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Supplementary Information

Multilayered silica-biopolymer nanocapsules with hydrophobic core and hydrophilic tunable shell thickness

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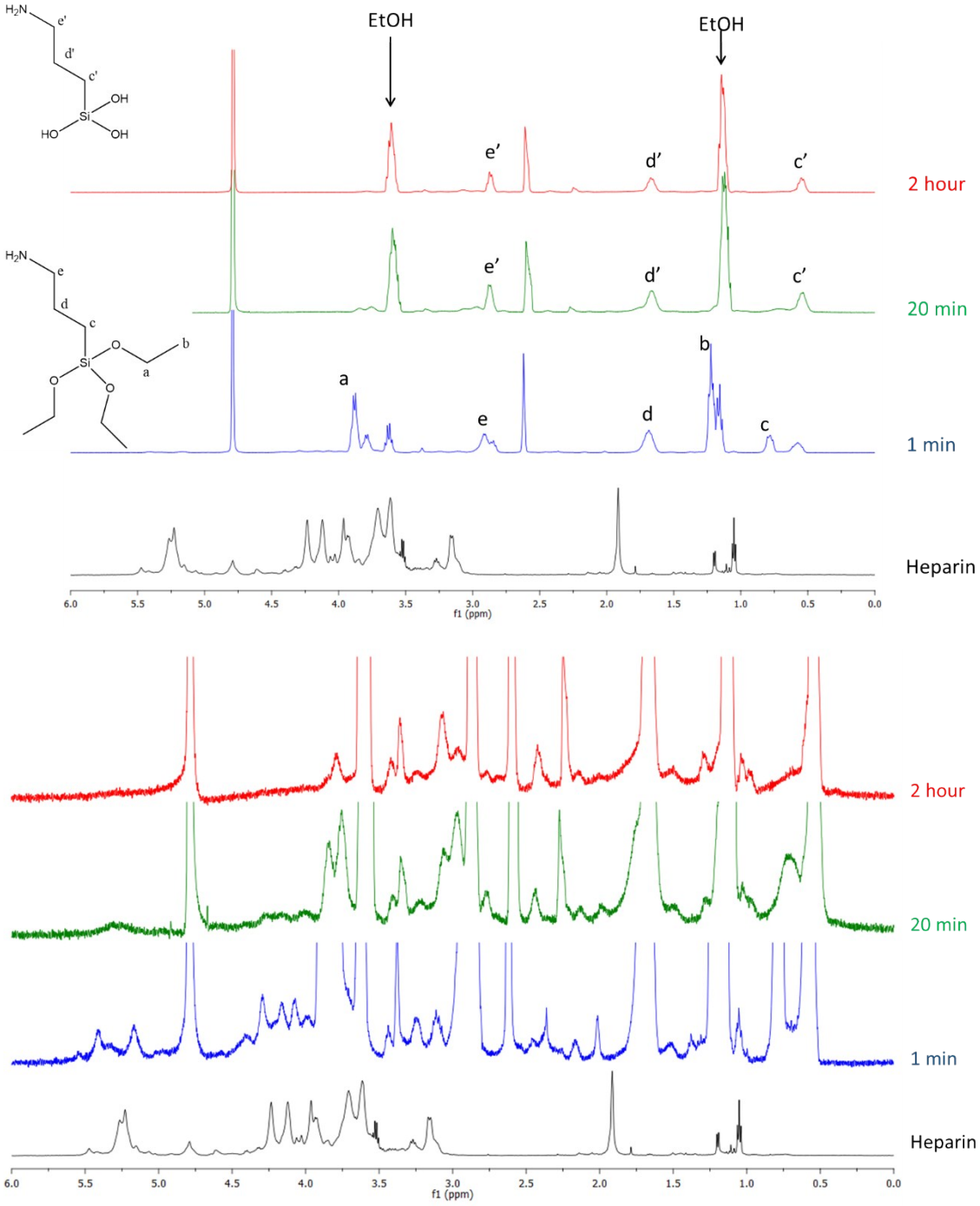

Figure S1 (a) 1H-NMR spectra of heparin (black line) and of the reacting mixture (heparin, EDC, NHS and APTS) at 3 different times. Mixture was kept at 4° C. (b) Expanded 1H-NMR spectra showing the characteristic heparin signals.

