Supporting Information

Shape Control of Mesoporous Silica Nanomaterials Templated with Dual Cationic Surfactants and Their Antibacterial Activities

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1. Materials

Cetyltrimethylammonium bromide (CTAB), tetrabutylammonium iodine (TBAI), tetraethyl orthosilicate (TEOS), ammonium hydroxide (25%), ethanol (200-proof), and hydrochloric acid (HCl, 37%) were purchased from Sigma-Aldrich. Water used was from a Milli-Q water ultrapure water purification system. Bromide and iodide standard solutions were purchased from Sigma-Aldrich prepared from high purity NaBr and KI, respectively. All chemicals were used as received without further purification.

Middlebrook 7H9 broth with oleic acid-albumin-dextrose-catalase (OADC) enrichment was prepared by mixing Middlebrook 7H9 (4.7 g, BD bioscience), glycerol (2.0 mL, Acros Organics), and water (900 mL). After sterilization by autoclaving (Tuttnauer EZ 10, Hauppauge, NY), OADC (100 mL, BD bioscience) and Tween 80 (0.05%, Sigma) were added.

2. Synthesis of mesoporous silica nanomaterials (MSNs)

FMSN, PMSN, SMSN, and RMSN were synthesized by tuning the mole ratio of CTAB to TBAI. Typically, CTAB and TBAI was dissolved in 140 mL of H_2O, and $NH_3 \cdot H_2O$ (25%, 2.5 mL) was added under stirring for 15 min at room temperature. TEOS (3 mL) was finally added with stirring for another 5 h at room temperature. The solid products were obtained by centrifugation and then washing three times with water.

3. Removing templates from MSNs

To remove the CTAB and TBAI templates, the as-synthesized MSNs were dispersed in 250 mL of ethanol solution containing 20 mL of HCl (37%). After stirring at 60 °C for 24 h, the mixture was centrifuged and the nanoparticles were washed with ethanol for three times.

4. Antibacterial assays

All bacteria handling and experimental protocols were performed in accordance with the University of Massachusetts Lowell guidelines. Procedures were conducted using approved Institutional Biosafety Committee (IBC) procedures (Registration No. 15-06-YAN). 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 12, 24, and 48 h with different concentrations of FMSN, PMSN, SMSN, and RMSN at 37 °C in a humidified incubator shaker (250 rpm). 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.

5. TEM characterization of interactions between MSNs and bacteria

Mycobacteria 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 were then harvested, centrifuged at 2000 rpm, and re-dispersed in the broth medium. FMSN, PMSN, SMSN, and RMSN (25 μg/mL) 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 and washed for several times with PBS (pH 7.4), and each time the supernatant containing free nanoparticles was discarded. A drop of the final bacteria cell suspension was deposited onto a Cu grid followed by vacuum drying overnight for TEM imaging.

6. Determination of the release profiles of surfactants from different shaped materials

The release profiles of CTAB and TBAI from FMSN, PMSN, SMSN, and RMSN were determined by a modified spectrophotometric method.[1] Briefly, calibration curves were obtained by measuring the absorbance of serially diluted solutions from the bromide and iodide standard solutions in quartz cuvettes. In this study, we used 210 nm and 240 nm to establish the standard curves of bromine and iodine, respectively. To determine the amount of released surfactants, 50 mg of FMSN, PMSN, SMSN, or RMSN were immersed in PBS buffer (pH 7.4), and the supernatant was collected at given time intervals. Absorbance of the supernatant at 210 nm or 240 nm was measured by UV-vis spectroscopy, and the values were compared to the standard calibration curves to determine the concentration of CTAB or TBAI released.

7. Characterization

The shape and pore properties of MSNs 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 10 kV. Samples were sputtered with Au prior to characterization. A DelsaNano C Zeta Potential analyzer (Beckman Coulter) was used to measure the particle surface charge. 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.

References

1. (a) N. Yonehara, T. Yamane, T. Tomiyasu and H. Sakamoto, *Anal. Sci.*, 1989, **5**, 175; (b) A. M. Zhang, S. H. Wang, L. Y. Du and H. Cui, *Anal. Lett.*, 2000, **33**, 2321; (c) H. F. Zhang, Q. Li, M. Guo, M. Z. Li and Z. J. Wu, *Patent*, CN103575685 A.

