Supporting Information

One-Step Synthesis of Amine-Functionalized Hollow Mesoporous Silica Nanoparticles as Efficient Antibacterial and Anticancer Materials

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1. Materials

Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), ammonium hydroxide (25%), ethanol (200-proof), hydrochloric acid (HCl, 37%), fluorescein isothiocyanate (FITC), doxorubicin hydrochloride (Dox), and isoniazid (INH) were purchased from Sigma-Aldrich. Water used was from a Milli-Q water ultrapure water purification system. 3-Aminopropyltriethoxysilane (APTES) was acquired from TCI America (Portland, OR). Cell-counting kit-8 (CCK-8) was obtained from Dojindo Molecular Technologies, Inc. (Rockville, MD). All chemicals were used as received without any further purification.

2. Synthesis of hollow mesoporous silica nanoparticles (HMSNs)

A mixture of APTES and TEOS (2 g) with a mole ratio of 1:4 was added dropwise to an aqueous solution of CTAB (4 mM, 140 mL). After gentle stirring, $NH_3 \bullet H_2O$ (25%, 0.5 mL) was added and the mixture was stirred for another 5 hours at room temperature. The solid products were obtained by centrifugation and then washed three times with ethanol.

To remove the CTAB template, the particles were dispersed in 120 mL of ethanol solution containing 12 mL of HCl (37%). After stirring at 60 °C for 12 h, the mixture was centrifuged and the nanoparticles were washed with water for three times. Finally, the nanoparticles were dried at 60 °C in air to give HMSNs (780 mg).

3. Synthesis of conventional mesoporous silica nanoparticles (MSNs)

In a typical synthesis, CTAB was dissolved in 140 mL H_2O to give 4 mM solution, and $NH_3 \bullet H_2O$ (25%, 1.5 mL) was added with stirring for 30 min at room temperature. TEOS (1.5 g) was then added with stirring for another 5 h. After centrifugation and followed by washing three times with ethanol, the resulting solid was treated in acidic ethanol using the same procedure as in the case of HMSNs to remove CTAB

templates. The sample was finally dried at 60 °C in air to give conventional MSNs.

4. Labeling HMSNs with FITC (HMSNs-FITC)

FITC-labeled HMSNs (HMSNs-FITC) were prepared as follows. A solution of FITC (2 mg) in acetone/water mixture (0.3 mL/2.7 mL) was added to a dispersion of HMSNs (20 mg) in PBS (10 mL, pH 7.4). The reaction was allowed to proceed for 24 h at room temperature in the dark. The mixture was centrifuged and the solid was washed four times with acetone and PBS (pH 7.4), respectively to remove non-conjugated FITC. The sample was finally dispersed in PBS (pH7.4) and stored in a dark place.

5. Loading and releasing of isoniazid (INH)

To load INH, HMSNs (30 mg) were suspended in an aqueous solution of INH (15 mg/mL, 1 mL). The solution was stirred for 48 h at room temperature, and the drug-loaded nanoparticles were collected by centrifugation and careful washing with water twice to remove INH adsorbed on the particle surface. All supernatants were collected and measured by UV-vis spectroscopy at 260 nm to determine the amount of INH that was not loaded in the nanoparticles, using a standard calibration curve constructed by measuring the absorbance of INH solutions at varying concentrations (Fig. S11). The amount of INH loaded in the nanoparticles was then computed by subtracting the unloaded from the initially added.

For drug release, the INH-loaded HMSNs sample (2 mg) was immersed in PBS buffer (pH 6.6), and the supernatant was collected at given time intervals. The supernatant was measured by UV-vis spectroscopy at 260 nm to determine the concentration of INH released.

6. Antibacterial assays

Mycobacteria (*M. smegmatis* strain mc^2 651) were inoculated overnight in enriched Middlebrook 7H9 broth at 37 °C while shaking at 200 rpm until an optical density (OD_{650}) of 0.3 (ca. 10^8 CFU/mL) was reached. An aliquot of this bacterial suspension (1 mL) was taken and was serially diluted 100 folds in Middlebrook 7H9 broth. From this dilution, 100 μL aliquots were incubated for 24 h and 72 h with different concentrations of HMSNs, HMSNs-INH, and free INH at 37 °C in a humidified incubator shaker (250 rpm). Colony counting was obtained from the microdiluted plate. An aliquot of 10 μL from each well of the bacterial suspension was taken, and serially diluted in Middlebrook 7H9 broth. From the dilution, 20 μL was spread out on Middlebrook 7H10 agar plates, and the plates were incubated at 37 °C for 72 h. Colonies were counted and reported as log CFU/mL.

