Electronic Supplementary Information (ESI)

Proper design of silica nanoparticles combine high brightness, lack of cytotoxicity and efficient cell endocytosis

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Contents

Photophysical measurements

UV-VIS absorption spectra were recorded at 25°C by means of Perkin-Elmer Lambda 45 spectrophotometer. The fluorescence spectra were recorded with a Perkin-Elmer Lambda LS 55 fluorimeter and with an Edinburgh FLS920 fluorimeter equipped with a photomultiplier Hamamatsu R928P. The same instrument connected to a PCS900 PC card was used for the Time Correlated Single Photon Counting (TCSPC) experiments. Quartz cuvettes with optical path length of 1 cm were used for both absorbance and emission measurements.

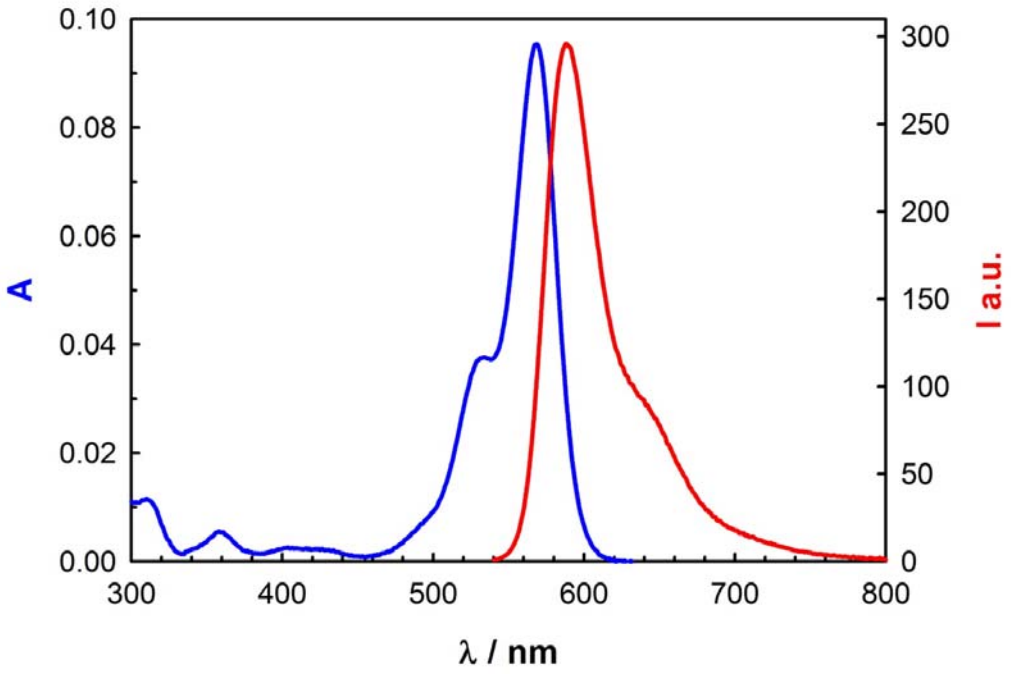


Fig. S1: Representative absorption and emission spectra of SiNPs in water.

Trasmission Electron Microscopy (TEM) images

A Philips CM 100 transmission electron microscope operating at 80 kV was used. For TEM investigations a 3.05 mm copper grid (400 mesh) covered by a Formvar support film was dried up under vacuum after deposition of a drop of nanoparticles solution diluted with water (1:50). The SiNP TEM images show that only the silica cores present sufficient contrast to appear in the images. The size distribution was obtained analyzing images with a block of several hundred

nanoparticles. The obtained histogram was fitted according to a Gaussian distribution obtaining the average diameter for the silica nanoparticles core.

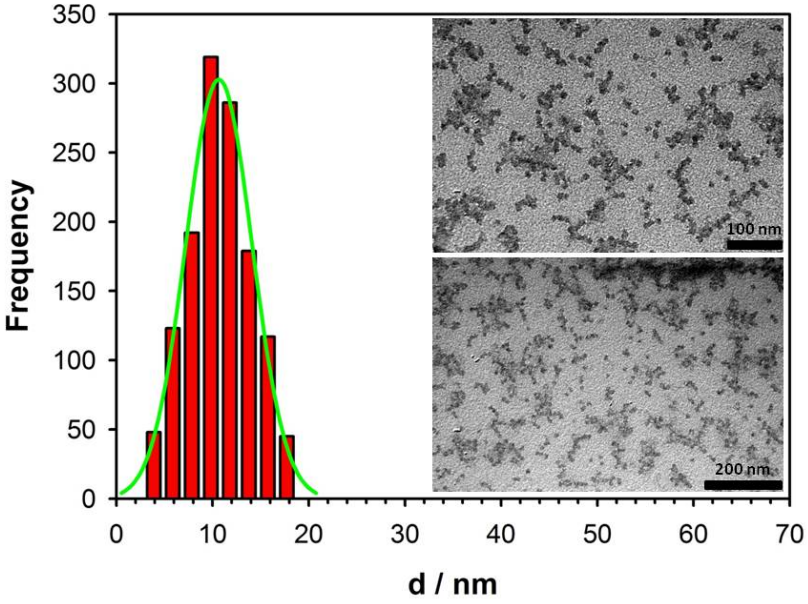


Fig. S2: TEM images of Core-shell NP-PEG (silica core size distribution, d = 11 ± 3 nm).

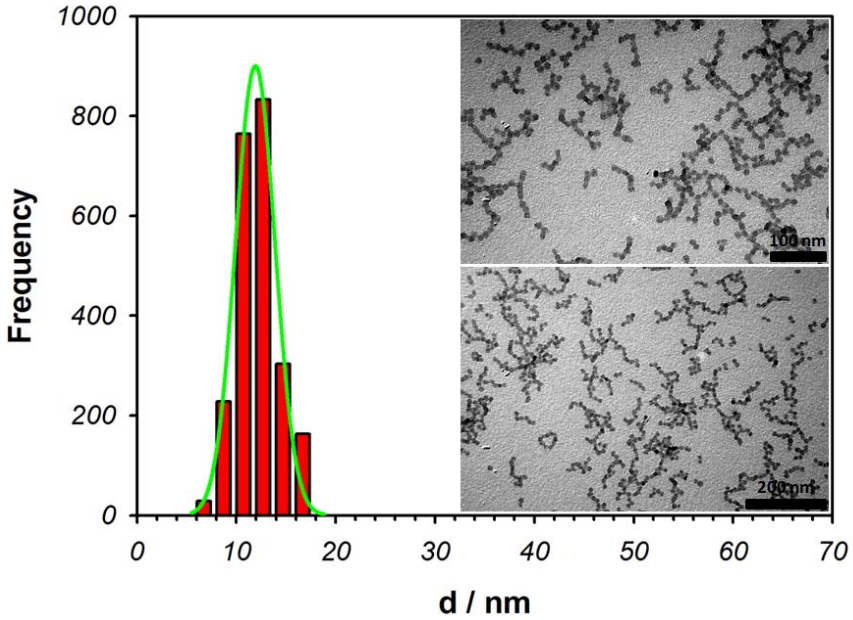


Fig. S3: TEM images of Core-shell NP-PEG-amino (silica core size distribution, d = 12 ± 2 nm).