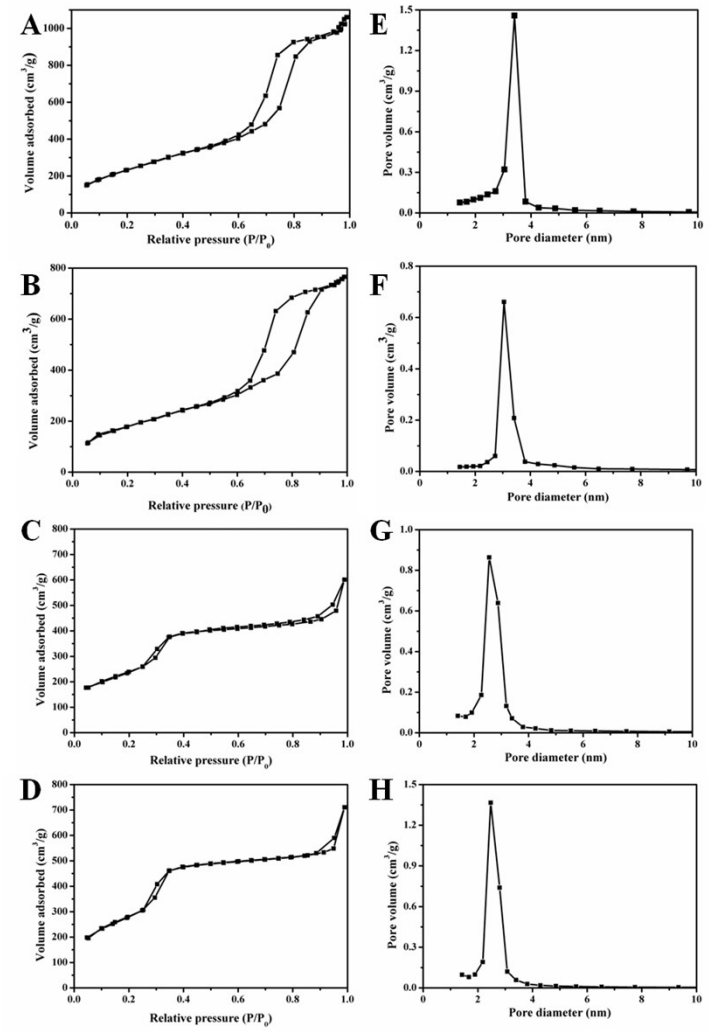

Figure S1. (A-D) N_2 adsorption-desorption isotherm of FMSN (A), PMSN (B),

SMSN (C), and RMSN (D). (E-H) The corresponding pore size distribution plot of FMSN (E), PMSN (F), SMSN (G), and RMSN (H), measured by BJH method from adsorption branches of the isotherms.

Table S1. Pore properties of template-removed PMSN, FMSN, SMSN, and RMSN.

Particle type	BET surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
FMSN	1039.8	0.97	3.4
PMSN	606.1	0.48	3.1
SMSN	916.4	0.89	2.9
RMSN	1121.7	1.03	2.8

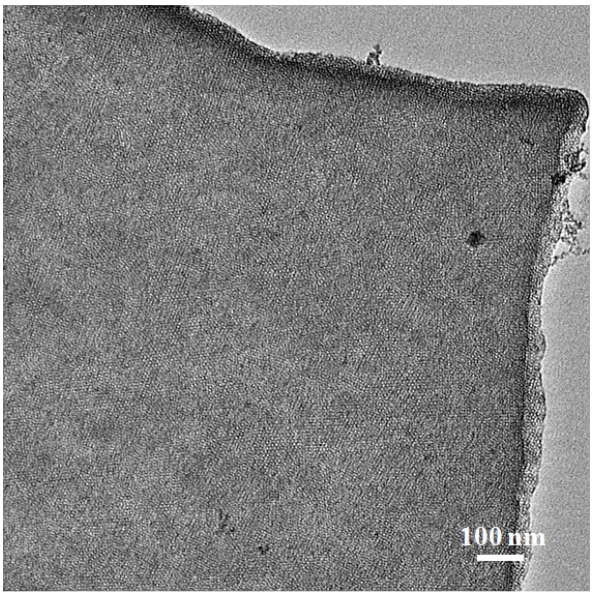

Figure S2. TEM image of nanomaterial synthesized following the same procedures as MSNs except that the mole ratio of CTAB to TBAI was increased to 1.0. The obtained mesoporous silica film had an average thickness of ca. 50 nm.

