Electronic Supplementary Information for:

Silica nanoparticle remodeling under mild conditions: versatile one step conversion of mesoporous to hollow nanoparticles with simultaneous payload loading

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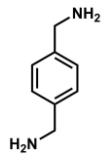
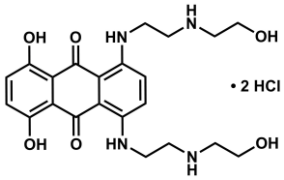
1. Materials and Chemical Additives Tested For Capacity To Convert Mesoporous SNPs Into Hollow SNPs Under Mild Conditions

Mesoporous silica nanoparticles (5 mg/mL in ethanol) were purchased from Nanocomposix, San Diego, USA (Mesoporous Silica Nanospheres, Hexagonal, NanoXact™, Product Number: SHSN100, Lot Number: JRC0610). Particle diameter (TEM): 105 ± 15 nm, Pore Diameter (nitrogen adsorption (BET): 3.74 nm, Particle surface: silanol, Calculated Particle Concentration: 1.2 x 10^{13} particles/mL.

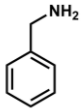
p-Xylylenediamine and benzylamine were purchased form Sigma-Aldrich. Mitoxantrone (hydrochloride) was purchased from Cayman Chemical Company and stored at -20 °C.



p-xylylenediamine

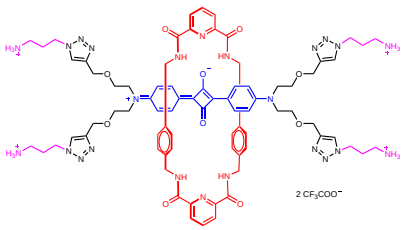


mitoxantrone

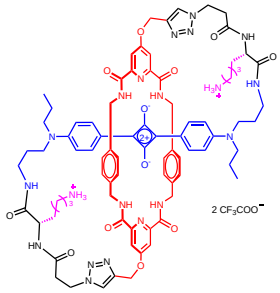


benzylamine

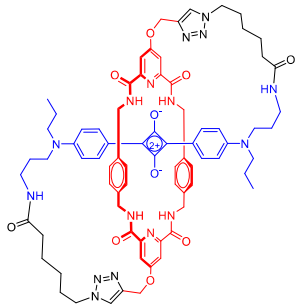
The project used the following deep-red fluorescent squaraine dyes **S4** (four ammonium groups), **S2** (two ammonium groups), and **S0** (zero ammonium groups). The synthesis and properties of these dyes have been reported previously by our group,[1] [2] and high purity of each sample was confirmed by NMR spectroscopy. The squaraine dyes have narrow and intense deep-red absorption and emission peaks. Surrounding the squaraine (colored blue) is a tetralactam macrocycle (colored red) that sterically protects the squaraine from chemical degradation; thus, the dyes survive overnight heating of mesoporous SNPs at 75-80 °C in PBS.



S4 (four ammonium groups)
(**λ**$_{abs}$ 652 nm, **λ**$_{em}$ 678 nm)



S2 (two ammonium groups)
(**λ**$_{abs}$ 660 nm, **λ**$_{em}$ 677 nm)



S0 (zero ammonium groups)
(**λ**$_{abs}$ 645 nm, **λ**$_{em}$ 660 nm)

2. Electron Microscopy

Silica nanoparticles were characterized by transmission and scanning electron microscopy. TEM images were obtained at 120 and 200keV on JEOL 2011 microscope equipped with CCD camera (AMT). STEM imaging and EDX mapping were done at 300keV on probe corrected Spectra 30-300 microscope (Thermo Fisher Scientific) equipped with segmented Panther STEM detector and Super-X EDX system. SEM imaging was performed at 10-30 keV on Magellan 400 microscope (Thermo Fisher Scientific) using secondary electron and annular STEM detectors. To minimize the charging effect during SEM imaging, some samples were sputtered with iridium using a Cressington sputter coater.

The following electron microscopy data (Figures S1 – S19) are presented as sets of two representative images out of a collection of about twenty images acquired for each sample (in addition, virtually all imaging experiments were independently replicated and thus generated another collection of twenty images). At the bottom of each figure is a conclusion statement that considers the structural information provided by all twenty images acquired for each sample, not just the two specific images in the figure.

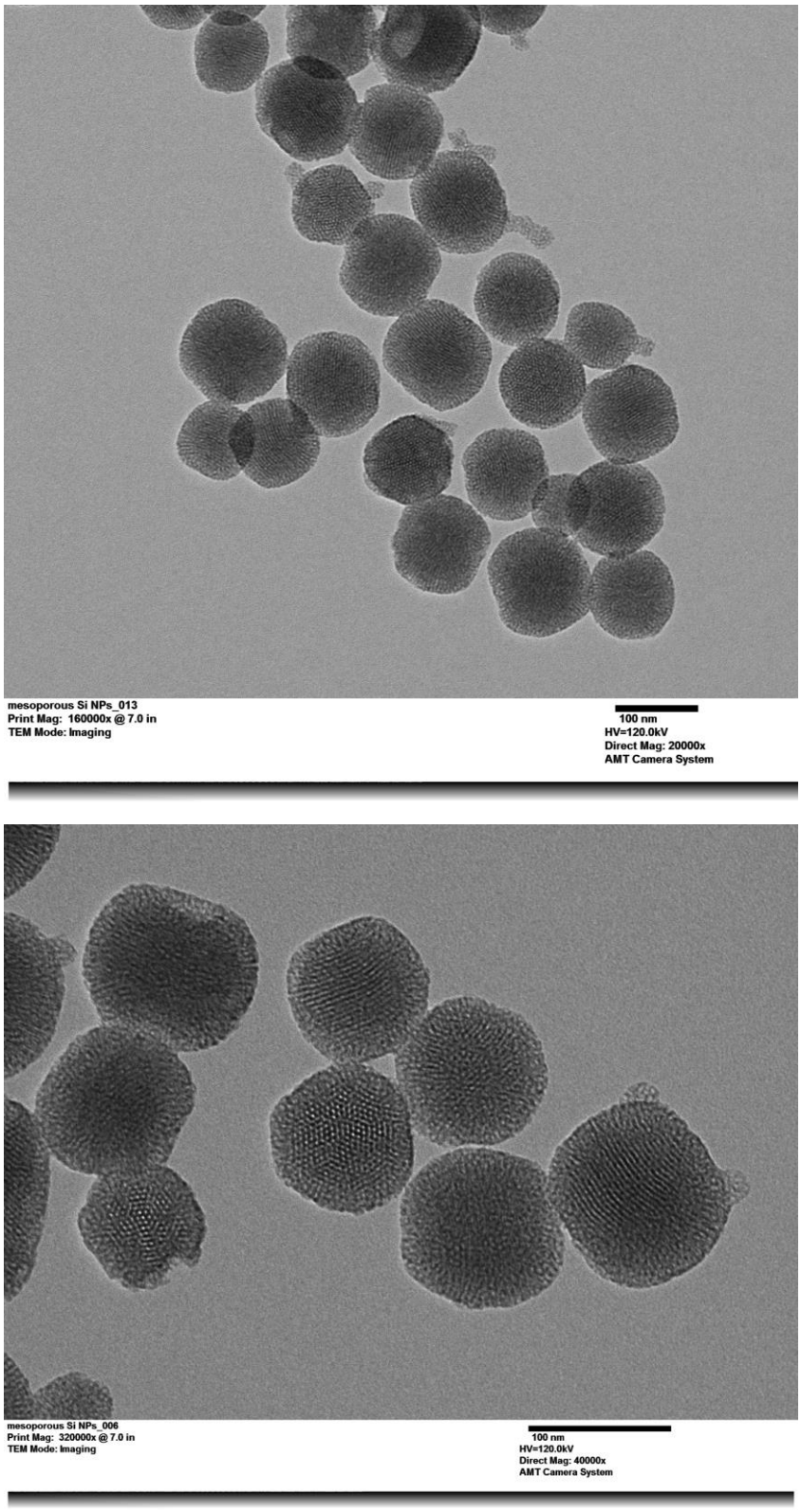


Fig. S1. TEM images of mesoporous silica nanoparticles (mesoporous SNPs) purchased from Nanocomposix; ~100 nm diameter, pore diameter 3.74 nm (nitrogen adsorption (BET).

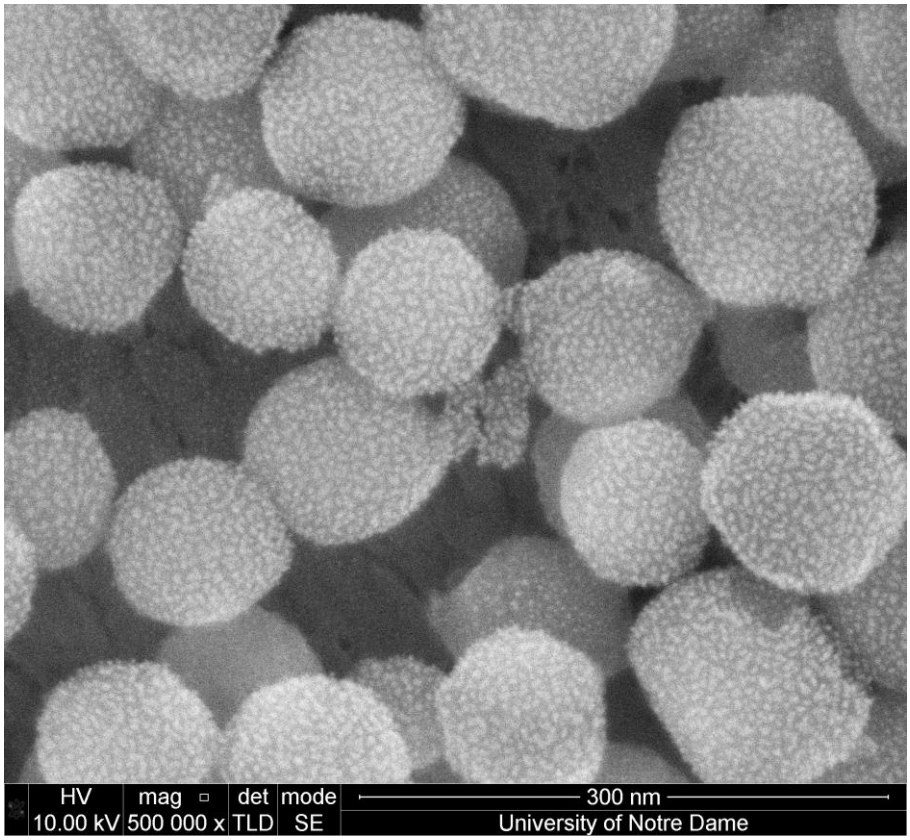
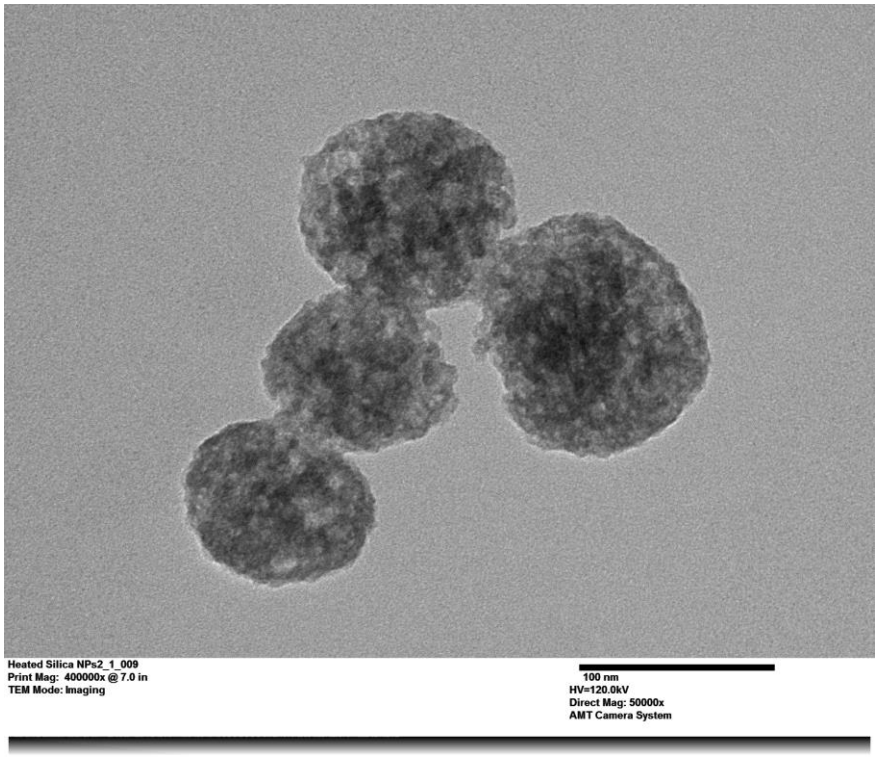


Fig. S2. SEM images of sputtered mesoporous silica nanoparticles (mesoporous SNPs) purchased from Nanocomposix. ~100 nm diameter, pore diameter 3.74 nm (nitrogen adsorption (BET).



