# Supplementary Information

# Mesoporous carbon nanoshells for high hydrophobic drug loading, multimodal optical imaging, controlled drug release, and synergistic therapy

Hui Wang,[a,#] Kui Wang,[a,#] Qingxin Mu,[a] Zachary R. Stephen,[a] Yanyan Yu,[b] Shuiqin Zhou,[b] and Miqin Zhang[a]

[a]Department of Materials Science and Engineering, University of Washington, Seattle, Washington 98195, United States

[b]Department of Chemistry of The College of Staten Island and The Graduate Center, The City University of New York, Staten Island, New York 10314, United States

*Corresponding Author: mzhang@u.washington.edu

# These authors contributed equally to this work.

## Materials and methods

### Materials

Paclitaxel (PTX) was purchased from LC Laboratories (Woburn, MA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide ($H_2O_2$, 30%), acetone ($C_3H_6O$, 99%), ethanol ($C_2H_6O$, 99%), tetraethyl silicate (TEOS, 98%), ammonia water ($NH_3 \cdot H_2O$, 27%), 3-Aminophenylboronic acid monohydrate (APBA) were used as received.

**Synthesis of $SiO_2$@Carbon shell**

Monodispersed $SiO_2$ nanospheres were synthesized following the reported Stöber method.[1, 2] Briefly, 75 mL ethanol and 6 mL ammonia water were mixed in conical flask (125 mL). The mixed solution was vigorously stirred for 20 min at 60 °C. 1.0 mL TEOS was quickly added, and the mixture solution was vigorously stirred for 2 h. The resulted $SiO_2$ nanospheres were purified with repeated centrifugation (12,000 rpm, 10 min) and redispersion in water for five cycles. Finally, the aqueous dispersion of $SiO_2$ nanospheres with a concentration of 2 mg/mL was collected.

Both 0.02 g of APBA and 0.3 mL of silica water solution (2 mg/mL) were dispersed in 30 mL of acetone. After the solution was intensely sonicated for 20 min, 1.0 mL of hydrogen peroxide (30 wt%) was dropped in. The solution mixture was then vigorously stirred for 20 min. Subsequently, the precursor solution was transferred to a Teflon-lined stainless autoclave (with a volume of 50.0 mL). The autoclave was maintained at 100 °C for 24 h and then heated to and maintained at 200 °C for 24 h. After cooling down to room temperature, a brown solution was obtained. The resulted $SiO_2$@carbon shell nanoparticles were purified with repeated centrifugation (10,000 rpm, 10 min) and redispersion in acetone after sonication (15 min) for three cycles. Finally, the solid $SiO_2$@carbon shell nanoparticles were collected.

**Synthesis of Hollow carbon nanoparticles**

The erosion of the $SiO_2$ core is performed using the reported method.[3] The as-prepared $SiO_2$@carbon shell nanoparticles (0.5 g) were re-dispersed in 30 mL ammonia water. After the solution was intensely sonicated for 30 min, the precursor solution was transferred to a Teflon-lined stainless autoclave (with a volume of 50.0 mL). The autoclave was heated to and maintained at 200 °C for 24 hours. After cooling down to room temperature, the resultant hollow carbon

nanoparticles were purified with repeated centrifugation (10,000 rpm, 20 min) and redispersion in distilled water for five cycles. Finally, the solid hollow carbon nanoparticles were collected.

**Drug loading and release of carbon nanoshells**

PTX was loaded into the carbon nanoshells by complexation method. FMP-CNSs dispersion (10 mL, 0.1 mg/mL) placed in a vial was stirred in a water bath (20 $^{o}$ C) for 30 min. PTX ethanol solution (1.0 mL, 1.0 mg/mL) was then added into the FMP-CNS and the mixture was continuously stirred for 48 h. The PTX-loaded FMP-CNS complexes were then separated from the dispersion by centrifugation (4 $^{o}$C, 10,000 rpm, 30 min) and washed four times with ethanol and distilled water to remove the unloaded PTX. All the supernatant was collected for the measurements of the drug loading content. The unloaded PTX presented in the supernatant was quantified by a HPLC-MS. The drug loading content of FMP-CNSs was calculated by $(M_0 - M_t)/M_N \times 100\%$, where $M_0$ and $M_t$ are the total mass of PTX dissolved in the initial solution and remained in the supernatant solution, respectively. $M_N$ is the mass of the FMP-CNSs used in the loading process.