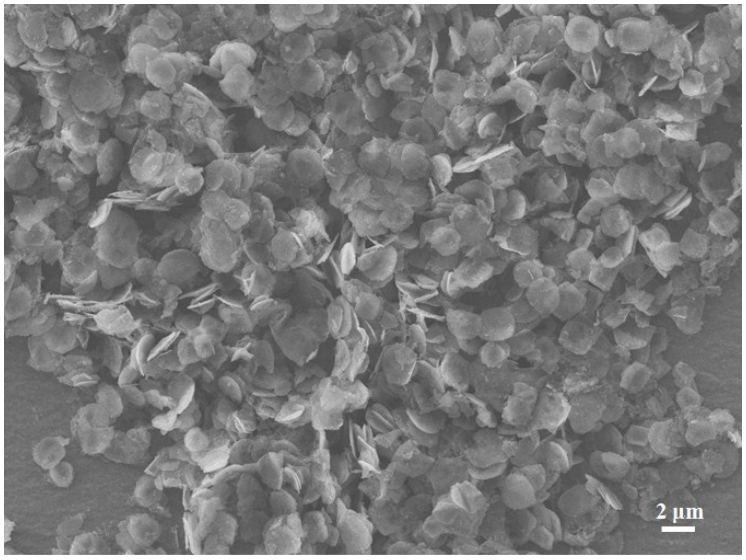

Figure S3. SEM image of nanomaterial synthesized following the same procedures as MSNs except that the mole ratio of CTAB to TBAI was increased to 1.3. The obtained mesoporous silica platelets have an average thickness of 100-200 nm.

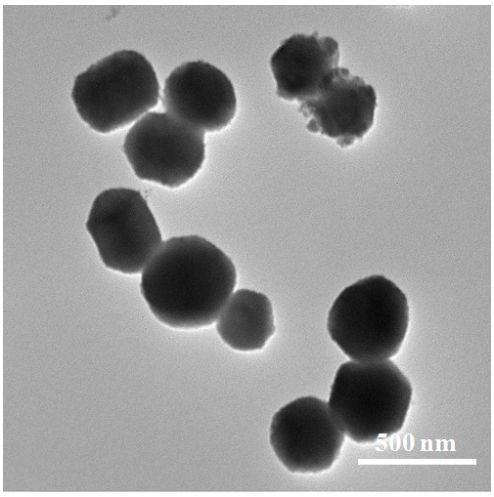

Figure S4. TEM image of nanomaterial synthesized following the same procedures as FMSNs except of replacing TBAI with tetrabutylammonium hydroxide (TBAH). In this case, platelet-like nanostructure could not be formed.

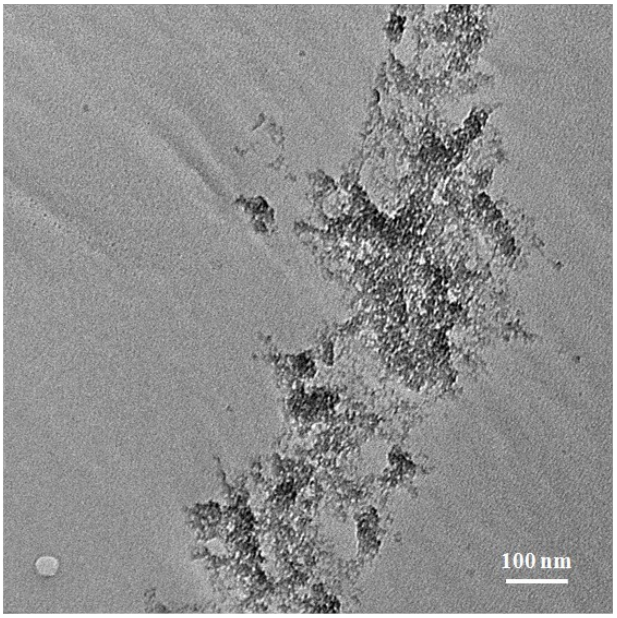

Figure S5. TEM image of product synthesized following the same procedures as MSNs except only TBAI surfactant was added. No ordered structure was obtained in the absence of CTAB as co-template.

