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Electronic Supplementary Information

A Facile Approach to Upconversion Crystalline $\text{CaF}_2\text{:Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$ Nanospheres for Tumor Therapy

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Experimental Method

Synthesis of crystalline $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$ nanospheres

In a typical procedure, 0.2 g cetyltrimethylammonium bromide (CTAB, $\geq 99\%$, Sigma–Aldrich Inc.), 25 mL deionized water, 5 mL ethanol (99.9 vol%, Sinopharm Chemical, China), 50 μL of triethanolamine (TEA, Sinopharm Chemical Reagent) were mixed and stirred at 60 °C for 30 min. Subsequently, 2 mL tetraethylorthosilicate (TEOS, Sigma–Aldrich Inc., USA) was added into the mixture solution under stirring until white precipitate forms. The precipitate was collected via centrifuging and calcined at 550 °C for 5 hours to remove organic additives. Three ethanol/TEOS volume ratios (2.5, 4, and 5) were set during the synthesis to control the particles size, denoted as MSNs-2.5, MSNs-4, MSNs-5, respectively.

The MSNs were then incorporated with $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals via a new approach we developed, namely chemical-assisted sol-gel growth method. A mixed ionic solution, consisting of 2.9774 g $\text{Ca}(\text{CH}_3\text{CO}_2)_2 \cdot \text{H}_2\text{O}$ ($\geq 99.0\%$, Sigma-Aldrich Inc.), 1.2667 g $\text{Yb}(\text{C}_2\text{H}_3\text{O}_2)_3 \cdot 4\text{H}_2\text{O}$ (99.9%, Sigma-Aldrich Inc.) and 0.0346 g $(\text{CH}_3\text{CO}_2)_3\text{Tm} \cdot x\text{H}_2\text{O}$ (99.9%, Sigma-Aldrich Inc.) was prepared in 34 ml deionized water, and then 6 ml trifluoroacetic acid (TFA, 99%, Sigma–Aldrich Inc.) was added dropwise into the mixed ionic solution under stirring for 24 hours at 40 °C. 400 mg MSN nanoparticles (MSNs-2.5, MSNs-4, MSNs-5) were added into 20 mL of the as-prepared solution and immersed for 24 hours under stirring at 40 °C. Subsequently, all three samples were dried at 80 °C for 3 hours, and calcined in air at 600 °C for 3 hours to obtain the composite upconversion nanoparticles labeled as UCNP-2.5, UCNP-4, and UCNP-5.

In vitro cytotoxicity assay

To determine the cytotoxicity of UCNPs, the Cell Counting Kit-8 (CCK-8; Dojindo, Kamimashiki-gun Kumamoto, Japan) was performed. Briefly, cells (HEK 293 cells) were seeded into 96-well plates and cultured in a 5% CO_2 incubator (Heraeus, Hanau,

Germany) for 12 hours. Then, the medium was removed and replaced with fresh medium containing varied concentrations of UCNPs. Subsequently, the 96-well plates were placed in the 5% CO₂ incubator for further 24 hours. To access the cytotoxicity, 100 μ L fresh medium mixed with 10 μ L CCK-8 solution was added to each well for 2 hours at 37 °C. Then, the absorbance was then measured at 450 nm using an universal microplate reader (BIO-TEK Instruments, Minneapolis, MN, USA)

Drug loading and release

Doxorubicin (DOX), an anticancer drug, was used as a model drug. The DOX loading and releasing characteristics were examined following a reported method.³⁴ UCNP-2.5, UCNP-4, and UCNP-5 samples (50 mg) were added into DOX aqueous solution (1mg/mL, 20 mL) at ambient temperature. The mixture was subsequently stirred in the dark condition for 24 hours to achieve the equilibrium state. The DOX loaded samples were collected by centrifugation and washed with deionized water to remove the adsorbed DOX at the outer surface. The loading amount of drug was determined using UV-Vis spectral analysis and calculated following the equation, Drug loading efficiency = (weight of DOX loaded)/(weight of total DOX used) $\times$ 100%