The *in vitro* tests of NIR-responsive release of PTX from PTX-loaded FMP-CNSs were evaluated by the dialysis method. The purified PTX-loaded FMP-CNSs were redispersed in 10 ml PBS solution (0.005 M, pH = 7.4). Two dialysis bags filled with 5 mL diluted PTX-loaded FMP-CNSs were immersed in 50 mL 0.005 M buffer solutions of pH = 7.4 at 37 $^{o}$C with one of them exposed to a NIR laser (825 nm) of an output power of 1.5 W/cm$^2$ for 5 min. The released PTX outside of the dialysis bag was sampled at defined time periods and assayed by HPLC-MS. Cumulative release is expressed as the total percentage of drug released through the dialysis membrane over time.

**Cell culture**

Human GBM cells SF-763 were kindly provided by Prof. John R. Silber at the University of Washington. Rat C6 glioblastoma cells were purchased from the American Type Culture Collecton (Manassas, VA, ATCC). Both cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic (Life technologies, Grand Island, NY). Cells were cultured in an incubator maintained at 37°C and 5% $CO_2$ with 95% humidity.

**Cellular uptake of FMP-CNSs by confocal laser scanning microscopy (CLSM)**

SF-763 cells were seeded onto glass cover slips in a 6-well plate. After overnight incubation, cells were incubated with FMP-CNSs (5 μg/mL) for 2 h. Cells were then washed with cold PBS 3 times and fixed with 4% paraformaldehyde for 15 min at 37°C. Cells were then mounted onto glass slides with ProLong® Gold Antifade Mountant (Life Technologies Inc., Gaithersburg, MD). The images of cells were acquired using a Laser Scanning Microscope Leica SP8X (Leica Microsystems GmbH, Germany). Three excitation wavelengths were used (405, 488 and 546 nm). The emission regions were 425–480 nm, 508–540 nm and 566–700 nm, respectively.

**Viability of Cells treated with FMP-CNSs and PTX-loaded FMP-CNSs with or without NIR irradiation**

Cells were seeded in a 96-well plate and incubated overnight in the aforementioned growth conditions. In the following day, the medium was replaced with a medium containing FMP-CNSs or PTX-loaded FMP-CNSs or with medium control. Three different drug concentrations (4, 2 and

1 μg/mL) were used, and samples at each concentration were ran in sextuplicate. The cells were incubated with FMP-CNSs or PTX-loaded FMP-CNSs for 72 h. Wells containing the normal medium without drugs were used as the control. For photothermal treatments, cells in the wells were irradiated with 1.5 W $cm^{-2}$ NIR light for 5 min. Cell viability was assessed using the alamar blue assay. Briefly, the medium was replaced with cell culture medium containing reagent and incubated for 2 h. Following the incubation, a microplate reader (SpectraMax i3, Molecular Devices, Sunnyvale, CA) was used to determine the fluorescence intensity of the dye (550ex/590em). The fluorescence intensity from FMP-CNSs and PTX-loaded FMP-CNSs was compared to those from untreated control cells to determine percent viability.

**In vivo NIR imaging of FMP-CNSs**

All animal studies were conducted in accordance with University of Washington's Institute of Animal Care and Use Committee (IACUC) approved protocols as well as with federal guidelines. FMP-CNSs (100 μL, 1mg/mL) were injected subcutaneously into athymic nude mice (Jackson Labs, Bar Harbor, ME). Fluorescence images as well as photographs were taken by a Xenogen IVIS imaging system (IVIS®Lumia II, PerkinElmer Inc) under different excitation wavelength including 605 nm, 640 nm, 675 nm, 710 nm and 745 nm with exposure time of 1 s, binning factor of 2 and f number of 8. The corresponding imaging detection channel wavelength ranges are 695−770, and 810−875 nm, respectively.