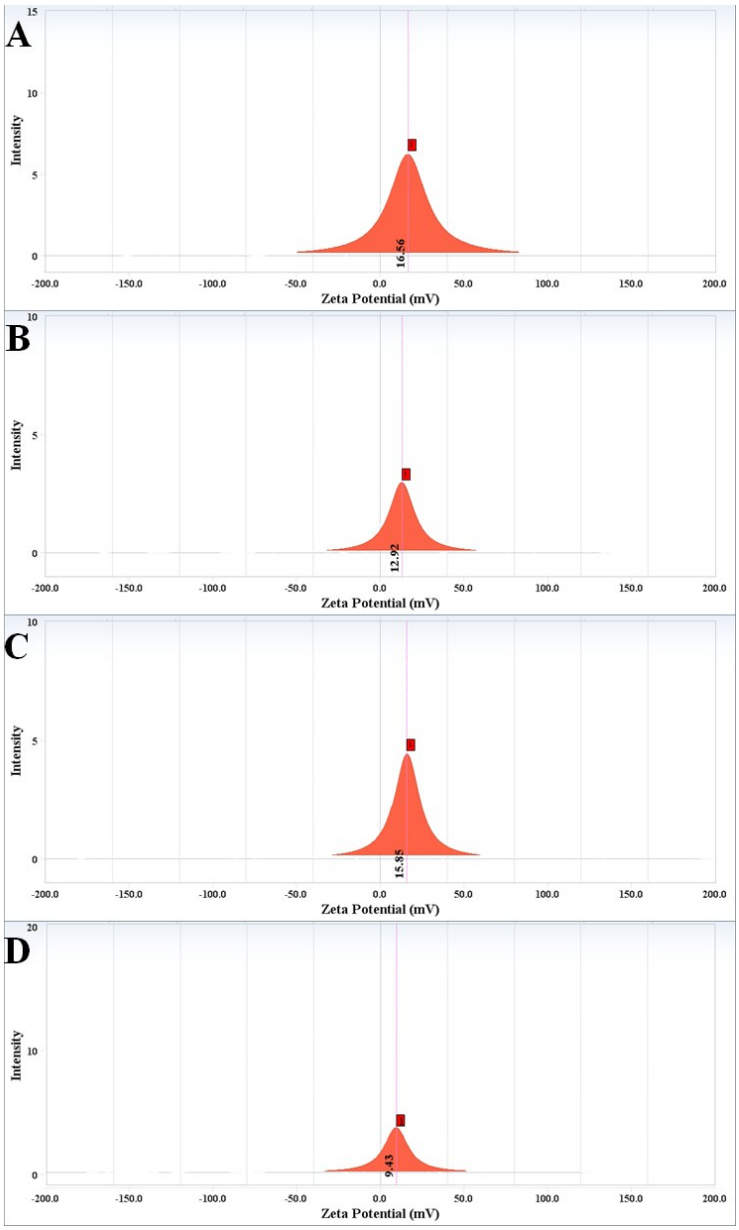

Figure S6. Zeta potential of FMSN (A, 16.56 mV), PMSN (B, 12.92 mV), SMSN (C, 15.85 mV), and RMSN (D, 9.43 mV) before removing CTAB and TBAI templates.