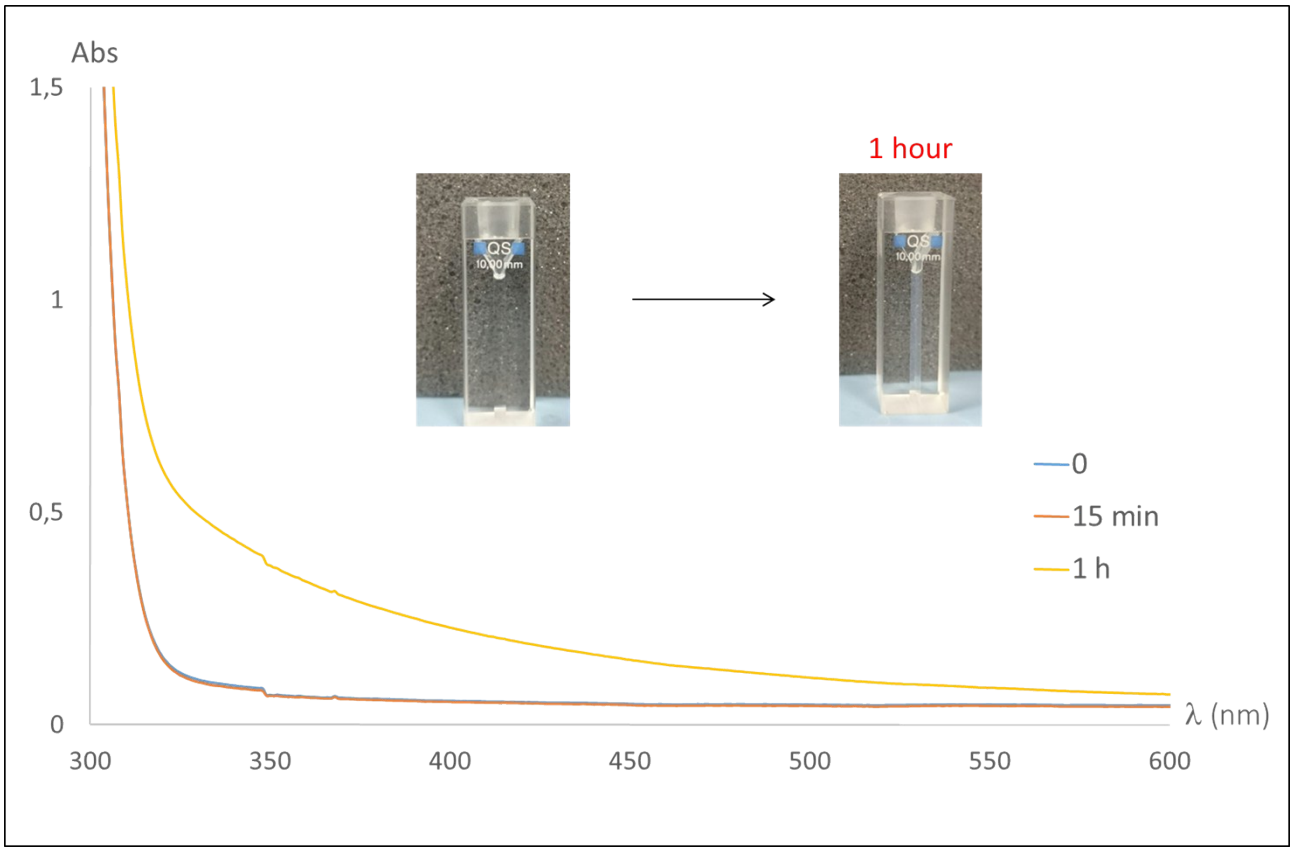

Figure S2 UV-Vis spectra of reaction mixture containing heparin, EDC, NHS and APTS at different times (blue and red line are overlapped). In the inset, the reaction batch just mixed and after 1 h are compared, thus showing the appearance of opalescence in the solution.

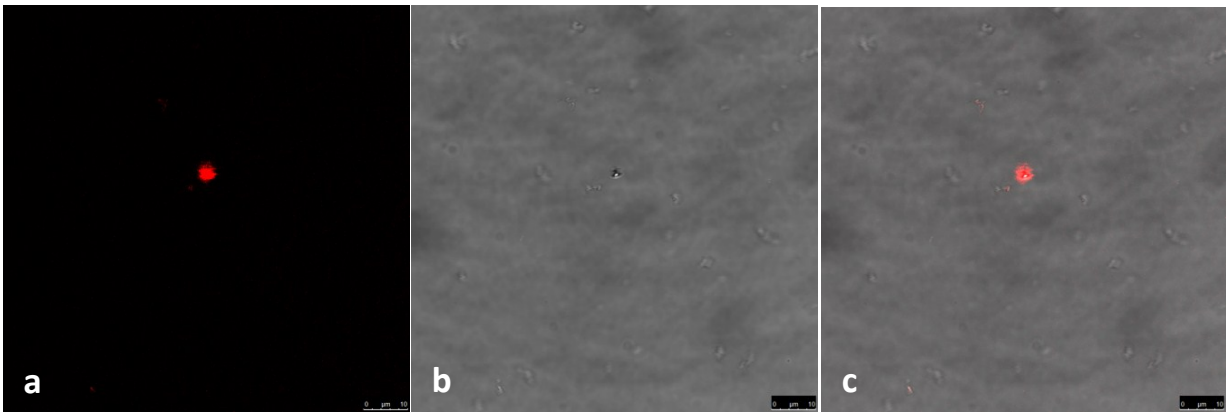

Figure S3 Confocal analysis of nanocapsules stained with Nile Red in the oil core: in fluorescence analysis (a), in bright field (b); matching of the two images.

