Supplementary Information

Clickable Multifunctional Large-Pore Mesoporous Silica Nanoparticles as Nanocarriers

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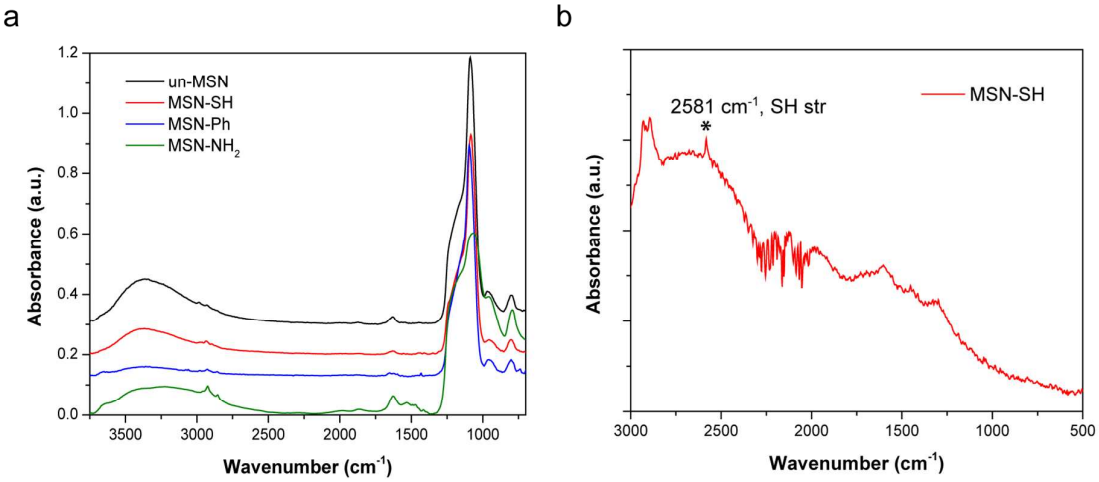


Figure S1 (a) Full range infrared spectra of MSNs. All curves are shifted along the y-axis by 0.1 units for clarity. (b) Raman spectrum of MSN-SH. The spectrum obtained from a Nd:YAG laser (λ = 1064 nm) at a laser power of 100 mW and 3000 scans.

