Journal of Materials Chemistry B

Paper

Supplementary information

Disulfide-Gated Mesoporous Silica Nanoparticles Designed for Two-Photon-Triggered Drug Release and Imaging

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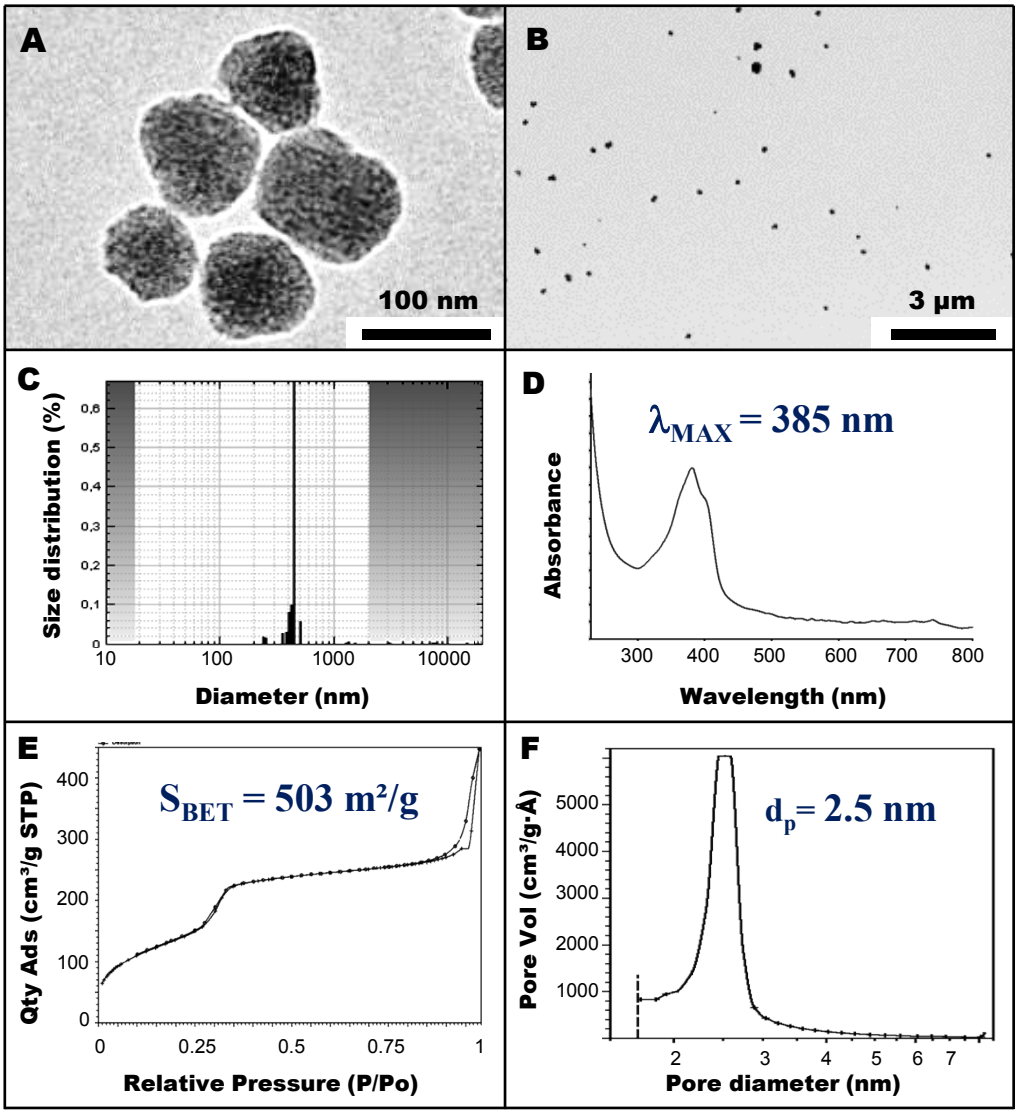


Figure S1. M2PS-35 nanogates characterization via TEM (A-B), DLS (C), UV-Visible spectroscopy (D), and N_2-adsorption-desorption isotherm (E) BJH pore size distribution (F).