(mesoporous SNPs)
+ heating

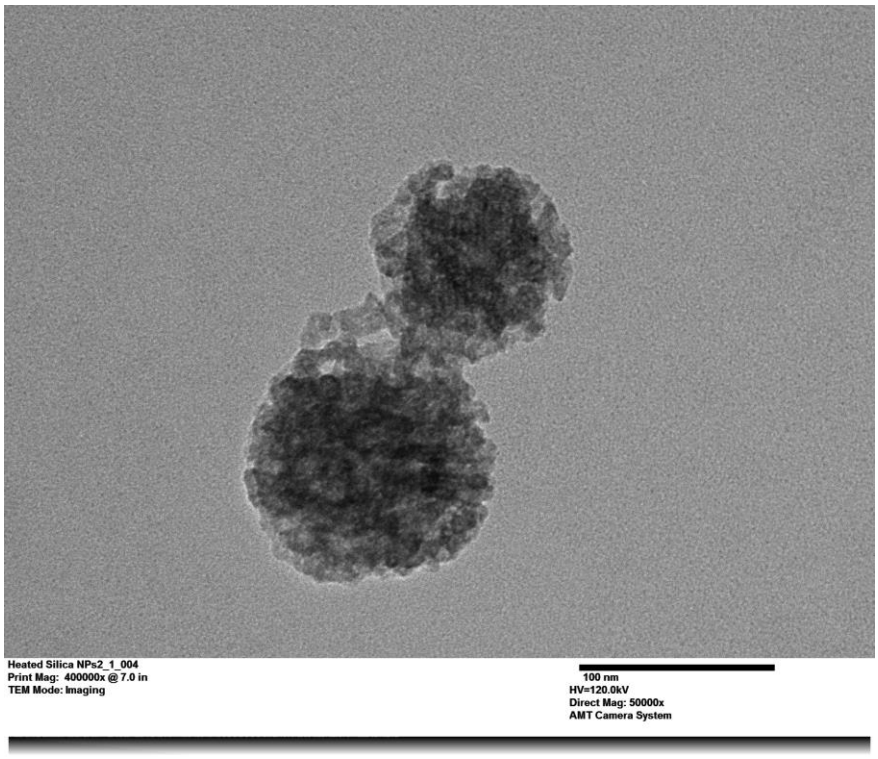


Fig. S3. TEM images. Mesoporous silica nanoparticles (mesoporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (3 mL). The particles were stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Moderate degradation of nanoparticles, no evidence for hollow structures.

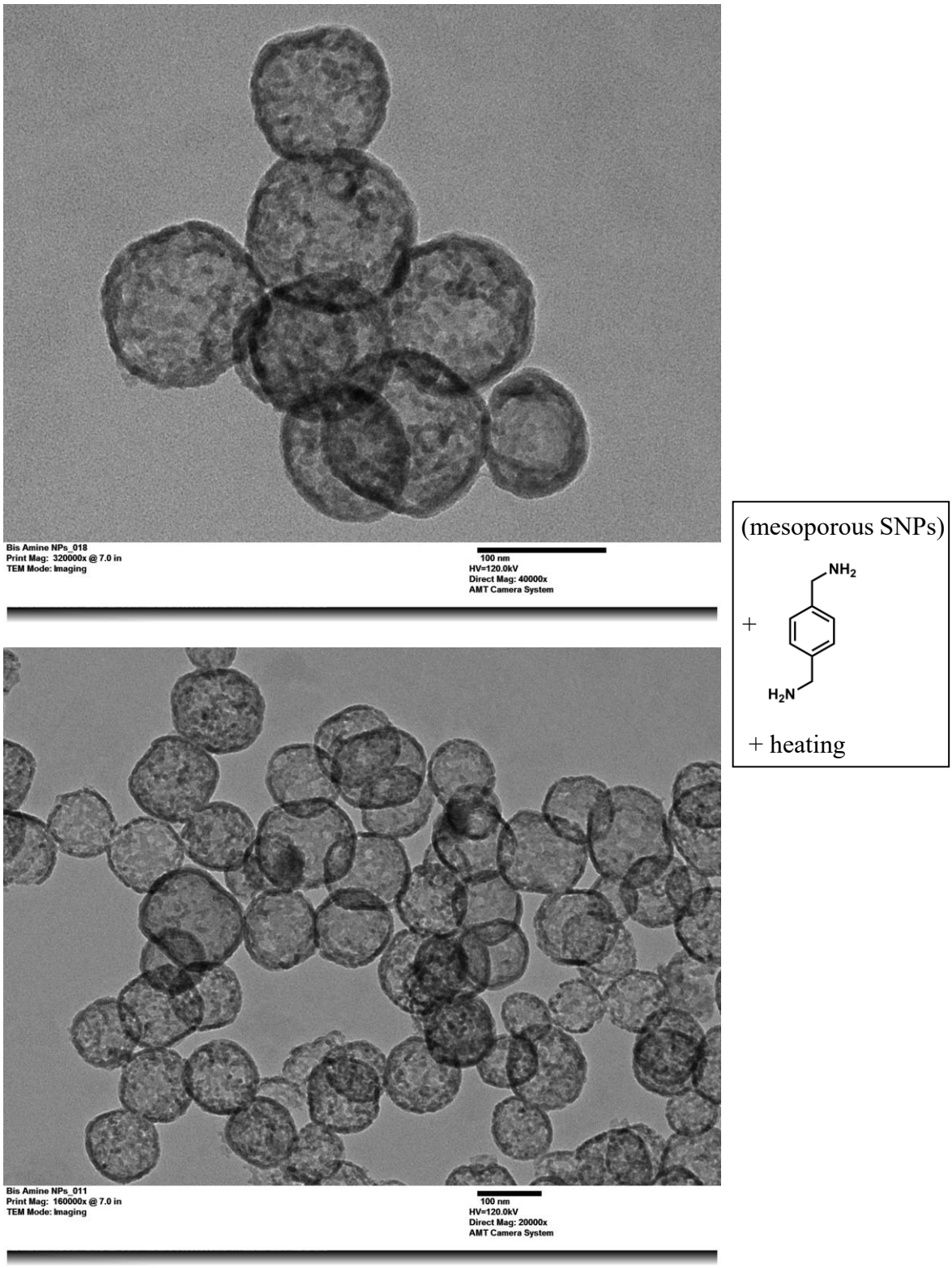


Fig. S4. TEM images. Mesoporous silica nanoparticles (mesoporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). *p*-Xylylenediamine (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing *p*-xylylenediamine and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).
Conclusion: Mostly hollow SNPs, a few unchanged or partially degraded nanoparticles

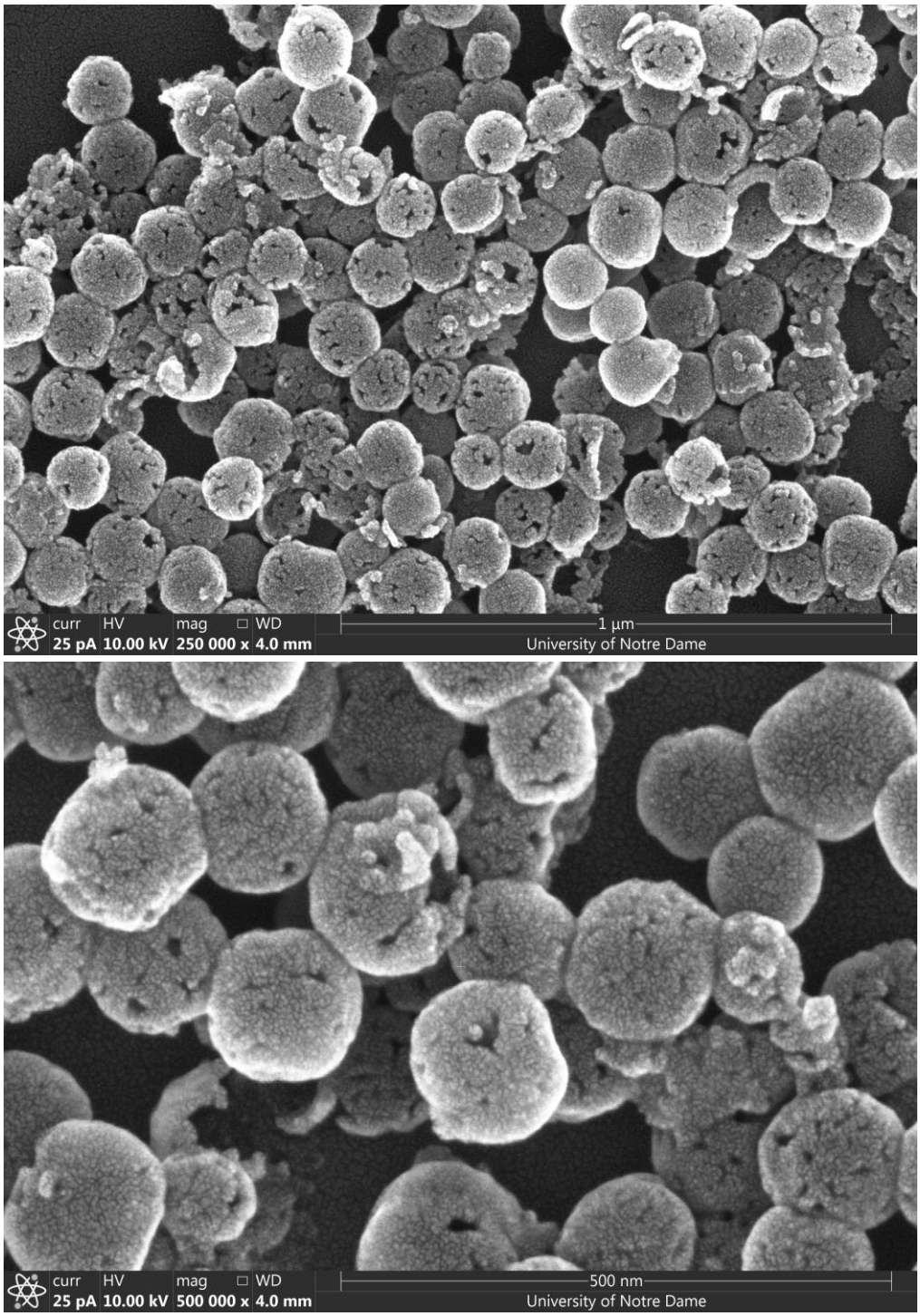


Fig. S5. SEM images of sputtered samples. Mesoporous silica nanoparticles (mesoporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). *p*-Xylylenediamine (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing *p*-xylylenediamine and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly hollow SNPs, a few unchanged or partially degraded nanoparticles

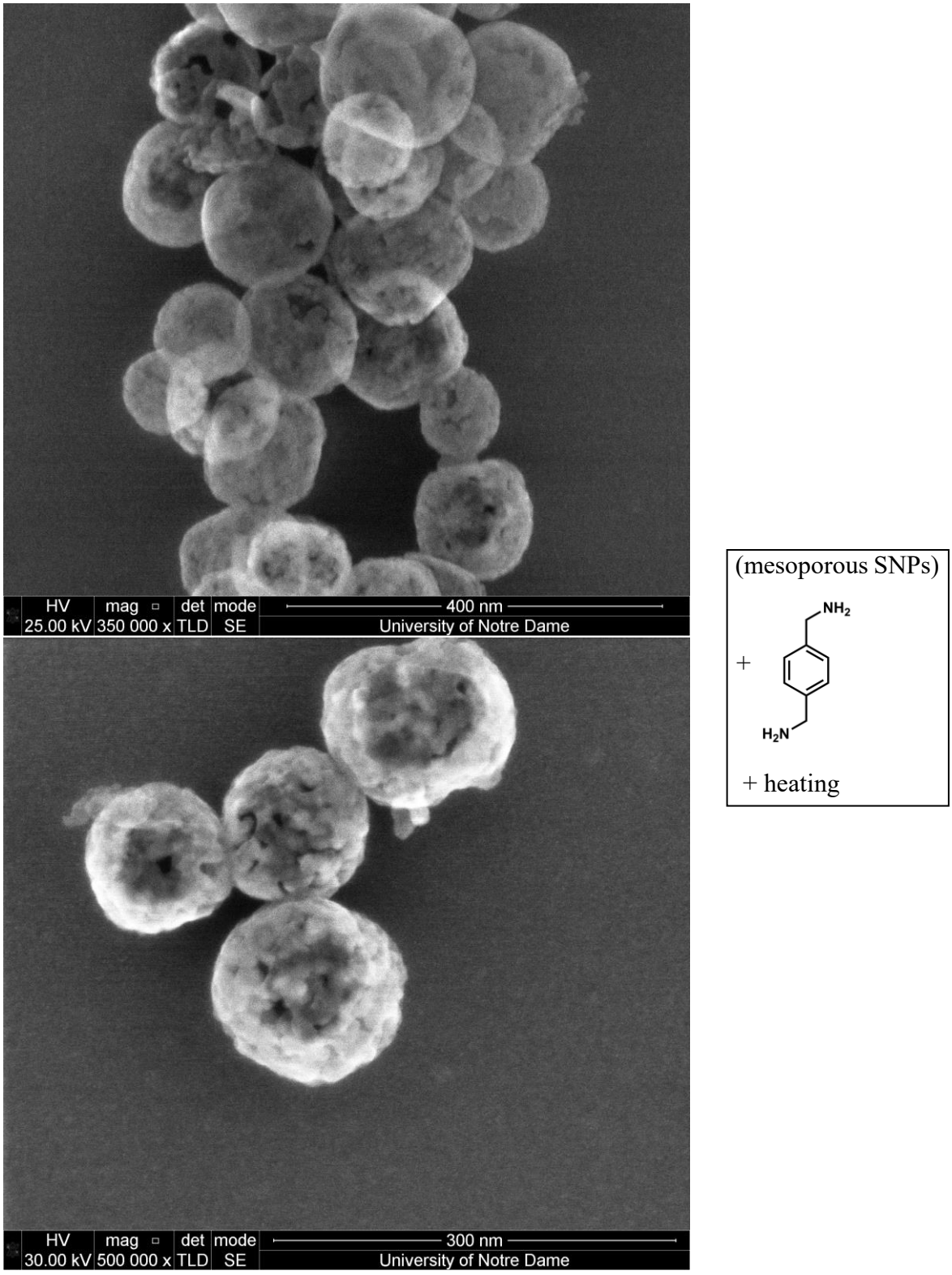
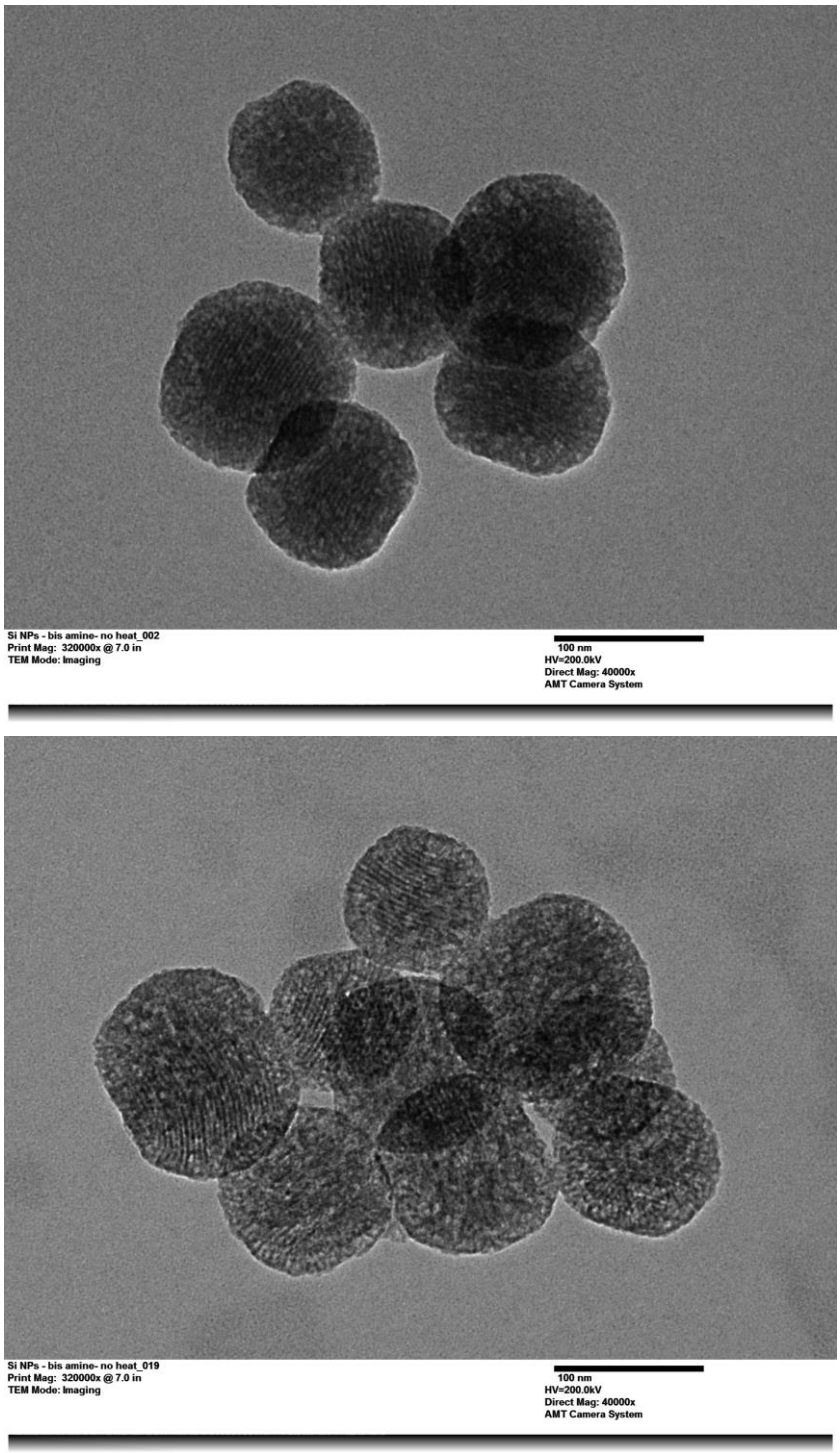


