

Supporting Information

Poly(propylene imine) dendrimer caps on mesoporous silica nanoparticles for redox-responsive release: smaller is better

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Experimental Section

Materials and methods

Chemicals were purchased from the following suppliers, purity is given in brackets: tetraethyl orthosilicate (TEOS) [98%], cetyltrimethylammonium bromide (CTAB) [$\geq 99.0\%$], dithiotreitol (DTT) [$\geq 99\%$], 3-mercaptopropionic acid [$\geq 99\%$], triethylamine [$\geq 99.5\%$], N-hydroxysuccinimide [98%], N,N'-dicyclohexylcarbodiimide [99%], (3-mercaptopropyl)trimethoxysilane (MPTMS) [95%], fluorescein disodium salt (FSS) [suitable for fluorescence], 5(6)-carboxyfluorescein (CF) [suitable for fluorescence], DMSO- d_6 [99.9 atom % D], chloroform-D [99.9 atom % D], anhydrous toluene [$\geq 99.7\%$], ethanol [$\geq 99.8\%$] from Sigma-Aldrich; methanol [$\geq 99.9\%$], sodium hydroxide [$\geq 99\%$], 2,2'-dipyridyl disulfide (DPDS) [$\geq 98\%$], acetic acid [99.8%], dichloromethane [$\geq 99.8\%$] from Merck; anhydrous acetonitrile [99.9%] from Acros; (3-aminopropyl)trimethoxysilane (APTMS) [97%] from ABCR; and dendrimers [approx. 95%] from DSM. BET (Brunauer–Emmett–Teller) surface area and BJH (Barrett–Joyner–Halenda) adsorption/desorption curves were acquired via N_2 adsorption/desorption isotherms using Micromeritics ASAP 2020 volumetric adsorption analyzer at 77 K. Scanning electron micrographs were acquired using Zeiss SUPRA 35VP scanning electron microscope. Low-angle diffractograms were acquired on PANalytical X-Pert PRO high-resolution diffractometer. UV-VIS spectroscopy measurements were performed using a PerkinElmer Lambda 900 instrument, while for fluorescence spectroscopy measurements a PerkinElmer LS 55 fluorescence spectrometer was used. 1H NMR spectra were acquired on a 300 MHz Varian and a 400 MHz Bruker NMR system. IR spectra were acquired on Bruker IFS 66/S instrument using KBr pellets. Mass spectroscopy measurements were carried out on Waters Q-TOF Premier instrument. Elemental analysis was performed on PerkinElmer Elemental Analyzer.

Linker syntheses

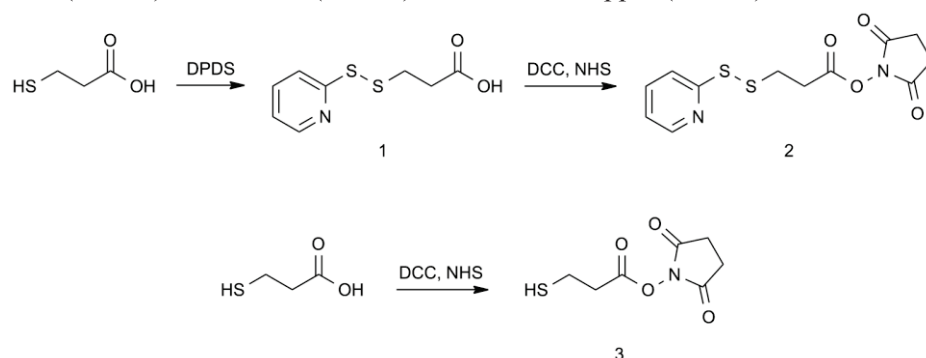
SPDP heterobifunctional linker¹ was synthesized according to the literature² in two steps depicted in Scheme S1.

3-(Pyridyldithio)propionic acid 1. 2,2'-Dipyridyl disulfide (DPDS) (2.08 g, 9.4 mmol, 2 equiv.) was dissolved in ethanol (17 mL) and acetic acid (0.27 mL, 4.7 mmol, 1 equiv.) was added and stirred at room temperature. 3-mercaptopropionic acid (0.50 g, 4.7 mmol, 1 equiv.) solution in EtOH (11 mL) was added slowly with development of yellow color. The solution was stirred overnight at room temperature. Next, the solvent was removed under reduced pressure and the remaining yellow oil was purified using silica gel flash chromatography. By-products were eluted with ethyl acetate:methanol:triethylamine 200:10:4 and the product (0.77 g, 76%) was eluted with ethyl acetate:methanol:acetic acid 200:20:1. δ_H (300 MHz, DMSO- d_6 , 25 °C) 2.63 (2H, t, $^3J(H,H)=7.00$ Hz), 3.01 (2H, t, $^3J(H,H)=6.87$ Hz), 7.21 - 7.29 (1H, m), 7.73 - 7.79 (1H, m), 7.79 - 7.87 (1H, m) and 8.44 - 8.48 ppm (1H, m).