The *in vitro* DOX releasing test was performed by immersing the DOX loaded UCNPs in 20 mL of PBS under gentle stirring at 37 $\pm$ 0.1 °C. At predetermined time intervals, a 10 mL buffer solution was collected and immediately replaced with an equal volume of fresh PBS. The amount of released DOX in PBS solution was measured using an UV-vis spectrophotometer (TU-1810, China) at a maximum wavelength (λ_{max}) of 480 nm. Three measurements were performed for each sample

***In vitro* cell viability during drug releasing**

Cell proliferation during DOX releasing was assessed by the Cell Counting Kit-8 (CCK-8; Dojindo, Kamimashiki-gun Kumamoto, Japan) according to the manufacturer's instruction. Briefly, 6 $\times$ 10³ cells (MCF-7 human breast cancer cells) were seeded

onto 96-well plate and treated with free DOX, UCNP-2.5-DOX, UCNP-4-DOX and UCNP-5-DOX. 100 μ L of CCK-8 solution was added into each chamber and incubated at 37 °C for 2 hours. Then, cell proliferation was assessed by measuring the absorbance at 450 nm using an universal microplate reader (BIO-TEK Instruments, Minneapolis, MN, USA).

MCF-7 cancer cells were seeded onto 8-chamber cell culture slide (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 2×10^4 cells/chamber and incubated with UCNP-2.5-DOX, UCNP-4-DOX, and UCNP-5-DOX samples for 4 hours. The cells were fixed in 4 % paraformaldehyde for 15 minutes. After washing with 6.7mM phosphate-buffered saline (PBS, pH 7.4, HyClone Laboratories Inc., Logan, Utah), MCF-7 human breast cancer cells were stained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma-Aldrich Co., St. Louis, MO, USA) for 10 minutes. The cell morphology after culturing was examined using a laser scanning confocal microscopy (LSCM, Fluoview OLYMPUS, JAPAN CO., LTD, Tokyo, Japan).

Characterization

The morphology and microstructures of the samples were characterized by field-emission scanning electron microscopy (FESEM, Hitachi SU-70, Japan) and high-resolution transmission electron microscopy (HRTEM, Tecnai F20, FEI, USA). The crystal structure was examined using an X-ray diffraction instrument (XRD, X'Pert PRO MPD, The Netherlands) operating at 40 mA and 40 kV using Cu K α radiation. The FTIR spectra were recorded on a PerkinElmer 580B infrared spectrophotometer on KBr pellets (Tensor 27, Bruker, Germany). The upconversion photoluminescence spectra were recorded at room temperature under the excitation of continuous laser with a wavelength of 980 nm from a fluorescence spectrophotometer (PL, FLSP920, Edinburgh). To minimize experimental uncertainties in the measurement, the sample positions and the spectra collection were maintained in identical conditions.