**Histopathological Evaluation of FMP-CNSs**

Five days after intravenous administration of FMP-CNSs at 1 mg/mL, whole organs (heart, kidney, liver, lung and spleen) of C57BL/6 mice (Jackson Labs, Bar Harbor, ME) were removed

through necropsy and preserved in 10% formalin for 48 h. Tissues were then embedded in paraffin, sliced into 5 μm sections, and stained with hematoxylin and eosin. Microscopic images of tissues were acquired using a Nikon ECLIPSE TE2000-S microscope.

**Biodistribution of FMP-CNSs**

Mice were injected via tail vein with 200 μL of 1 mg/mL of FMP-CNSs. Mice receiving no injection were used as controls. At 24 h after injection, mice were euthanized and whole organs of liver, spleen, kidney, lung, and heart were harvested. Fluorescence was acquired for each tissue type using a Xenogen IVIS imaging system (IVIS®Lumia II, PerkinElmer Inc) at a excitation wavelength of 745 nm.

**In vivo therapy of FMP-CNSs and PTX-loaded FMP-CNSs with or without NIR irradiation**

Flank xenograft C6 tumors were prepared by subcutaneous injection of one million cells suspended in serum free media and Matrigel (BD Biosciences, San Jose, CA) into athymic nude mice (Jackson Labs, Bar Harbor, ME). Tumors were allowed to grow for 2 weeks before mice were injected intratumorally with nanoparticles. Mice were divided into four groups (n = 5 per group) injected with 200 μL of PBS, FMP-CNSs and PTX-loaded FMP-CNSs (1 mg/ mL, dose of PTX = 7.94 mg/kg). After 24 h, tumors were applied with or without NIR irradiation (825 nm, 1.5 $W/cm^2$) for 5 min. Tumor sizes were monitored every 2 days for 12 days. The length and width of the tumors were measured by a digital caliper. The tumor volume was calculated based on the following formula: $width^2 * length/2$.

**Characterization**

Transmission electron microscopy (TEM) images were taken on a FEI TECNAI transmission electron microscope at an accelerating voltage of 100 kV. Field emission scanning electron microscopy (FE-SEM) images were acquired on an AMRAY 1910 scanning electron microscope. High-resolution transmission electron microscopy images were acquired with JEM 2100 at an acceleration voltage of 200 kV. UV-vis absorption spectra were obtained on a Thermo Electron Co. Helios β UV-vis Spectrometer. The Raman spectrum was taken on a LABRAM-HR Confocal Laser Micro-Raman spectrometer using an $Ar^+$ laser with 514.5 nm at room temperature. FT-IR spectra were recorded with a Nicolet Instrument Co. MAGNA-IR 750 Fourier transform infrared spectrometer. The UV light was provided by a 250 W high-pressure fluorescent Hg lamp. photoluminescence spectra were obtained on a JOBIN YVON Co. FluoroMax®-3 Spectrofluorometer equipped with a Hamamatsu R928P photomultiplier tube, calibrated photodiode for excitation reference correction from 200 to 980 nm, and an integration time of 1 s. The hydrodynamic size of FMP-CNSs was determined using Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) at the room temperature. Nitrogen adsorption-desorption measurements were carried out on a Micromeritics ASAP 2020 instrument. HPLC-MS spectra were collected on a Bruker Esquire ion trap mass spectrometer (Bruker Daltonics, Billerica, MA) using the positive ion mode. The two photon fluorescence image was obtained using Olympus FV1000 MPE BX61 Multi-photon Microscope. X-ray photoelectron spectroscopy (XPS) experiments were carried out at the National ESCA and Surface Analysis Center. Powder X-ray diffraction (XRD) patterns were acquired from lyophilized samples using D8 Bruker X-ray diffractometer with Cu Kα radiation.

*Quantum Yield (QY) Measurements of FMP-CNSs:* Rhodamine B in water (QY = 0.31) was selected as a control standard. The QY of FMP-CNSs was estimated by the follow equation:

$$\Phi_s = \Phi_r \times (I_s/I_R) \times (A_R/A_S) \times (n_s^2/n_R^2)$$

where the Φ is the QY, I is the integrated PL emission intensity (excited at 514 nm for FMP-CNSs and Rhodamine B), n is the refractive index of water (1.334), and A is the absorbance value at the excitation wavelength of 514 nm (distilled water). The subscript "R" refers to the standard. The subscript "s" refers to the FMP-CNSs.