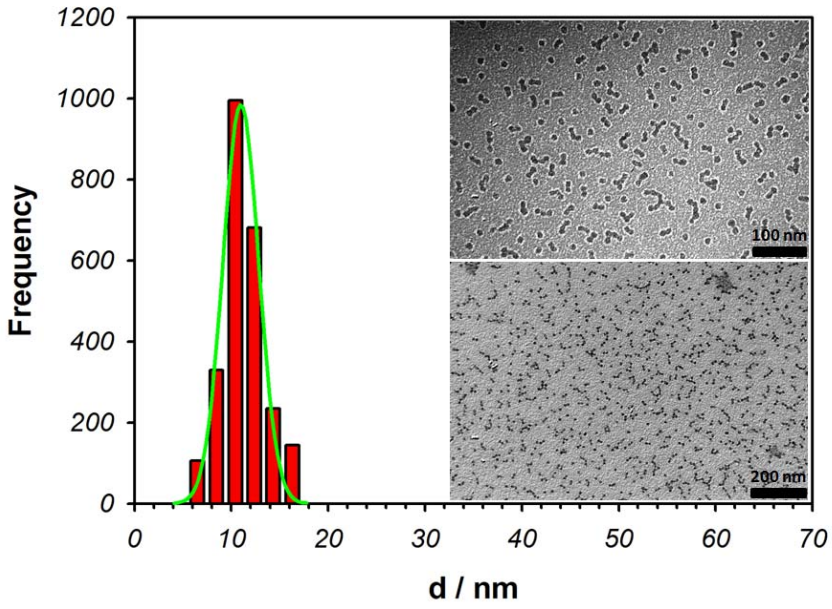


Fig. S4: TEM images of Core-shell NP-PEG-carbo (silica core size distribution, d = 11 ± 2 nm)

Dynamic Light Scattering

SiNP hydrodynamic diameter (d_H) distributions determination was carried out through Dynamic Light Scattering measurements with a Malvern Nano ZS instrument equipped with a 633 nm laser diode. Samples were housed in disposable polystyrene cuvettes of 1 cm optical path length, using water as solvent. The width of DLS hydrodynamic diameter distribution is indicated by Polydispersion Index (PdI). In case of a mono-modal distribution (gaussian) calculated by means of cumulant analysis, $PdI=(\sigma/Z_{avg})^2$, where σ is the width of the distribution and Z_{avg} is average diameter of the particles population respectively. DLS measurements showed no aggregation of the SiNPs even after several months.

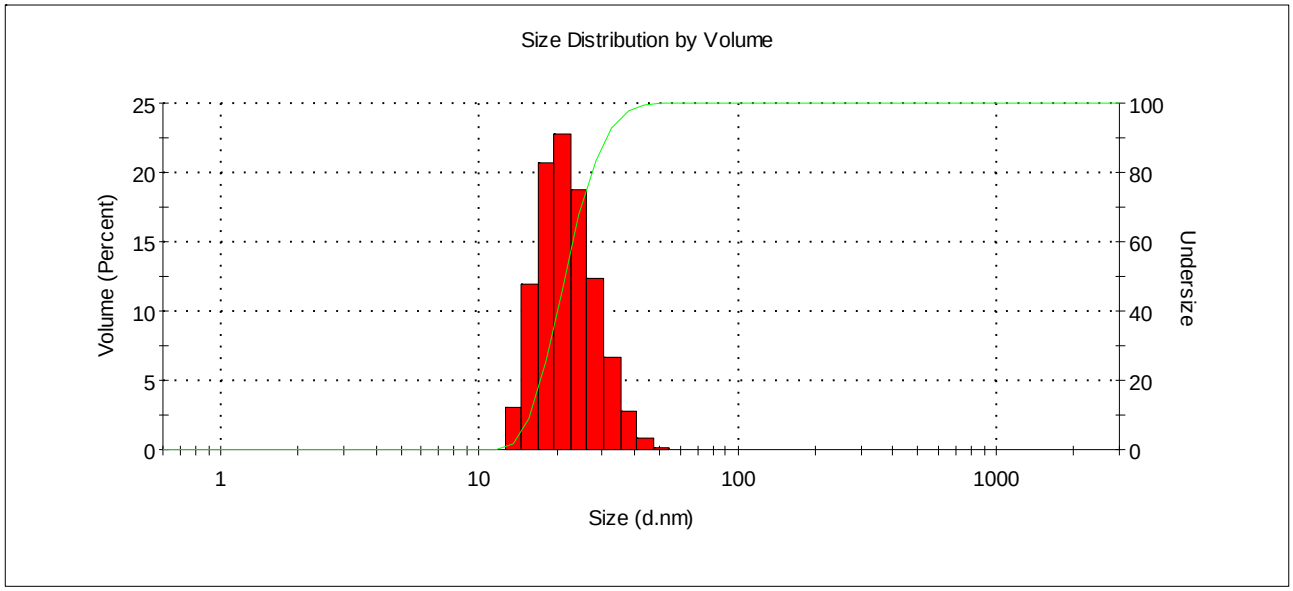


Fig. S5: Dynamic light scattering diameter distribution by volume and undersize curve for nanoparticles NP-PEG (d_H = 25 nm; PdI = 0.04, water, 25°C).

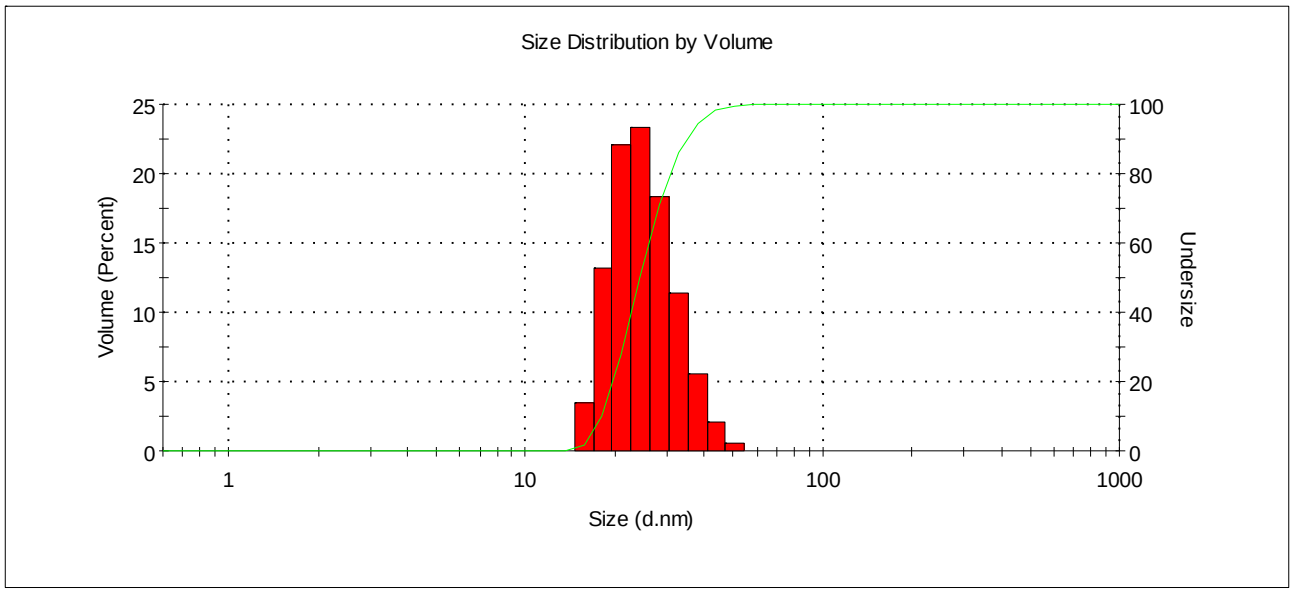


Fig. S6: Dynamic light scattering diameter distribution by volume and undersize curve for nanoparticles NP-PEG-carbo (d_H = 28 nm, PdI = 0.08, water, 25°C).

