Supplementary Material (ESI) for Chemical Communications

One-Pot Self-Assembly of Mesoporous Silica Nanoparticles-Based pH-Responsive Anti-Cancer Nano Drug Delivery System

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Experimental Methods

Preparation of CPT@CTAB@MSNs

CPT (0.5 mmol) was solubilized in 500 mL of CTAB solution (10 mM) at 80 °C under intensive stirring and light-sealed environment. After several minutes, a clear and homogeneous solution was obtained, indicating insoluble CPT solubilized/encapsulated uniformly into CTAB (CPT@CTAB), as illustrated in Scheme 1. Then NH_4F (0.04 mol) which was used as a catalyst for accelerating the hydrolyzation and condensation of silicon sources was added. Immediately, tetraethyl orthosilicate (TEOS, 0.04 mol) was added dropwise for 20 minutes. After 1 hour, the reaction solution in a semi-transparent colloidal state was centrifugated for 10 min with the centrifugal force of 18000*g* in a high speed refrigerated centrifuge. The upper clear solution was collected and it was found that its UV characteristic adsorption peak positon and intensity kept accordance with that of initial solution. This suggests that insoluble CPT was always solubilized/encapsulated uniformly into CTAB during reaction. Therefore, it can be inferred that the final product has the inclusion structure of CPT@CTAB@MSNs, as illustrated in Scheme 1. Nanoparticles were also collected and washed with ethanol and super-pure water in turn for three times to

completely remove residual reactants and the catalyst NH_4F. Finally, products were dispersed in super-pure water and the freeze drying power was used for measurements. The control sample CTAB@MSNs without loading CPT drug was synthesized *via* the similar method without the addition of CPT in the initial reaction solution. To obtain pure MSNs carrier, sample CTAB@MSNs was extracted several times with a mixed solution of ethanol (150 mL) and hydrochloric acid (36–38%, 2 mL) to completely remove CTAB.

Nanoparticles Characterization

The UV adsorption spectra of clear reaction solution before adding TEOS and upper clear solution after 1-hour reaction and 10-min centrifugation were collected on a Shimadzu UV-3101PC UV-vis absorption spectrophotometer. According to the adsorbance difference, the drug loading capacity of CPT@CTAB@MSNs was calculated by the Beer–Lambert law. The drug loading capacity of CPT@CTAB@MSNs was measured by dissolving CPT@CTAB@MSNs into a clear aqueous solution of HF and then determining the concentration of free drugs (~ 1.5 mg CPT and 600 mg CTAB per gram MSNs).

The morphology and mesostructure of nanoparticles were observed *via* transmission electron microscopy (TEM). TEM micrographs were obtained on a JEM–2010 electron microscope with an accelerating voltage of 200 kV. The mesostructure ordering was characterized by small-angle X-ray diffraction (SA-XRD). SA-XRD data were recorded on Rigaku D/Max-2550V diffractometer using Cu Kα radiation (40 kV and 40 mA) at a scanning rate of 0.4°/min over the range of 0.5–6.0° with a step width of 0.002°. The particle size distribution data were collected by a DLS method in a Mastersizer 2000 analyzer (Malvern Instruments Ltd. UK).

In vitro drug release behaviors of drugs@CTAB@MSNs in buffer solutions of different pH values

Sterilized dialysis bags with dialyzer molecular-weight cut-off 10,000 Dalton were used to carry out the drug release experiments. These dialysis bags were pretreated prior to use as follows. These dialysis bags were fully immersed into 50% aqueous solution of ethanol and boiled 1 h, and then washed with 50% ethanol, 10 mmol L^{-1} $NaHCO_3$ and 1 mmol L^{-1} EDTA in turn. Phosphate buffered saline (PBS) of pH = 7.4 and acetic buffer solutions (ABS) of pH = 4, 5 and 6.5 were used as the drug release

media to simulate normal blood/tissues and tumor environments. The sample CPT@CTAB@MSNs (60 mg) was dispersed into 2 mL release media, and then the solutions were put into pretreated dialysis bags. The sealed dialysis bags were put into brown bottles and then 58 mL release media was added. These bottles were shaken at a speed of 100 rpm at 37 °C under a light-sealed condition. At certain time intervals, 3 mL of the release media were taken out for measuring the released drug concentrations by the UV-vis absorption technique, and then were returned to the original release media. The concentrations of released drug were calculated by the Beer–Lambert law according to the absorbances of the release media at a certain characteristic adsorption wavelength. Each release experiment was replicated three times under the same environment, and average values and standard deviation were calculated.