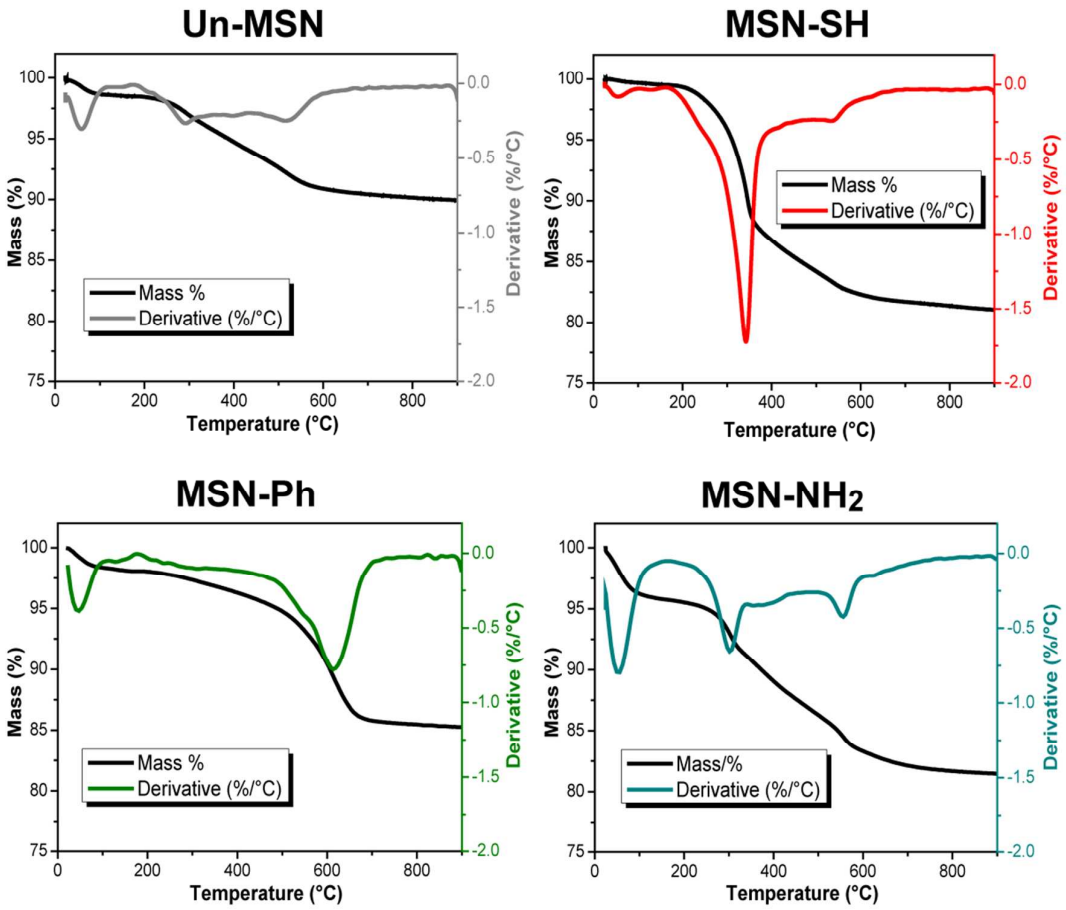


Figure S2 Thermogravimetric analysis of MSNs functionalized with different organic groups.

Table S1 TGA mass losses and the estimated degree of organic functionalizations of the functionalized MSNs.

Sample	Formulation of functional group (R)	Molecular weight of functional group (MW_R)	TGA mass loss[a] (%)	Estimated degree of organic functionalization[b] (%)
MSN-SH	C_3H_6SH	75.2	18.6	15.4
MSN-Ph	C_6H_5	77.1	13.1	10.5
MSN-NH_2	$C_3H_6NH_2$	58.1	15.0	15.4

[a] Calculated based on the mass differences between 150 °C and 900 °C.

[b] Calculated based on the formulation:

$$Mass\ loss\ (\%) = \frac{\mathrm{x} \times MW_R}{\mathrm{x} \times MW_R + (100 - x)MW_{SiO2}}$$

,x (%) = degree of organic functional groups.

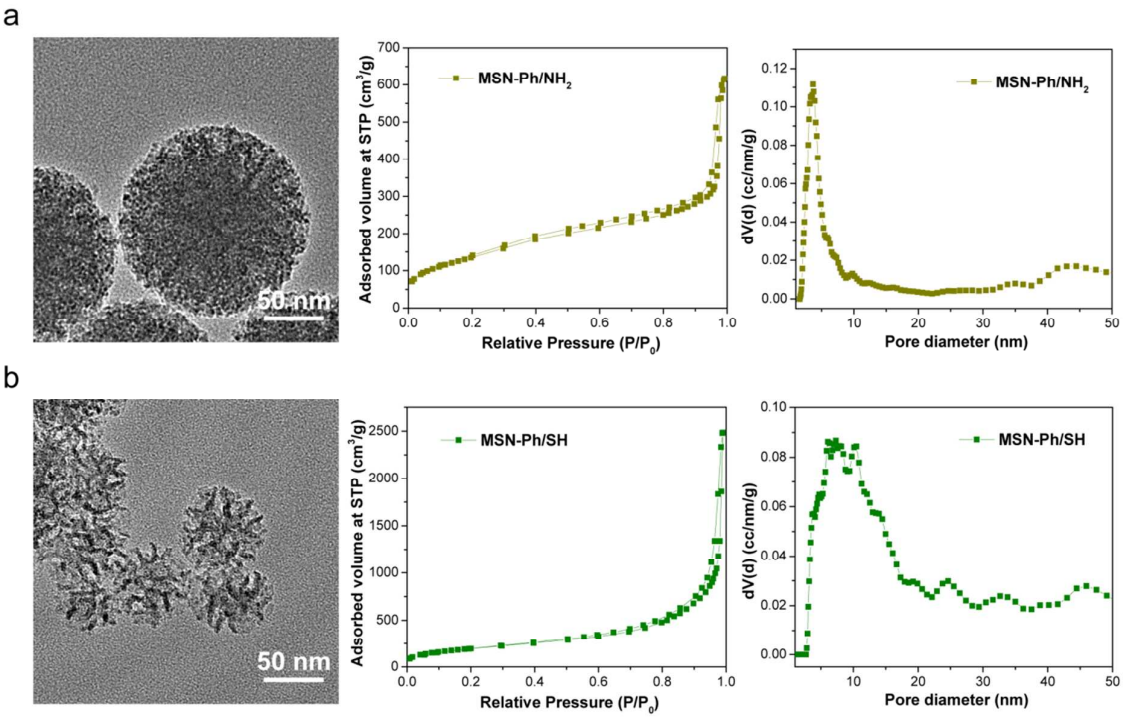


Figure S3 Characterization of (a) MSN-Ph/NH_2 and (b) MSN-Ph/SH. From left to right: TEM images, N_2 sorption isotherms and NLDFT pore size distributions obtained from adsorption branches.