7. Interactions of HMSNs with bacteria by confocal fluorescence microscopy

Mycobacteria (*M. smegmatis* strain mc^2 651) were inoculated overnight in the Middlebrook 7H9 broth at 37 °C and 200 rpm until an OD_{650} of 0.3 was attained. The bacteria cells (30 mL) were then harvested, centrifuged, and re-dispersed in pH 7.4 PBS buffer. HMSNs-FITC (0.25 mg) were added to an aliquot of bacteria (ca. 10^6

CFU), and the mixture was incubated at 37 °C for 4 h while shaking at 150 rpm. The mixture was then centrifuged at 2000 rpm for 8 minutes, and the supernatant containing nanoparticles was discarded. A drop of bacteria dispersion was spread on the cover slip and examined by a confocal fluorescence microscope (Olympus FV300).

8. Interactions between HMSNs and bacteria by TEM

HMSNs-treated bacteria cells were prepared following the same procedure as above. A drop of bacteria cell suspension was placed onto a Cu grid followed by vacuum drying overnight.

9. Loading and releasing of doxorubicin (Dox)

To load Dox into HMSNs or HMSNs-FITC, HMSNs or HMSNs-FITC were dispersed in an aqueous solution of Dox (20 mg mL^{-1}) while keeping the weight ratio of nanoparticles to drug at 2:1. The mixture was stirred at 37 °C for 48 h, followed by centrifugation and washing twice with water to obtain the drug-loaded hollow mesoporous silica nanoparticles (HMSNs-Dox, or HMSNs-FITC-Dox). The loading amount of Dox was determined by UV/vis spectroscopy at 233 nm using a standard calibration curve of Dox (Fig. S17). For the drug release, Dox-loaded HMSNs samples were immersed in PBS (pH 4.5 and pH 7.4), and the supernatant was collected at given time intervals. The absorbance at 233 nm was measured to determine the amount of Dox released.

10. Cellular uptake and cell imaging analysis

A549 cells (Human Lung Carcinoma Epithelial Cells, ATCC) were maintained in high glucose DMEM (Dulbecco's Modified Eagle's Medium, ATCC) supplemented with 10% FBS (ATCC) and 1% penicillin-streptomycin (Sigma) in a humidified incubator at 37 °C with 5% CO_2 and 95% air. For intracellular localization, 10^5 A549 cells per well were seeded in 6-well plates containing a cover glass and were allowed to adhere for 24 h. After incubation with 50 μg/mL Dox-loaded FITC-labeled HMSNs for 4 hours, the cover glass containing A549 cells were washed with PBS and was then mounted onto a glass slide. The slide was examined under a confocal fluorescence microscope (Olympus FV300).

11. *In vitro* cytotoxicity evaluation

The cytotoxicity of HMSNs, HMSNs-Dox, and free Dox was evaluated using the WST-8 viability assay. For 24 h and 72 h cytotoxicity evaluation, A549 cells were seeded at a density of 8000 and 2000 cells per well in 96-well plates, respectively. After incubating the A549 cells with HMSNs, HMSNs-Dox, or free Dox for 24 h or 72 h, 10 μL WST-8 reagent was added to each well and incubated for 4 h. The absorbance of the resulting solution in each well was recorded at 450 nm with a microplate reader (Bio-Rad, USA). Before reading, the plate was gently shaken on an orbital shaker for one minute to ensure homogeneous distribution of color.

12. Characterization

The morphology of the hollow mesoporous silica nanoparticles were examined by TEM using a Phillips EM-400 TEM microscope operating at an accelerating voltage of 100 kV. Scanning electron microscopy (SEM) images were obtained on a JEOL JSM 7401F FE-SEM instrument operated at 15 kV. Samples were sputtered with Au prior to characterization. Dynamic light scattering (DLS) measurements were taken using a Horiba SZ-100 analyzer (Horiba Scientific Ltd., Kyoto, Japan). A DelsaNano C Zeta Potential analyzer (Beckman Coulter) was used to measure the particle surface charge. Fluorescence spectra were obtained on a Cary Eclipse fluorimeter (Agilent Technologies, Walnut Creek, CA). Nitrogen adsorption–desorption measurements were carried out using a Quantachrome Autosorb-3B surface area analyzer at −196 °C. The specific surface area was calculated by the Brunauer-Emmett-Teller (BET) method. Pore-size distributions were estimated using the Barrett–Joyner–Halenda (BJH) method. Pore volumes were determined from the amounts of N_2 adsorbed at the single point of P/P_0 =0.98. The infrared spectra were recorded on a Nicolet 6700 FT-IR spectrometer from Thermo Scientific Corporation. Thermogravimetric analysis (TGA) was carried out on Q50 (TA Instrument, DE); samples were heated from room temperature to 1000 °C at a heating rate of 20 °C/min.