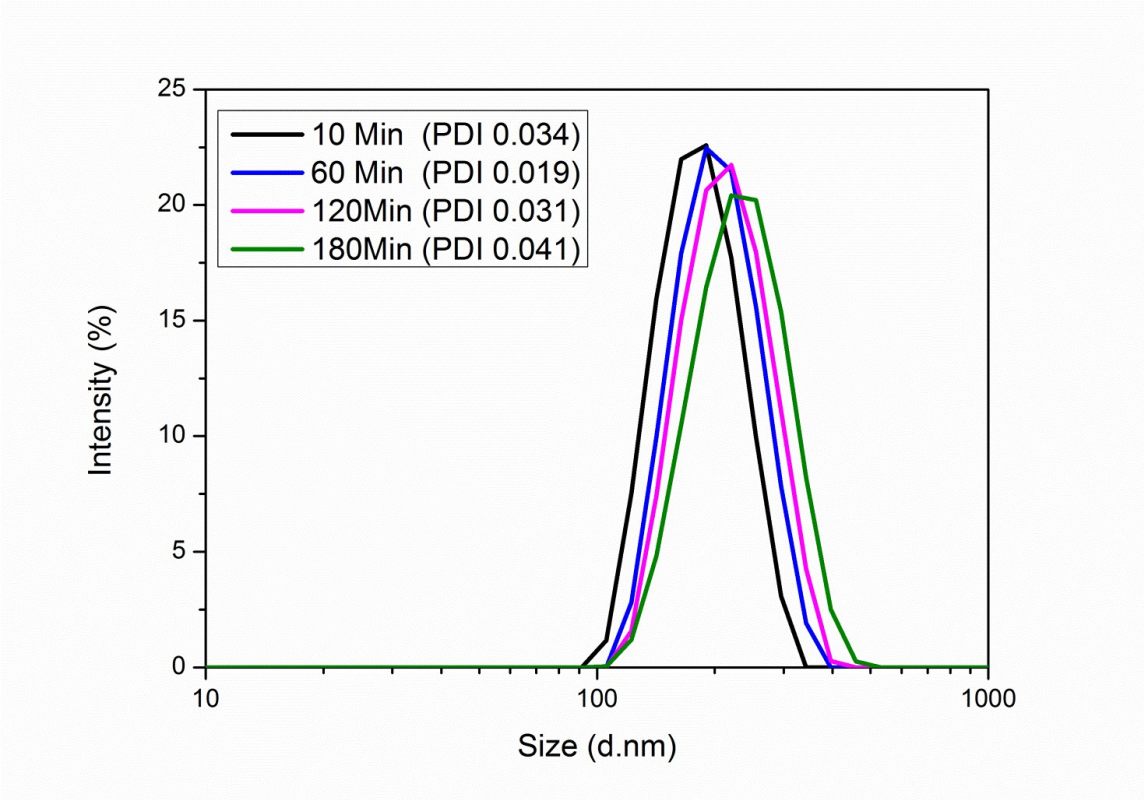

Figure S4 DLS graph of silica nanocapsules collected at different reaction time intervals.

Table S1. DLS data of silica nanocapsules collected at different reaction time intervals.

Time (min)	Size 1 (nm)	PDI	Size 2 (nm)	PDI	Size 3 (nm)	PDI
7,5	153,3	0,042	152,3	0,036	153,9	0,031
15	165,1	0,028	161,2	0,015	161,9	0,057
30	175,4	0,019	172,2	0,017	173,0	0,045
45	182,3	0,044	181,0	0,018	176,9	0,042
60	187,8	0,043	186,9	0,037	185,5	0,035
90	198,7	0,036	199,1	0,022	195,9	0,055
120	198,4	0,060	203,6	0,050	203,9	0,030
150	203,8	0,054	209,4	0,036	204,5	0,054
180	205,7	0,045	209,8	0,044	209,8	0,057