Table S2 Synthesis and characterization information of MSN-Ph/NH_2 and MSN-Ph/SH.

Sample	Precursors	DFT Pore size distribution (nm)	Pore volume (cm^3/g)	BET surface area (m^2/g)	Particle size (nm)	pH value after silane addition
MSN-Ph/NH_2	95 mol% TEOS 2.5 mol% PTES 2.5 mol% APTES	2.5-5.0	0.9	496	190 ± 54	9.92
MSN-Ph/SH	90 mol% TEOS 5 mol% PTES 5 mol% MPTES	3.5-22	2.6	712	73 ± 15	9.33

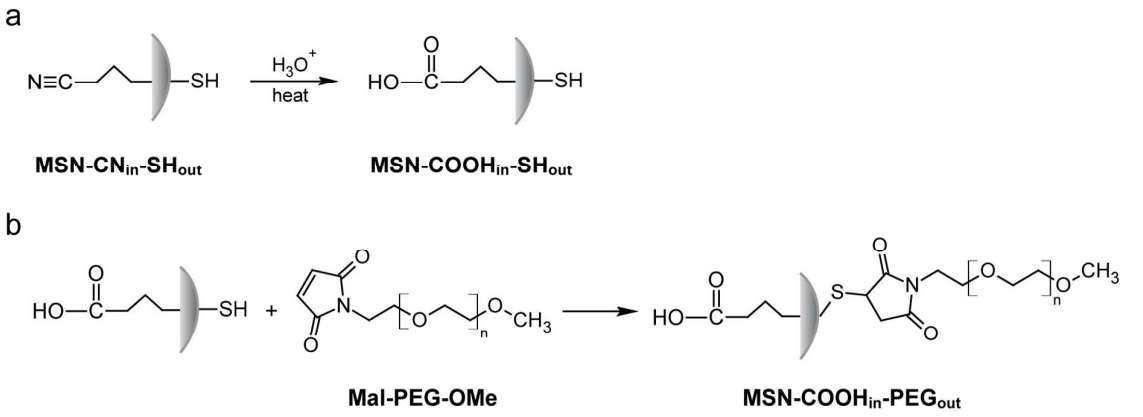
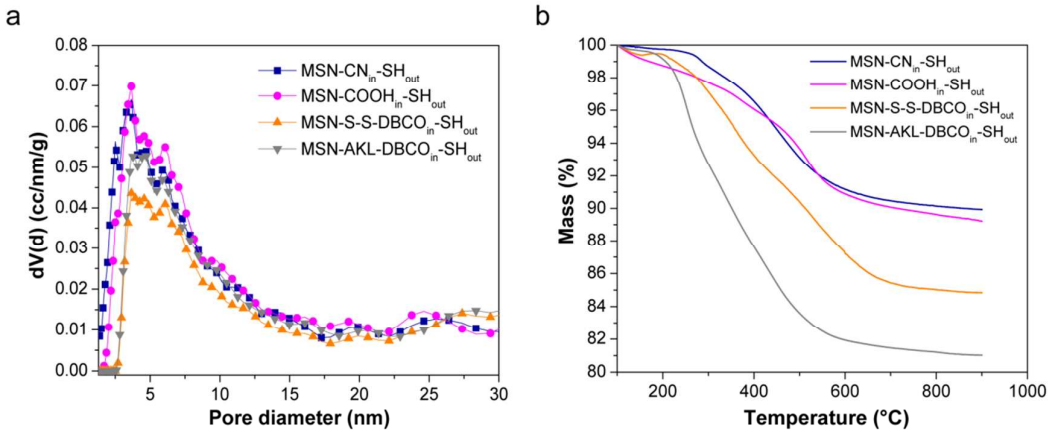


Figure S4 Surface modification of core-shell MSNs. (a) Hydrolysis of MSN-CN_{in}-SH_{out} particles in acidic condition at 90 °C. (b) PEGylation of MSN-$COOH_{in}$-SH_{out}.