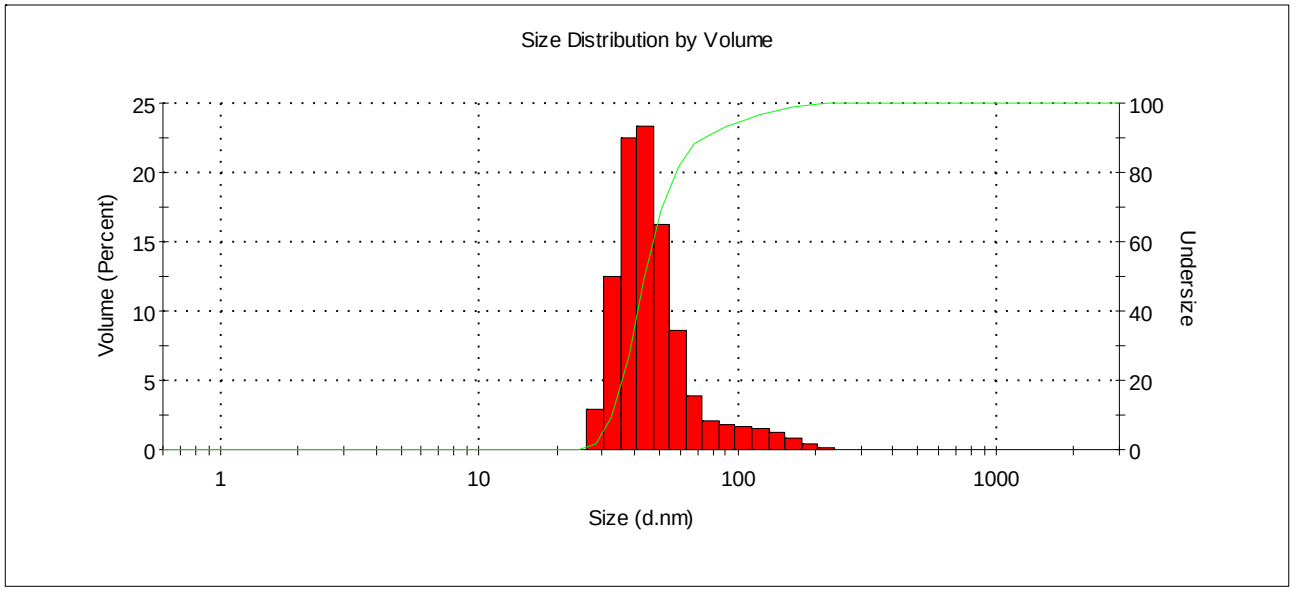


Fig. S7: Dynamic light scattering diameter distribution by volume and undersize curve for nanoparticles NP-PEG-amino (d_H = 69 nm; PdI = 0.20, water, 25°C). Main population Hydrodynamic diameter is 50 nm.

Materials

Pluronic F127, tetraethyl orthosilicate (TEOS, 99.99 %), chlorotrimethylsilane (TMSCl, ≥ 98 %), acetic acid (HOAc, ≥ 99.7 %), HCl (≥ 37 %), 4-(Dimethylamino)pyridine (DMAP, ≥ 99 %), N,N'-disuccinimidyl carbonate, (DSC, ≥ 98 %), 1,6-Diaminohexane **(**98 %), fluorescamine (Sigma, ≥ 98 %), reagent grade dimethylformamide (DMF), diethylether (Et_2O), dichloromethane and acetone were purchased from Sigma-Aldrich. Methoxypolyethylene glycol amine (PEG-NH_2, MW 750 g/mol), triethylamine (≥ 99.5 %), NaCl and Silica on TLC Alu foils (4 × 8 cm, with fluorescent indicator 254 nm) were purchased from Fluka. UF tubes Amicon Ultra-0.5mL, cut-off 100 KDa, were purchased from Millipore.

Dialysis was performed vs. water at room temperature under gentle stirring with regenerated cellulose dialysis tubing (Sigma, mol wt. cut-off > 12 KDa, avg. diameter 33 mm).

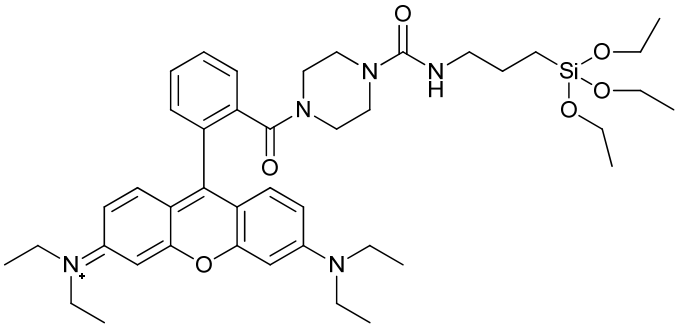
NMR measurements

NMR measurements were carried out with a Varian 200, 300 and 400 MHz spectrometer, using $CDCl_3$ as solvent. The ^{1}H-NMR and ^{13}C-NMR spectra of Pluronic F127 are reported as references.

Synthesis

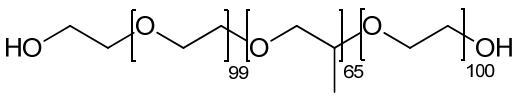
Triethoxysilane derivative R of Rhodamine B

The triethoxysilane derivative of Rhodamina B, **R**, was synthesized following reported procedures. [E. Rampazzo, S. Bonacchi, R. Juris, M. Montalti, D. Genovese, N. Zaccheroni, L. Prodi, D. C. Rambaldi, A. Zattoni, P. Reschiglian, *J. Phys. Chem. B*, 2010, **114**, 14605]



Scheme S1: chemical structure of triethoxysilane Rhodamine B derivative **R**.

Pluronic F127 (BASF)



Scheme S2: chemical structure of Pluronic F127.