**Supplementary Tables**

Table S1. PL quantum yields of Rhodamine B and FMP-CNSs

| Sample | I | A | N | Φ |
|---|---|---|---|---|
| Rhodamine B | 125643 | 0.0514 | 1.34 | 0.31 |
| FMP-CNSs | 35807 | 0.0478 | 1.34 | 0.095 |

Table S2. Comparison of *in vitro* cytotoxicity of FMP-CNSs and PTX-loaded FMP-CNSs with or without exposure to 1.5 W/cm$^2$ NIR light for 5 min.

| Concentration μg/mL | FMP-CNSs / % | PTX-FMP-CNSs / % | FMP-CNSs+NIR / % | PTX-FMP-CNSs+NIR / % |
|---|---|---|---|---|
| 1 | 104.6±5.4 | 90.6±8.5 | 93.5±5.3 | 85.2±7.4 |
| 2 | 99.9±6.2 | 73.5±5.9 | 85.4±4.3 | 58.3±7.8 |
| 4 | 95.1±4.5 | 58.4±6.1 | 72.4±5.2 | 33.3±7.2 |

## Supplementary Figures

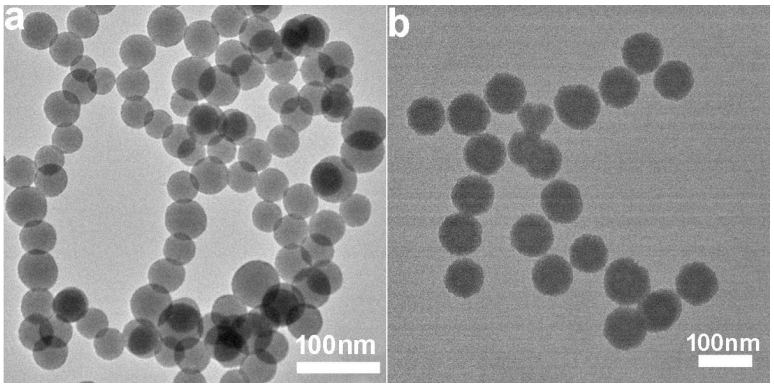


**Fig. S1** (a and b) Representative TEM images of the $SiO_2$ template and $SiO_2$@FMP-CNSs, respectively.

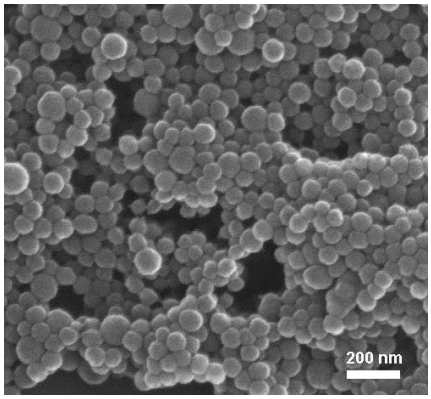


**Fig. S2** Representative SEM images of FMP-CNSs.

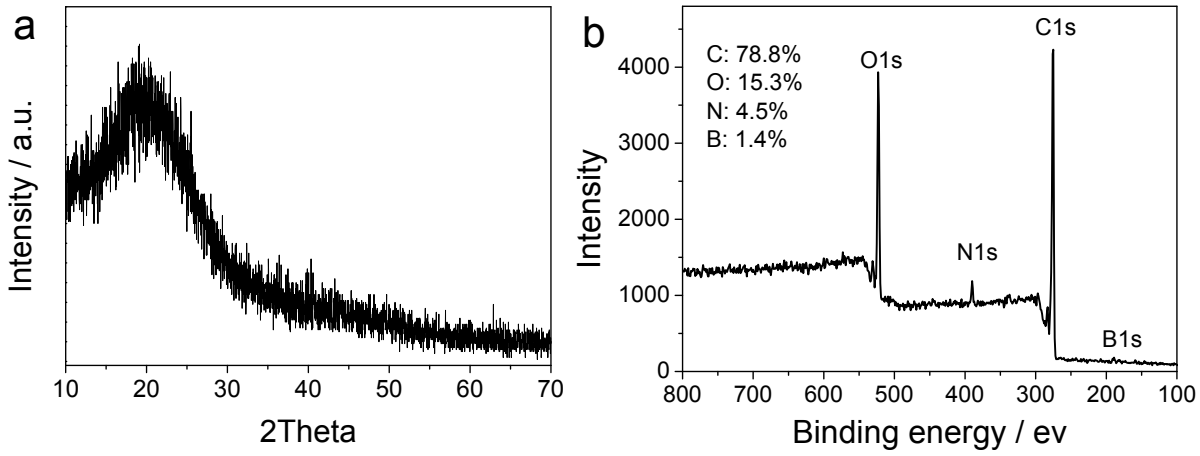

**Fig. S3** (a) X-ray diffraction patterns and (b) XPS scanning spectra of as-synthesized FMP-CNSs.

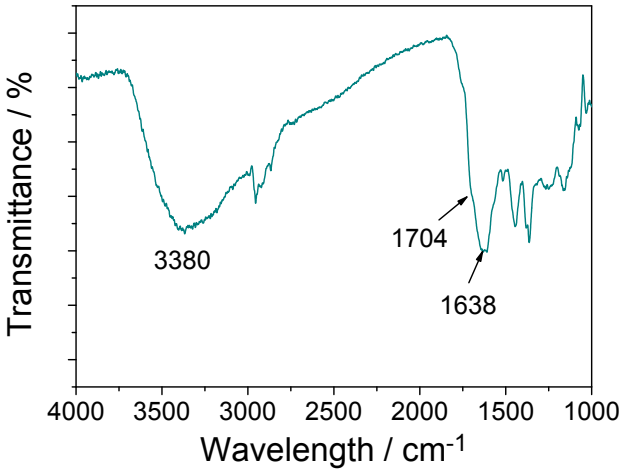


**Fig. S4** FT-IR spectrum of as-synthesized FMP-CNSs.

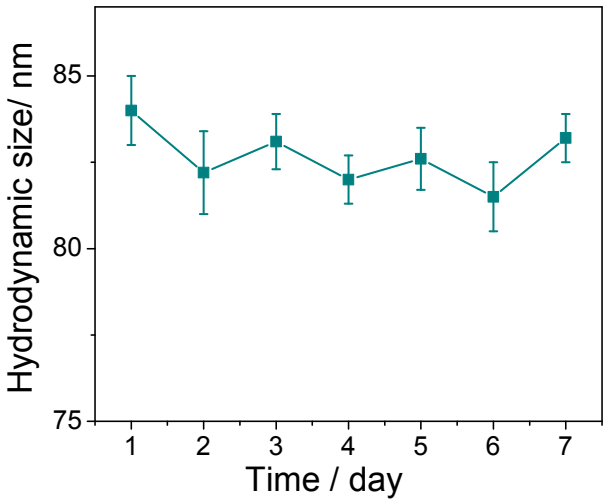