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Sample	Pore size distribution (nm)	Pore volume (cm^3/g)	Surface area (m^2/g)	TGA mass loss at 900 °C (%)
MSN-**CN$_{in}$**-SH$_{out}$	2.5-12	1.5	573	10.1
MSN-**COOH$_{in}$**-SH$_{out}$	3.0-12	1.5	535	10.8
MSN-**S-S-DBCO$_{in}$**-SH$_{out}$	3.5-12	1.2	339	15.2
MSN-**AKL-DBCO$_{in}$**-SH$_{out}$	3.5-12	1.4	416	19

Figure S5 Characterization of core-shell MSNs. (a) NLDFT pore size distribution of core-shell MSNs calculated from adsorption branches of N_2 sorption isotherms. (b) Thermogravimetric analysis. (c) Summary of the core-shell MSNs characterization. The TGA mass loss was calculated based on the mass differences between 100 °C and 900 °C.

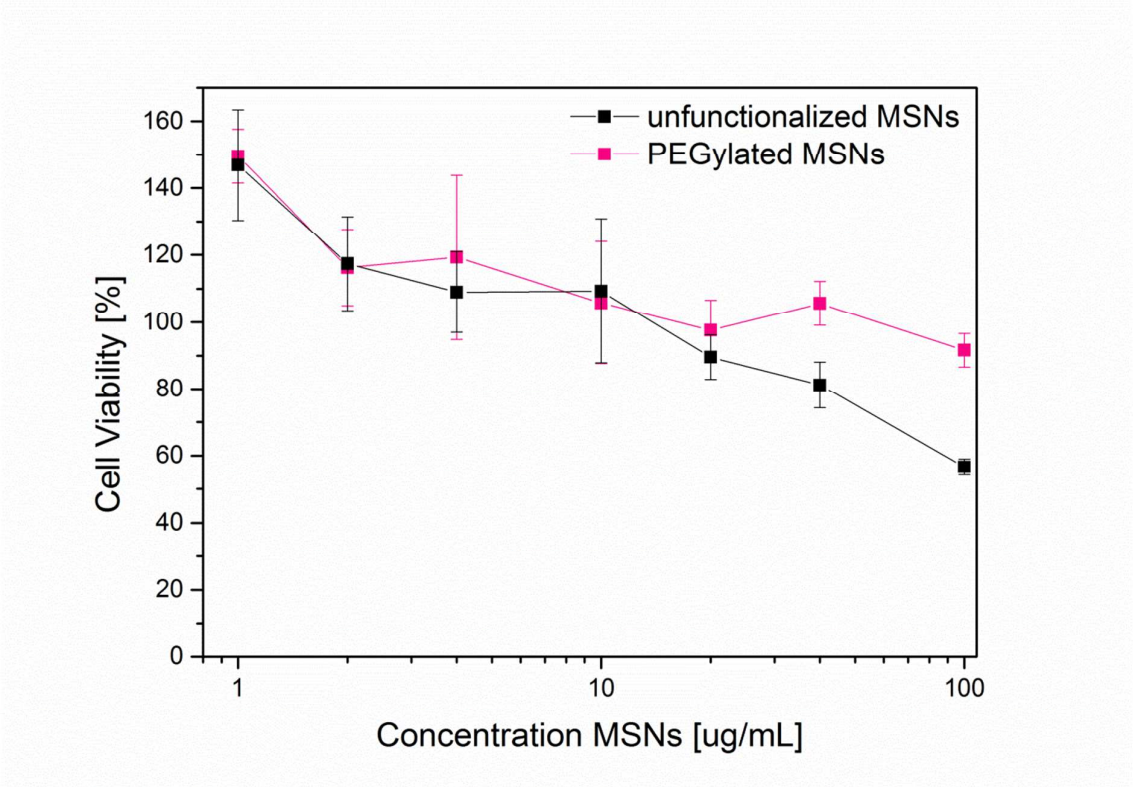

Figure S6 Cytotoxicity of MSNs. CCK-8 assay for cytotoxicity studies of unfunctionalized and PEGylated core-shell MSNs after 24 h incubation on HeLa cells.

The CCK-8 (Cell Counting Kit 8) was used to determine the cytotoxicity of the here used large-pore MSNs. HeLa cells were seeded on a 96-well microplate (5 x 10^3 cells per well) in DMEM (100 µL $well^{-1}$) and incubated at 37 °C and 5% CO_2. 24h after cell seeding, 10 µL of MSNs (with various particle concentrations) were added to each well. The control group consisted of 10 µL water and was used as reference (100%). The cells were incubated with MSNs for 24 h. Afterwards 10 µL of CCK-8 solution were added to each well of the plate and after incubating the plate for another 4 h, the absorbance at 450 nm was measured in a microplate reader (SPARK 10M, Tecan Austria GmbH) with 600 nm as reference wavelength. Experiments were performed in triplicates. Error bars show the standard deviations.