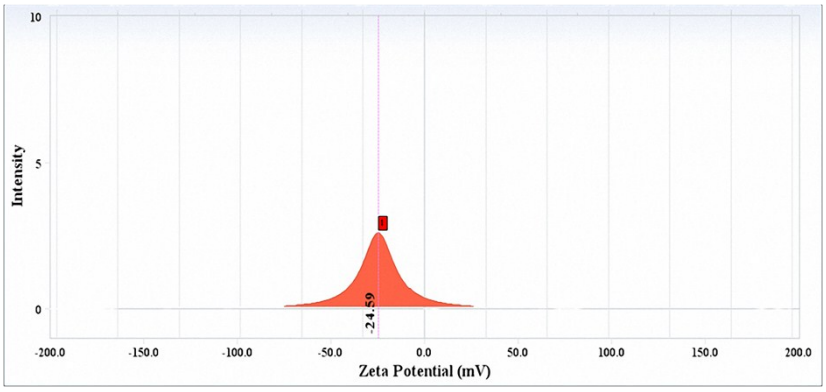

Figure S7. Zeta potential of mycobacteria dispersed in water (-24.59 mV).

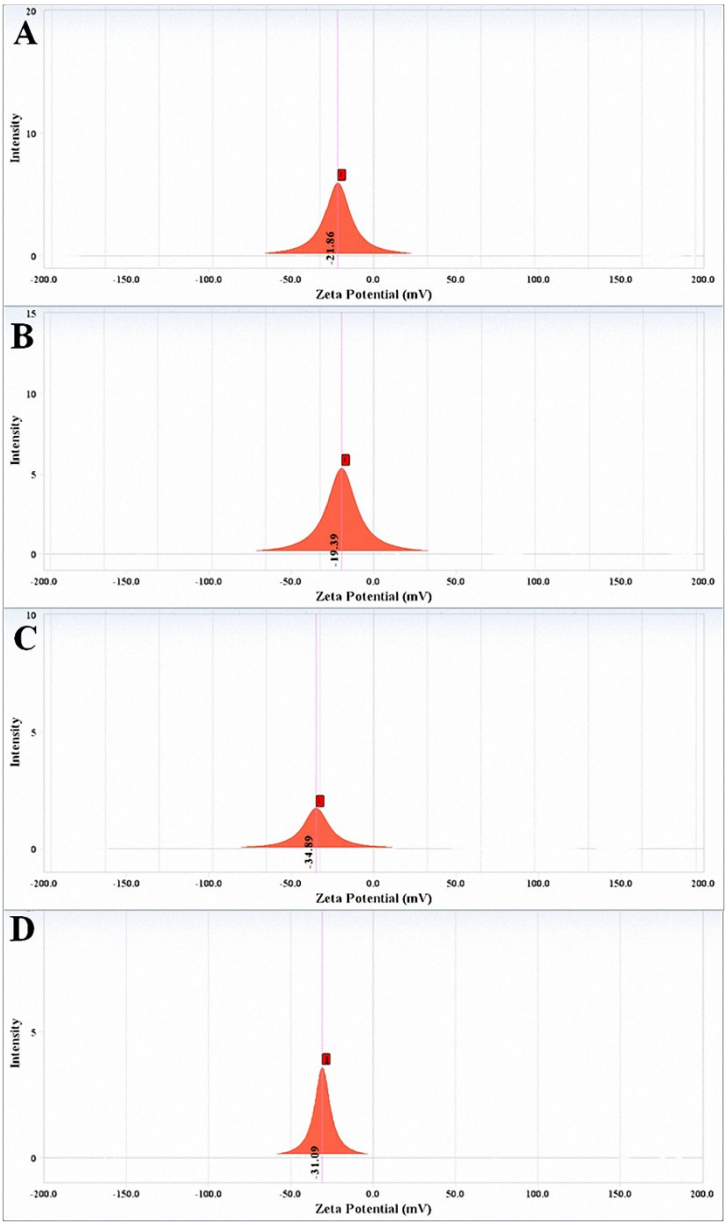

Figure S8. Zeta potential of FMSN (A, -21.86 mV), PMSN (B, -19.39 mV), SMSN (C, -34.89 mV), and RMSN (D, -31.09 mV) after removing CTAB and TBAI templates.