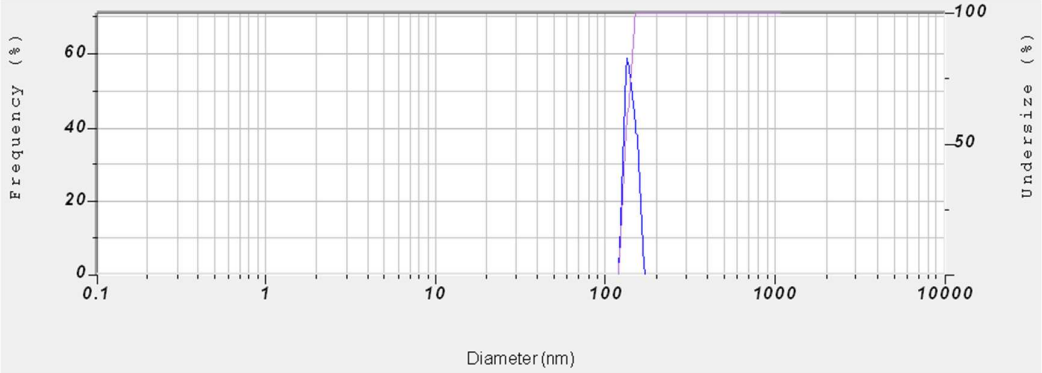

Figure S1. DLS histogram of HMSNs in water. (Hydrodynamic size=131.7 ± 8.1 nm, PDI=0.379)

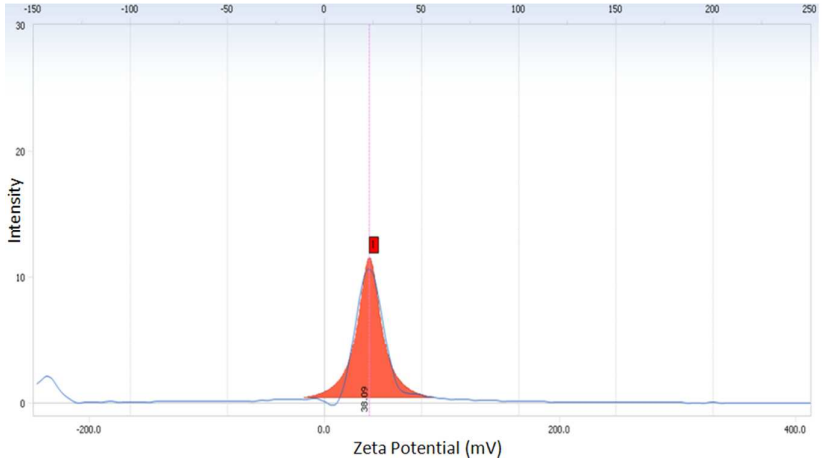

Figure S2. Zeta potential measurement result of HMSNs in water. (+38.09 mV)

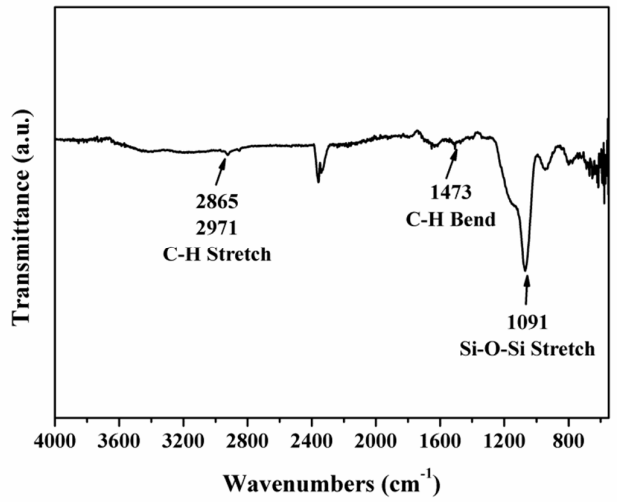

Figure S3. FTIR spectrum of hollow mesoporous silica nanoparticles after removing CTAB.