Fig. S6. SEM images of unpsuttered samples. Mesoporous silica nanoparticles (mesoporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). *p*-Xylylenediamine (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing *p*-xylylenediamine and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly hollow SNPs, a few unchanged or partially degraded nanoparticles



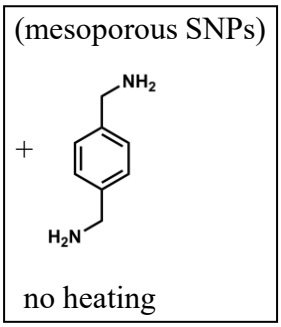


Fig. S7. TEM images. Mesoporous silica nanoparticles (mesoporous SNPs) (200 μL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). *p*-Xylylenediamine (0.4 mg in 50 μL DMSO) was added; another 1 mL PBS was used to rinse the tube containing *p*-xylylenediamine and combined with the reaction mixture. The reaction was stirred at r.t. for 48 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly unchanged or very slightly degraded mesoporous SNPs

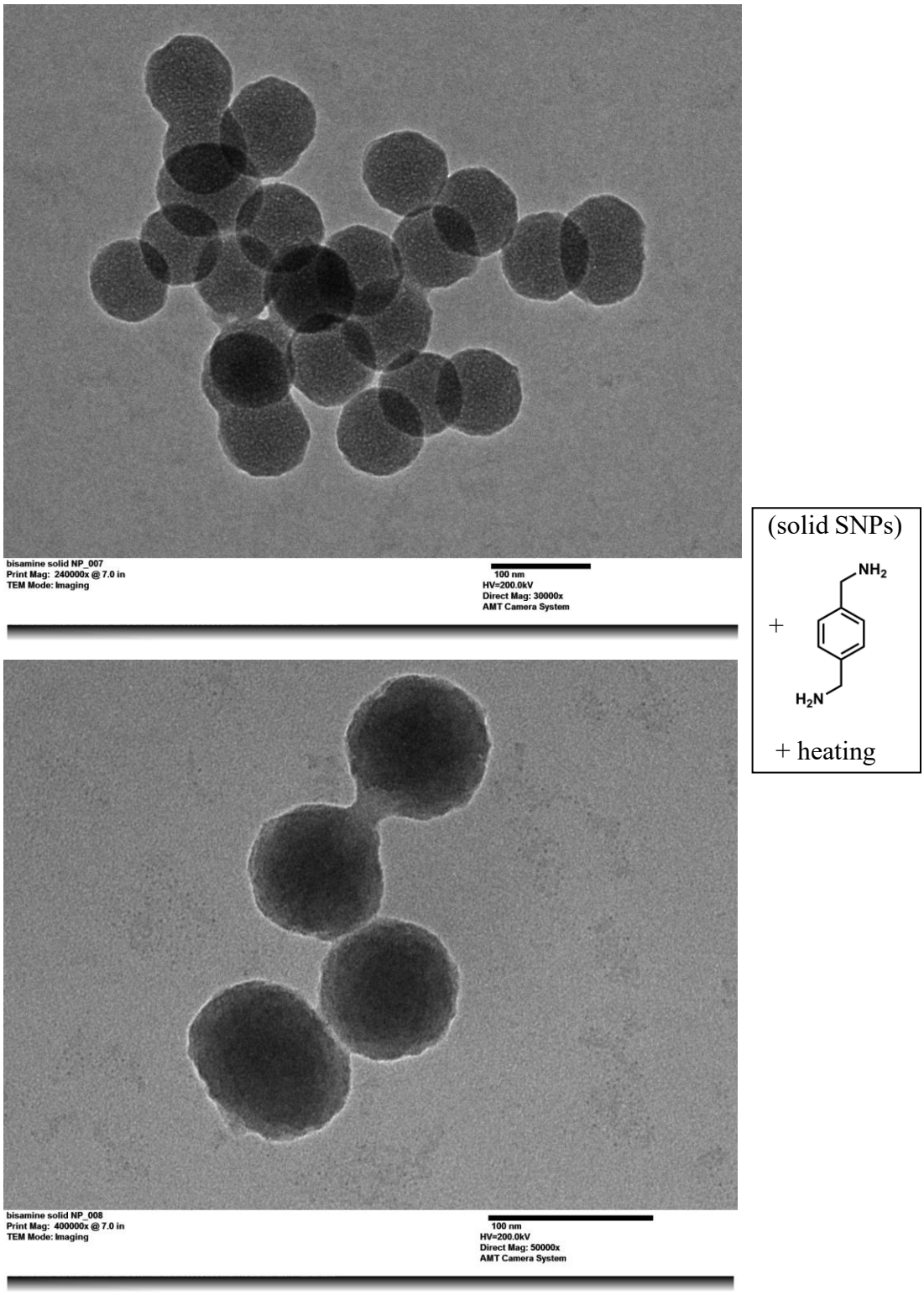
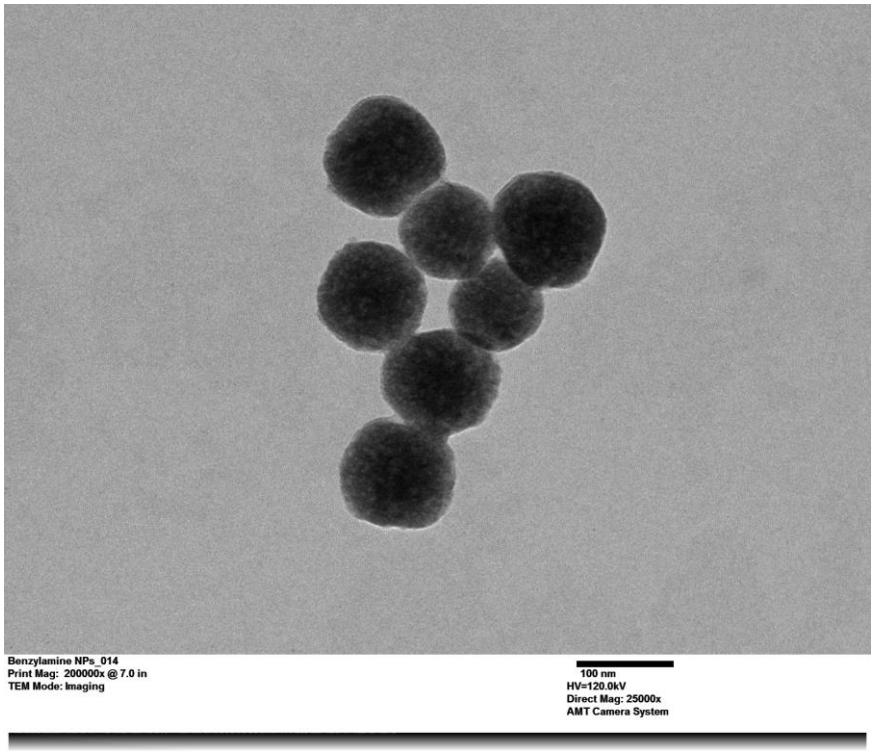
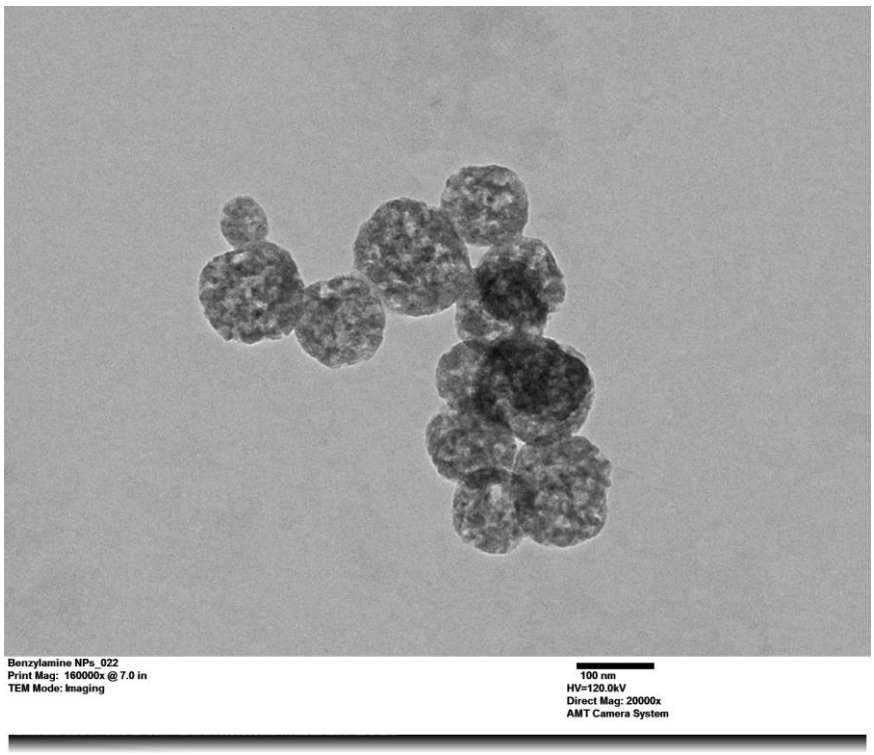


Fig. S8. TEM images. Solid silica nanoparticles (200 µL, 10 mg/mL in water) were washed with PBS then suspended in PBS (2 mL). *p*-Xylylenediamine (0.8 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing *p*-xylylenediamine and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly unchanged solid SNPs, a few partly fused nanoparticles