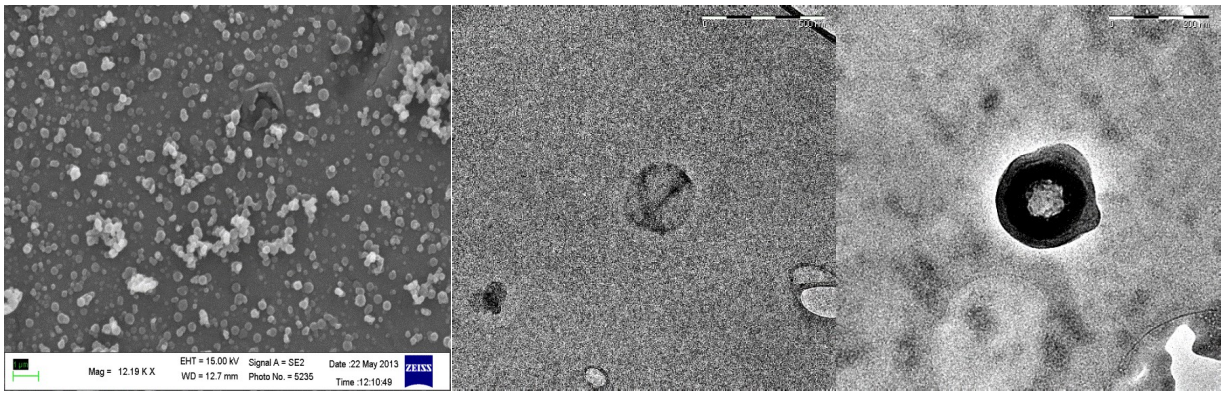

Figure S5 SEM micrographs (a) of silica nanocapsules after 1 h of seeded growth; TEM tomography of a single silica nanocapsule after 1h (scale bar 500 nm) (b) and 3h (scale bar 200 nm) (c) of seeded growth.

Video S5 TEM tomography of a single silica nanocapsule after 1h (a) and 3h (b) of reaction time

1) Video S5a
2) Video S5b

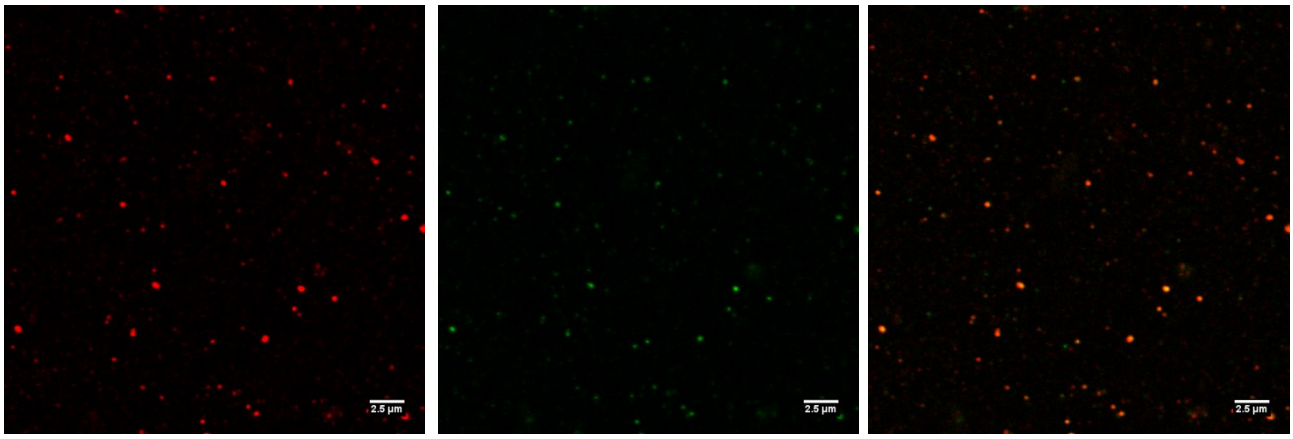

Figure S6 Confocal image of silica nanocapsules by exciting sulforhodamine B in the shell (a) and FITC on the chitosan (b) and the overlapped image of a and b (c).

External sink release method

Silica nanocapsules (2 ml) were collected during silica deposition process (30, 60, 180 min of seeded growth time) to get different shell diameters and placed in vials. In each of the vials 2 ml hexane was added and the samples were stirred vigorously at 1500 rpm. After predetermined times hexane layer was collected and analyzed by UV-vis spectroscopy for the absorbance of soybean oil between 200-300 nm and then replaced back. With the growth of silica shell the release of soybean oil diminished and in case of thick silica shell (after 180 min of seeded growth time) only a slight increase in absorbance was observed with time even after 8 h of rigorous stirring.