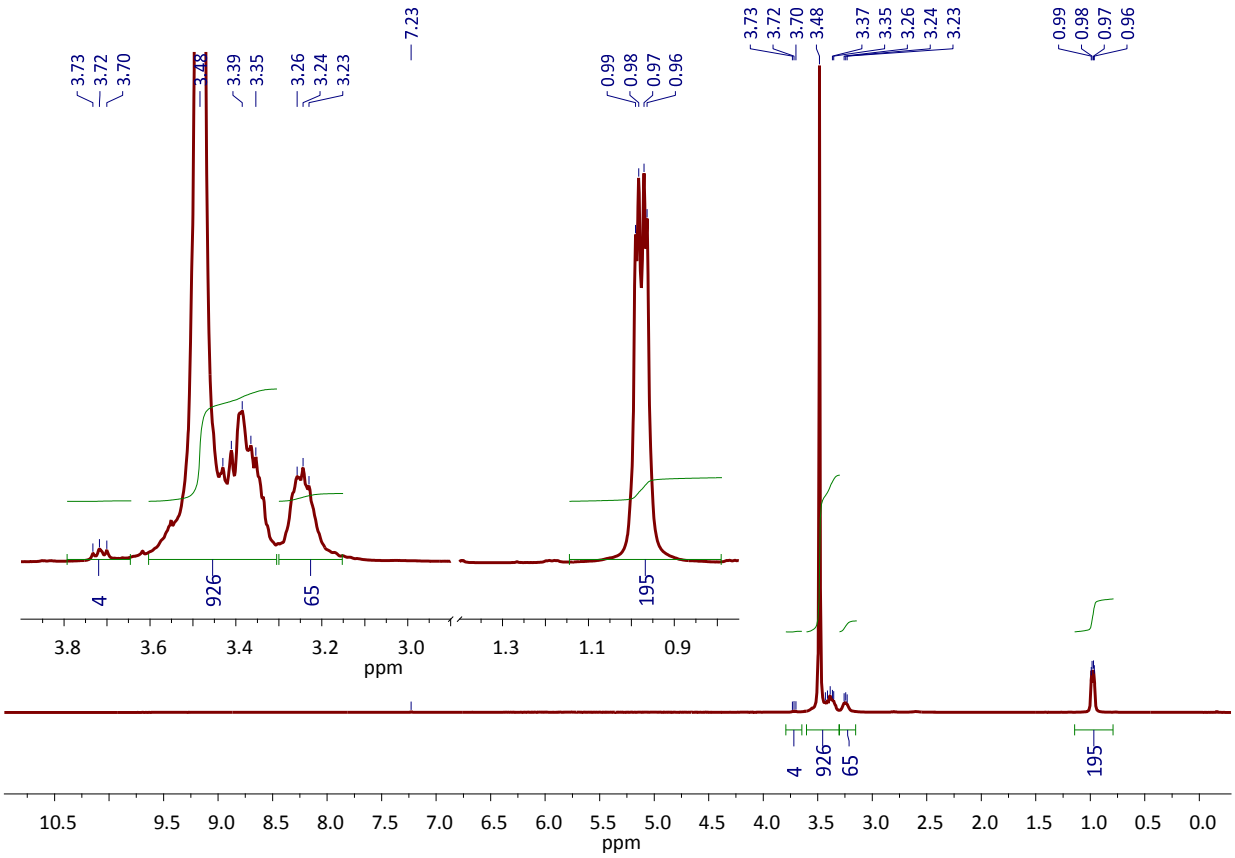


Fig. S8: ^{1}H-NMR spectra of Pluronic F127 ($CDCl_3$, 300 MHz).

^{1}H NMR (300 MHz, $CDCl_3$, 25°C, δ ppm): 3.71−3.74 (m, 4H, −CH_2CH_2OH); 3.49 (s, −OCH_2CH_2O−) and 3.36−3.42 (m, −OCH_2C−CH_3O−) ~926 H, 3.24−3.27 (m, −OCH$_2$CHCH$_3$O−) 65 H, 0.96-0.99 (q, −OCH$_2$CHCH_3O−) ~190H.

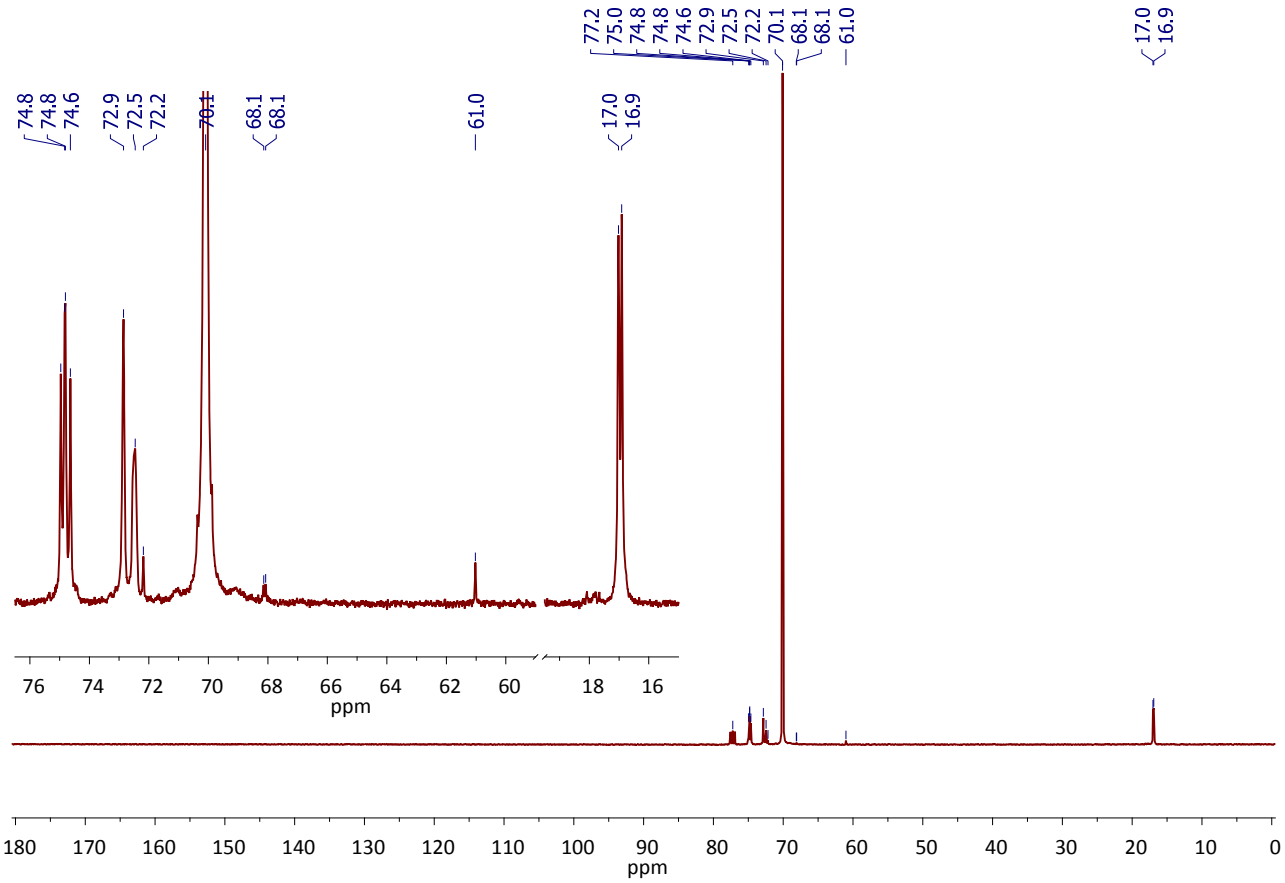
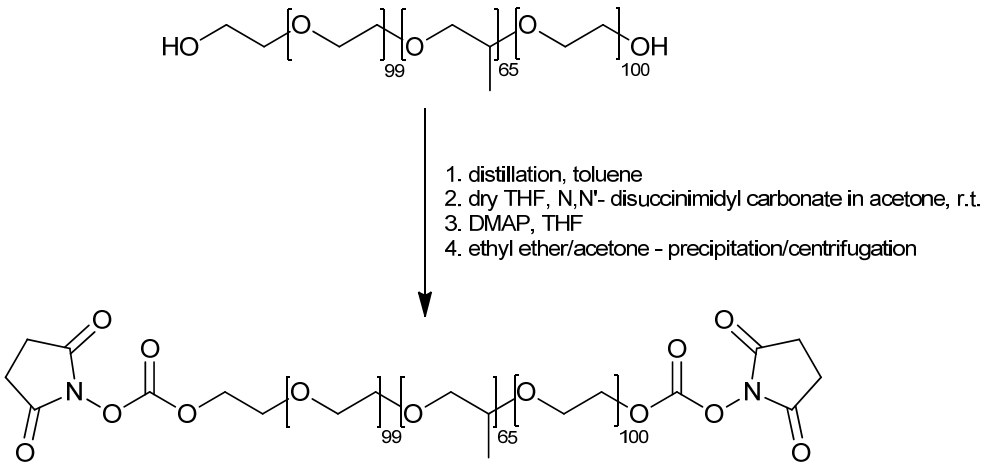