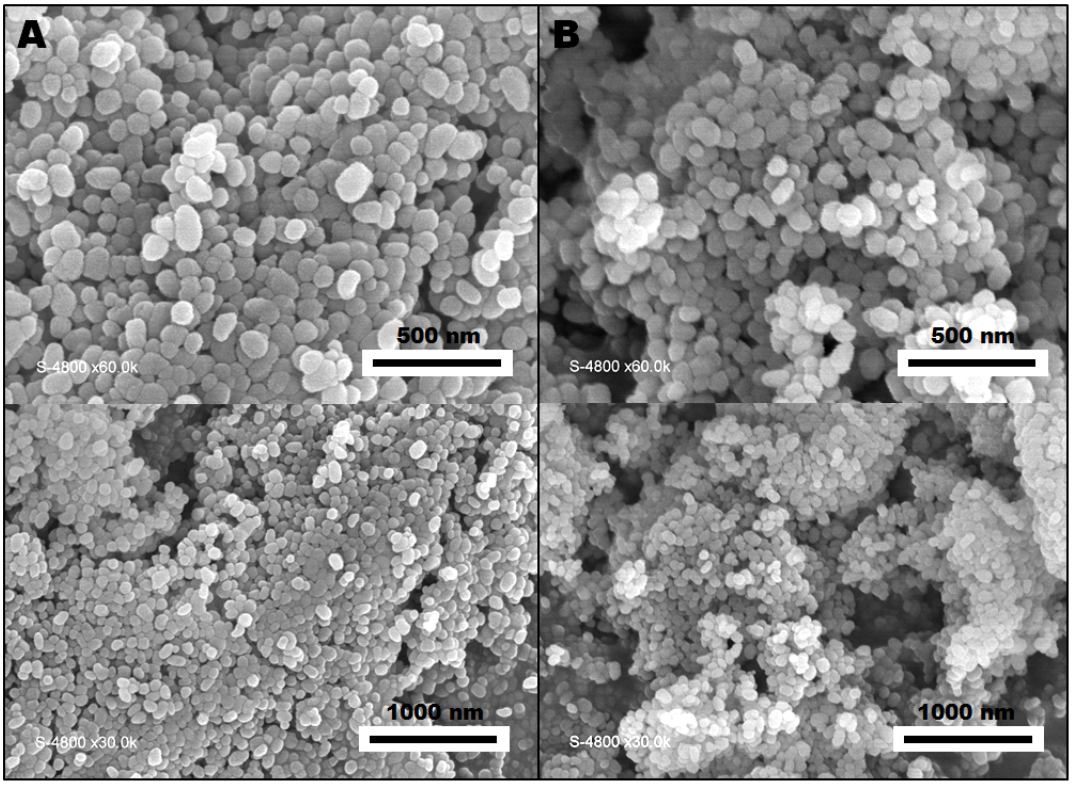


Figure S2. SEM images of M2PS-35 (A), and M2PS-16 NPs (B).