Cytotoxicity against MCF-7 and MCF-7/ADR cells

MCF-7 cells were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS). MCF-7/ADR cells were cultured in RPMI 1640 containing 10% FBS and 1 μg mL^{-1} of DOX. Cells were maintained at 37 °C in a humidified and 5% CO_2 incubator. For all experiments, cells were harvested by the use of D-Hank's-trypsin solution and resuspended in fresh medium before plating. *In vitro* cytotoxicity against MCF-7 and MCF-7/ADR cells was assessed by the standard Cell Counting Kit-8 (CCK-8) assay. The statistical evaluation of data was performed using a two-tailed unpaired Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant. Each data point is represented as mean ± standard deviation (SD) of eight independent experiments (n = 8, n indicates the number of wells in a plate for each experimental condition). The time and dose dependences of the cytotoxicity were investigated at different time points of incubation (1 day, 2 days and 3 days) at different concentrations.

MCF-7 cells were seeded in 96-well plates at a density of 10^4 cells per well. After incubation for 24 h at 37 °C in 100 μL RPMI 1640 medium containing 10% FBS, culture medium was discarded and then cells were treated with 100 μL pH 7.4 D-Hank's solution of drugs at different concentrations. At the end of each incubation (1 day, 2 days or 3 days), 10 μL of CCK-8 solution was added into each wells. After cells were incubated for another 4 h, the absorbance was monitored at 450 nm on a micro-plate reader (Bio-Tek ELx800). A culture medium without nanoparticles was

used as the blank control. The cytotoxicity was expressed as the percentage of the cell viability as compared with the blank control.

Intracellular uptake and drug release of CPT@CTAB@MSNs in MCF-7 and MCF-7/ADR cells

Red emitting rhodamine-B-isothiocyanate (RITC) was grafted onto CPT@CTAB@MSNs (denoted by 'CPT@CTAB@MSNs-RITC') by a facile co-condensation approach as described in our previous report [Qianjun He, Jianlin Shi, Xiangzhi Cui, Jinjin Zhao, Yu Chen, Jian Zhou, Rhodamine B-co-condensed Subsphaeroidal SBA-15 Nanoparticles: Facile Co-condensation Synthesis and Excellent Fluorescence Features, Journal of Materials Chemistry, 2009, 19, 3395–3403]. In a typical procedure, 2×10^4 MCF-7 and MCF-7/ADR cells were cultured for 12 h at 37 °C in RPMI 1640 medium supplemented with 10% FBS in each petri dish. Then cells were gently washed twice with D-Hank's solution of pH 7.4, and subsequently D-Hank's-buffered CPT@CTAB@MSNs-RITC at a final concentration of 80 μg mL^{-1} (or free CPT at a final concentration of 8 μg mL^{-1}) added into petri dishes. After incubation for 4 h, cells were washed for several times with D-Hank's solution to remove the residual nanoparticles, and then directly visualized *via* a Leica TCS confocal microscope (Leica Microsystems, Germany). In the assay, all experiments were carried out under a light-sealed condition to avoid photo-bleaching.

In vivo biodistribution of CPT@CTAB@MSNs in tumor

The tumor-bearing mouse model was induced by injecting MCF-7/ADR cells into the right infra-axillary dermis of nude mice. MCF-7/ADR tumor-bearing mice were intravenously injected with CPT@CTAB@MSNs-RITC at a dose of 10 μg g^{-1} through the tail vein. The administrated mice were maintained daily under a 12 h light/dark cycle at the Animal Care Facility, and fresh distilled water and food for all animals were available ad libitum. After mainline administration for one day, mice were sacrificed *via* the cervical dislocation. The tissues such as heart, liver, spleen, lung, kidney and tumor were cut into slices of 5 μm in thick following polyoxymethylene fixing and actin staining (Actin-Tracker Green Kit, Beyotime) for directly observing the localization of CPT@CTAB@MSNs-RITC under an Olympus FV1000-IX81 confocal microscopy. The *in vivo* animal experiments were carried out under the guideline approved by the International Animal Care and Use Committee (IACUC) of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