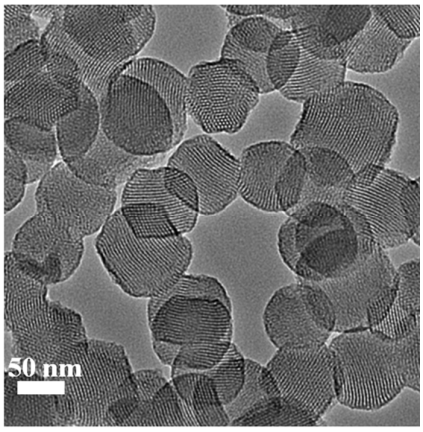

Figure S4. TEM image of conventional mesoporous silica nanoparticles (MSNs).

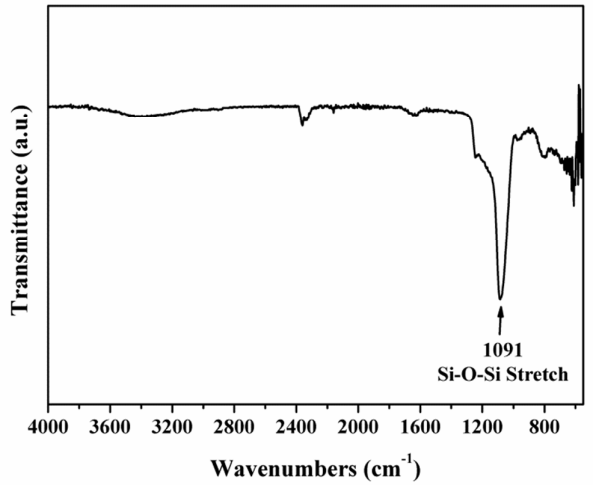

Figure S5. FTIR spectrum of conventional mesoporous silica nanoparticles after

removing CTAB.

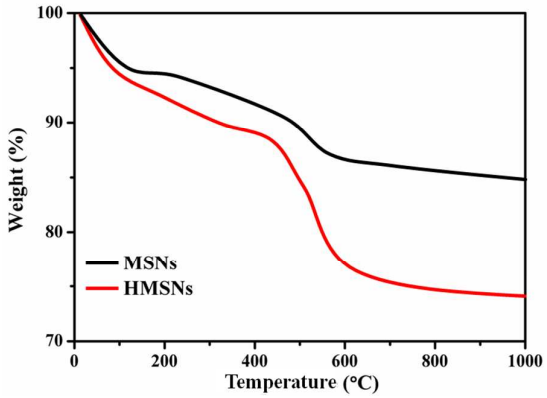

Figure S6. TGA decomposition plots of conventional mesoporous silica nanoparticles (weight loss ~15.1%) and hollow mesoporous silica nanoparticles (weight loss ~25.6%).

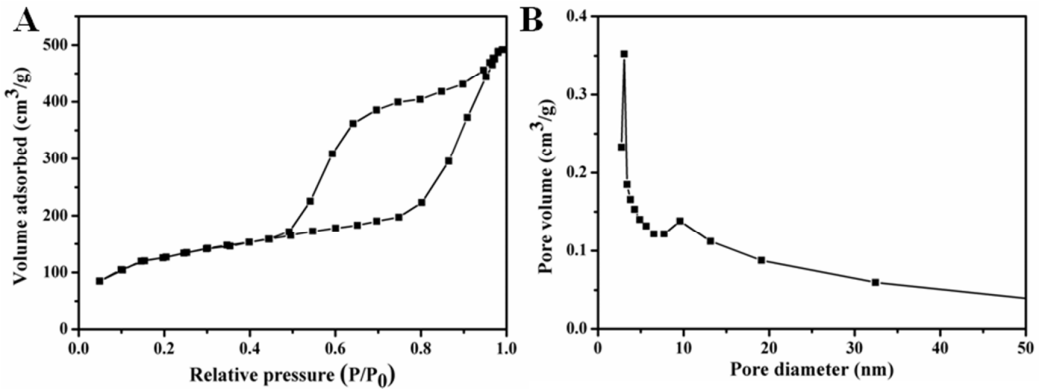

Figure S7. (A) N_2 adsorption-desorption isotherm of hollow mesoporous silica nanoparticles; (B) The corresponding adsorption pore size distribution plot by BJH method.