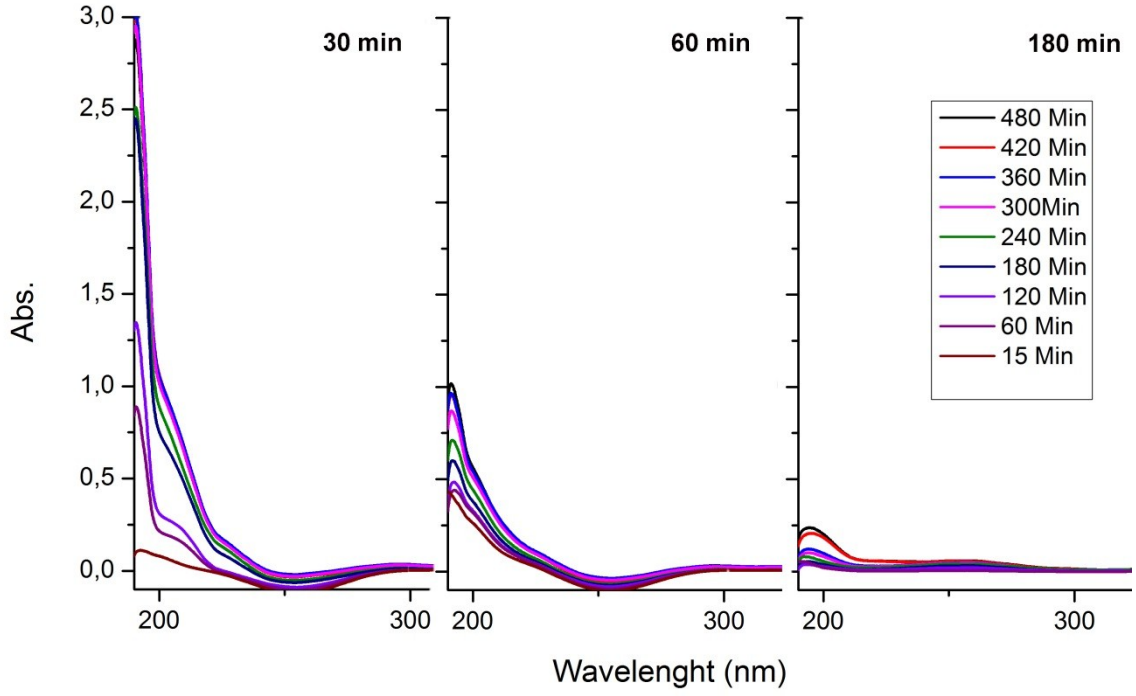

Figure S7 UV absorbance of soybean oil released from nanocapsules at different silica shell thickness (corresponding to 30, 60 and 180 min of seeded growth time) over stirring time in the mixture with hexane.

Synthesis of Fluorescein PEG

PEG_{5000}-COOH (Methoxypolyethylene glycol 5000 acetic acid, 90.0 mg) and NHS (12.0 mg) were dissolved in 2.50 ml of MilliQ water. After 10 min EDC (10.0 mg) was added. In the meantime, 7.0 mg of 6-Aminofluorescein were dissolved in 100 µl of DMSO. After 30 min the fluorescein solution was added dropwise to the PEG_{5000}-COOH solution. pH was corrected to 6.7 adding NaOH 1 M. After 24 h the solution was dialyzed (dialysis tubing RCE, 3.5 kDa MWCO) against Milli Q water to remove solvent and unreacted reactants and freeze dried. An absorbance spectrum was recorded in order to select the correct excitation wavelength (480 nm) and, then, the emission spectrum was recorded between 500 and 600 nm using an excitation wavelength of 480 nm and a flashes number of 1000 (Figure S6).