**Fig. S5** The stability of FHMPs-CNS in culture medium containing 10% serum under different time points.

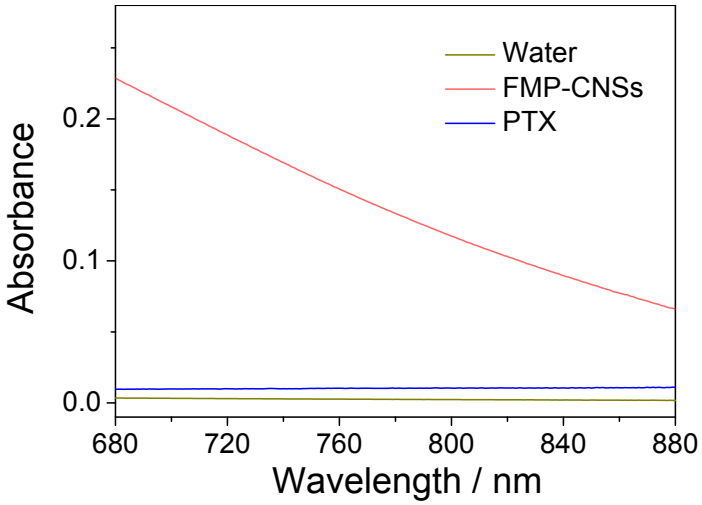


**Fig. S6** Vis-NIR absorption spectra (650–900 nm) of control (water), PTX and FMP-CNSs (1 μg/mL).

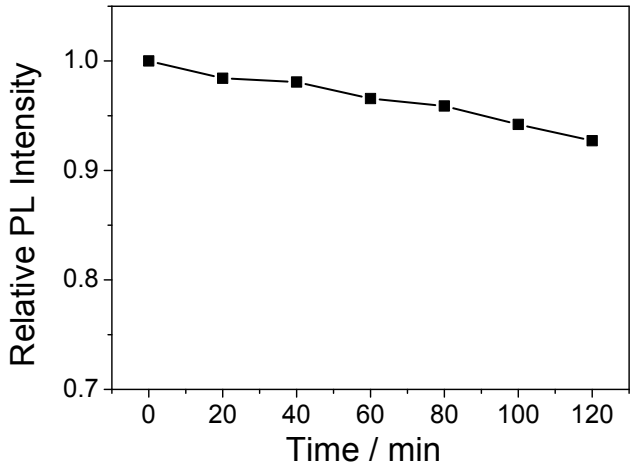


**Fig. S7** Fluorescence intensity variation of FMP-CNSs under different excitation times from 0 to 120 min. Excitation wavelength = 360 nm.

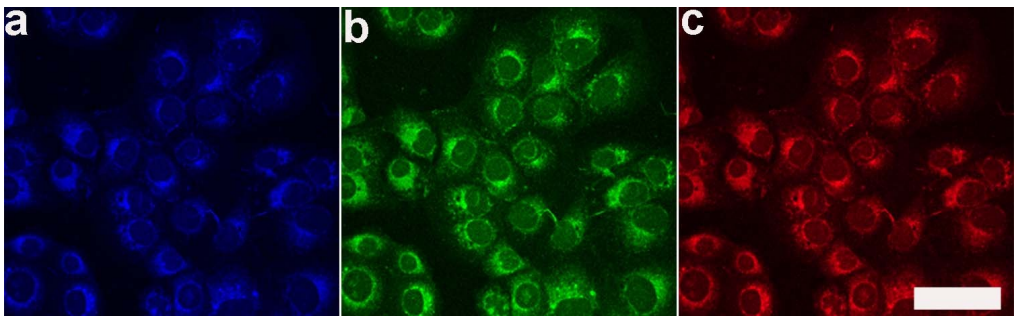

**Fig. S8** Laser scanning confocal microscopy images of SF-763 cells incubated with FMP-CNSs for 14 days under different excitation wavelengths: (a) 405 nm; (b) 488 nm; (c) 546 nm. Scale bar=50 μm.

