM images of 4T1 cells after co-cultured with p-PB@d-SiO<sub>2</sub>-SS-PEG suspension (10 mg/mL) and irradiated by 808 nm laser light at 2 W/cm<sup>2</sup> (a1, a2,a3 and a4, control; a5, a6, a7 and a8, 5 min; a9, a10, a11 and a12, 10 min; a13, a14, a15 and a16, 20 min; under corresponding 403 nm, 488 nm single-photon and 750 nm two-photonexcitation). (all scale bars: 50  $\mu$ m).

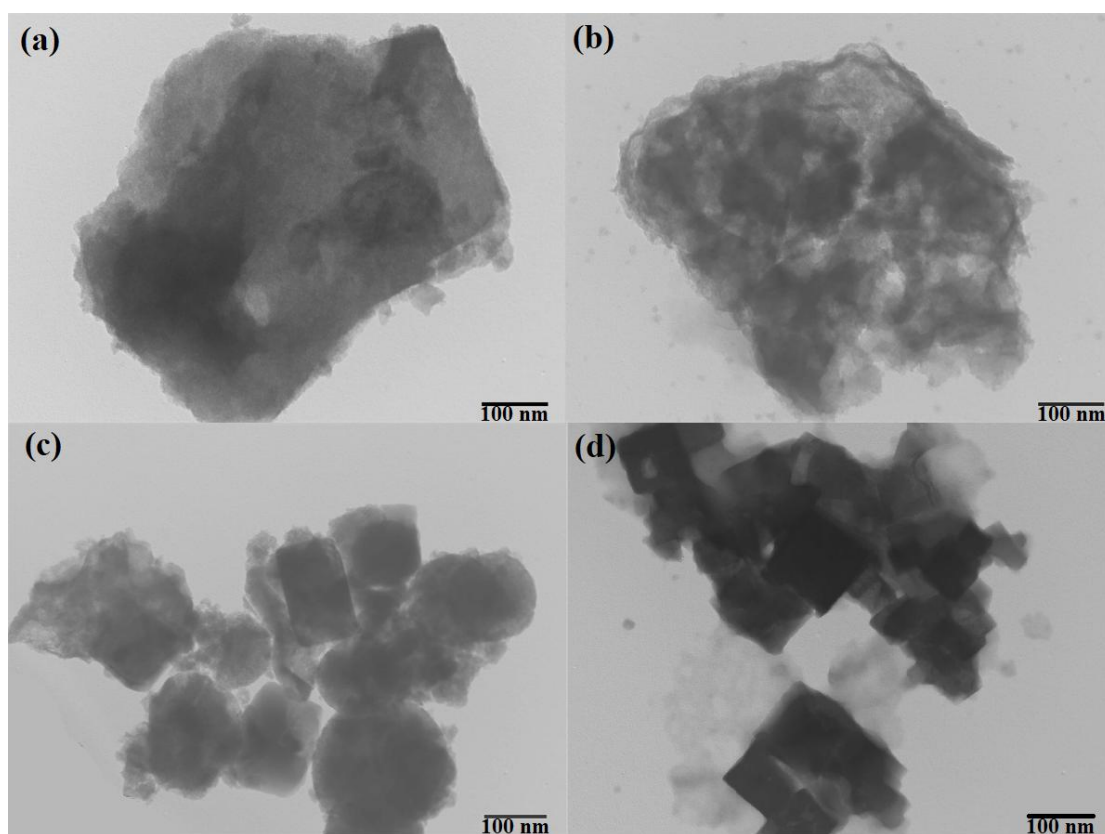




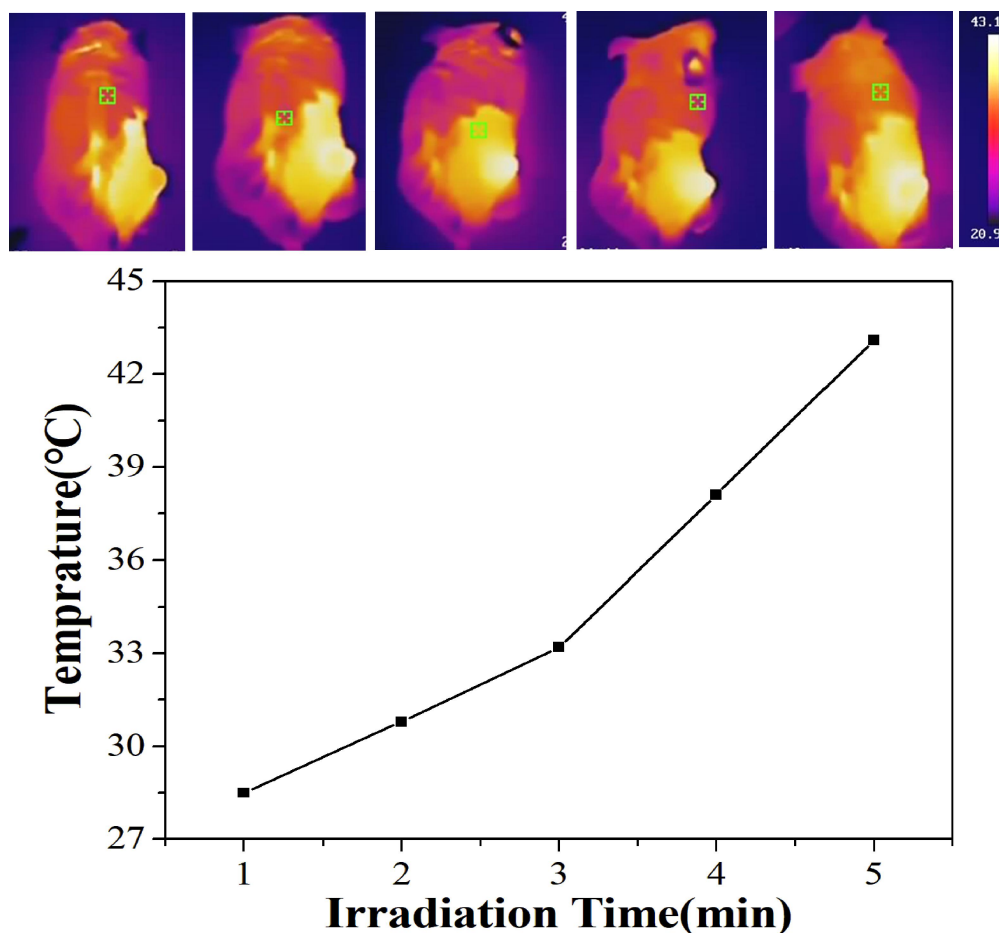
**Figure S8.** DLS measurement of IBU and DOX.



**Figure S9.** UV-vis spectroscopy of (a) IBU and (b) DOX before and after loading.



**Figure S10.** TEM images of dual drugs loaded p-PB@d-SiO-SS-PEG nanoparticles in different conditions (after 24h). (a) pH 7.4 and low GSH; (b) pH 5.8 and low GSH; (c) pH 7.4 and high GSH; (d) pH 5.8 and high GSH



**Figure S11.** In vivo photothermal images of the experimental group with the p-PB@d-SiO<sub>2</sub>-SS-PEG injection for 4h.(With the excitation of 808 nm laser at 1.5 W/cm<sup>2</sup> , NIR irradiation time was 1min, 2min, 3min, 4min and 5min respectively)

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