N-Succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) 2. Pyridyldithiopropionic acid (0.40 g, 1.9 mmol, 1.0 equiv.) and N-hydroxysuccinimide (0.24 g, 2.0 mmol, 1.1 equiv.) were dissolved in anhydrous dichloromethane (9 mL). Next, N,N'-dicyclohexylcarbodiimide (0.42 g, 2.0 mmol, 1.1 equiv.) in anhydrous dichloromethane (3 mL) was added over 60 minutes and the mixture was stirred for 4 hours. The urea by-product was filtered off and the filtrate concentrated under reduced pressure and purified using a silica gel column (dichloromethane:methanol 50:1), yielding 0.29 g (49%). δ_H

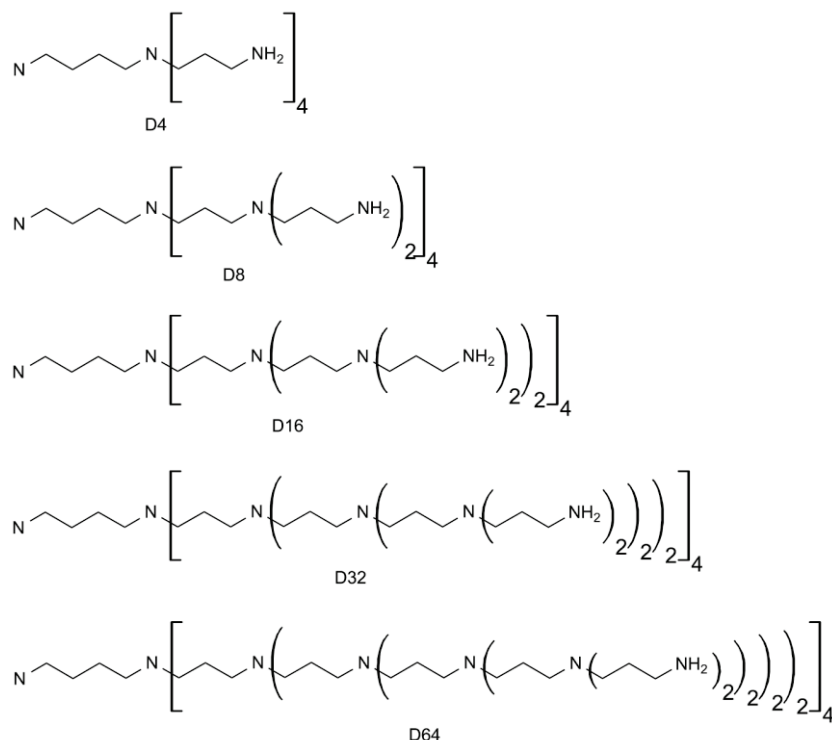
(300 MHz, DMSO-*d*₆, 25 °C) 2.78 - 2.86 (4 H, m), 3.10 - 3.28 (4 H, m), 7.23 - 7.31 (1 H, m), 7.73 - 7.88 (2 H, m) and 8.46 - 8.51 ppm (1 H, m).

N-succinimidyl-3-mercaptopropionate (SMP) 3. SMP was synthesized according to the literature³ as depicted in Scheme S1. N-hydroxysuccinimide (0.73 g, 6.3 mmol, 1.1 equiv.) was dissolved in anhydrous dichloromethane (150 mL) with stirring over 30 minutes. 3-mercaptopropionic acid (0.61 g, 5.7 mmol, 1 equiv.) was introduced into solution and N,N'-dicyclohexylcarbodiimide (1.30 g, 6.3 mmol, 1.1 equiv.) in dichloromethane (7 mL) was added slowly over 30 minutes at room temperature and stirred overnight. The urea by-product was filtered off and the filtrate concentrated under reduced pressure. The oily residue was purified using silica gel flash chromatography (dichloromethane:methanol 50:1). δ_{H} (400 MHz, CDCl₃, 25 °C) 1.85 (1 H, t, $^3J(\text{H,H})=8.66$ Hz), 2.83 - 2.88 (4 H, m), 2.88 - 2.91 (2 H, m) and 2.96 - 3.00 ppm (2 H, m).