Fig. S9: ^{13}C-NMR spectra of Pluronic F127 ($CDCl_3$, 75.7 MHz).

^{13}C NMR (75.7 MHz, $CDCl_3$, 25°C, δ ppm): 75, 74.8, 74.6, 72.9, 72.5, 72.2, 70.1, 68.1, 61.0, 17, 16.9.

Pluronic F127 N-disuccinimidyl carbonate (F127-carbonate)

Pluronic F127-carbonate was synthesized adapting reported procedures [K. Huang, B. Lee, P. B. Messersmith, *Polymer Preprints*, 2001, **42**, 147]. A toluene solution of Pluronic F127 (4.01 g, 0.32 mmol, 1.0 eq, 100 mL) was distilled under reduced pressure at 50-60°C in a 250 mL round bottom flask. The residue was dried under vacuum and finally solubilized with 15 mL of dry THF. N,N'-disuccinimidyl carbonate (0.80 g, 3.1 mmol, 10 eq) solubilized in 7.0 mL di acetone was added. DMAP (0.41 g, 3.32 mmol, 10 eq) was then slowly added at room temperature under stirring by a dropping funnel.



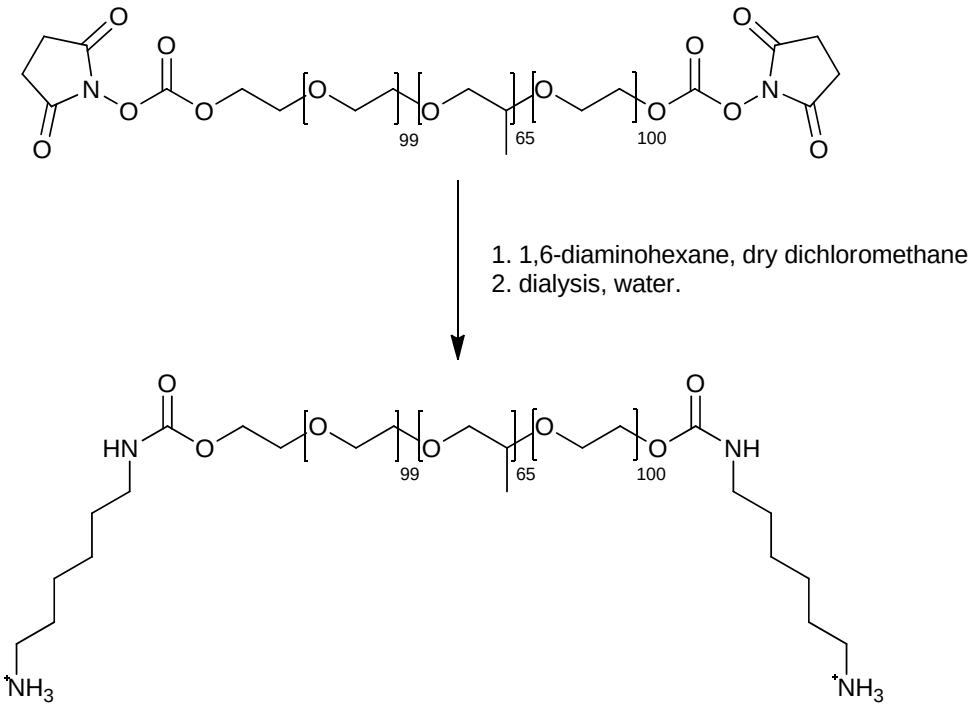
Scheme S2: synthesis of F127-carbonate (Pluronic F127 N-disuccinimidyl carbonate).

After 12 hours, Pluronic F127-carbonate was precipitated with ethyl ether and recovered by centrifugation (25 minutes, 5000 rpm). The precipitate was resuspended and centrifugated several times with acetone (20 mL), until no trace of the excess reagent (TLC, chloroform-methanol 5:1) was present. The product was finally dried under vacuum and directly used for amine functionalization.

F127-amino (Pluronic F127-di-yl bis((6-aminoesyl)carbamate))

Pluronic F127-amino was synthesized adapting reported procedures [K. Huang, B. Lee, P. B. Messersmith, *Polymer Preprints* 2001, **42**, 147]. In a flamed 100 mL round bottom flask dried under vacuum, 1,6-Diaminohexane (1.908 g, 16.4 mmol, 100 eq) was solubilized with 10 mL of dry dichloromethane. A 10 mL dichloromethane solution of F127-carbonate (2.0 g, 0.16 mmol, 1 eq) was then slowly added under stirring at room temperature.

After 3 hours the reaction mixture was distilled under reduced pressure and the residue was solubilized with 20 mL of water. The resulting solution was dialyzed vs. Milli-Q water (regenerated cellulose, 12 KDa) for about 20 h under gentle stirring. The dialyzed solution was evaporated under reduced pressure and finally dried under vacuum, obtaining 0.84 g of white solid (yield 42%).



Scheme S3: synthesis of F127-amino.