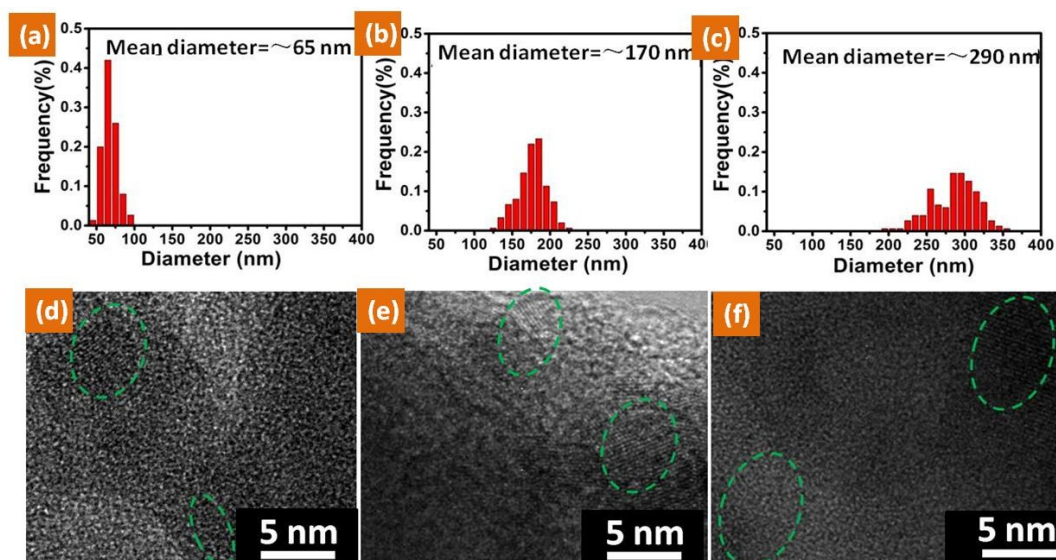


Figure S1 (a-c) the particle size distribution of UCNP-2.5, UCNP-4, and UCNP-5. HRTEM images of UC nanoparticles obtained with different Ethanol/TEOS volume ratio: (d) UCNP-2.5, (e) UCNP-4, and (f) UCNP-5.

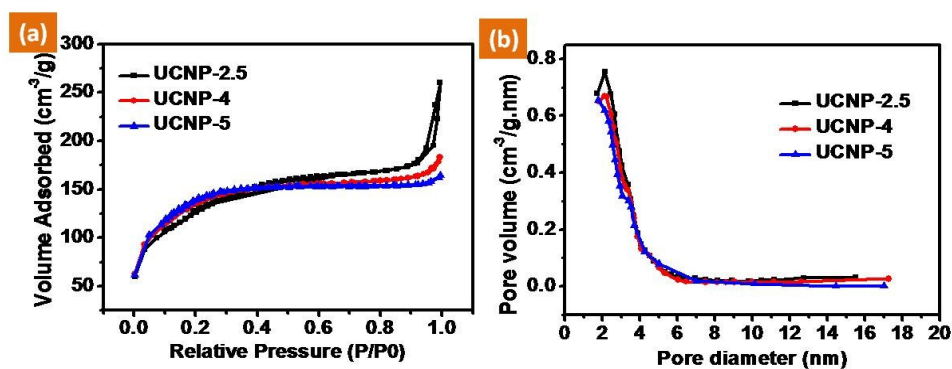


Figure S2. (a) N₂ adsorption/ desorption isotherm of particles, and (b) the corresponding pore size distributions.

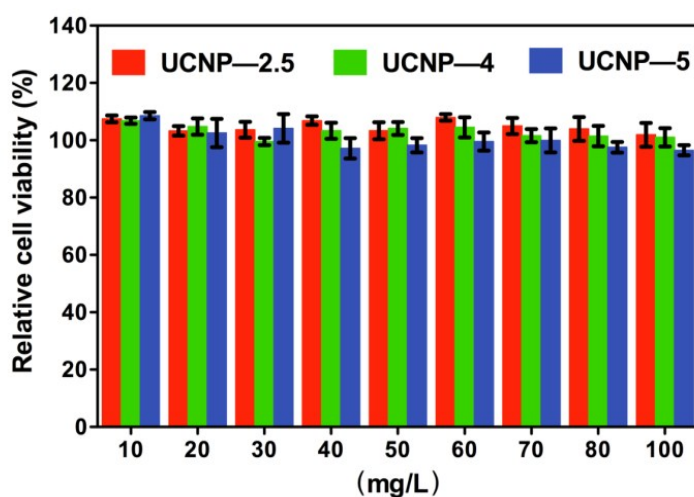


Figure S3 Relative cell viability of MCF-7 human breast cancer cells incubated with UCNP-2.5, UCNP-4, UCNP-5 of different concentrations for 24 hours.