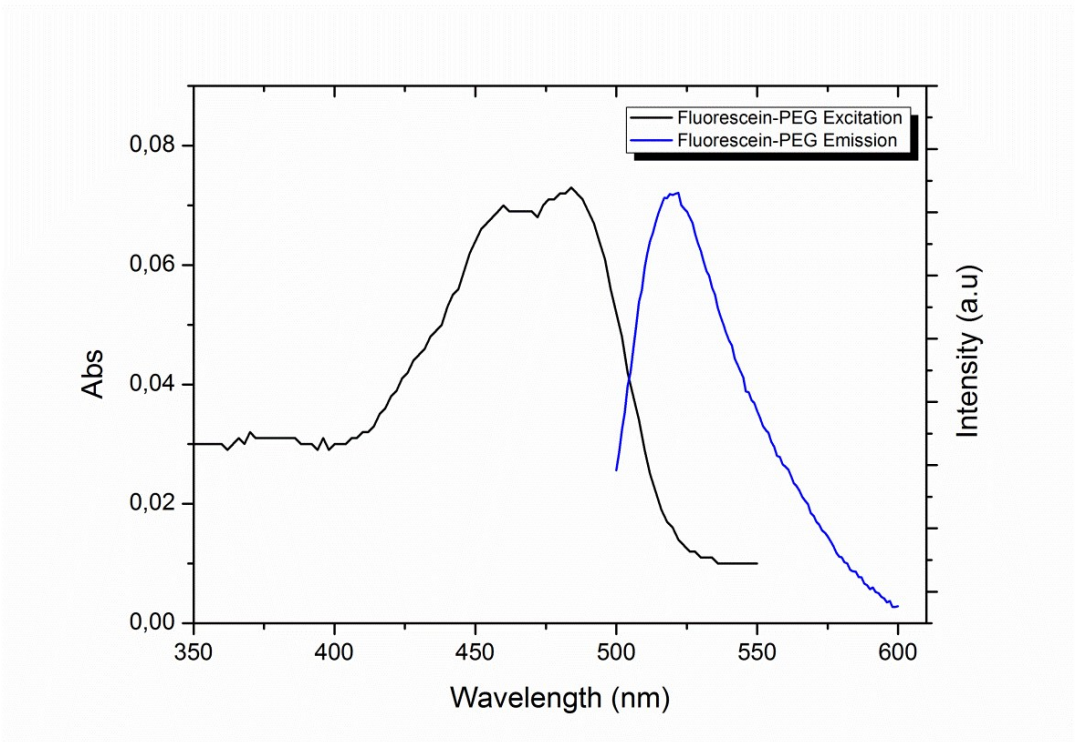

Figure S8 Excitation (black curve) and emission (blue curve) spectrum for FA modified PEG_{5000}-COOH.

Spectrofluorimetry Analysis and Confocal imaging of PEGylated nanocapsules

To confirm that shell PEGylation occur an experiment was performed using the FA modified PEG described in the previous paragraph. The so obtained marked sample was dialyzed (Dialysis tubing, RC, 25 kDa MWCO) against water to remove the excess of PEG_{5000}-COOH and dialysis was monitored recording the fluorescent emission of fluorescein in the washing water (EnSpire Multimode Plate Reader 2300-0000, Perkin Elmer). 10 µl of NaOH solution 1 M were added to 300 µl of water and loaded in a multi-well (3 replicates for each sample were prepared) and irradiated with a light source at 480 nm. Dialysis is kept until no significant emission is recorded in the water, also after sonication of the sample. Moreover, to show that PEG-FA is still present on the sample and, in particular on the shell, an emission spectrum of sample was recorded (10 µl of sample were added to 10 µl of NaOH solution 1 M and dilute to 300 µl, Figure S9) together with a confocal images of silica nanocapsules with Nile Red in the core and PEG-FA on the shell surface (Figure S10).

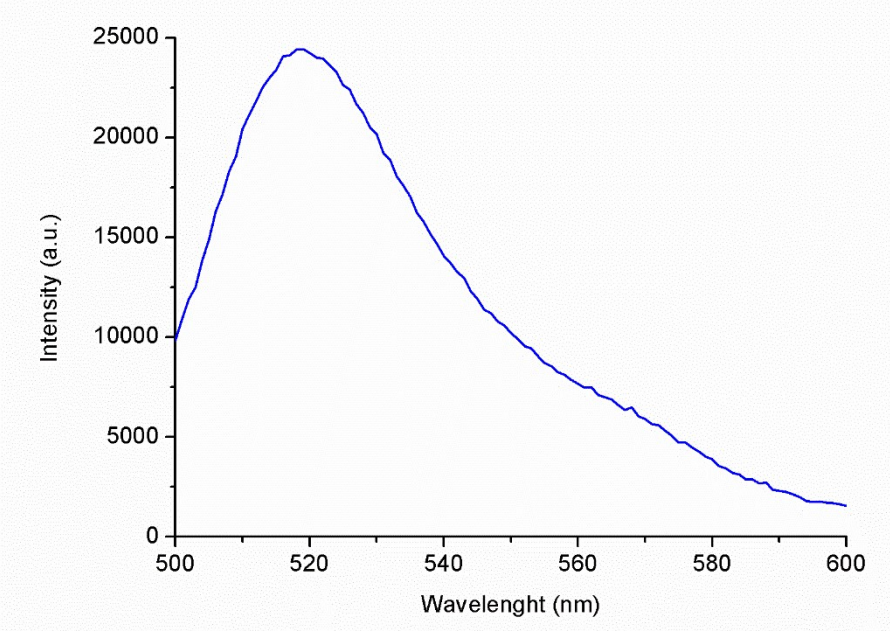

Figure S9 Emission spectra of silica nanocapsules coated with FA-labeled PEG_{5000}-COOH after dialysis.