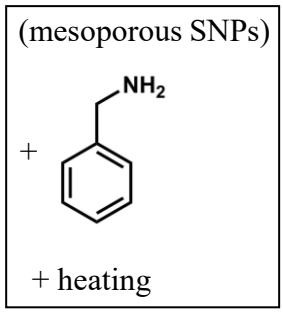
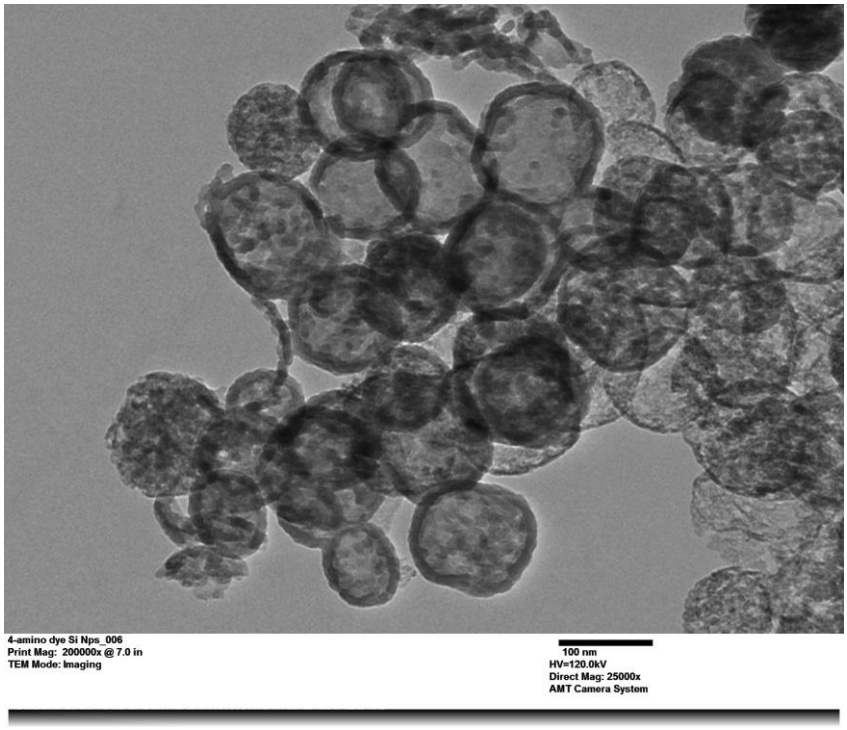


Fig. S9. STEM images. Mesoporous silica nanoparticles (mesoporous SNPs) (200 μL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Benzylamine (0.4 mg in 50 μL DMSO) was added; another 1 mL PBS was used to rinse the tube containing benzylamine and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Partially degraded mesoporous SNPs



(mesoporous SNPs)

+ **S4**

+ heating

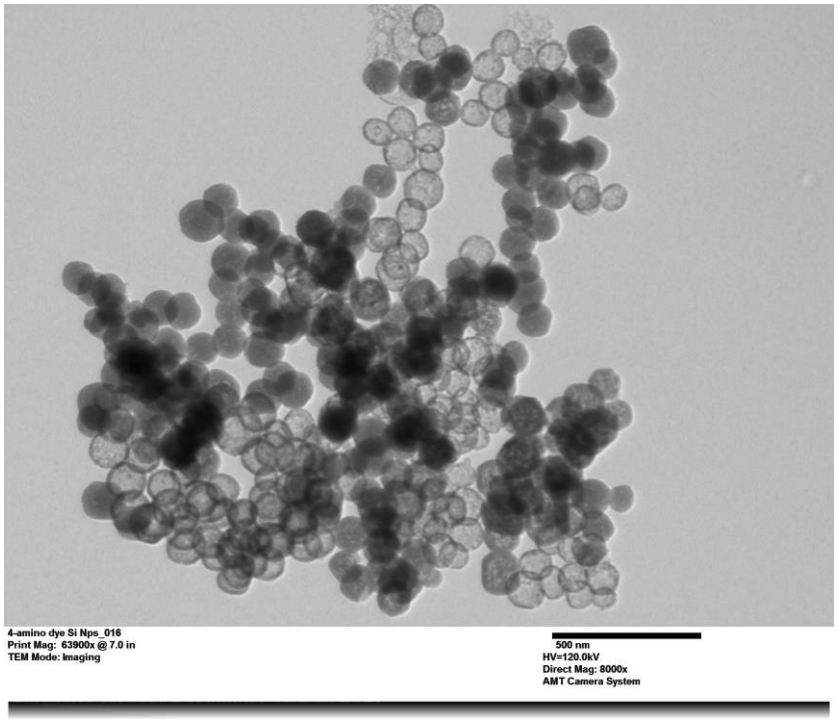
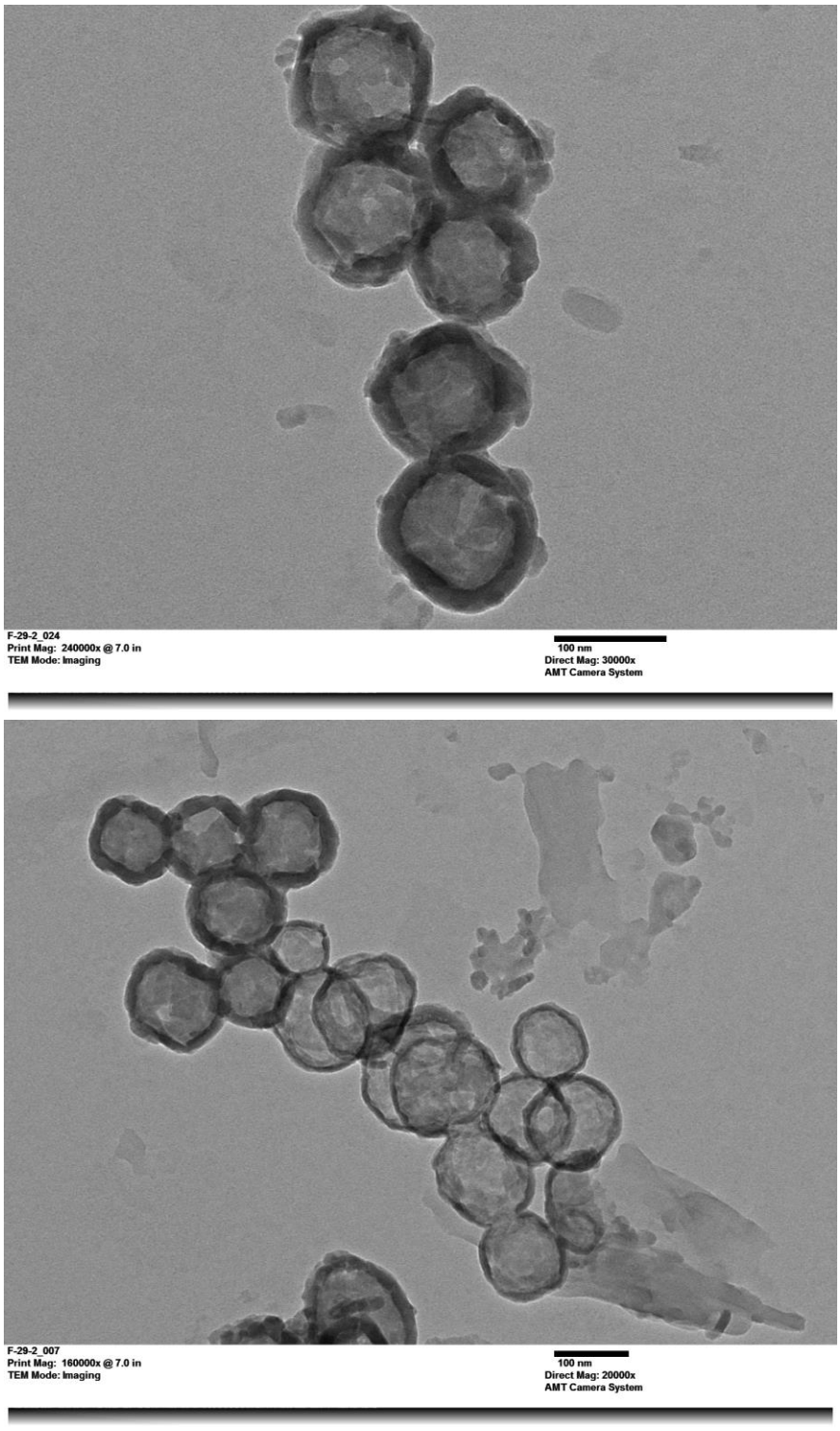


Fig. S10. TEM images. Mesoporous silica nanoparticles (microporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Dye **S4** (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing **S4** and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly hollow SNPs, some unchanged nanoparticles



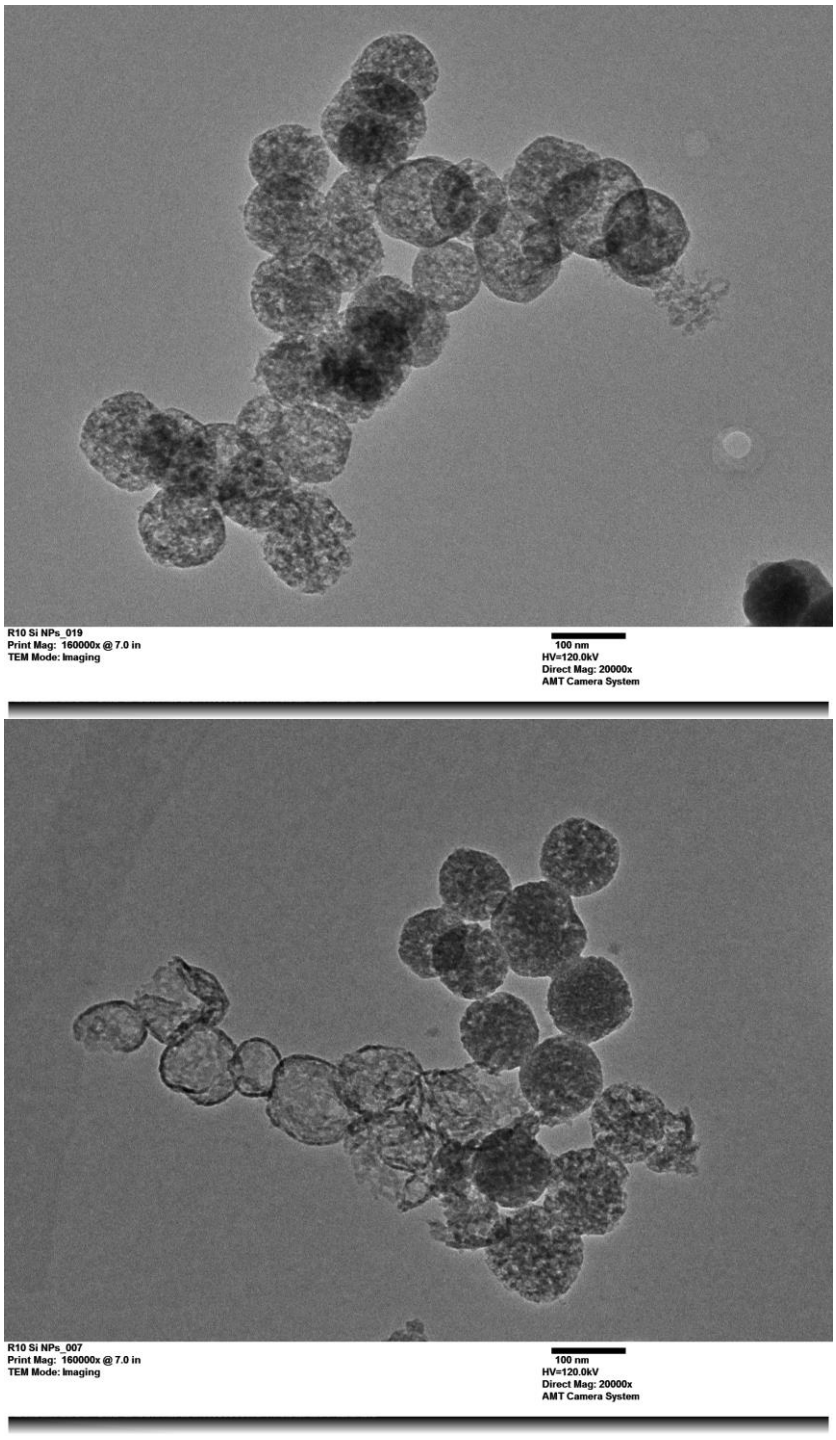
(mesoporous SNPs)

+ **S2**

+ heating

Fig. S11. TEM images. Mesoporous silica nanoparticles (mesoporous SNPs) (200 μL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Dye **S2** (0.4 mg in 50 μL DMSO) was added; another 1 mL PBS was used to rinse the tube containing **S2** and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly hollow SNPs, a few unchanged or partially degraded nanoparticles



(mesoporous SNPs)

+ **S0**

+ heating

Fig. S12. TEM images. Mesoporous silica nanoparticles (mesoporous SNPs) (200 μL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Dye **S0** (0.4 mg in 50 μL DMSO) was added; another 1 mL PBS was used to rinse the tube containing **S0** and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Moderate degradation of SNPs, negligible hollow nanoparticles