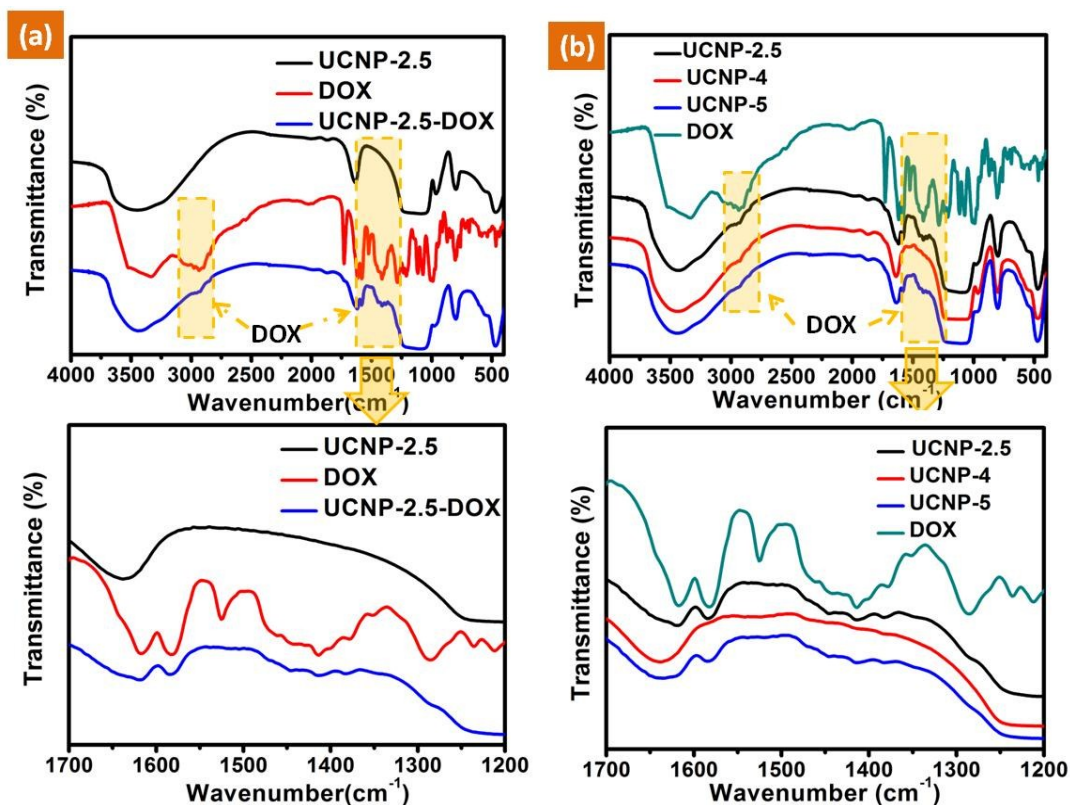


Figure S4. (a) FTIR spectra of UCNP-2.5 before and after DOX loading, and (b) the comparison of FTIR spectra of particles after DOX loading.

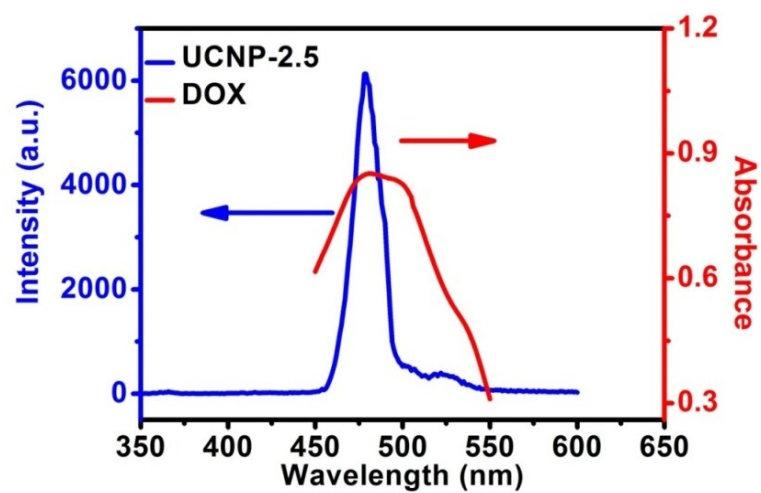


Figure S5. The emission spectrum of UCNP-2.5 and the UV-vis absorption spectrum of DOX drug.