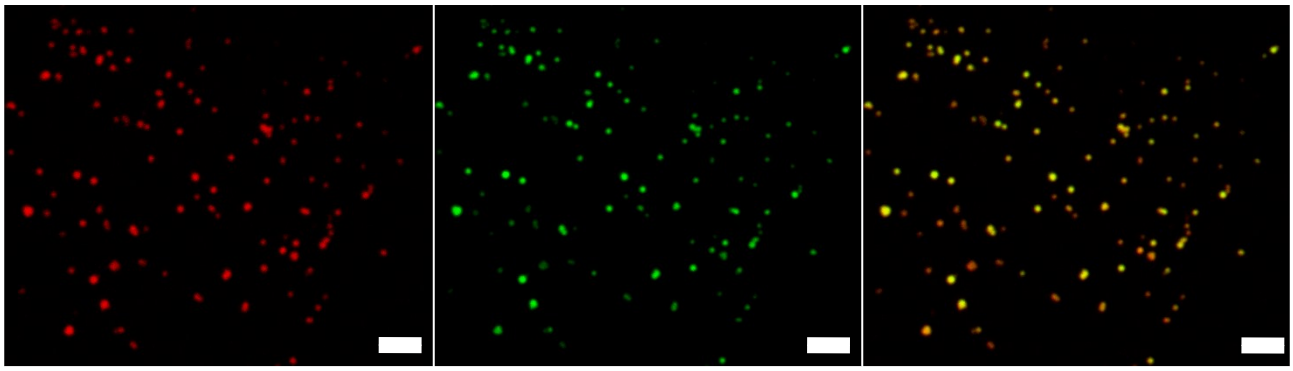

Figure S10 Confocal image of silica nanocapsules by exciting Nile Red in the oil core (a) and PEG-FA on the shell (b) and the overlapped image of a and b (c). Scale bar 2.5 μm.

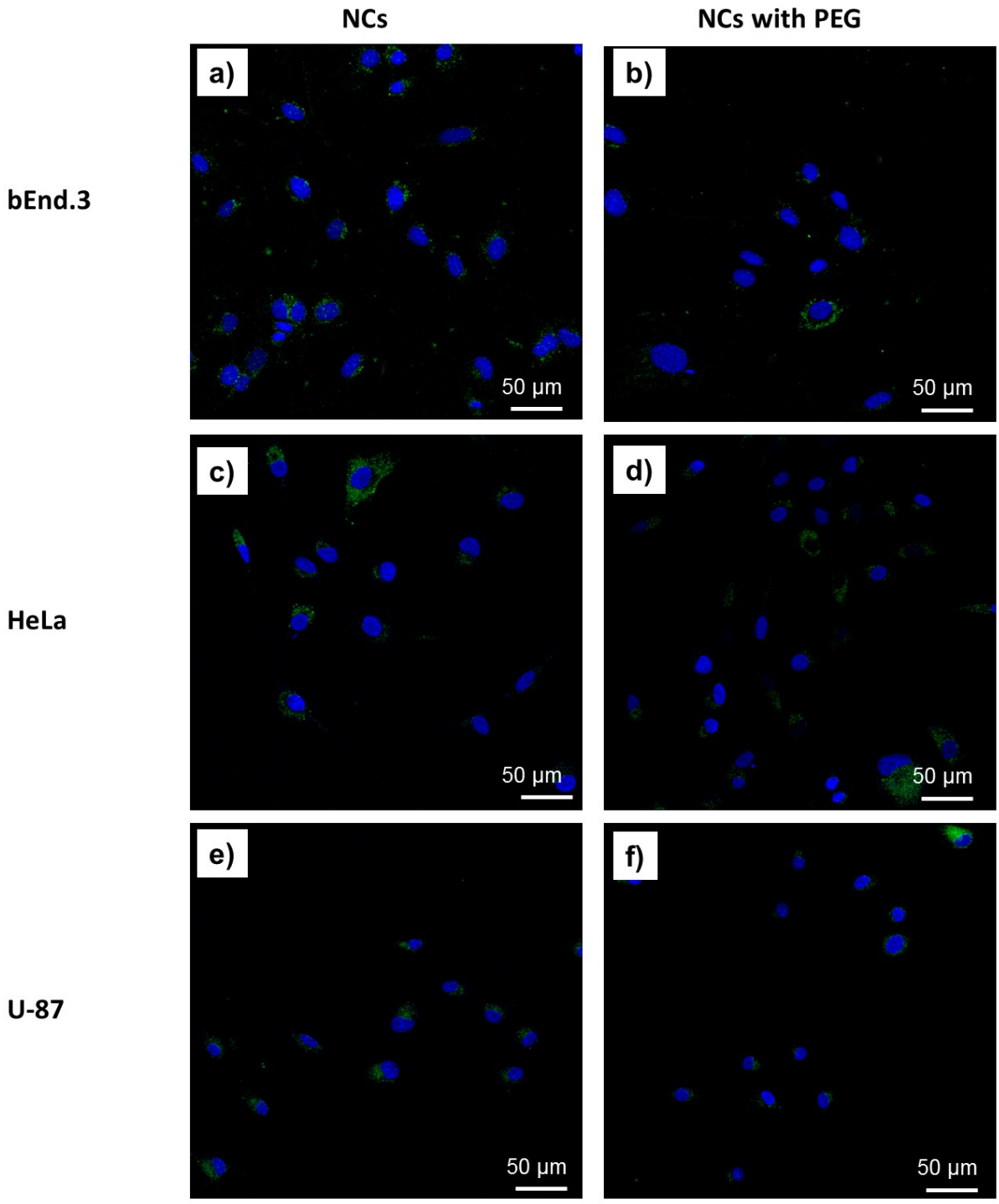

Figure S11 Confocal images of bEnd.3 (a and b), HeLa (c and d) and U87 (e and f) cells incubated with FITC-doped nanocapsules and PEGylated nanocapsules at the final concentration of 14.2 µg/ml for 2 h at 37 °C.