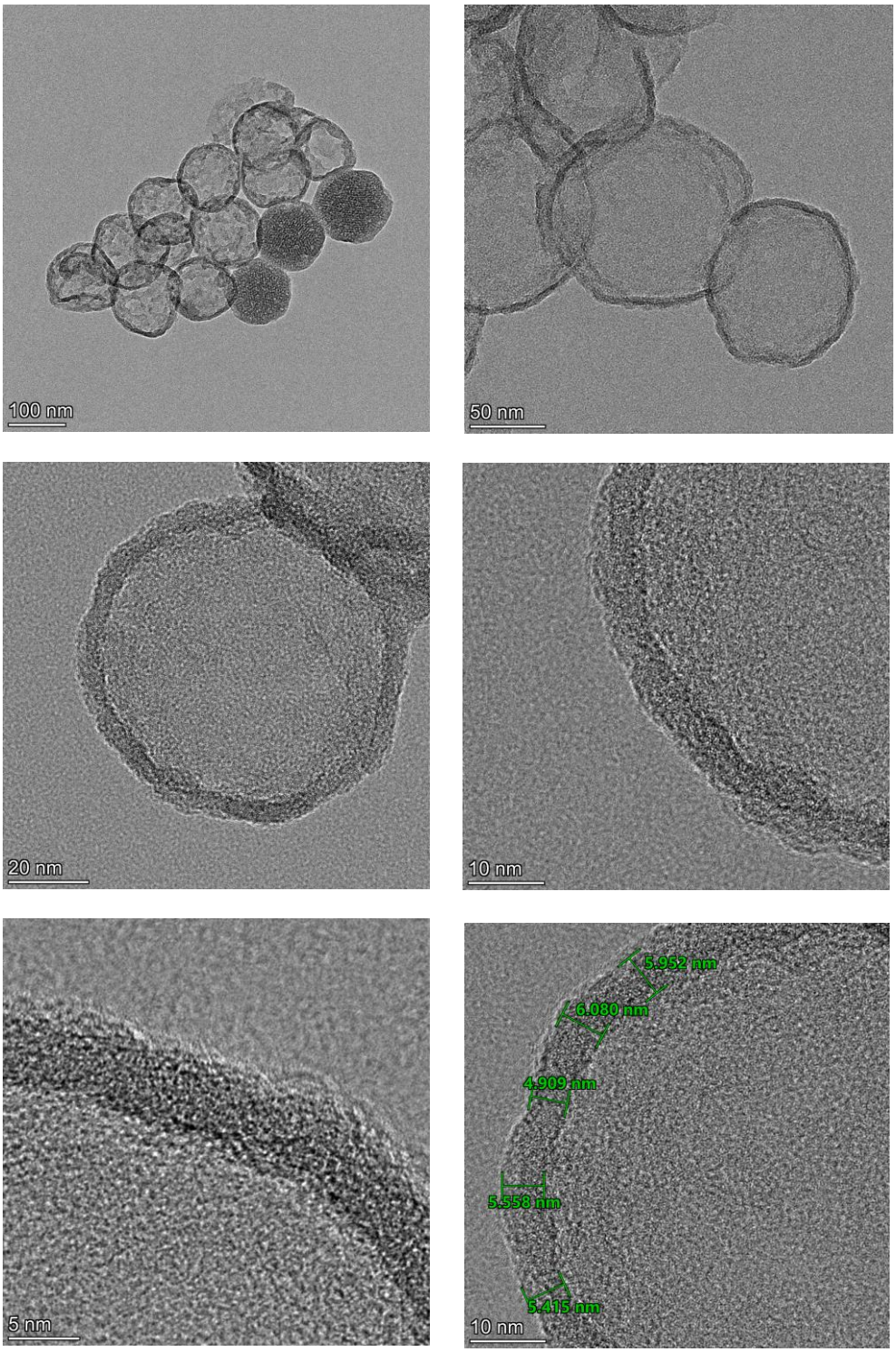
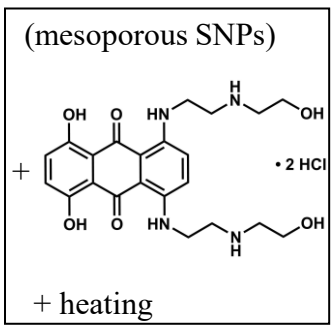
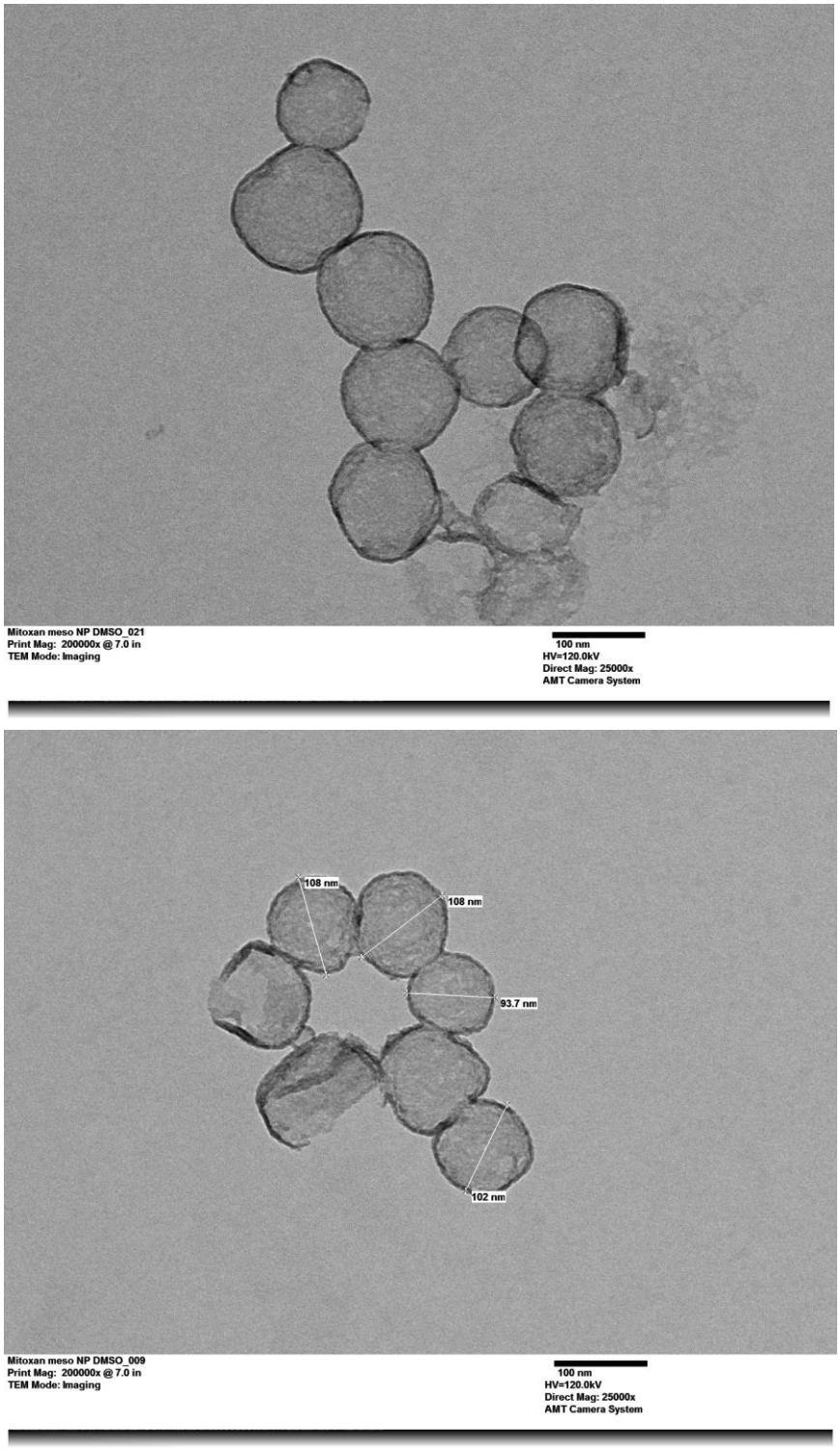
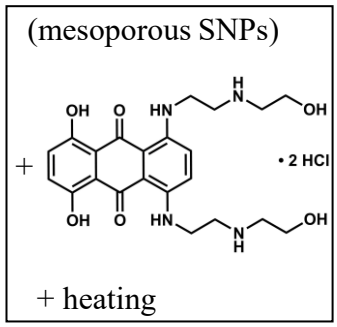


Fig. S13. TEM images. Mesoporous silica nanoparticles (mesoporous SNP) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Mitoxantrone (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing mitoxantrone and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).



Conclusion: Mostly hollow SNPs, and a few unchanged nanoparticles





No DMSO

Fig. S14. TEM images. Mesoporous silica nanoparticles (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). mitoxantrone (0.4 mg in 50 µL water) was added; another 1 mL PBS was used to rinse the tube containing mitoxantrone and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly hollow SNPs, and some partially degraded nanoparticles

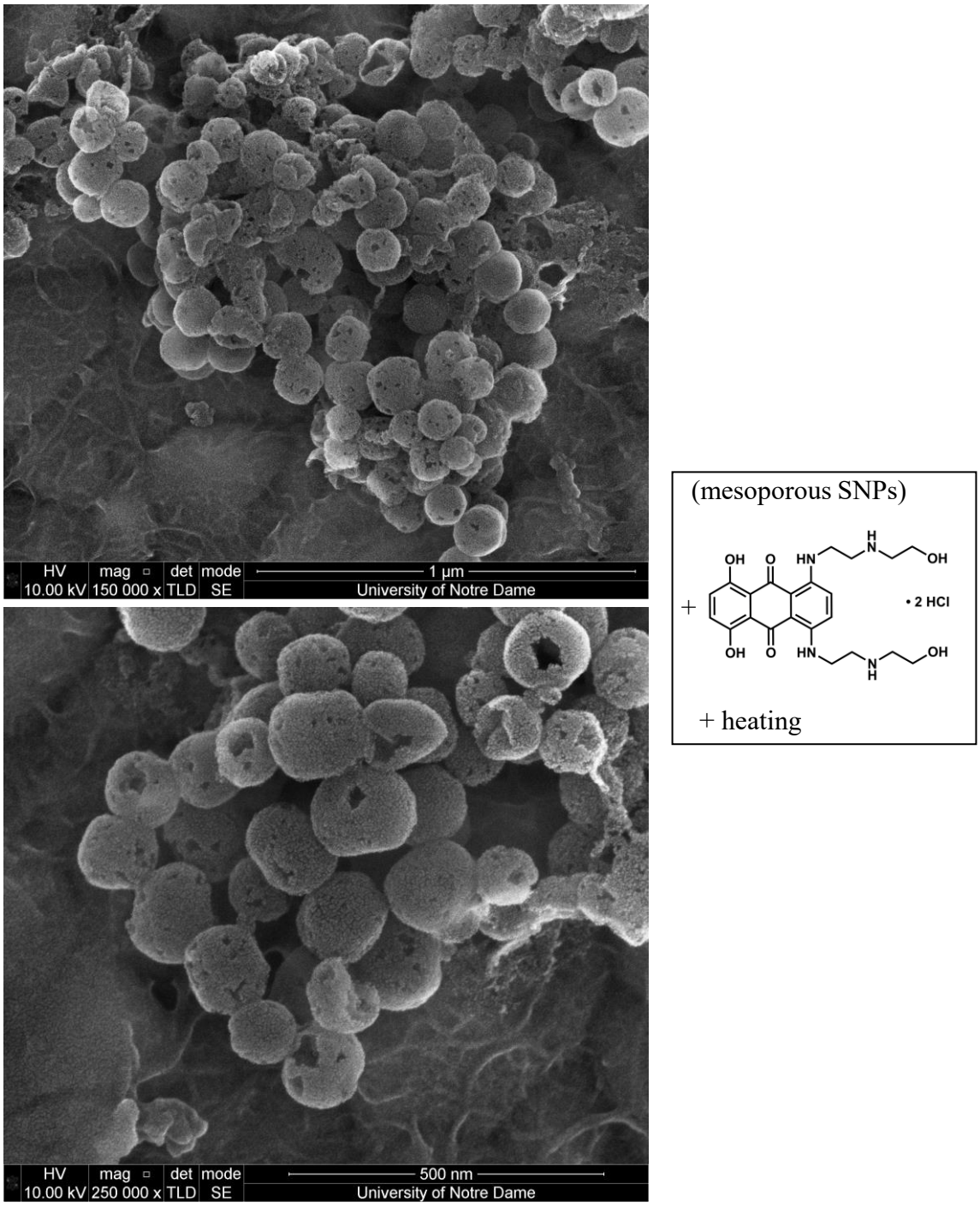
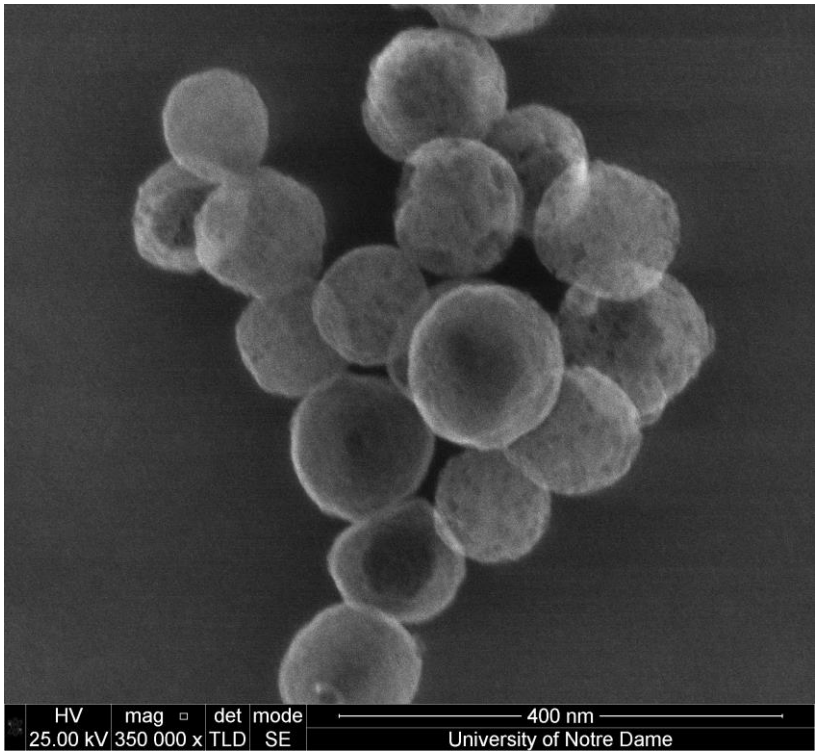


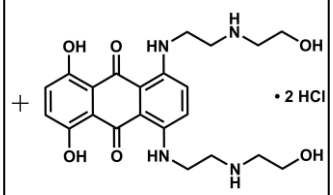
Fig. S15. SEM images of sputtered samples. Mesoporous silica nanoparticles (mesoporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Mitoxantrone (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing mitoxantrone and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly hollow SNPs, a few unchanged mesoporous SNPs