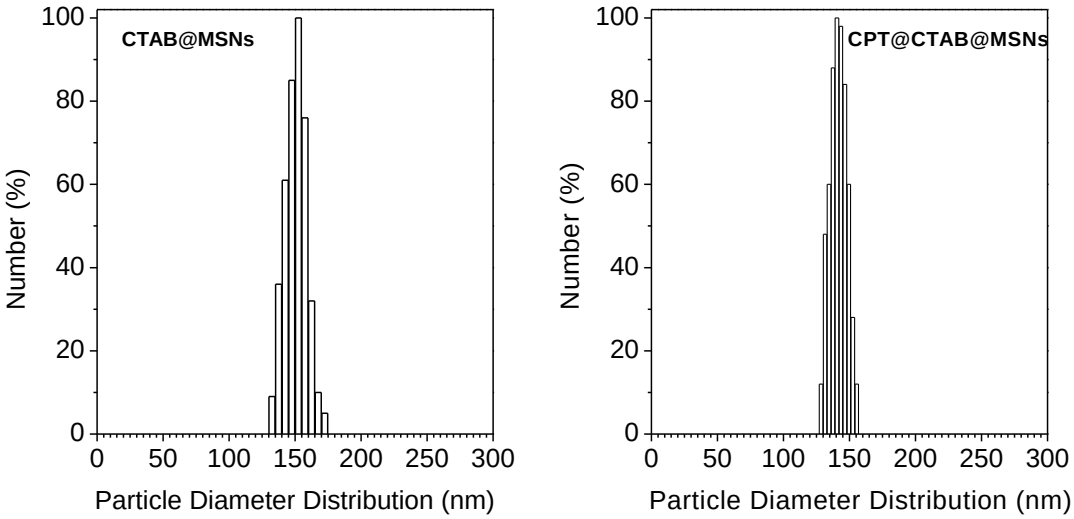

Figure S1. Particle diameter distributions of CTAB@MSNs and CPT@CTAB@MSNs measured by the DLS method. The particle diameter distributions of CTAB@MSNs and CPT@CTAB@MSNs are narrow, in accordance with the TEM measurement results (Figure 1). The particle diameters measured by the DLS method represent the hydrodynamic sizes, and therefore are slightly larger than those observed by TEM imaging (Figure 1).

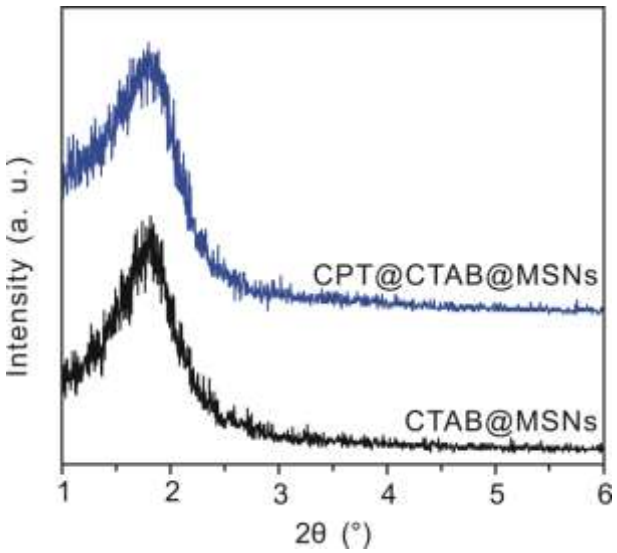

Figure S2. SA-XRD patterns of CTAB@MSNs and CPT@CTAB@MSNs. The presence of a clear peak at about 1.8º indicates that both CTAB@MSNs and CPT@CTAB@MSNs have a distinct mesoporous structure with the partial ordering in accordance with the TEM measurement results (Figure 1).