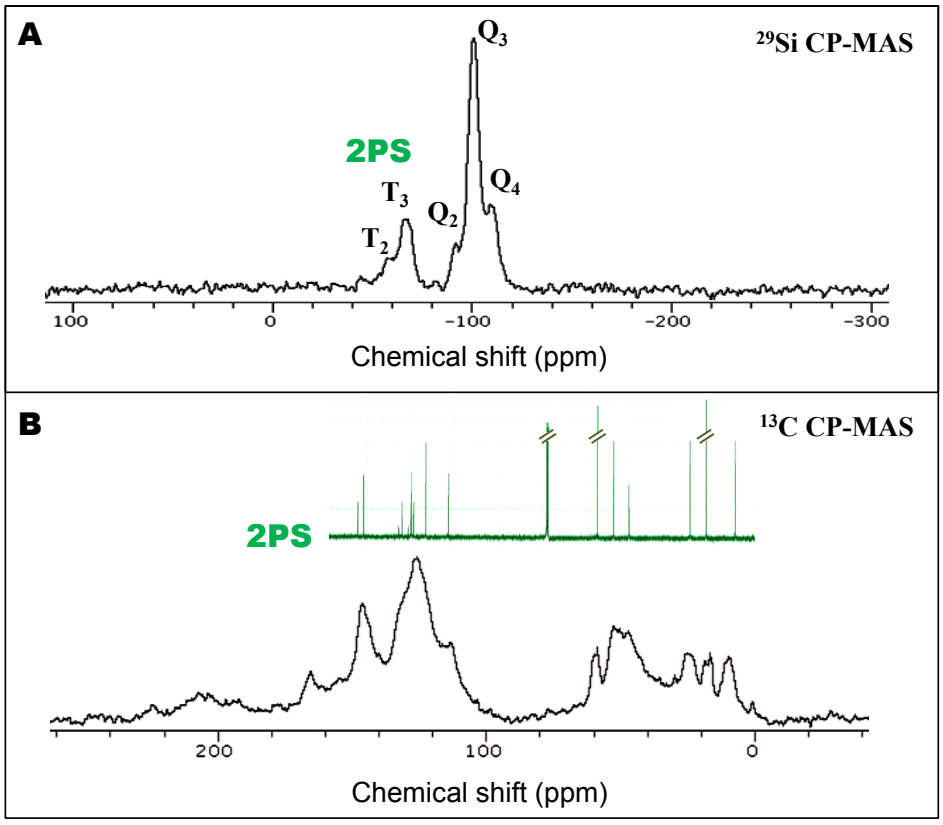


Figure S3. Solid state NMR CPMAS spectra of ^{29}Si (A) and ^{13}C (B) for M2PS-35 NPs.

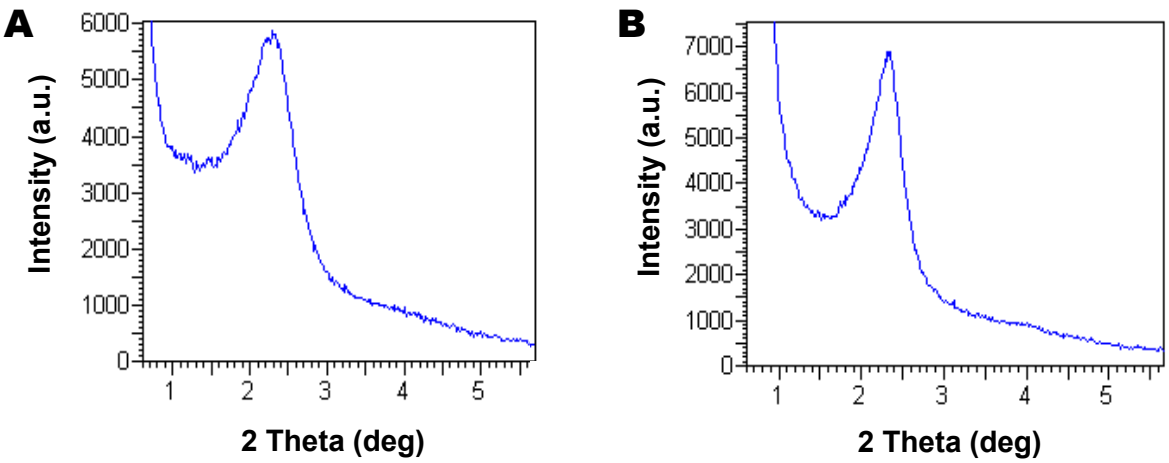


Figure S4. Small angle XRD patterns of M2PS-35 (A), and M2PS-16 NPs (B).

Sample Name	M2PS-35	M2PS-16
Nitrogen dosage (wt%)	7.3	3.4
2PS Calculations (wt%)	34.8	16.2

Table S1. 2PS weight percent (wt%) determination in the M2PS-35 and M2PS-16 nanogates. The 2PS content is calculated by combustion measurements of nitrogen in the M2PS-35, and M2PS-16 NPs, via the nitrogen content (21%) in $O_{1.5}Si\text{-}R\text{-}SiO_{1.5}$.

Sample Name	M2PS-35	M2PS-16
$\lambda_{abs}/\lambda_{em}$ (nm)	385/450	384/448
Φ_F [a]	0.26	0.41
σ_2^{max} [b] (GM)	180	200
σ_2^{max} [b] $*\Phi_F$ (GM)	46	82

Table S2. Photophysical properties of M2PS-35 and M2PS-16 NPs. The wavelength absorption and emission maxima are listed ($\lambda_{abs}/\lambda_{em}$), the fluorescence quantum yield (Φ_F), the maximum two-photon cross sections (σ_2^{max}), and the brightness ($\sigma_2^{max} *\Phi_F$). [a] Quinine bisulfate standard at 0.5 M in H_2SO_4. [b] per chromophore.