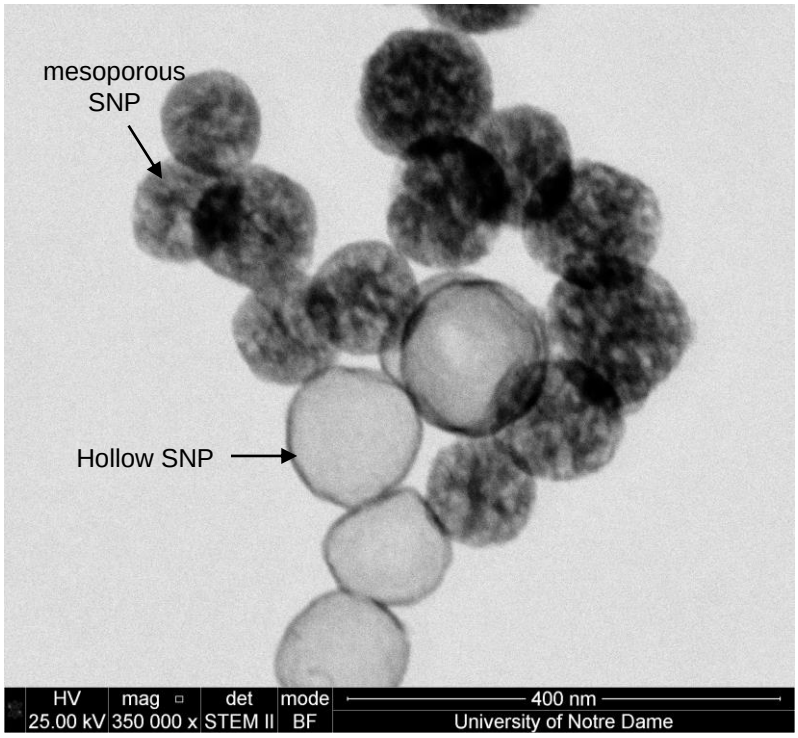


SEM

(mesoporous SNPs)



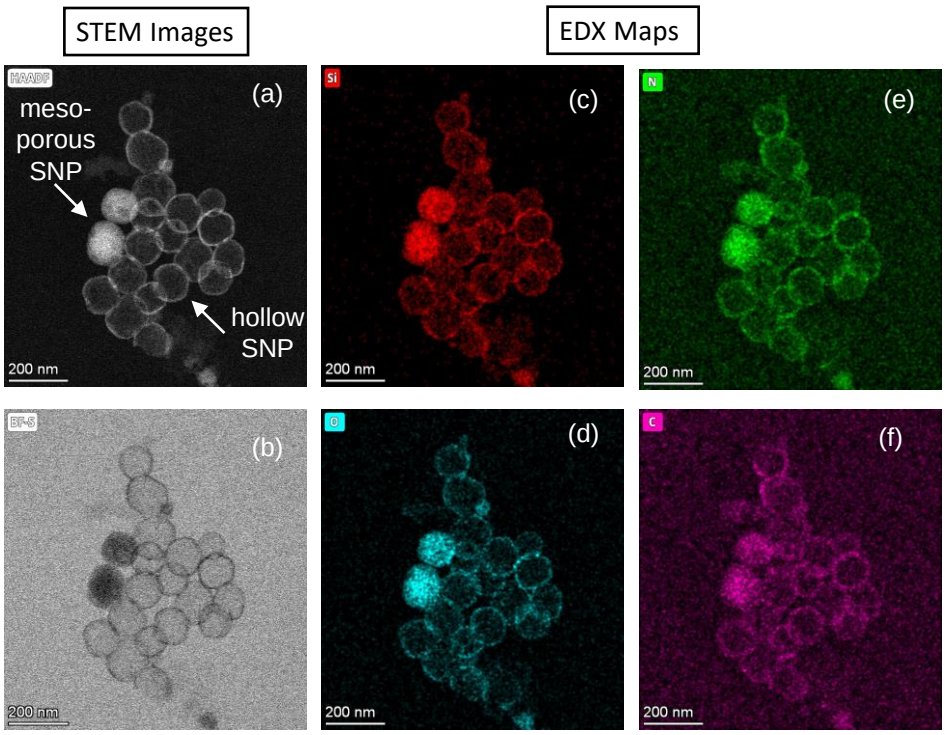
+ heating

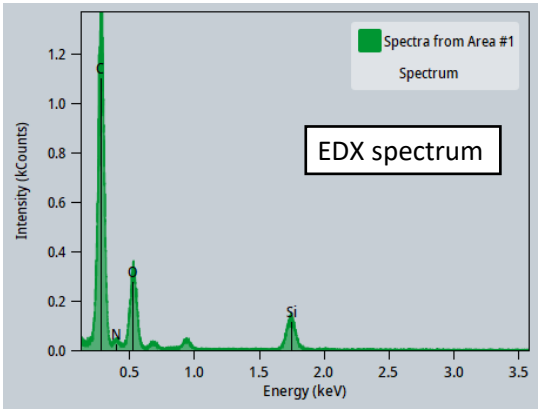


STEM

Fig. S16. SEM and STEM images of unsputtered samples. Mesoporous silica nanoparticles (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Mitoxantrone (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing mitoxantrone and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL).

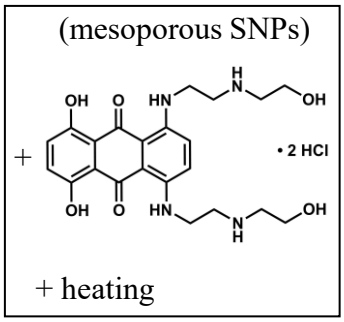
Conclusion: Mixture of hollow SNPs and unchanged mesoporous SNPs





Quantification of the EDX Spectrum

	At (%)	Error
C	84.23	4.27
N	1.19	0.25
O	11.65	2.40
Si	2.93	0.59



Panels (a-b): **STEM Images** (high angle annular dark field, HAADF and bright field scanning, BFS) of a nanoparticle population comprised of hollow SNPs and two unconverted mesoporous SNPs. Panels (c-f): **EDX Maps** of the image showing distribution of Silicon, Oxygen, Nitrogen, and Carbon. There is also an **EDX Spectrum** and associated table with **Quantification of the EDX Spectrum** for each element in the image.

Fig. S17a. Mesoporous silica nanoparticles (mesoporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Mitoxantrone (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing mitoxantrone and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then washed with water and stored as a suspension in water (~1 mg/mL). **See Figure S17b for EDX spectra of two replicate samples, all spectra show the same results.**

Conclusion: Mitoxantrone is localized in the shell of hollow SNPs

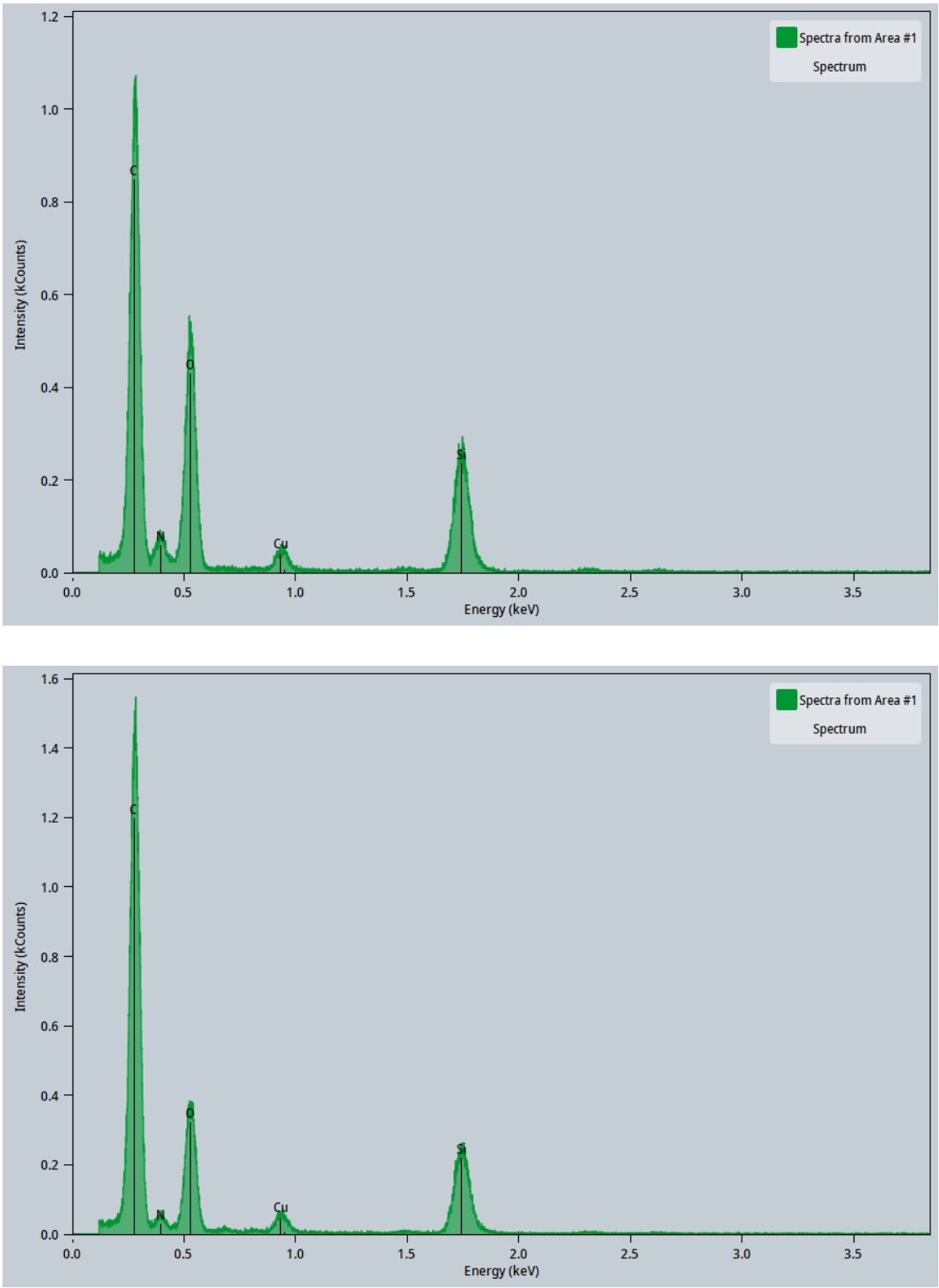
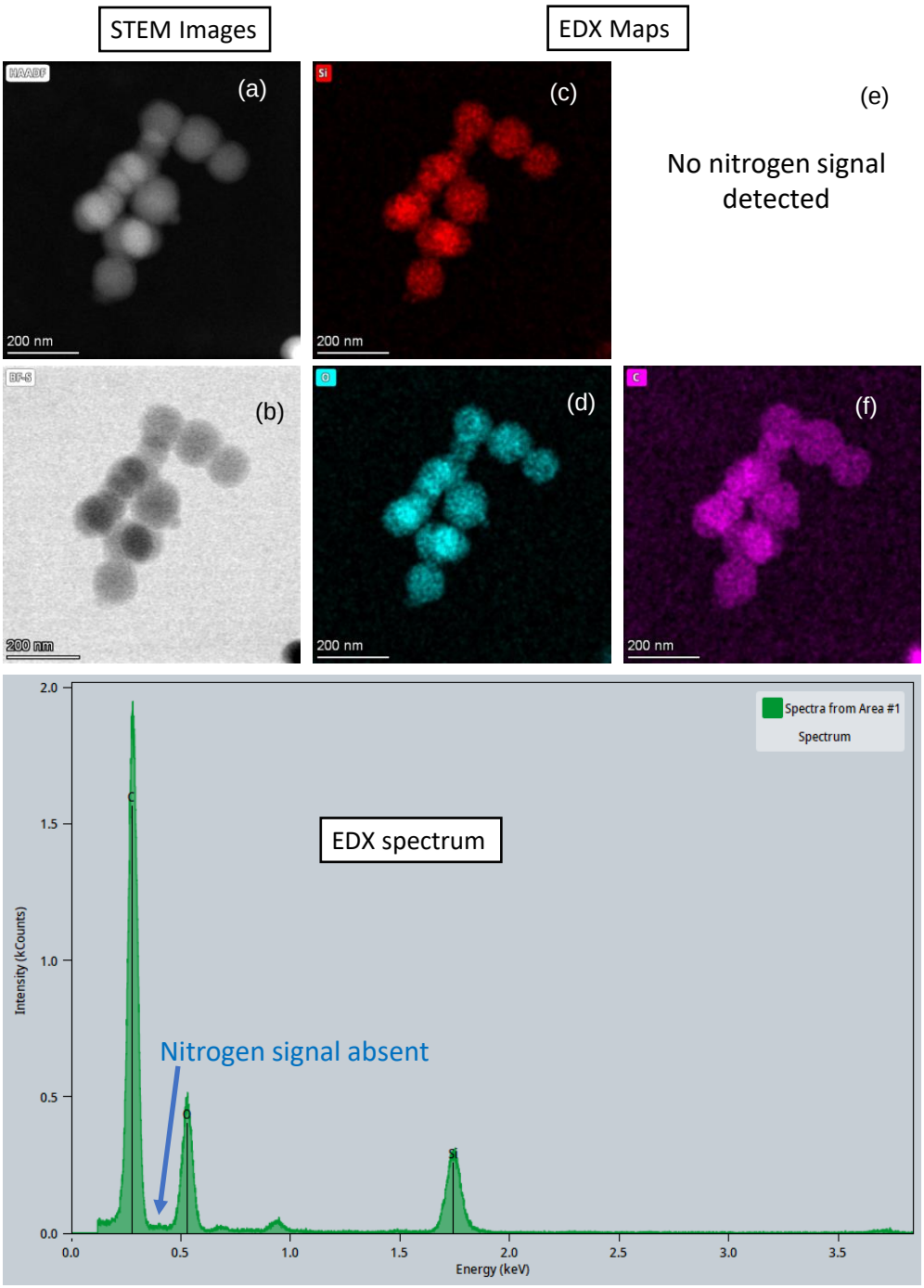


Fig. S17b. EDX spectra of two independent replicates of the experiment described in Figure S17a, all spectra show the same results.