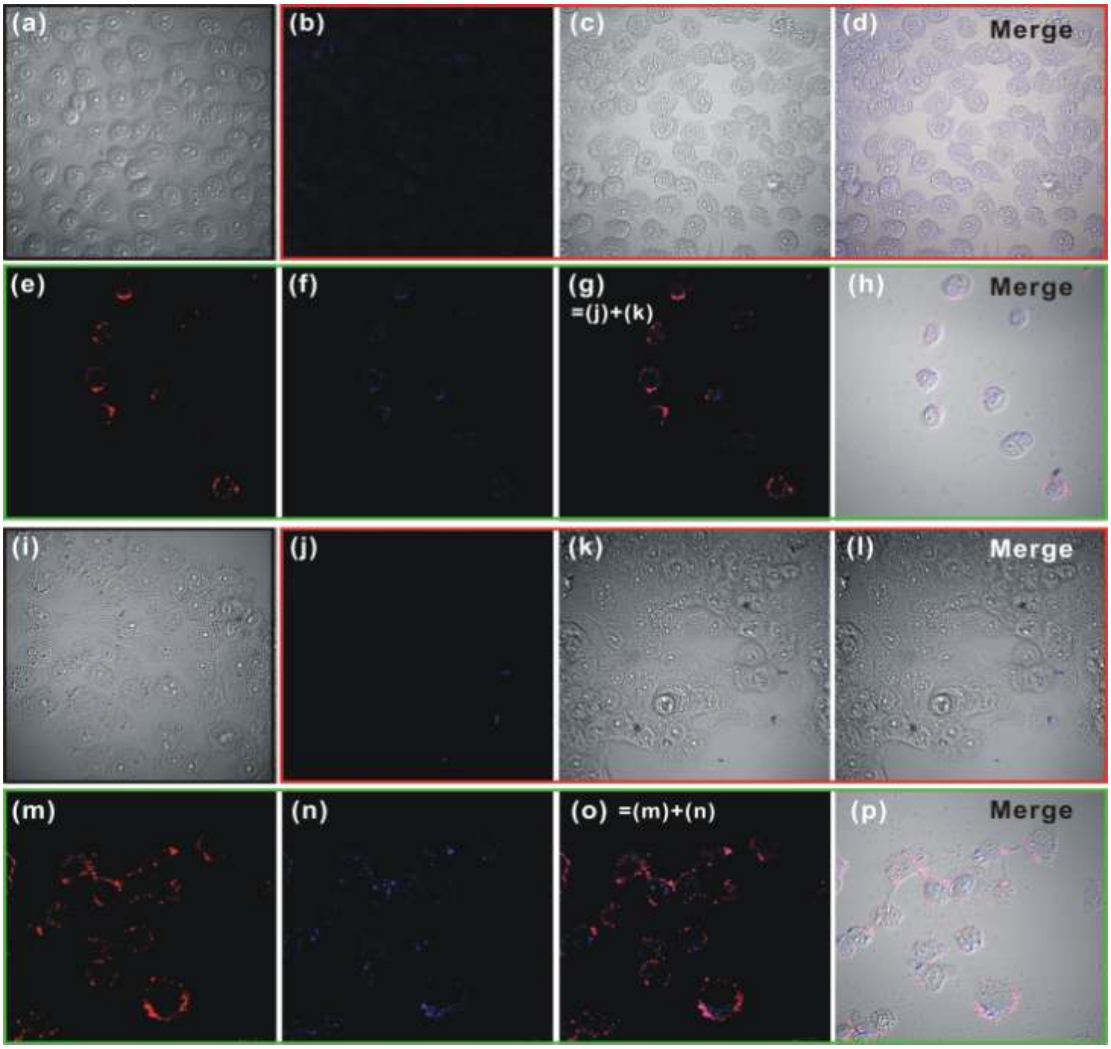

Figure S3. Intracellular drug uptake behaviors of the nano-DDS CPT@CTAB@MSNs in MCF-7 and MCF-7/ADR cells observed via the confocal microscopic imaging. Blue and red represent CPT drug and RITC-labeled MSNs cargo, respectively. Figures a, b–d and e–h represent MCF-7 cells treated with blank medium, free CPT and CPT@CTAB@MSNs, respectively. Figures i, j–l and m–p represent MCF-7/ADR cells treated with blank control, free CPT and CPT@CTAB@MSNs, respectively. Figures d, h, l and p are the merge of corresponding red, blue, light channels. Blue zones which did not overlap with red in Figures g and o indicate the intracellular CPT release.