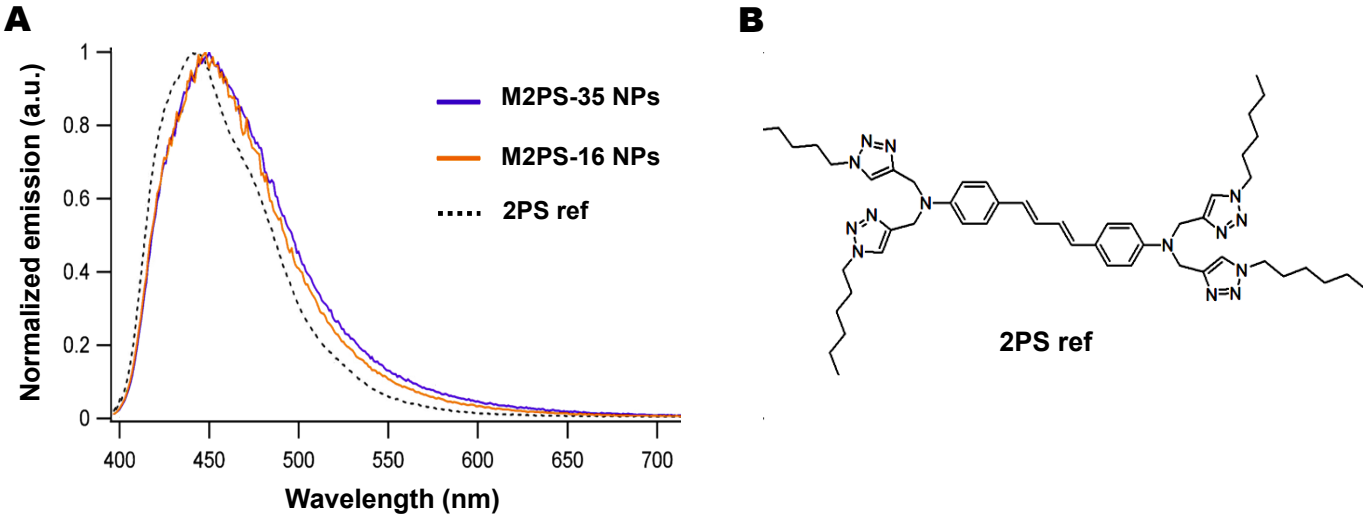


Figure S5. Normalized fluorescence emission spectra of M2PS-16 and M2PS-35 NPs (A), compared with a reference of the 2PS precursor in ethanol (B). The redshift of the peak of the 2PS in the NPs indicates the aggregation of the chromophore.

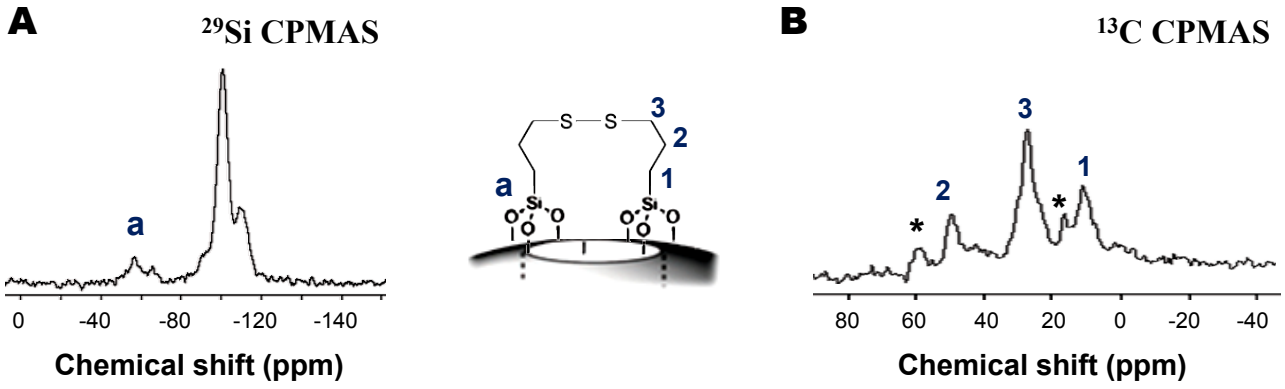


Figure S6. Solid state NMR ^{29}Si (A) and ^{13}C (B) CPMAS spectra of MSN nanogates.