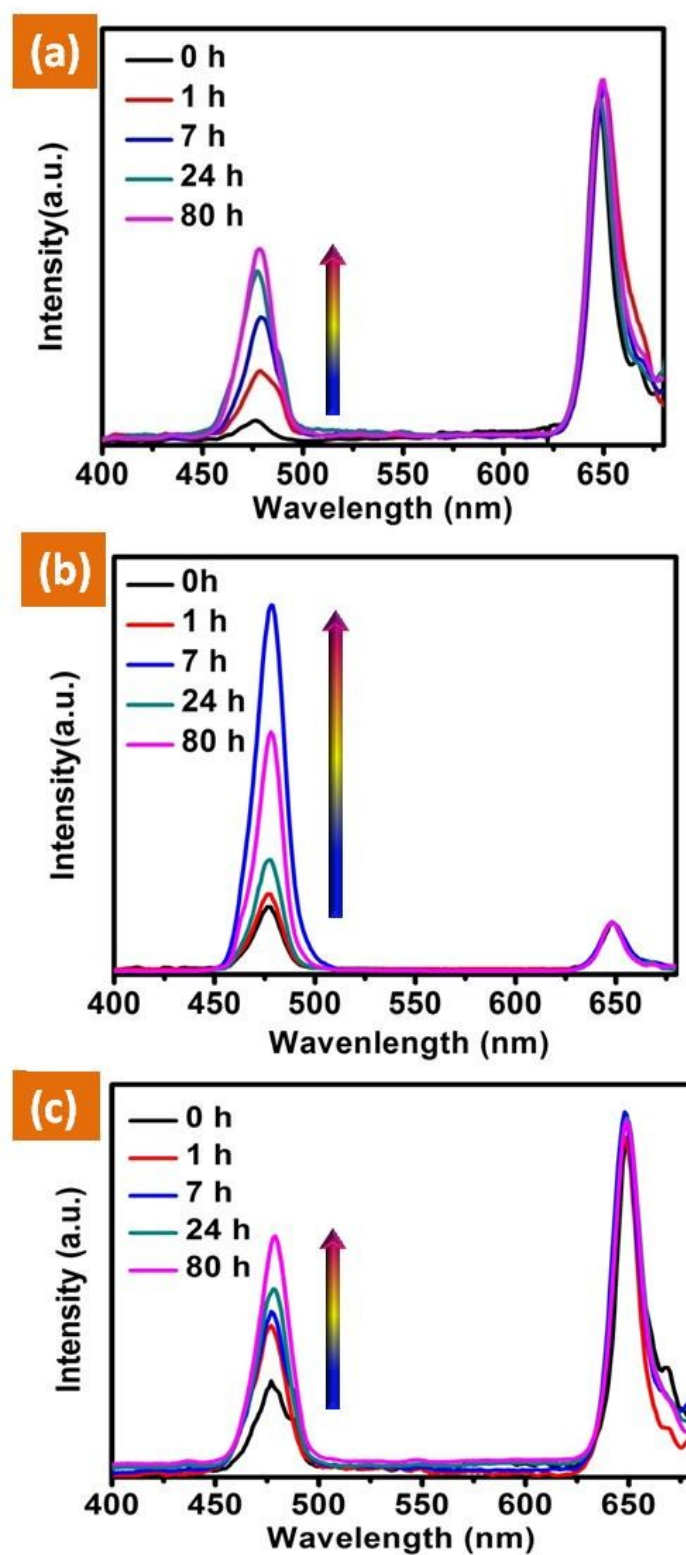


Figure S6. The photoluminescence spectra of (a) UCNP-2.5, (b) UCNP-4 and (c) UCNP-5 samples during DOX releasing for 80 hours.