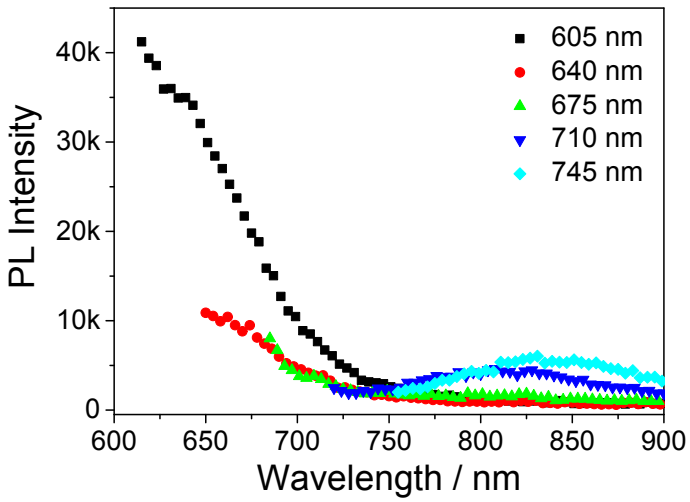


**Fig. S9** PL spectra of FMP-CNSs obtained with different excitation wavelengths.

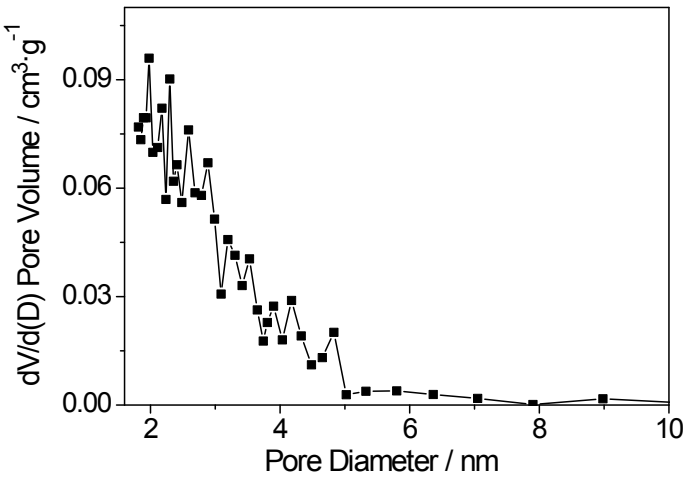


**Fig. S10** The pore size distribution of the as-obtained FMP-CNSs.

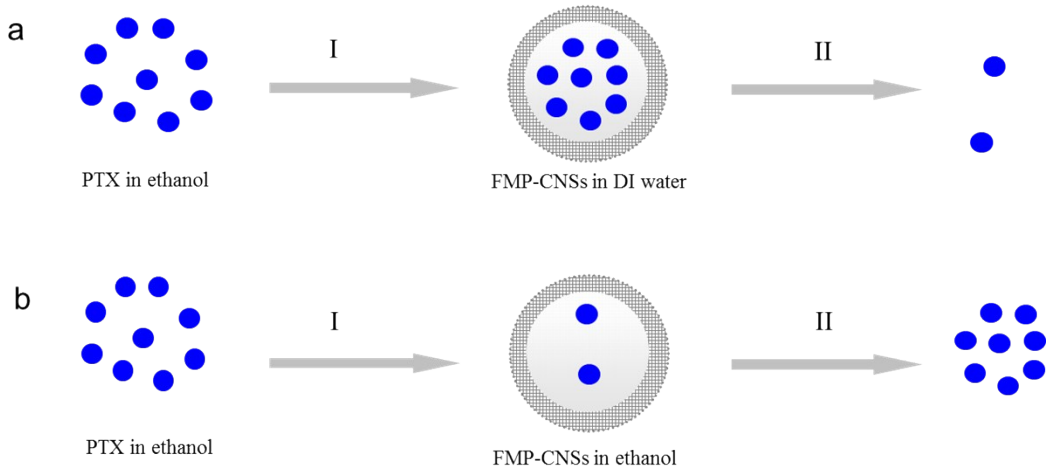


**Fig. S11** Drug loading of FMP-CNSs in different sovlent. (a) Schematic representation of drug loading in mixing solvents. I: PTX molecules enter into FMP-CNSs in DI water by diffusion. II: the entered PTX molecules will release from FMP-CNSs; (b) Schematic representation of drug loading in an ethanol solvent. I: PTX molecules in ethanol enter into FMP-CNSs in ethanol. II: the entered PTX molecules will release from FMP-CNSs by diffusion.