Element	C	N	O	Si	S	TOTAL
M2PS Nanogates	**43.13**	5.52	34.76	16.47	**0.13**	100.00
MSN Nanogates	**6.91**	0.00	55.51	37.43	**0.15**	100.00

Table S3. EDS elemental analysis of M2PS-35 and MSN nanogates. The presence of sulfur and carbon confirmed the nanogates surface mechanization.

Sample	Zeta Potential (mV)
MSN	-20
MSN Nanogates	**-12**
MSN Nanogates + ME	-27
M2PS	-23
M2PS Nanogates	**-14**
M2PS Nanogates + ME	-31

Table S4. Zeta potential analysis on M2PS and MSN before and after nanogates functionalization, and after disulfide cleavage via mercaptoethanol addition. The zeta potential trends correlates the activation mechanism of the disulfides.

Sample Name	M2PS-35	M2PS-16
DOX Loading capacity (wt %)	2.0	2.5

Table S5. DOX loading capacities in the M2PS-35 and M2PS-16 nanogates. The loading capacities were calculated from the UV-Visible analysis of the supernatant of the mercaptoethanol-triggered release of doxorubicin in solution.

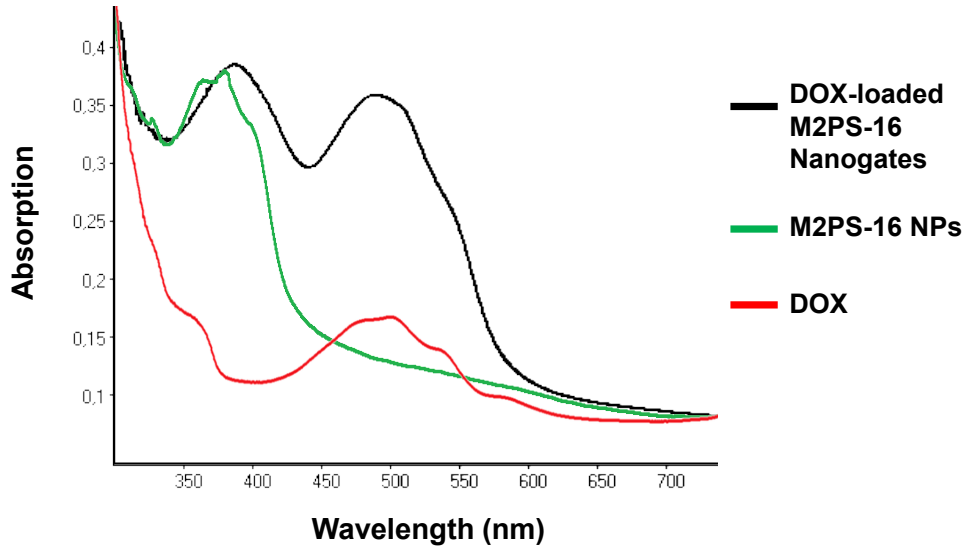


Figure S7. Absorption spectra comparison of DOX, M2PS-16, and DOX loaded M2PS-16 nanogates in water, depicting the DOX encapsulation in the M2PS mesopores.