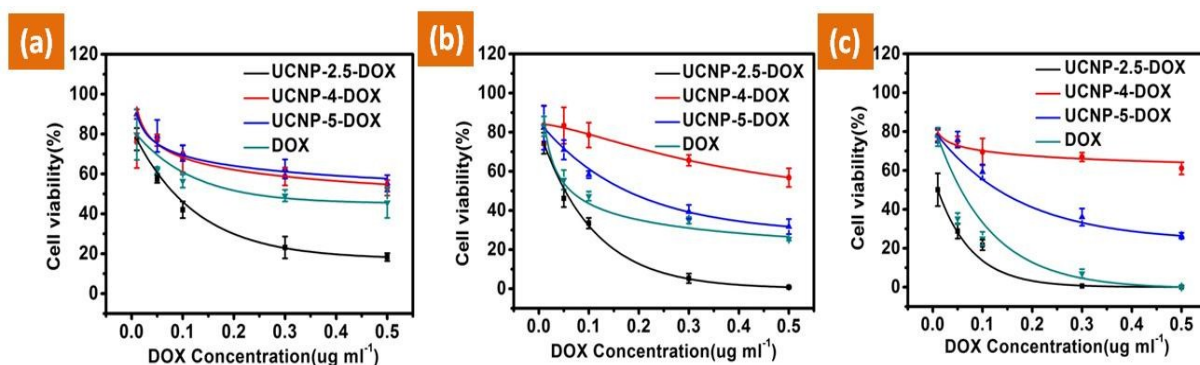


Figure S7 *In vitro* cellular viability of MCF-7 human breast cancer cells treated with free DOX, UCNP-2.5-DOX, UCNP-4-DOX and UCNP-5-DOX for (a) 24 hours, (b) 48 hours, and (c) 72 hours.

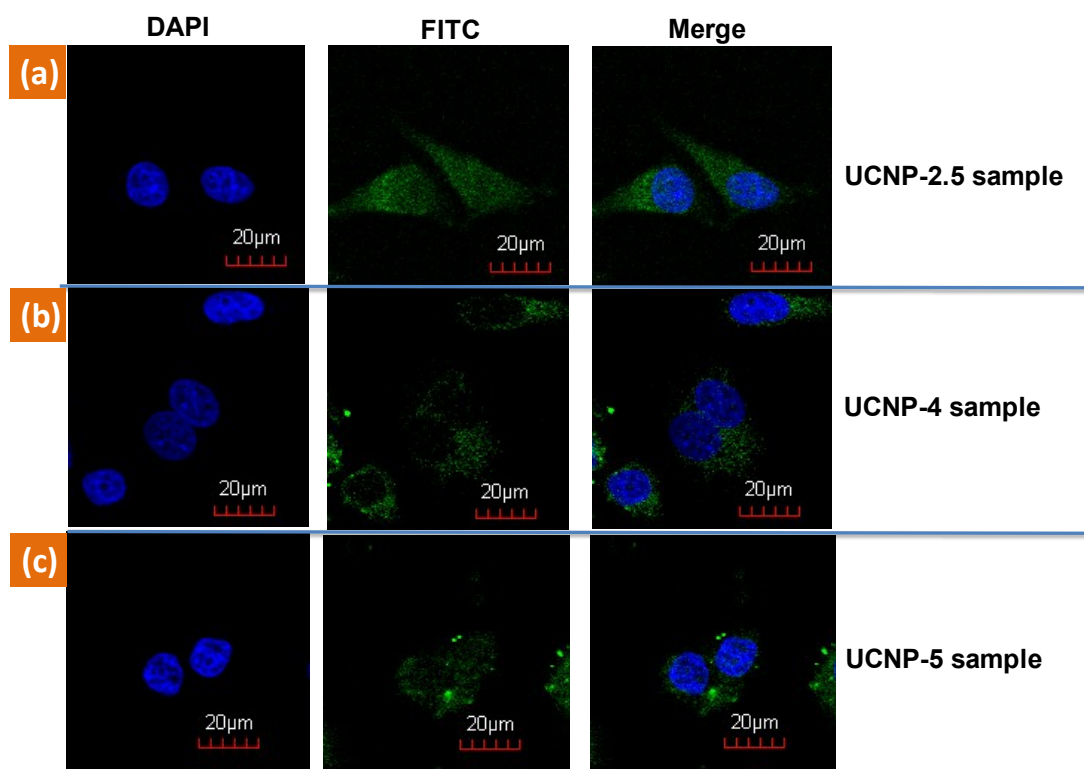


Figure S8. CLSM images of MCF-7 cell cells incubated with (a) FITC-UCNP-2.5, (b) FITC-UCNP-4, (c) FITC-UCNP-5 for 4 hours. For each panel, images from left to right show the MCF-7 cell nuclei stained by DAPI (blue), FITC fluorescence in cells (green), and overlay of both images