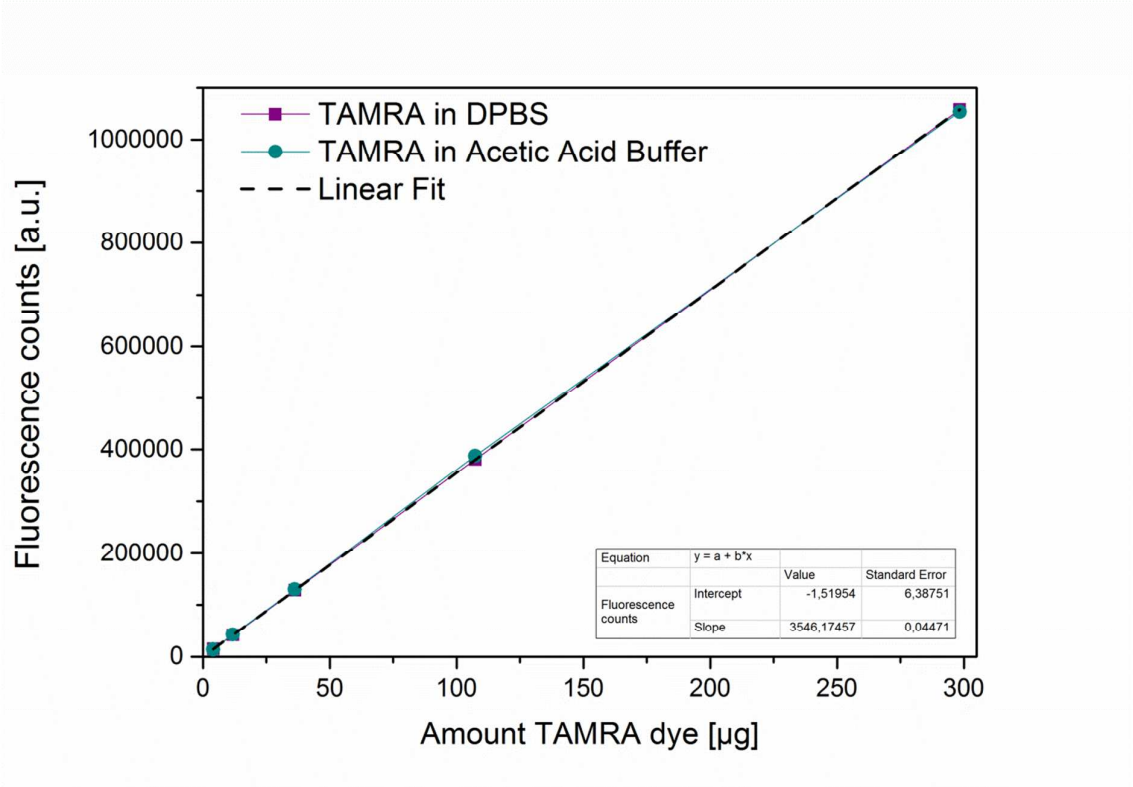

Figure S7 Calibration curve of TAMRA dye for quantification of released amount. Different molar concentrations of TAMRA dye are measured with a fluorescence spectrometer and fitted linearly. As expected, TAMRA does not show a pH-dependent fluorescence.

To make sure that the successful click reaction is not hindered due to lack of TAMRA supply, we used TAMRA dye in excess. The supernatants were measured before and after the click reaction to determine the percentage of loaded dye. For MSN-AKL-DBCO-TAMRA 2% of the dye was loaded into the pores (100% correlates to 200 µg TAMRA dye used in the solution for the click reaction). After hydrolysis of the AK-linker, 60% of TAMRA dye were released. In the pores of MSN-S-S-DBCO-TAMRA, 10% of the provided dye was loaded and 57% thereof was released after reductive cleavage of the disulfide-bridges.