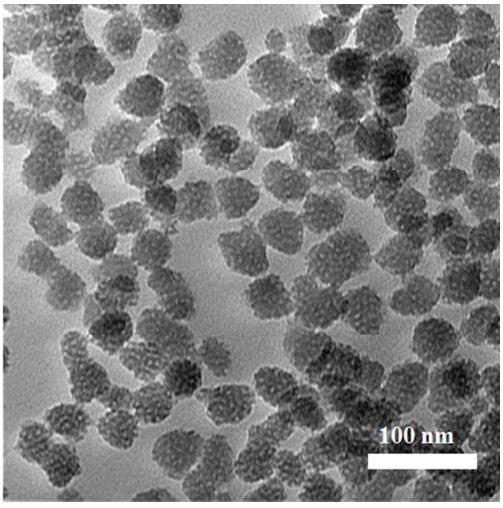

Figure S8. TEM image of nanoparticles synthesized following the same procedures

as HMSNs without APTES.

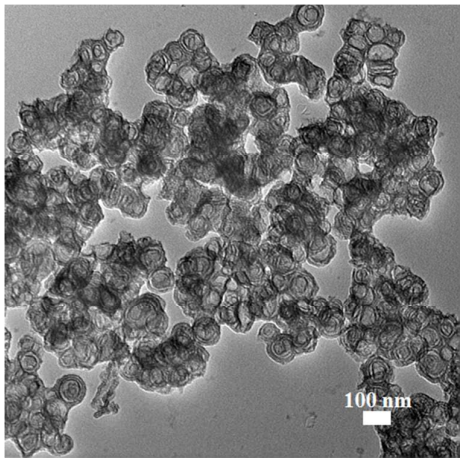

Figure S9. TEM image of nanoparticles synthesized following the same procedures as HMSNs without CTAB.

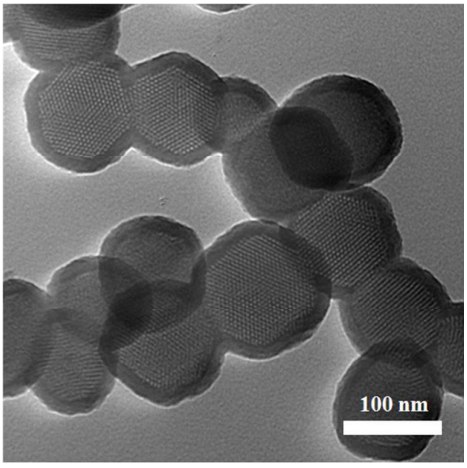

Figure S10. TEM image of nanoparticles synthesized following the same procedures as HMSNs except that the addition order of ammonia and the mixture of APTES and TEOS was changed.

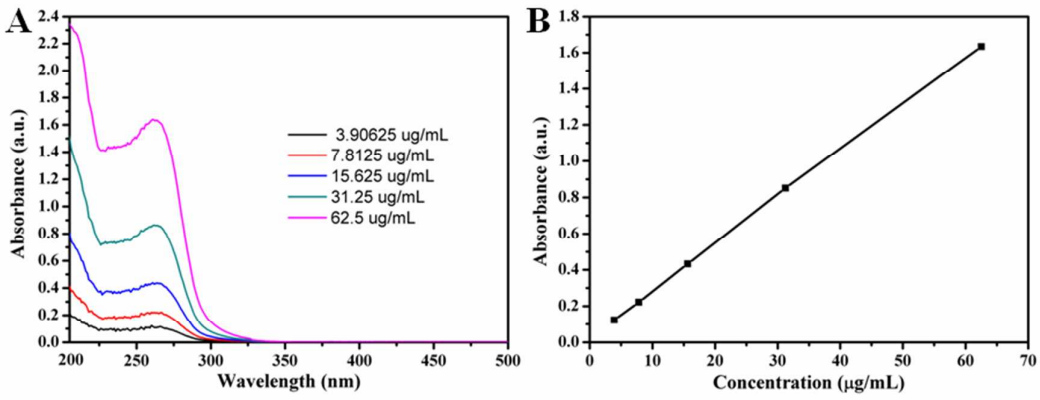

Figure S11. (A) UV-vis spectra of different concentrations of INH in water; (B)

Standard calibration curve of INH in water at 260 nm (R^2=0.9996).