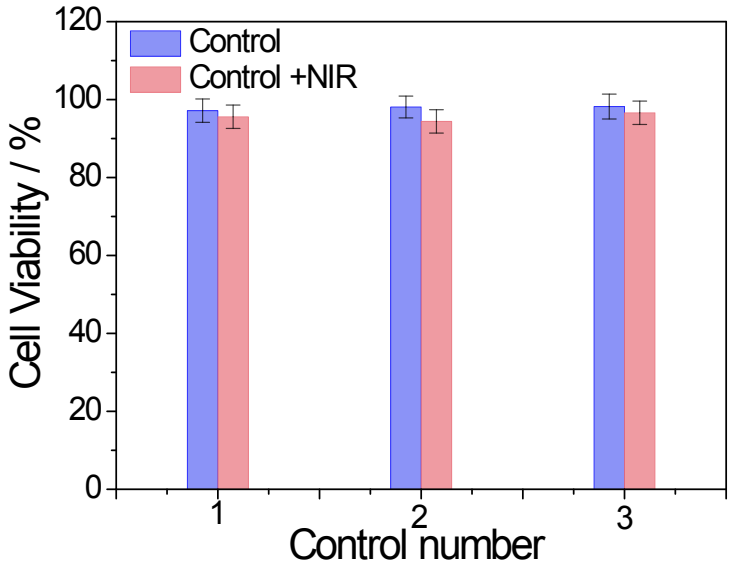


**Fig. S12** Cell viability in the culture medium in the absence and presence of 1.5 W/cm$^2$ NIR for 5 min.

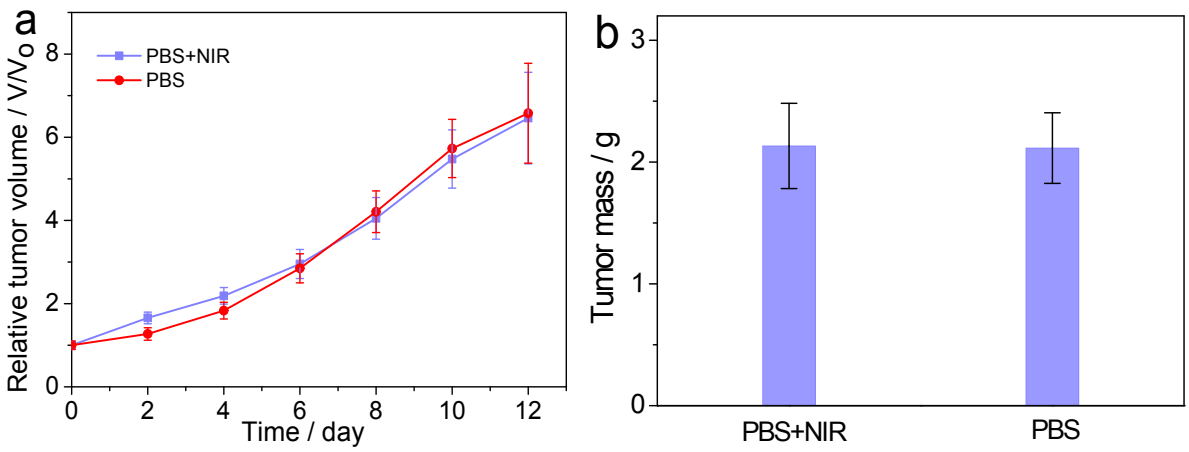


**Fig. S13** In vivo tumor grown of mice without/with NIR irradiation for 5 min at an intensity of 1.5 W/cm$^2$. (a) The tumor volume growth curves of mice after various treatments (four mice for each group. (b) Average of tumors collected from mice at the end of treatments (day 12).

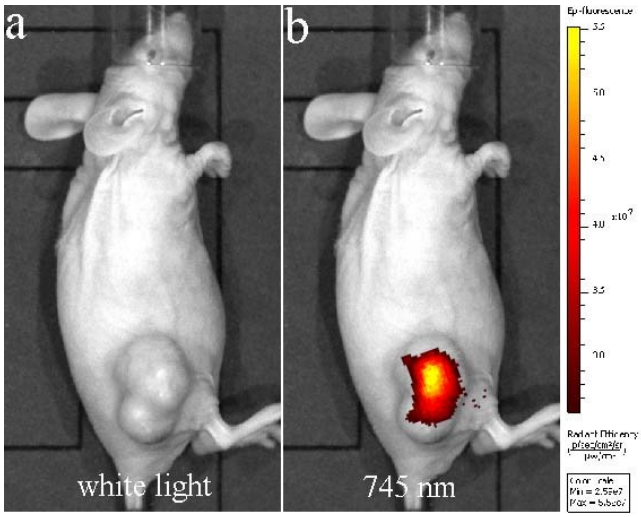


**Fig. S14**. *In vivo* NIR fluorescence images of FMP-CNSs-treated mice by intratumoral injection at various excitation conditions: (a) white light and (b) 745 nm.


1. K. Cheng, Q. Chen, Z. Wu, M. Wang and H. Wang, *CrystEngComm*, 2011, **13**, 5394-5400.
2. W. Stöber, A. Fink and E. Bohn, *J. Colloid Interface Sci.*, 1968, **26**, 62-69.
3. K. Cheng, Y.-M. Zhou, Z.-Y. Sun, H.-B. Hu, H. Zhong, X.-K. Kong and Q.-W. Chen, *Dalton Transactions*, 2012, **41**, 5854-5861.