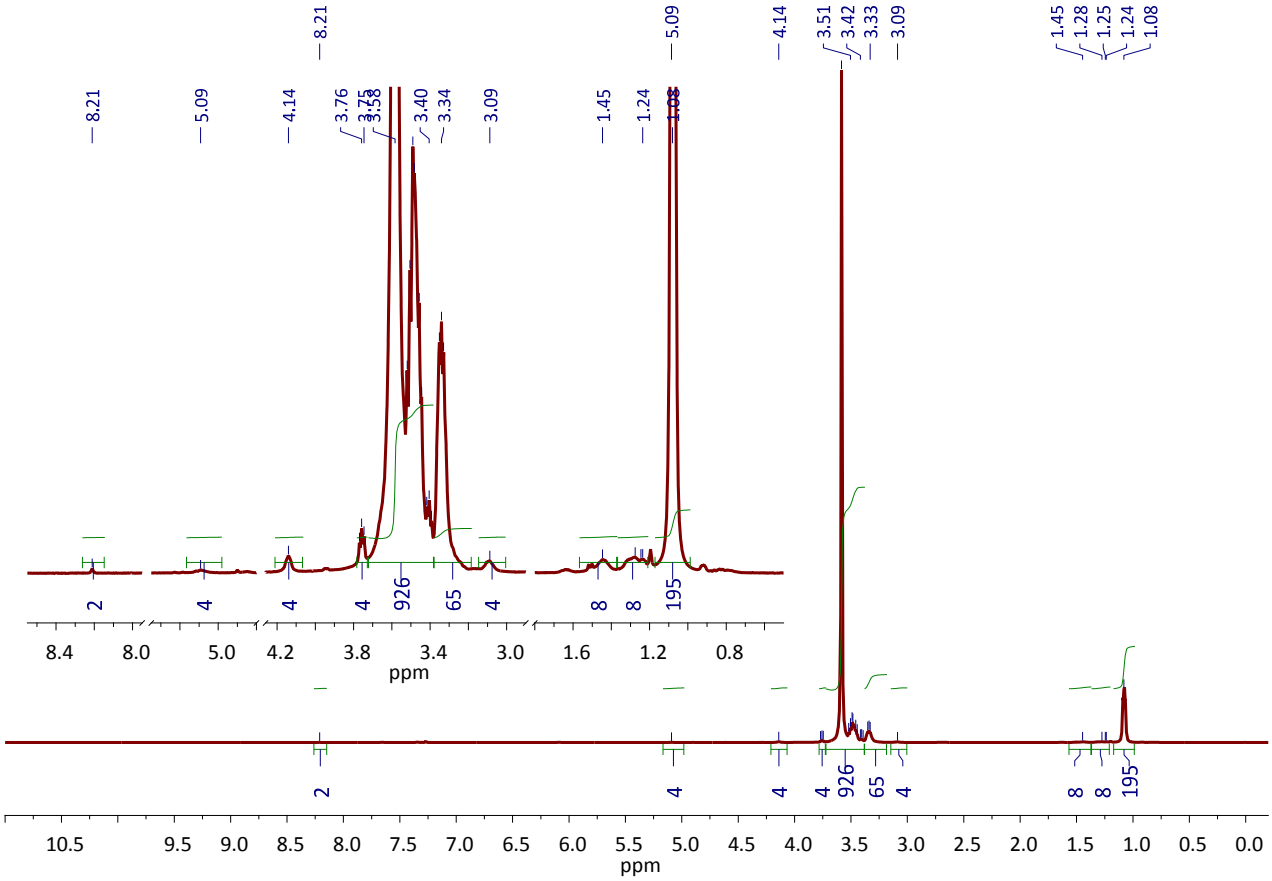


Figure S10: ^{1}H-NMR spectra of F127-amino ($CDCl_3$, 400 MHz).

^{1}H NMR (300 MHz, $CDCl_3$, 25°C, δ ppm): 8.21 (s, 2H, −N*H*CO−); 5.09 (s, 4H, −NH_2); 4.14 (s, 4H, −CH_2O−CO−); 3.75−3.76 (m, 4H, −CH_2CH$_2$OH); 3.58 (s, −OCH_2CH_2O−) and 3.42−3.51 (m, −OCH_2C−CH$_3$O−) ~ 926H, 3.33 (m, −OCH$_2$C*H*CH$_3$O−) 65 H, 3.09 (s, 4H, −CH_2NH$_2$); 1.45(s, 8H, −NHCH$_2$CH_2CH$_2$CH$_2$CH_2CH$_2$NH$_2$); 1.24−1.25 (s, 8H, −NHCH$_2$CH$_2$CH_2CH_2CH$_2$CH$_2$NH$_2$); 1.08 (s, −OCH$_2$CHCH_3O−) ~195H;

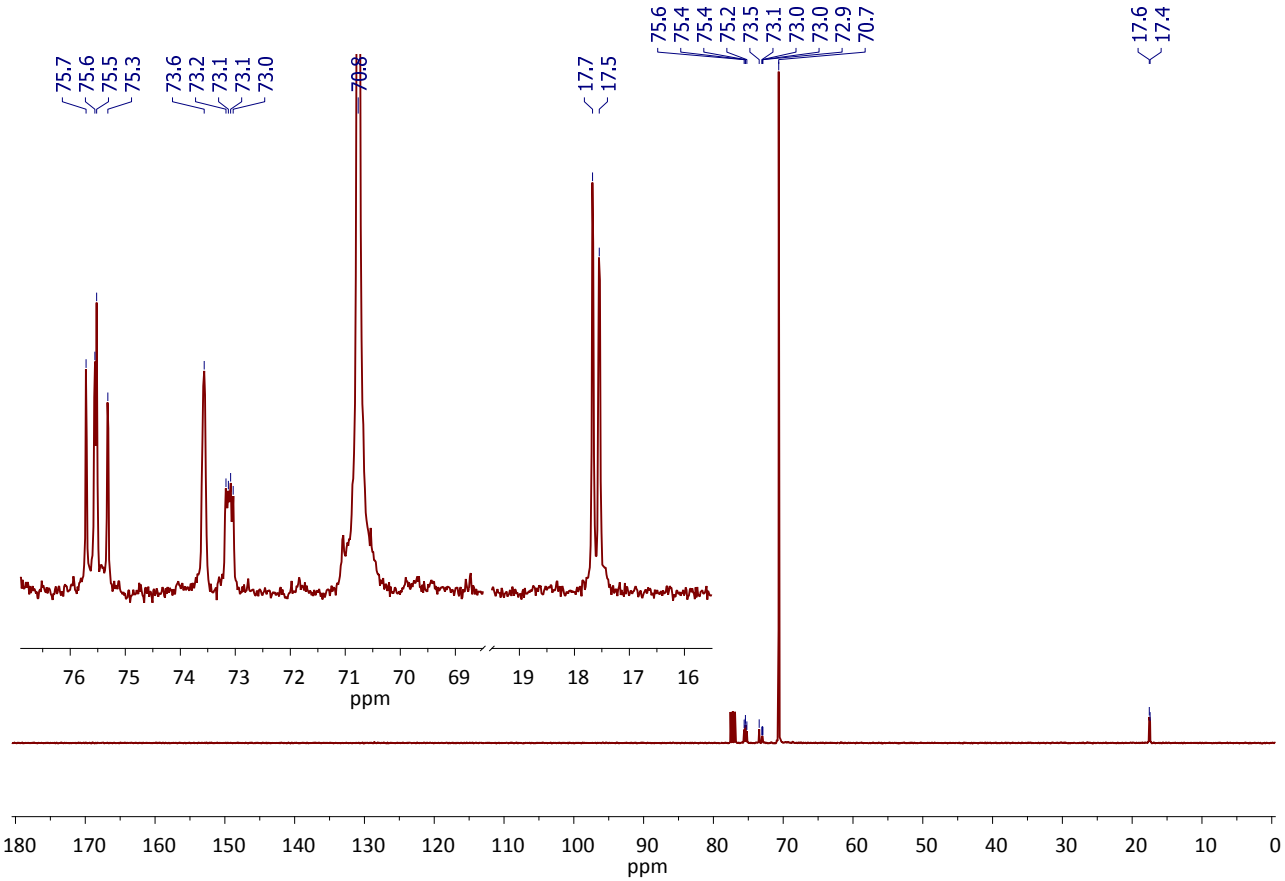
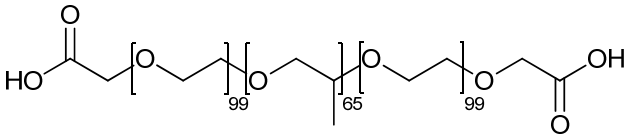


Figure S11: ^{13}C-NMR spectra of F127-amino ($CDCl_3$, 100 MHz).