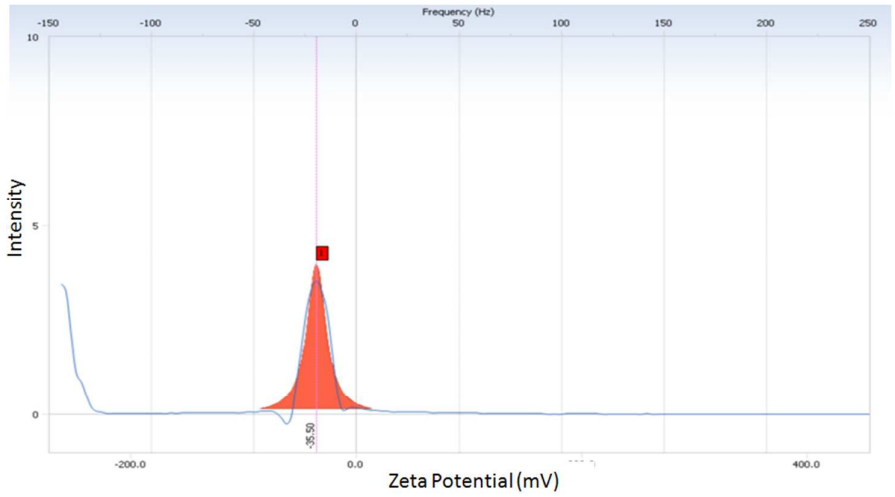

Figure S12. Zeta potential measurement result of conventional mesoporous silica nanoparticles (MSNs) in water. (-35.50 mV)

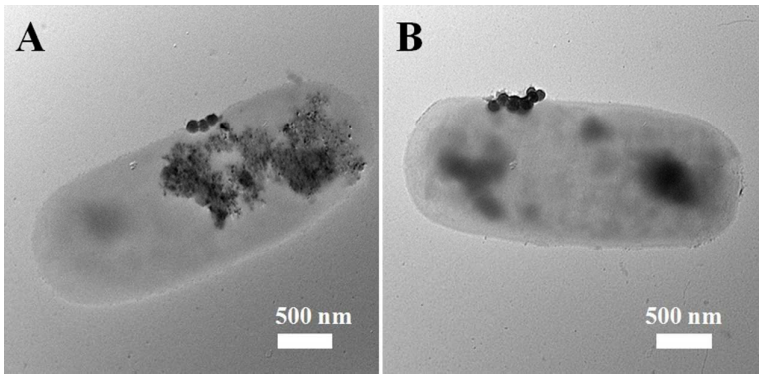

Figure S13. TEM images of bacteria after *M. smegmatis* strain mc^2 651 mycobacteria were treated with conventional MSNs.

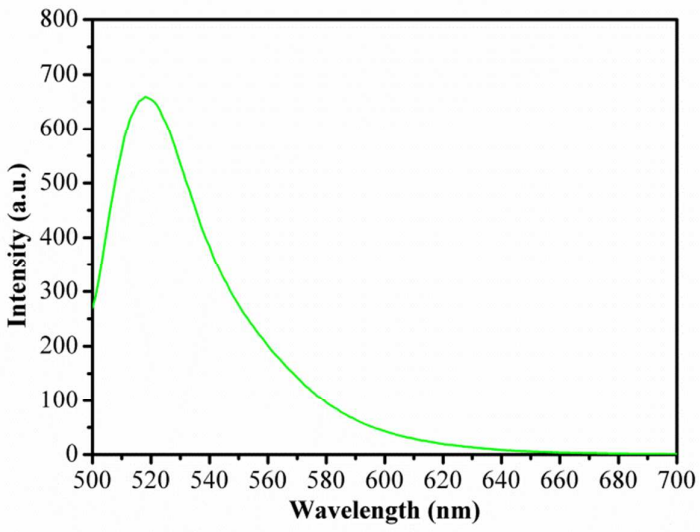

Figure S14. Fluorescence spectrum of HMSNs-FITC at particle concentration of 50

μg/mL.