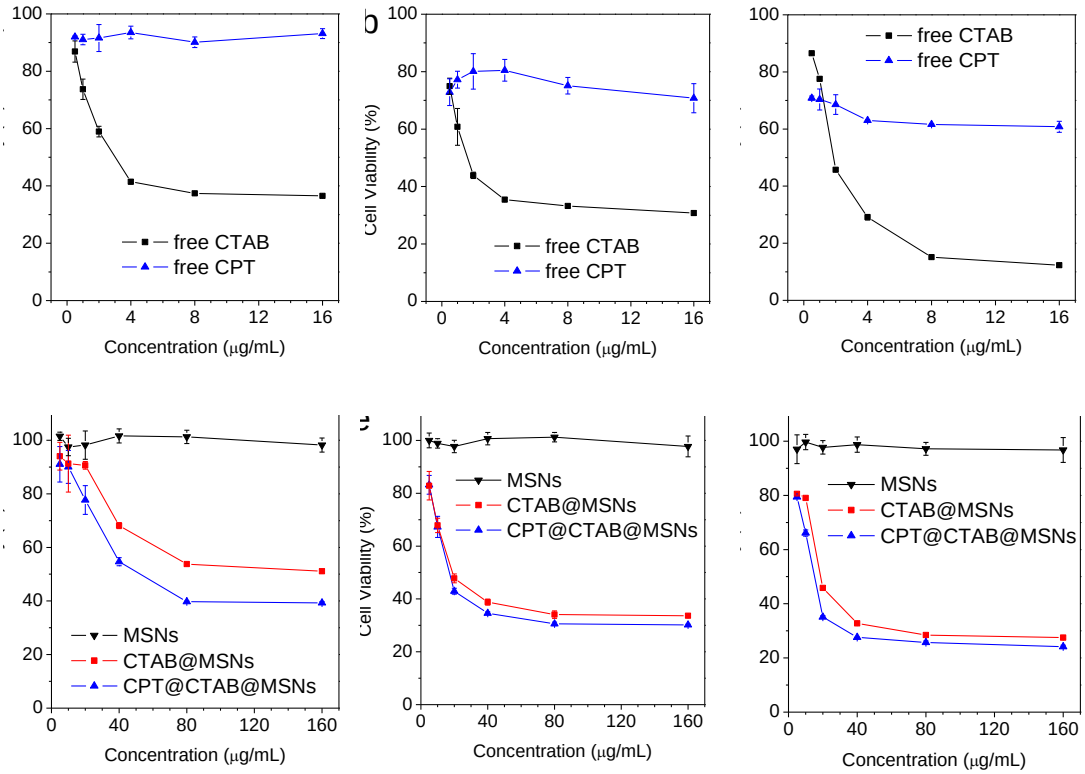

Figure S4. Cytotoxicity of free drugs (a–c) and the nano-DDSs (d–f) against MCF-7 cells treated for 1 day (a, d), 2 days (b, e) and 3 days (c, f).