^{13}C NMR (75.7 MHz, $CDCl_3$, 25°C, δ ppm): 75.7, 75.6, 75.4, 75.3, 73.6, 73.5, 72.2-73.0 (m), 70.0, 17.7, 17.5.

F127-carbo (dicarboxylic acid derivative of Pluronic®F127)

The pluronic F127 derivative F127-carbo was synthesized following reported procedures [M. Soster, R. Juris, S. Bonacchi, D. Genovese, M. Montalti, E. Rampazzo, N. Zaccheroni, P. Garagnani F. Bussolino, L. Prodi, S. Marchiò, *Int. J. Nanomed.* 2012, 74797].



Scheme S4: F127-carbo (dicarboxylic acid derivative of Pluronic®F127).

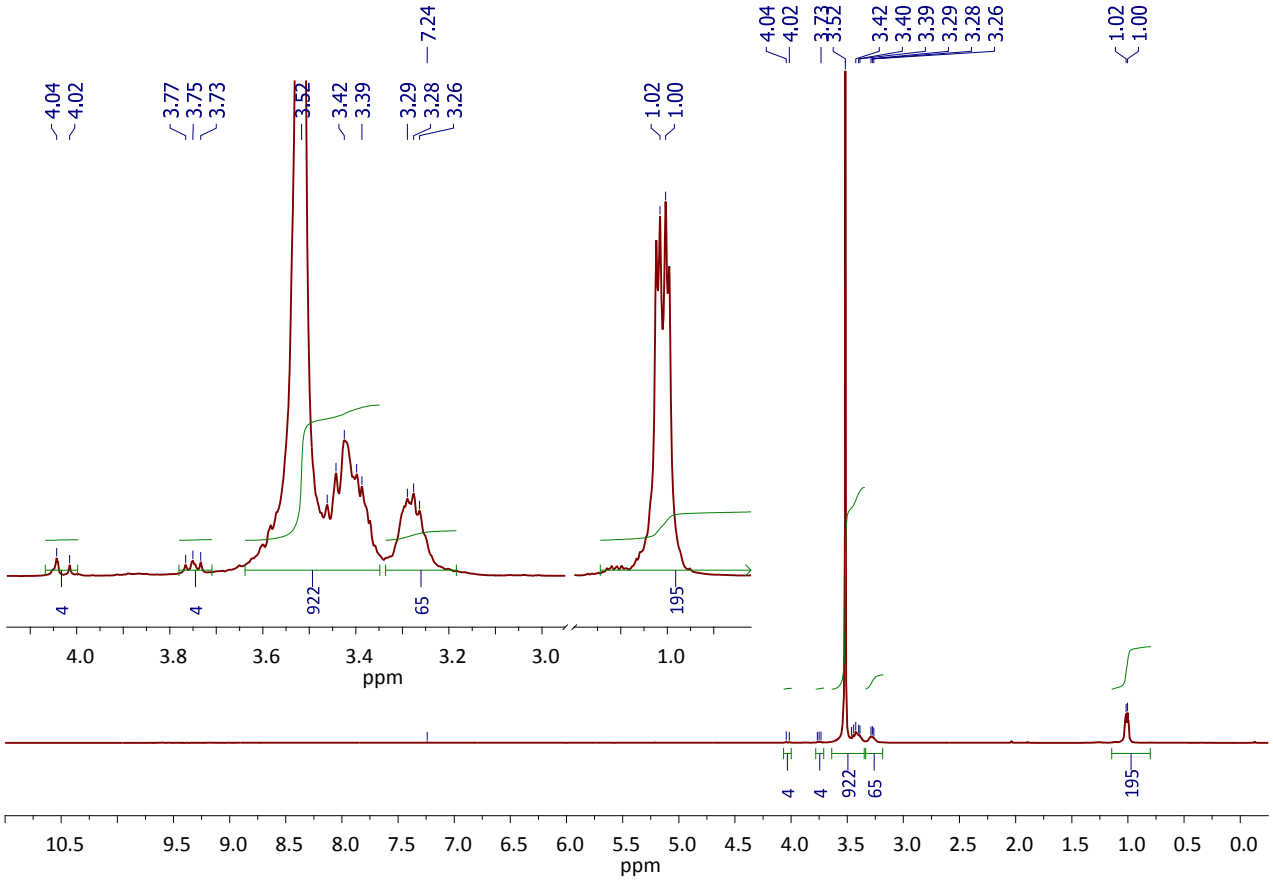


Figure S12: ^{1}H-NMR spectra of F127-carbo ($CDCl_3$, 300 MHz).

^{1}H NMR (300 MHz, $CDCl_3$, 25°C, δ ppm): 4.02−4.04 (d, 4H, −C*H*$_2$COOH); 3.73−3.77 (t, 4H, −OCH$_2$C*H*$_2$OCH$_2$COOH); 3.52 (s, −OC*H*$_2$C*H*$_2$O−) and 3.39−3.42 (m, −OC*H*$_2$C−CH$_3$O−) ~ 922H; 3.26−3.29 (m, −OCH$_2$C*H*CH$_3$O−) 65H, 1.00-1.02 (d, −OCH$_2$CHC*H*$_3$O−) ~ 195H;