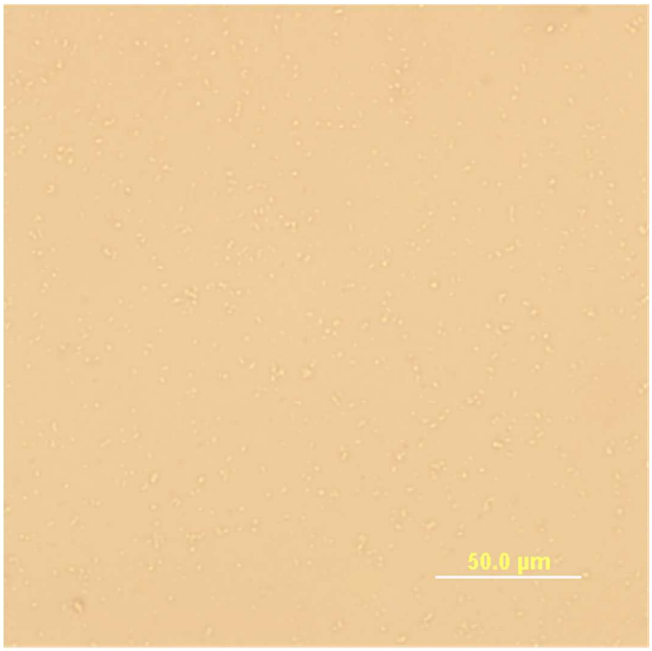

Figure S15. Bright-field image of *M. smegmatis* strain mc^2 651 treated with HMSNs-FITC.

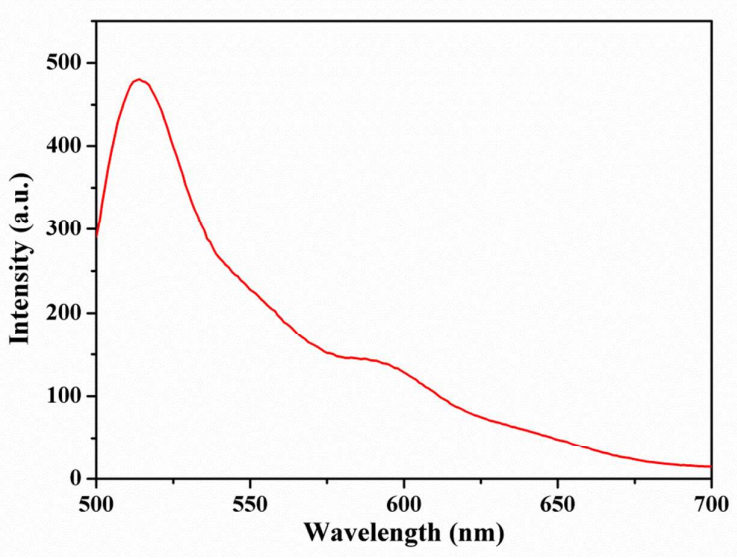

Figure S16. Fluorescence spectrum of Dox-loaded HMSNs-FITC at particle concentration of 50 μg/mL.

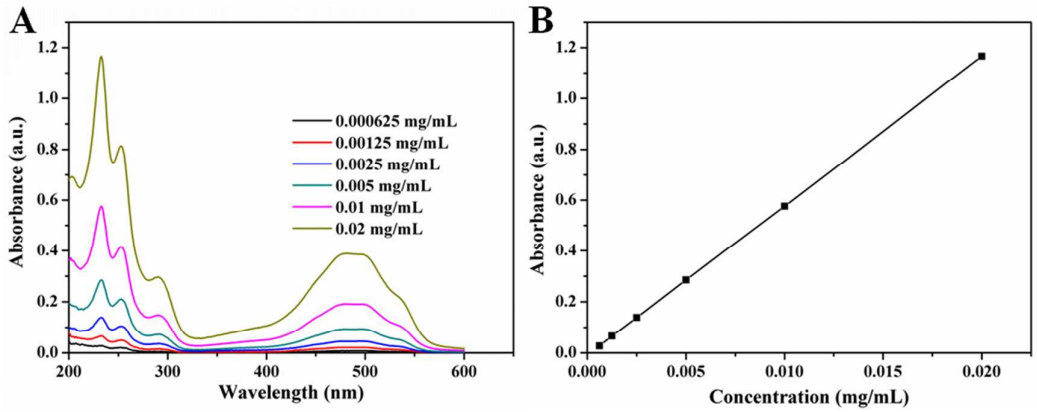

Figure S17. (A) UV-vis spectra of different concentrations of Dox in water; (B) Standard calibration curve of Dox in water at 233 nm (R^2=0.9998).