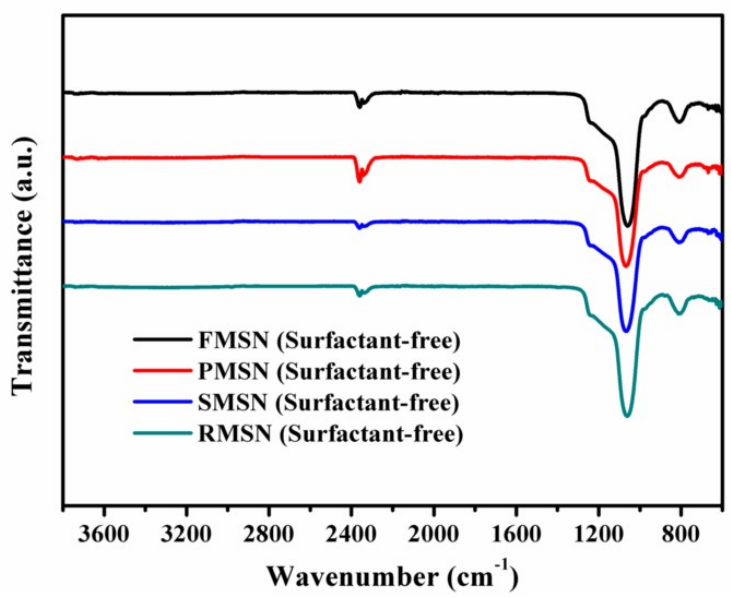

Figure S9. FTIR spectra of surfactant-free FMSN, PMSN, SMSN, and RMSN.

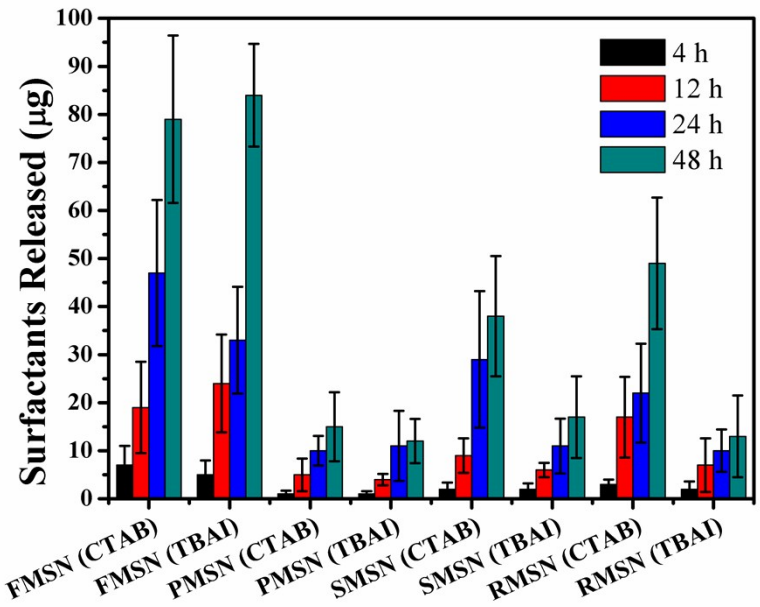

Figure S10. Release profiles of CTAB and TBAI from FMSN, PMSN, SMSN, and RMSN at different time durations.

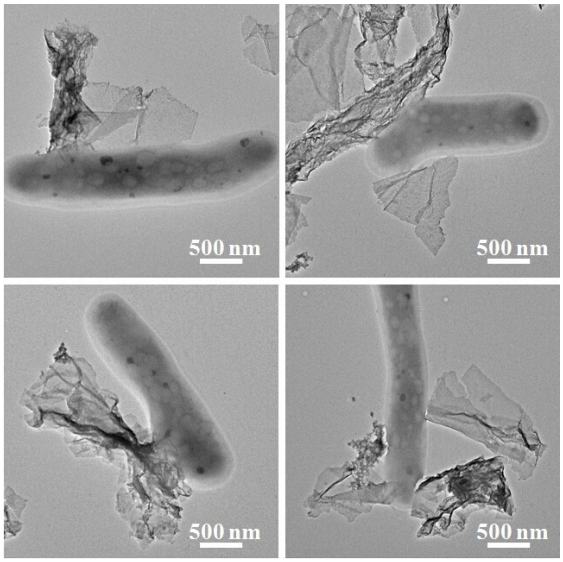

Figure S11. Additional TEM images showing the interactions of as-synthesized FMSN with mycobacteria after 4 h. Most of interaction loci were at the film edges.