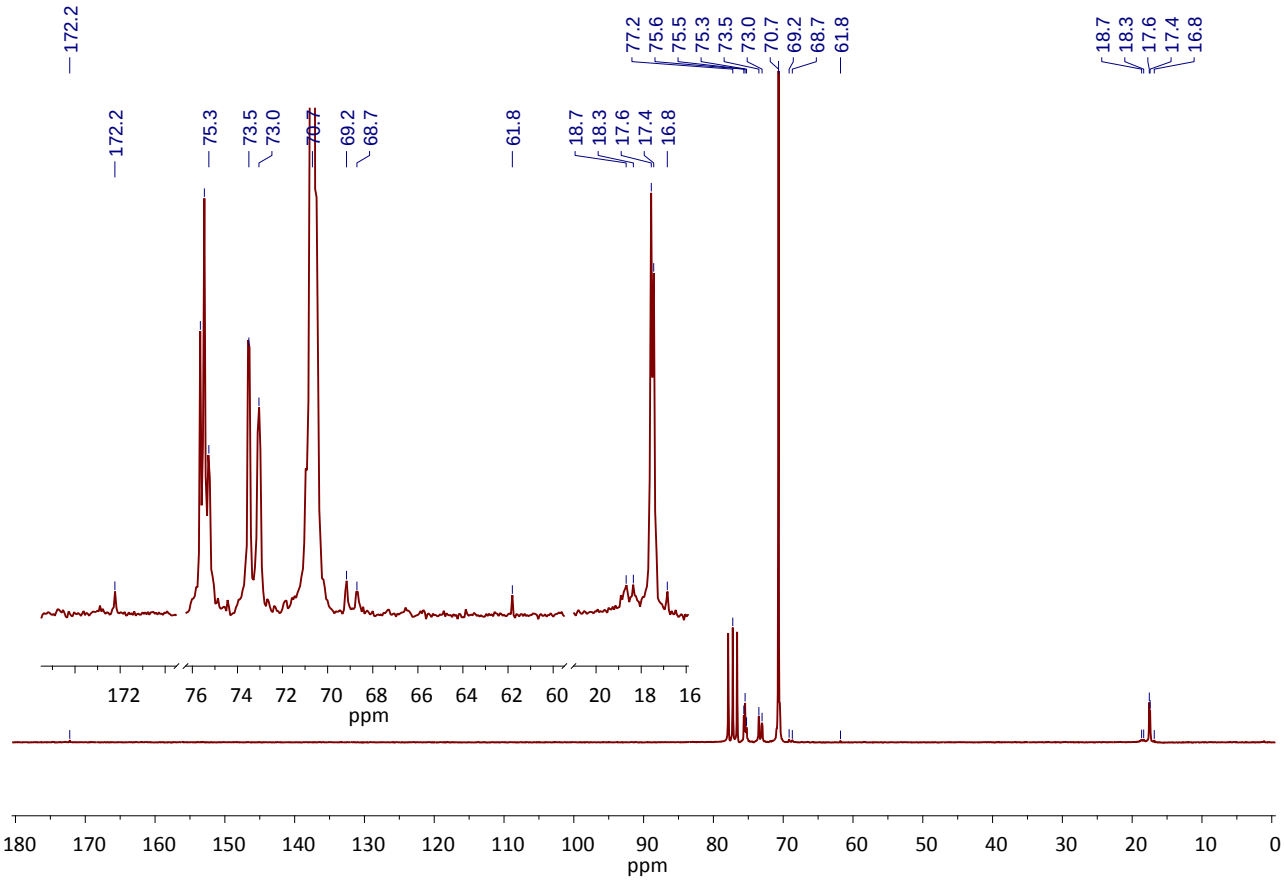


Figure S13: ^{13}C-NMR spectra of **F127-carbo** ($CDCl_3$, 50 MHz).

^{13}C NMR (75.7 MHz, $CDCl_3$, 25°C, δ ppm): 172.2, 75.6, 75.5, 75.3, 73.5, 73.0, 70.7, 69.2, 68.7, 61.8, 18.7,18.3, 17.6,17.4, 16.8.

IR (NaCl, thin solid film): −COOH 1735 cm^{-1}.

Determination of amine groups in F127-amino

Amine determination in the modified F127 surfactants and in the NPs solutions was made using fluorescamine as labeling reagent [R. Adamou, A. Coly, S. E. Douabalé, M. L. Ould Cheikh Ould Saleck, M. D. Gaye-Seye, A. Tine, *J. Fluoresc.*, 2005, **15**, 679].

To minimize the influence of the fluorescamine hydrolysis during the measurements, the fluorescamine reaction was carried out in organic solvent (DMSO). The following DMSO stock solutions were used: [Fluorescamine] = 10 mM, [PEG-NH_2] = 2 mM, [F127-amino] = 1 mM. The calibration curves were made using PEG-NH_2 (MW 750 g/mol) as standard reagent with a

concentration in the range 3-17 mM using the following procedure: the desidered amount of PEG-NH_2 solution (5-25 µL), 180 µL of DMSO and 50 µL of Fluorescamine solution were added in a polypropylene vial and mixed thoroughly for 8-10 min at room remperature. A Pluronic F127 aqueous solution was added (10 µL, 22 mg/mL) and the final mixture was then diluted to a total volume of 3000 µL with phosphate buffer (pH=8). Fluorescence spectra were recorded in the 400-650 nm range (λ_{ex} = 380nm). To measure the ratio of amine groups per molecule the measurements were carried with the same procedure, introducing 20 µL of F127-amino instead of PEG-NH2. This method provided a value of 1.8 ± 0.3 –NH_2 groups per surfactant molecule, corresponding to a derivatization yield of roughly 75 %. To check the stability of the carbamate moieties of derivatized F127-amino the measurements were carried out on a different set of experimental conditions that was used to simulate the NPs synthesis conditions. The procedure was the following: 70 mg of Pluronic F127, and 30 mg of F127-amino were dissolved in 1500 µL of HOAc 1 M/NaCl 0.85 M aqueous solution. The resulting mixture was stirred for 12 hours at 25°C, extracted with dichloromethane, dried with anhydrous Na_2SO_4, evaporated under reduced pressure and finally dried under vacuum. The reaction with fluorescamine was carried as already described on 60 µL of 1.0 mM surfactants mixture, giving similar results as previously reported.

Determination of amine groups in NP-PEG-amino

The fluorimetric determination of amino groups was carried out on an aliquot (50 µL, $2.2x10^{-5}$M) of SiNPs dispersion following a procedure similar to the one used for functionalized surfactants. To minimize water concentration in the reaction mixture and then the influence of fluorescamine hydrolysis, the SiNPs samples were concentrated to 15 µL (Amicon Ultra-0.5mL Millipore, cut-off 100 KDa, 10000 rpm). In a typical determination the concentrated aliquot of SiNPs (20 µL) was resuspended in DMSO (185 µL) and 40 µL of Fluorescamine solution as added. After incubation under stirring at room temperature (10 min) the mixture was diluted to 3000 µL with phosphate

buffer solution (pH 8.0) for fluorimetric measurements. The fluorimetric titration gave an average of 13 amine groups per SiNP.

ζ-Potential experiments

SiNPs ζ-Potential values were determined using a Malvern Nano ZS instrument. Samples were housed in disposable polycarbonate folded capillary cell (750 µL, 4 mm optical path length). Electrophoretic determination of ζ-Potential was made under Smoluchowski approximation in acqueous media at moderate electrolyte concentration.

Table S1: Values of ζ-Potential ± SD (n = 6) for SiNPs samples. Conditions: [NPs] = 2 µM, [PB] = 1 mM, [KCl] = 1 mM, pH 7.4, 25°C.

SiNPs sample	**ζ-Potential ± SD (mV)**
NP-PEG-amino	-4.3 ± 0.3
NP-PEG	-6.1 ± 0.5
NP-PEG-carbo	-9.6 ± 0.4

The introduction of functional groups in the SiNPs, by means of the modified surfactants Pluronic F127-amino and F127-carbo, seems to have a rather small influence on the overall surface charge of the SiNPs, as represented by the ζ-Potential values. This is probably due by the mobility of the surface charges and by the fact that the negative silica core is shielded by the PEG shell. The SiNPs architecture, in fact, place the charged functional groups outstretched outside from the nanoparticle surface, probably at the periphery of the diffuse layer surrounding the nanoparticle, where they are not able to influence thenanoparticle ζ-Potential.

However, as the experimental data suggest, the charged functional groups are still able to interact with cells membrane, conferring different internalization capabilities to these SiNPs.