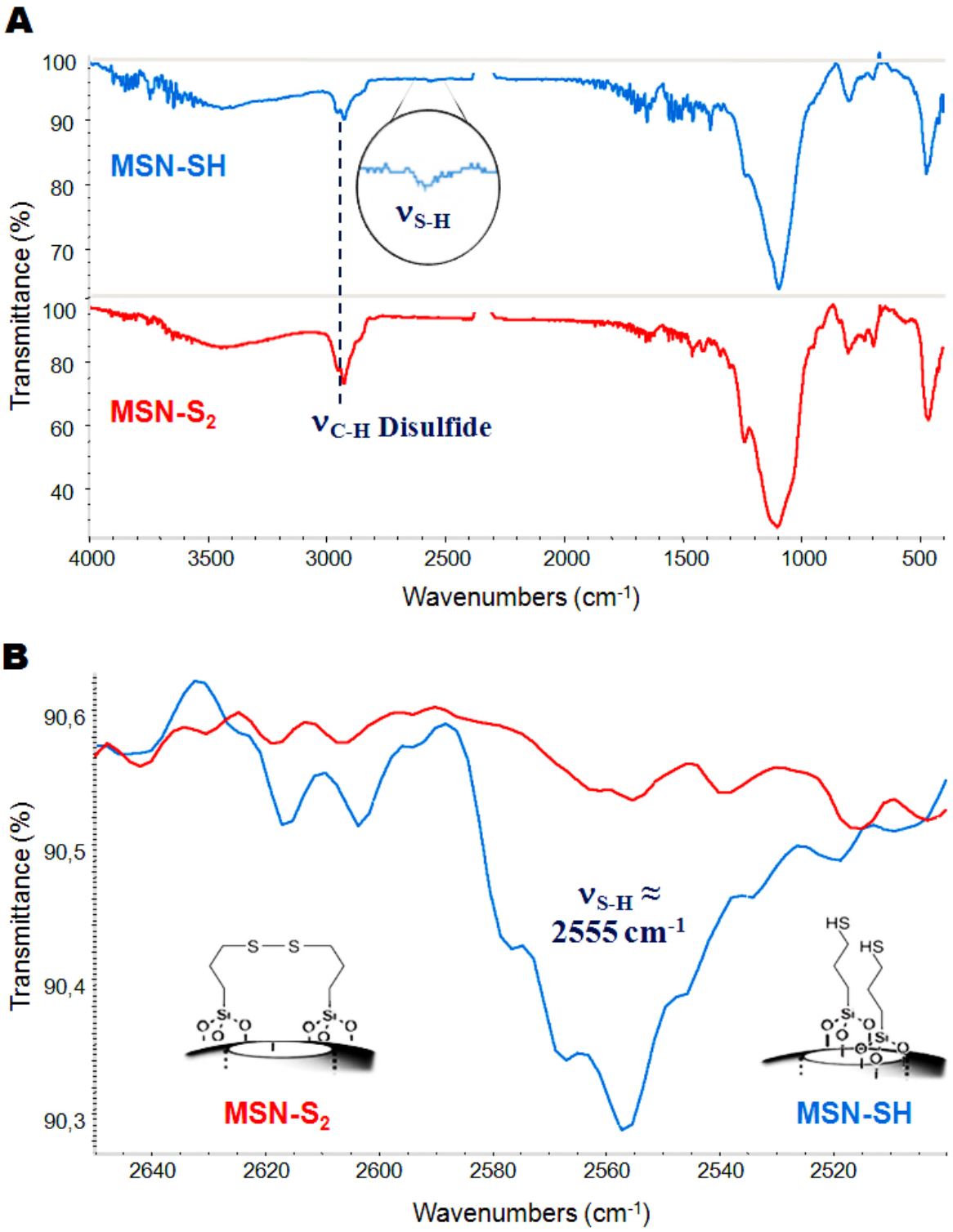
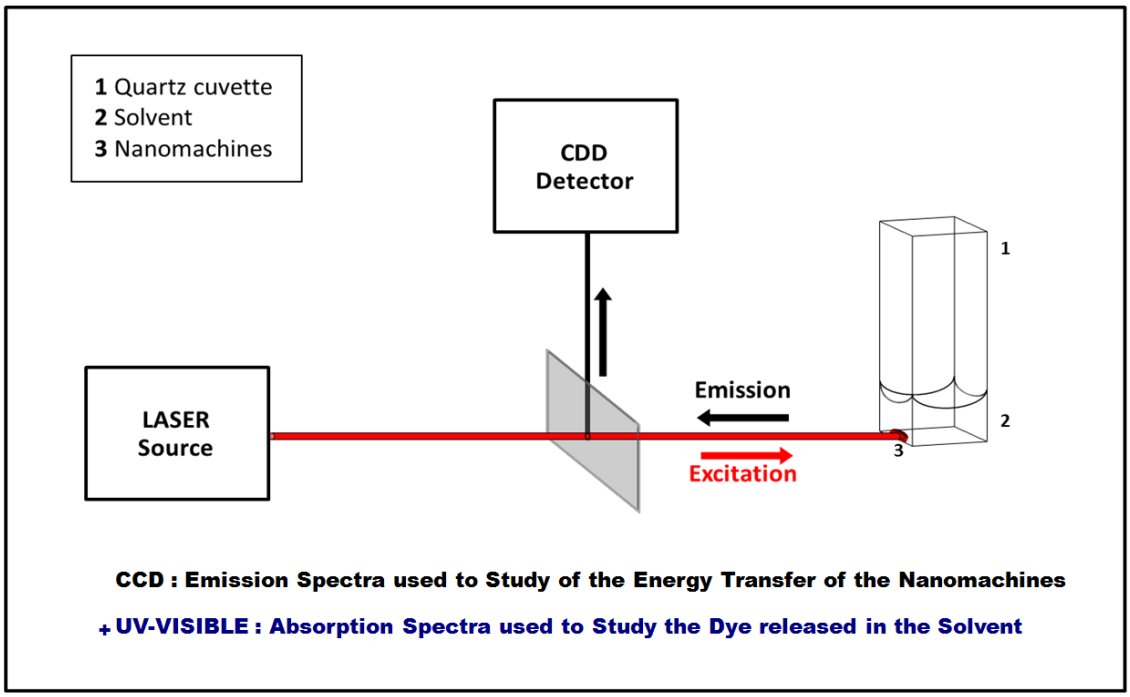