Scheme S1. Synthesis path of SPDP **2** and SMP **3** linkers.

Dendrimer derivatization

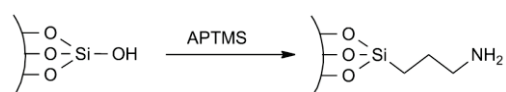


Scheme S2. Structural formulae of dendrimers D4 through D64.

MSN synthesis and grafting

MSN synthesis (S-0). MSNs were synthesized according to the procedure described by Li et al.⁴ CTAB (1.0 g) was dissolved in water (480 ml) with stirring. Afterwards, 2 M NaOH solution (3.5 ml) was added and the temperature adjusted to 353 K. TEOS (5 ml) was added dropwise in order to form a milky solution. After 2 hours the suspension was filtered and washed with water and methanol. As-synthesized MSNs were dried at room temperature overnight. The template was then removed using a solvent extraction. The nanoparticles were suspended in methanol (160 ml) and fuming hydrochloric acid (9 ml) was added dropwise. The suspension was stirred and refluxed overnight under inert atmosphere. After cooling to room temperature, the particles were filtered and washed with methanol. Solvent-extracted MSNs were dried at 353 K under high vacuum for 24 hours.

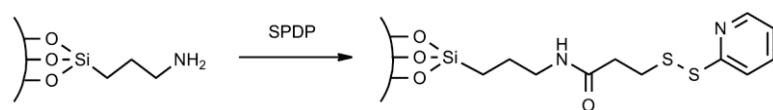
MSNs grafting with (3-aminopropyl)trimethoxysilane (APTMS) (S-1). The synthesis was performed following a well-known procedure.⁵ Solvent-extracted MSNs (S-0) (1.00 g) were suspended in anhydrous toluene (100 mL) and APTMS was added (see Scheme S3). The suspension was refluxed under inert atmosphere for 16 hours. The grafted nanoparticles were collected using filtration and washed with toluene and methanol, followed by drying under high vacuum. The product S-1 (1.04 g) was obtained.



Scheme S3. Grafting MSNs with APTMS.

MSNs grafting with (3-mercaptopropyl)trimethoxysilane (MPTMS) (S-4). The protocol described for the synthesis of APTMS nanoparticles was used also for MPTMS nanoparticles.

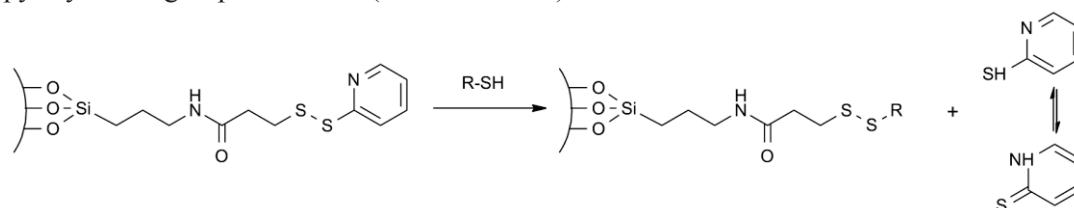
Functionalization with SPDP (S-2). The synthesis was in a similar manner as in the literature.⁵ A solution of SPDP (0.14 g, 0.44 mmol) in anhydrous acetonitrile (10 mL) was added to a suspension of amino-functionalized MSNs (S-1, 0.40 g) in anhydrous acetonitrile (30 mL) (see Scheme S4). The suspension was stirred at room temperature overnight. The material was then filtered and washed with acetonitrile and methanol. The product, denoted as S-2, was dried under high vacuum.



Scheme S4. Functionalization of S-1 with SPDP.

Loading with FSS and capping with dendrimer.

After the addition of dendrimer with mercapto groups disulfide exchange reaction took place with pyridyldithio groups on MSNs (see Scheme S5).



Scheme S5. Disulfide exchange reaction on silica surface. RSH - dendrimer with mercapto group.

Non-porous silica synthesis and grafting

NPS synthesis. NPS was synthesized via a modified Stöber protocol.⁴ 28% solution NH_4OH (8.00 mL) was diluted with ethanol (100 mL). TEOS (8.00 mL) was mixed with ethanol (20 mL) and added dropwise into the solution of NH_4OH while stirring. The reaction mixture was stirred overnight. The resulting precipitate was washed with centrifugation and dried under reduced pressure.