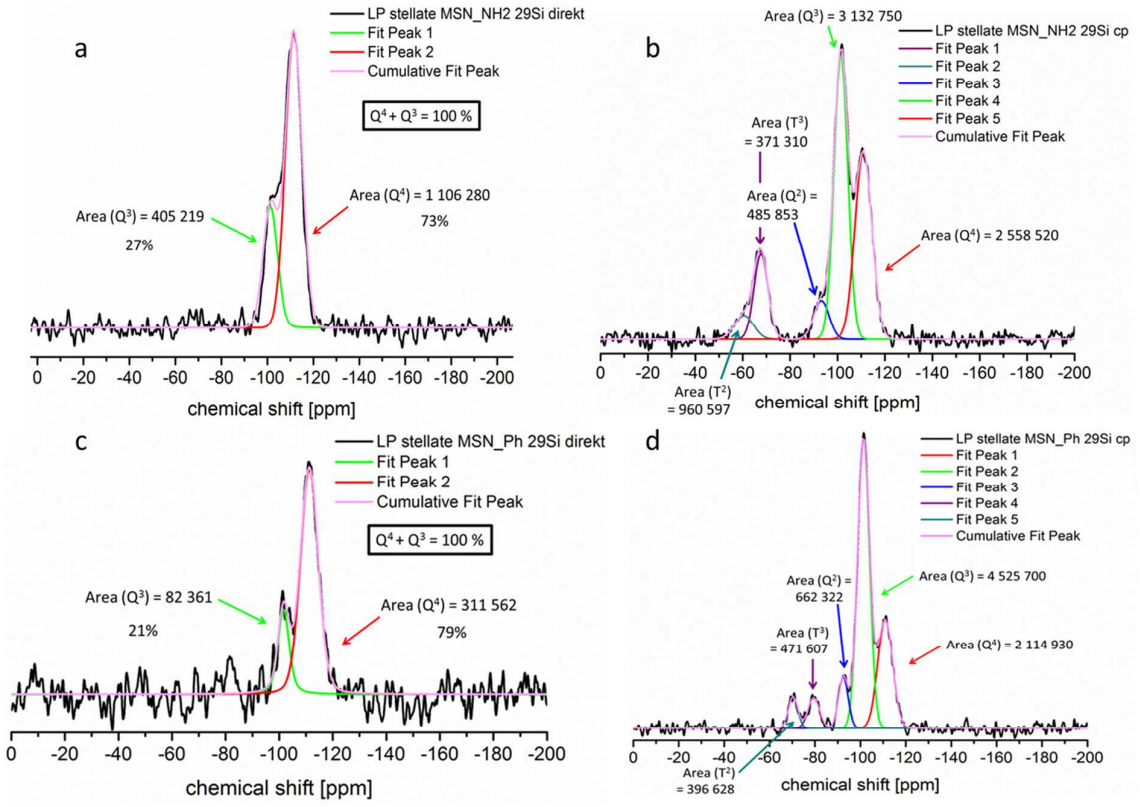

Figure S8 ^{29}Si MAS-NMR spectra (a) MSN-NH_2 (direct excitation), (b) MSN-NH_2 (cross-polarized), (c) MSN-Ph (direct excitation), (d) MSN-Ph (cross-polarized).

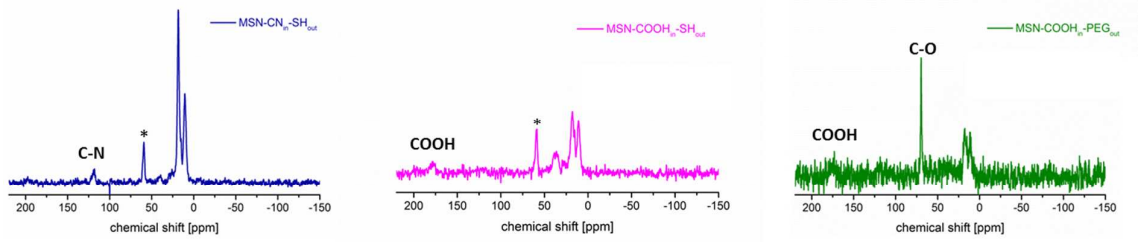

Figure S9 ^{13}C MAS-NMR spectra (a) MSN-CN_{in}-SH_{out} (blue), (b) MSN-$COOH_{in}$-SH_{out} (magenta) and (c) MSN-$COOH_{in}$-PEG_{out} (green). Hydrolysis of the cyano-groups (indicated with C-N at 118 ppm, blue spectrum) results in the resonance of carboxyl groups (COOH) appearing at 180 ppm (magenta and green spectra). Successful attachment of the PEG linker can be seen by C-O resonances appearing at 70 ppm (green spectrum). The asterisked peaks denote residual surfactants. The other resonances are assigned to functionalized silanes of the MSNs.