Conclusion: Mitoxantrone is localized in the shell of hollow SNPs



Panels (a-b): **STEM Images** (high angle annular dark field, HAADF and bright field scanning, BFS) of untreated mesoporous SNPs. Panels (c-f): **EDX Maps** of the image showing distribution of Silicon, Oxygen, Nitrogen, and Carbon. There is also an **EDX Spectrum** for each element in the image.

Fig. S18a. Untreated mesoporous silica nanoparticles (mesoporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with water and stored as a suspension in water (~1 mg/mL). See Figure S18b for EDX spectrum of a separate replicate sample, all spectra show the same results.

Conclusion: EDX analysis indicates absence of nitrogen in the untreated mesoporous SNPs

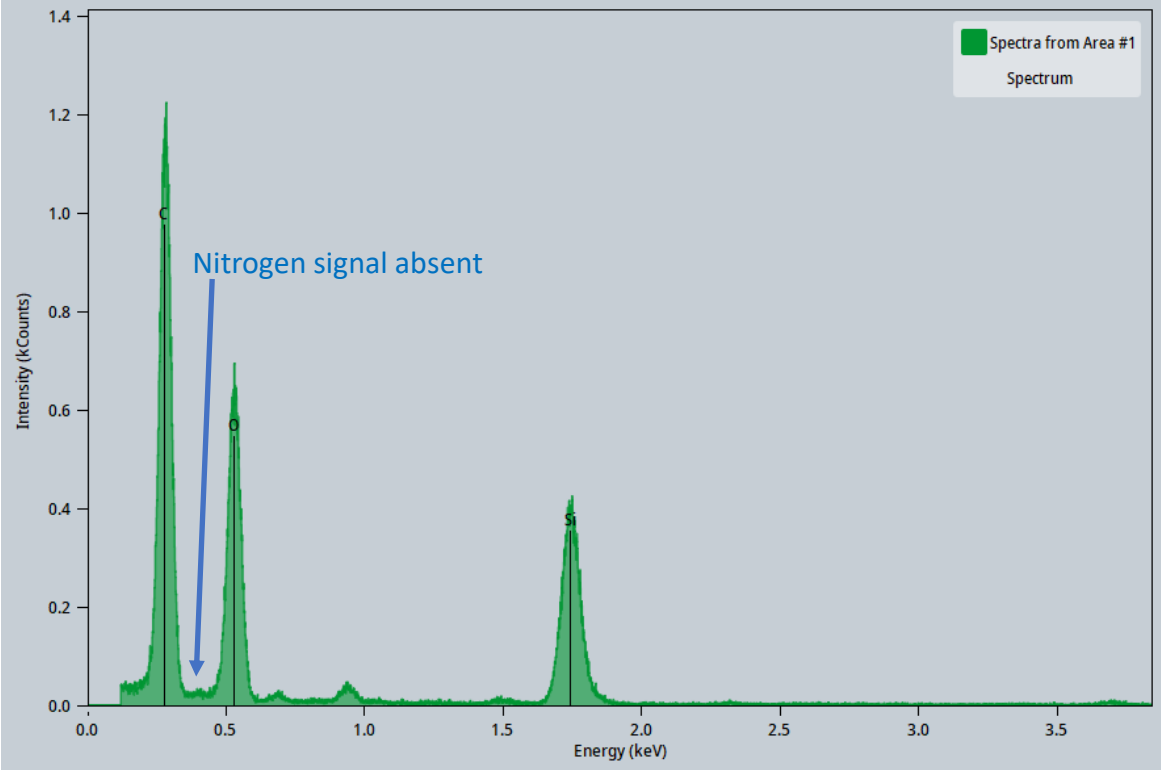
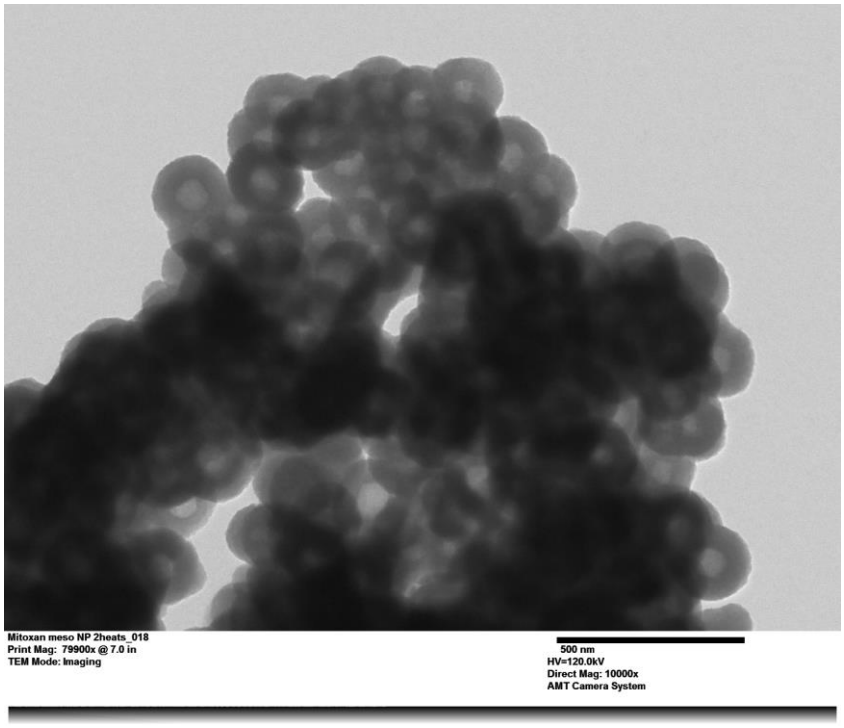
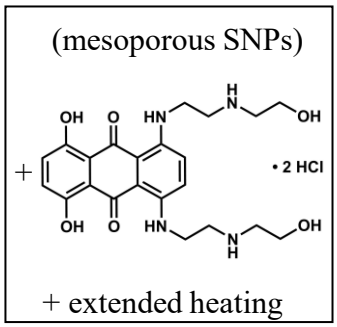


Fig. S18b. EDX spectrum of a separate sample of the mesoporous SNPs described in Figure S18a, all spectra show the same results.

Conclusion: EDX analysis indicates absence of nitrogen in the untreated mesoporous SNPs





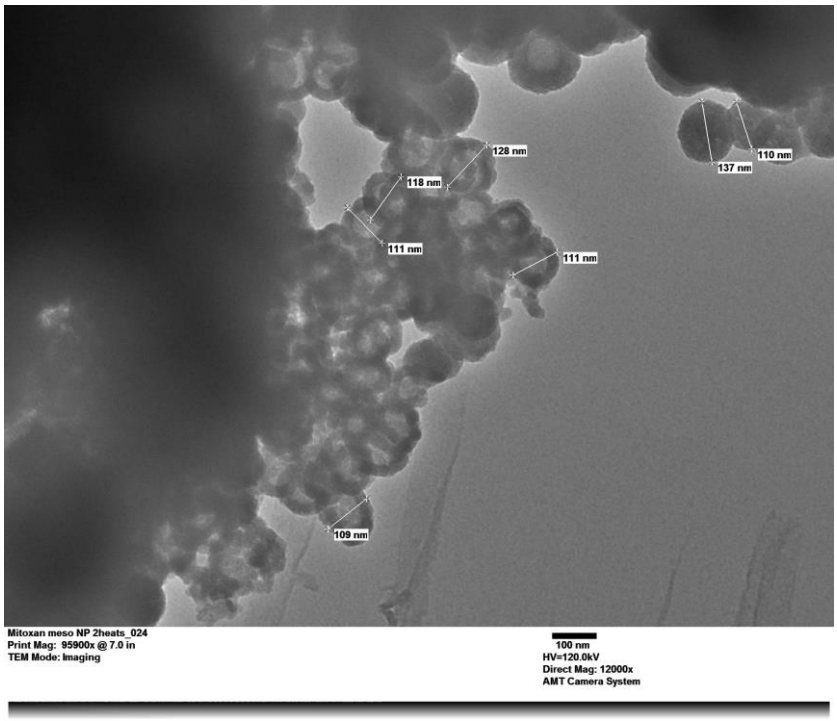


Fig. S19. TEM images. Mesoporous silica nanoparticles (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Mitoxantrone (0.4 mg in 50 µL DMSO) was added; another 1 mL PBS was used to rinse the tube containing mitoxantrone and combined with the reaction mixture. The reaction was stirred at 75 - 80 °C in an oil bath for 48 h, then washed with water and stored as a suspension in water (~1 mg/mL).

Conclusion: Mostly hollow SNPs, thicker shell and slightly larger diameter, a few unchanged mesoporous SNPs

3. Dye and Drug Loading onto Hollow SNPs

*Spectral Evidence for Dye **S4** Loading onto SNPs*

Mesoporous silica nanoparticles (microporous SNPs) (200 µL, 5 mg/mL in EtOH) were washed with PBS then suspended in PBS (2 mL). Dye **S4** (0.4 mg in 50 µL DMSO) was added to the nanoparticle sample; another 1 mL PBS was used to rinse the tube containing the dye and then combined with the nanoparticle sample. The reaction (exposed to air) was stirred at 75 - 80 °C in an oil bath for 18 h and at r.t. for another 24 h, then the **S4**@SNP was washed with water and stored as a suspension in water (~1 mg/mL). Absorption spectra (ex. 640 nm, 2 nm slit width) spectra of the stored dispersion of **S4**@SNP were acquired after 1 and 4 days.

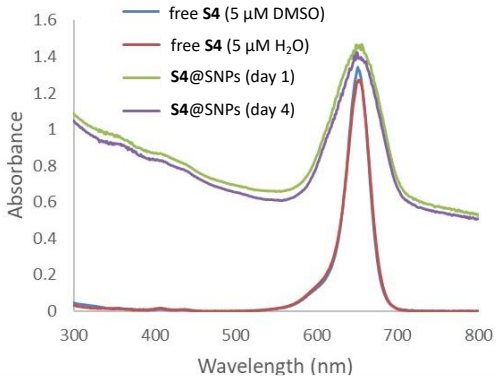


Figure S20. Absorbance spectra for solutions of free **S4** (5 µM, DMSO or H_2O), or hollow SNPs (**S4**@SNP, 1 mg/mL, H_2O) that had been created by heating mesoporous SNPs with **S4**. Spectra of **S4**@SNP acquired after storage for 1 or 4 days.

Efficiency for Mitoxantrone Drug Loading onto SNPs

Two separate, but otherwise identical, samples of mesoporous SNPs (1 mg) were washed with PBS and then suspended in 2 mL 1X PBS. An aliquot of mitoxantrone (0.4 mg) in 1 mL of 1X PBS was added to each nanoparticle dispersion. One sample was stirred at 75-80 °C (exposed to air) for 18 hours and then stirred at room temperature for another 24 hours. The second sample was simply stirred at room temperature for 36 hours. The SNPs were removed by centrifugation and the solvent removed by freeze drying. In each case, the mitoxantrone residue was redissolved in PBS and the mitoxantrone absorption at 610 nm was measured to determine the molar amount of mitoxantrone not taken up by the SNPs (mitoxantrone molar extinction coefficient $\varepsilon = 15{,}163\ mol^{-1}cm^{-1}$). The loading efficiency was calculated using the equation of Grund et al,[3] and was found to be 87% for the mesoporous SNPs and 67% for the hollow SNPs.

4. Cell Studies

A549 (human lung adenocarcinoma) and MCF-7 (human breast adenocarcinoma) cells were purchased from ATCC. The A549 cells were grown in F-12K media (supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin/penicillin) in a humidified incubator at 37°C at 5% CO_2. The MCF-7 cells were grown in EMEM media (supplemented with 10% fetal bovine serum, 0.1 mM non-essential amino acids, 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, and 1% penicillin/streptomycin) in a humidified incubator at 37 °C at 5% CO_2.

Cell Metabolic Activity Measurements using MTT Assay

A549 or MCF-7 cells were seeded at 5500 cells per well in a 96-well plate and grown to 80% confluency. Media was then removed and replaced with either free mitoxantrone (0 - 22.5 µM), mitoxantrone loaded mesoporous SNPs (mitoxantrone@mesoporousSNP) (0 – 0.5 mg/mL), mitoxantrone loaded hollow SNPs (mitoxantrone@hollowSNP) (0 – 0.5 mg/mL), p-xylyenediamine loaded hollow SNPs (p-xylyenediamine@hollowSNPs) (0 – 0.5 mg/mL) in complete media in a humidified incubator at 37 °C at 5% CO_2 for 72 hours. Media containing the treatment was carefully removed and the cells were rinsed once with warm 1x PBS buffer. Cells were then placed in 100 µL of growth medium containing [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, 1.1 mM). After 4 hours of incubation at 37 °C and 5% CO_2, SDS detergent solution was added to the MTT growth medium. Cells were then incubated overnight, and absorbance of each well was measured at 570 nm. Readings were normalized to untreated cells for each treatment and all measurements were repeated in triplicate. (see Figure S21 for representative data).