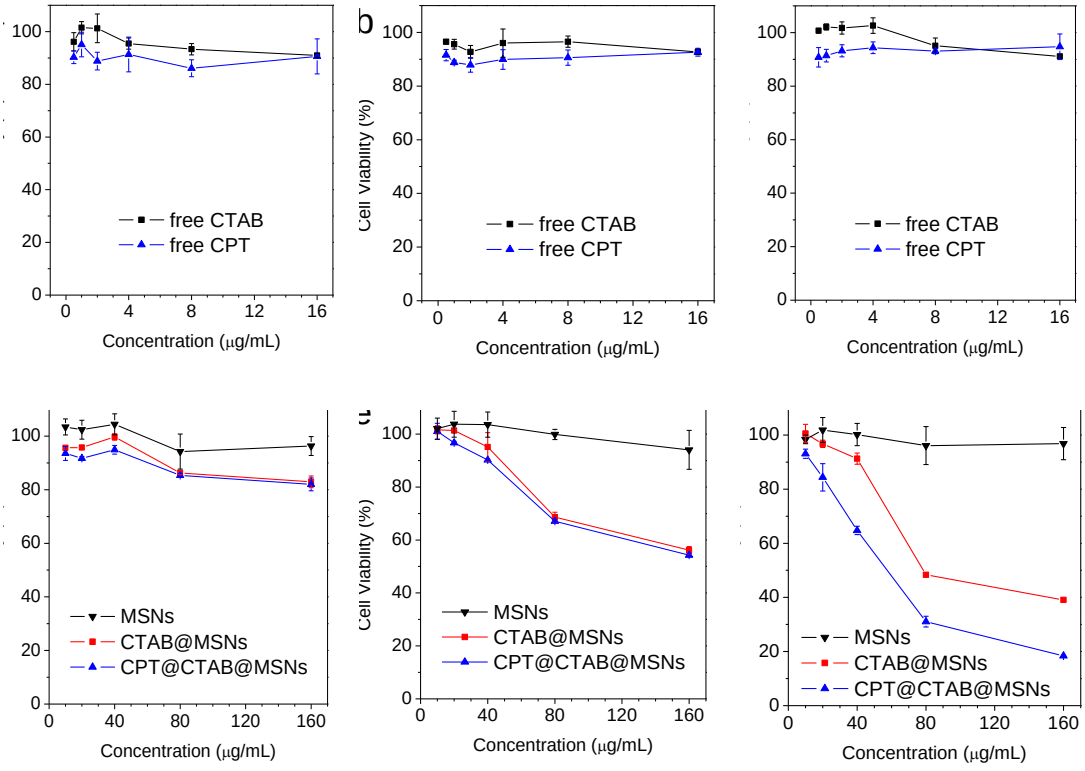

Figure S5. Cytotoxicity of free drugs (a–c) and the nano-DDSs (d–f) against MCF-7/ADR cells treated for 1 day (a, d), 2 days (b, e) and 3 days (c, f).

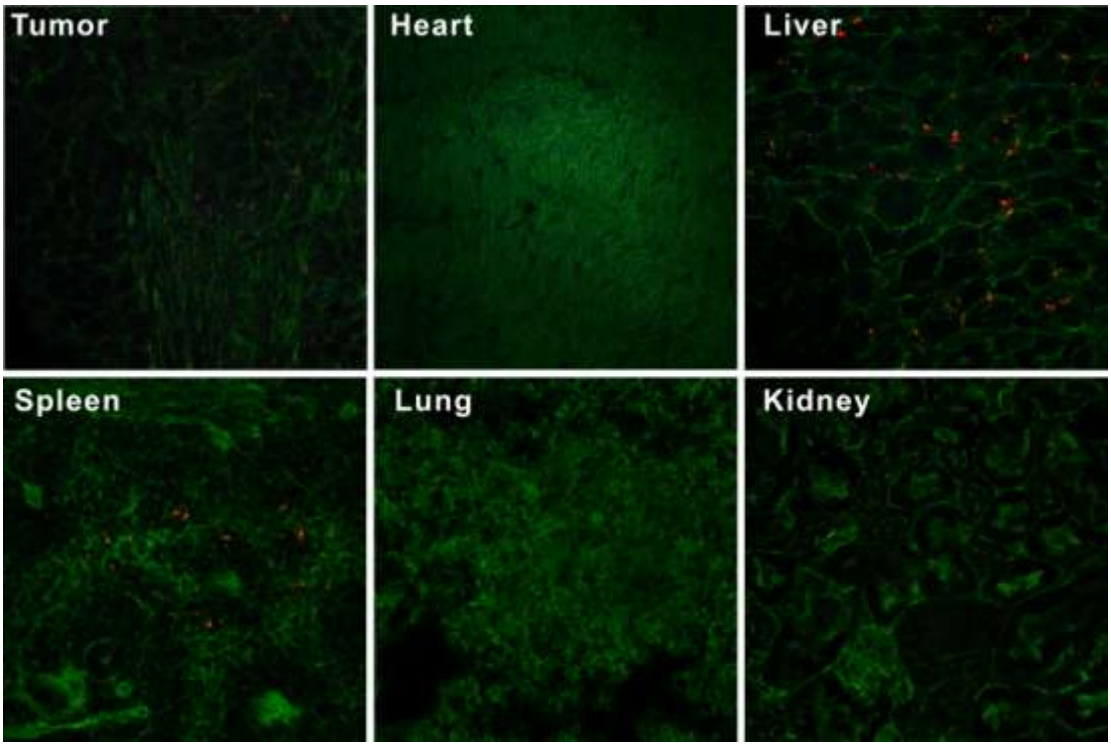

Figure S6. Biodistribution of the nano-DDS CPT@CTAB@MSNs in tumor and several normal tissues of the MCF-7/ADR tumor-bearing nude mice after mainline administration for one day. Red represents the RITC-labeled CPT@CTAB@MSNs.