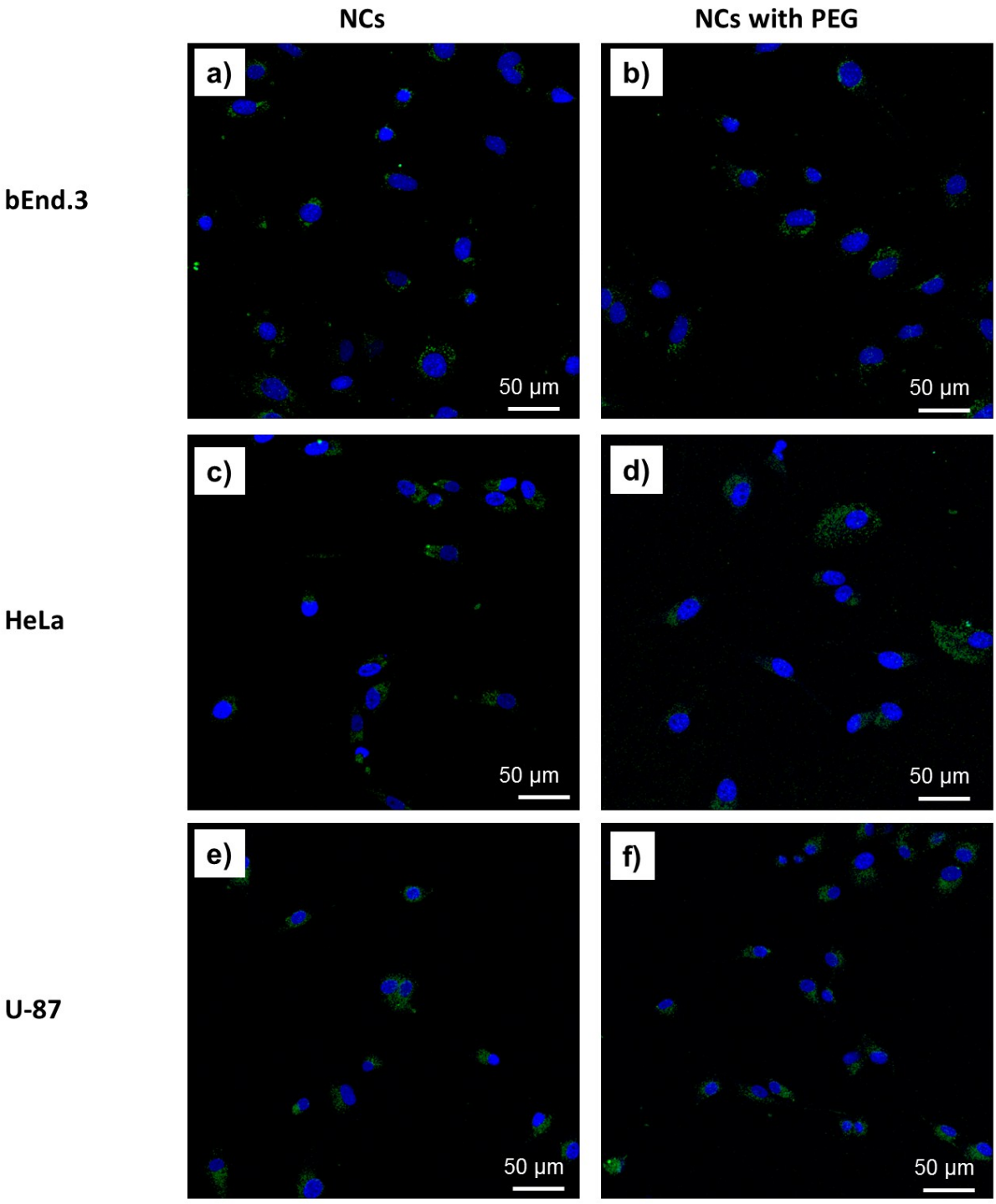

Figure S12 Confocal images of bEnd.3 (a and b), HeLa (c and d) and U87 (e and f) cells incubated with FITC-doped nanocapsules and PEGylated nanocapsules at the final concentration of 14.2 µg/ml for 4 h at 37 °C.