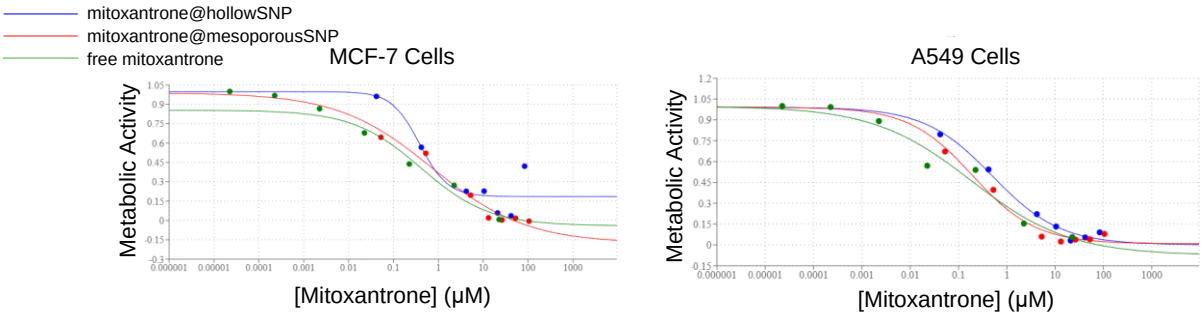


Figure S21. Representative MTT assay data for metabolic activity of MCF-7 or A549 cells that were incubated for 72 hours with free mitoxantrone, or mitoxantrone loaded into mesoporous silica nanoparticles (mitoxantrone@mesoporousSNP), or mitoxantrone used to create hollow SNPs (mitoxantrone@hollowSNP). IC_{50} values listed in Table 1 are the average and standard deviation of triplicate measurements.

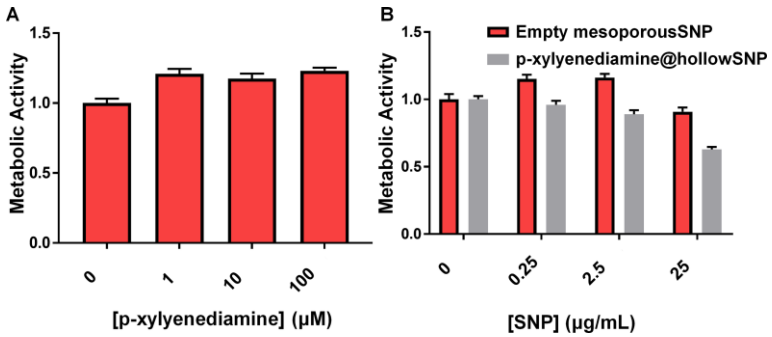


Figure S22. Metabolic activity measured using MTT assay for MCF-7 cells that were incubated for 72 hours with: (A) free p-xylyenediamine, the data indicate no cell toxicity, or (B) p-xylyenediamine that has been loaded into mesoporous silica nanoparticles (p-xylyenediamine@hollowSNPs), or empty mesoporous SNPs, the data indicate no cell toxicity at low microgram doses of SNP. Values are the average and standard deviation of triplicate measurements.

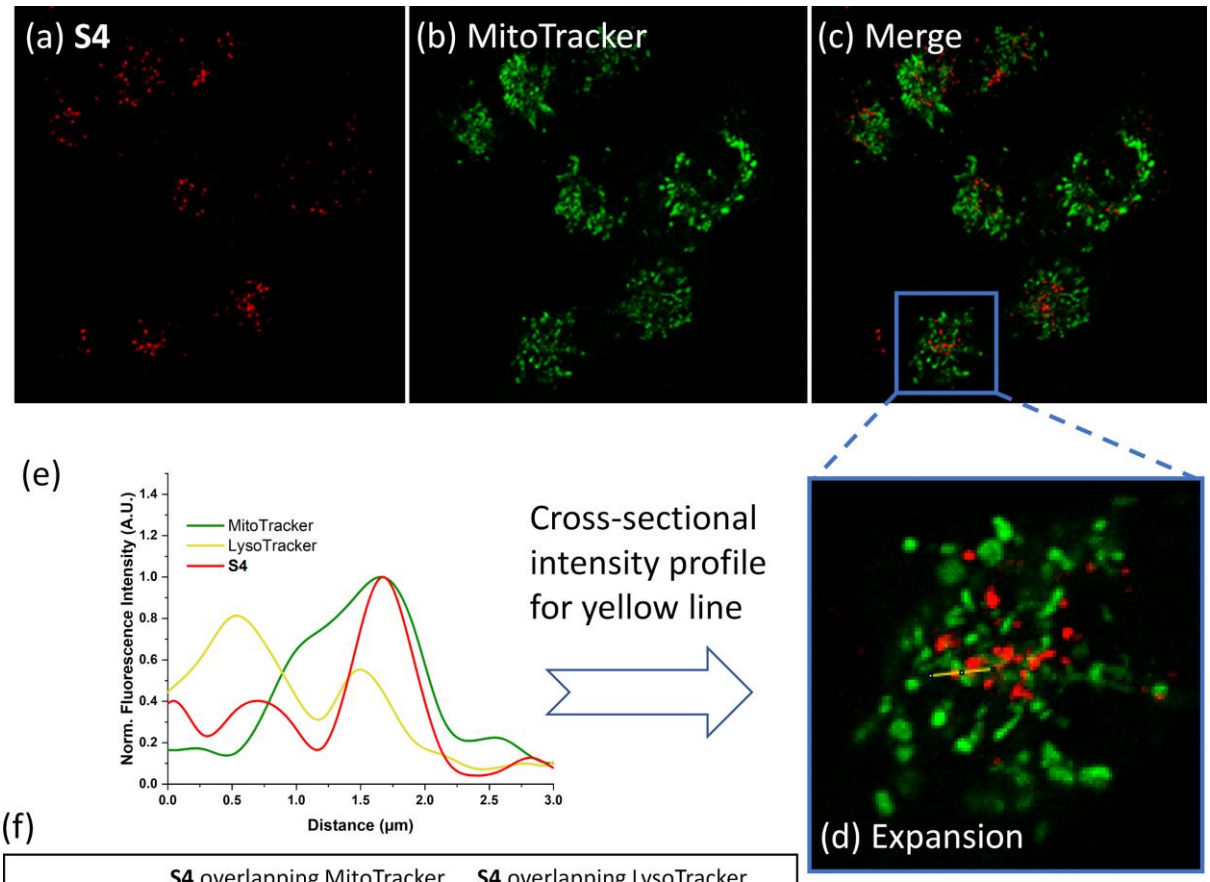
Cell Microscopy

A549 cells were seeded into 8-well chambered slides and were grown to 70% confluency. Cells were incubated with dye **S4** or **S4**@hollowSNP in media at 37 °C, then co-stained with MitoTracker Green FM and LysoTracker Red DND-99. The living cells were washed three times with phosphate buffered saline and imaged using a Nikon A1R confocal fluorescence microscope equipped with three laser lines 488 nm, 561 nm, and 633 nm. MitoTracker Green FM was viewed under FITC filter (ex: 488), Lysotracker Red DND-99 was viewed under TxRed filter (ex: 561), and **S4** was viewed under Cy5 filter (ex: 638). The confocal imaging acquired multicolor fluorescence images of an isolated Z-stack (60X oil lens, N.A. 1.4) that traversed the cells. Each multicolor micrograph was analyzed using the colocalization threshold program (JaCoP) within ImageJ2 software to determine the Manders correlation coefficient. Representative microscopy colocalization data are shown in Figures S23 and S24, along with a listing of the Manders correlation coefficients.

Observations and Conclusions of Cell Microscopy Studies

(1) **S4** localizes selectively in the cell mitochondria where the Manders correlation coefficient with MitoTracker is 0.99 compared to 0.37 for LysoTracker. Mitochondrial accumulation is expected for a cationic hydrophobic dye like **S4**.

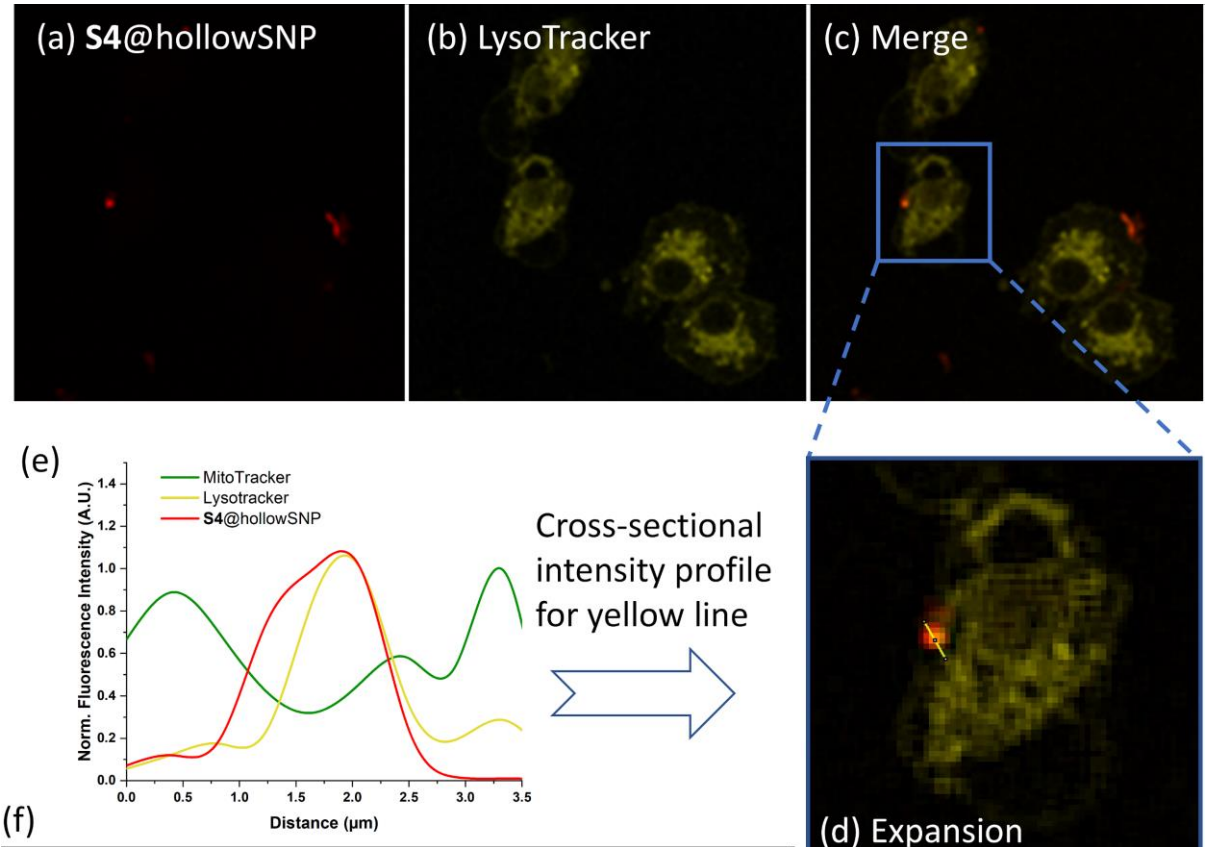
(2) A small fraction of the **S4**@hollowSNPs formed agglomerates in the cell culture media (not unexpected for non-passivated silica nanoparticles) and a large fraction was taken up by cell endocytosis over 60 minutes. The **S4**@hollowSNPs localize selectively in the cell lysosomes as quantified by a Manders correlation coefficient of 0.94 for LysoTracker and 0.28 for MitoTracker. Lysosome accumulation is expected for a non-targeted silica nanoparticle such as **S4**@hollowSNP. The very low level of mitochondrial staining suggests that the **S4**@hollowSNPs remain largely intact within the lysosomes after the 60 minute time-frame of the cell incubation experiment with negligible intracellular release of mitochondrial-targeting **S4**. This picture is not in conflict with the findings of the cell toxicity experiments above which incubated cells with mitoxantrone@hollowSNP for 72 hours and observed decreased cell metabolic activity indicating the active drug had escaped the SNP and reached its intracellular enzyme target. A goal of future studies is to elucidate the underlying cell uptake and payload release mechanisms.



(f)

	S4 overlapping MitoTracker	**S4** overlapping LysoTracker
Manders coefficient	0.99	0.37

Figure S23. Representative confocal fluorescence microscopy evidence for selective colocalization of fluorescent dye **S4** within mitochondria of living cells. A549 cells were incubated with dye **S4** (10 μM) for 30 min and co-stained with MitoTracker Green FM (1μM) and LysoTracker Red DND-99 (1μM) for 15 min. Panel (a): Red fluorescence of dye **S4**; Panel (b): Green fluorescence of MitoTracker Green FM; Panel (c): Merge of dye **S4** and MitoTracker; Panel (d): Expansion of one section within merged image, with yellow scale bar = 3.0 μm; Panel (e): Cross-sectional intensity profile for yellow line; Panel (e) Summary of Manders correlation coefficients for entire image. For improved clarity, the cell micrographs for LysoTracker staining are not shown.



(f)

	S4@hollowSNP overlapping MitoTracker	S4@hollowSNP overlapping LysoTracker
Manders coefficient	0.28	0.94

Figure S24. Representative confocal fluorescence microscopy evidence for selective colocalization of fluorescent **S4**@hollowSNP within the lysosomes of living cells. A549 cells were incubated with **S4**@hollowSNP (20 μg/mL) for 60 min and co-stained with MitoTracker Green FM (1μM) and LysoTracker Red DND-99 (1μM) for 15 min. Panel (a): Red fluorescence of **S4**@hollowSNP; Panel (b): Orange fluorescence of LysoTracker; Panel (c): Merge of **S4**@hollowSNP and LysoTracker; Panel (d): Expansion of one section within merged image, with yellow scale bar = 3.5 μm; Panel (e): Cross-sectional intensity profile for yellow line; Panel (f): Summary of Manders coefficients for entire image. For improved clarity, the cell micrographs for MitoTracker staining are not shown.

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3 S. Grund, T. Doussineau, D. Fischer and G. J. Mohr, *J. Colloid Interface Sci.*, 2012, **365**, 33–40.