Figure S8. IR spectra comparison of MSN-SH and MSN-S_2 (after iodine addition) controls confirming the disulfide redox mechanism.

TWO-PHOTON SOLUTION RELEASE STUDY



Scheme S1. Representation of the two-photon irradiation settings for the cargo release in solution. The quartz cuvette was taken out of this setting for UV-Visible measurements during the experiment.

TWO-PHOTON IN-VITRO STUDIES

Two-Photon Fluorescence Imaging. The day prior to the experiment, MCF7 cells were seeded onto bottom glass dishes (World Precision Instrument, Stevenage, UK) at a density of 10^6 cells.cm^{-2}. Adherent cells were then washed once and incubated in 1 mL culture medium containing nanovalves at a concentration of 40 µg.mL^{-1} for 20 h. Fifteen min before the end of incubation, cells were loaded with Cell Mask (Invitrogen, Cergy Pontoise, France) for membrane staining at a final concentration of 5 µg.mL^{-1}. Before visualization, cells were washed gently with phenol red-free DMEM. Cells were then scanned with a LSM 780 LIVE confocal microscope (Carl Zeiss, Le Pecq, France), at 760 nm with a slice depth (Z stack) of 0.62 µm.

Two-Photon-triggered drug delivery: Human breast cancer cells MCF-7 (purchased from ATCC) were cultured in DMEM supplemented with 10% fetal bovine serum and 50 μg.mL^{-1} gentamycin. All cells were allowed to grow in humidified atmosphere at 37°C under 5% CO_2. For in vitro phototoxicity, MCF-7 cells were seeded into a 384 multiwell glass-bottomed plate (thickness 0.17 mm), with a black polystyrene frame, 2000 cells per well in 50 μL of culture medium, and allowed to grow for 24 h. M2PS NPs were then dispersed under ultrasounds in PBS at a concentration of 1 mg.mL^{-1} and cells were then incubated for 20 h with or without M2PS NPs at a final concentration of 40 μg.mL^{-1} in DMEM. After incubation with M2PS NPs, cells were washed twice, maintained in fresh culture medium, and then submitted (or not) to laser irradiation; with the Carl Zeiss Microscope (laser power input 3 W). Half of the well was irradiated at 760 nm by three scans of 1.57 s duration in 4 different areas of the well. The laser beam was focused by a microscope objective lens (Carl Zeiss 10x/0.3 EC Plan-Neofluar). The scan size does not allow irradiating more areas without overlapping. After 2 days, the MTS assay was performed (as previously described[1]) and was corrected according to the following formula: Abs control –2 x (Abs control - Abs M2PS).