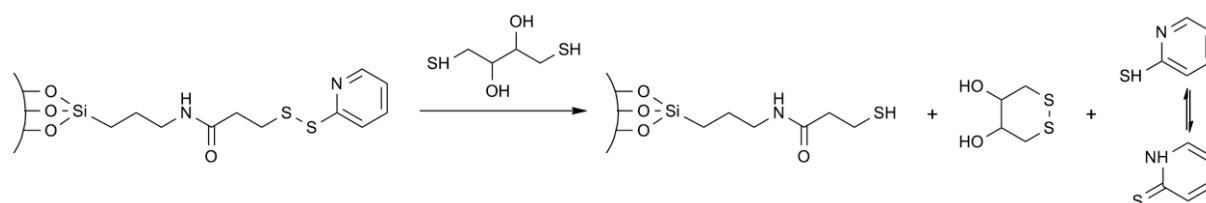
NPS grafting with AMPTS (NPS-1). NPS was decorated with aminopropyl moieties via procedure similar to MSN grafting. NPS (0.30 g) was suspended in anhydrous toluene (27 mL) and APTMS (0.053 mL) in anhydrous toluene (3 mL) was added. The suspension was refluxed under inert atmosphere for 16 hours. It was then allowed to cool down, filtered and washed with toluene and methanol. Product NPS-1 was dried under high vacuum.

Functionalization with SPDP (NPS-2). Amino-functionalized NPS (0.50 g) was suspended in anhydrous acetonitrile (3.75 mL), while SPDP (0.019 g) in anhydrous acetonitrile (1.25 mL) was added over 30 minutes with stirring. The suspension was stirred overnight and the precipitate washed with centrifugation to yield NPS-2.

Loading NPS with FSS (NPS-D8-(SH)₁). NPS-2 (5 mg) was suspended in methanol 2 mM solution of FSS and stirred for 24 hours. Then, a methanol solution of the D8-(SH)₁ was added and stirred for 3 days. Product NPS-D8-(SH)₁ was washed with H_2O and immediately used in release test.

Quantification of pyridyldithio groups

Accurately weighted material with pyridyldithio groups (~5 mg) was suspended in H_2O and excess of DTT (15 mg) was added (see Scheme S6). After stirring for 24 hours, the suspension was centrifuged, the supernatant diluted and analyzed spectrometrically. The absorption of released 2-thiopyridine was measured at 343 nm and the quantity calculated using molar extinction coefficient $\epsilon_{343} = 8.08 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$.⁶



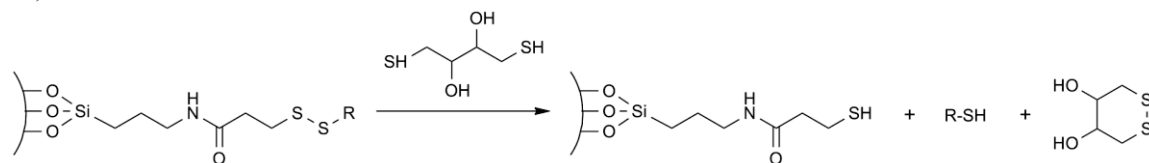
Scheme S6. Quantification of pyridyldithio groups on silica by reduction of disulfide bond with DTT.

Quantification of the dendrimer on MSNs

Systems S-D4-(SH)₂ through S-D64-(SH)₂ were synthesized in absence of the dye following the same procedure. After extensive washing and drying the obtained solid was characterized by elemental analysis.

Release test

Reducing agent DTT was used to cleave disulfide bonds connecting dendrimer and MSN (see Scheme S7).



Scheme S7. Reduction of disulfide bond connecting dendrimer to MSN with DTT, R = dendrimer.

Release test with pH increase was performed with addition of dilute NaOH solution to suspension of dye-loaded MSNs up to final pH of 9.5 and volume of 6.0 mL. After 20 minutes aliquots were taken, centrifuged and subjected to fluorescence measurement at 520 nm using excitation wavelength of 490 nm.

Quantification of dye

Dye-loaded MSNs were dissolved in concentrated NaOH solution. Next, the obtained solution was neutralized, pH adjusted to 7.6 and diluted to final volume of 6.0 mL. Aliquots were taken, diluted with water and concentration of the dye determined with fluorescence measurement at 520 nm using excitation wavelength of 490 nm.

Results

MSNs characterization

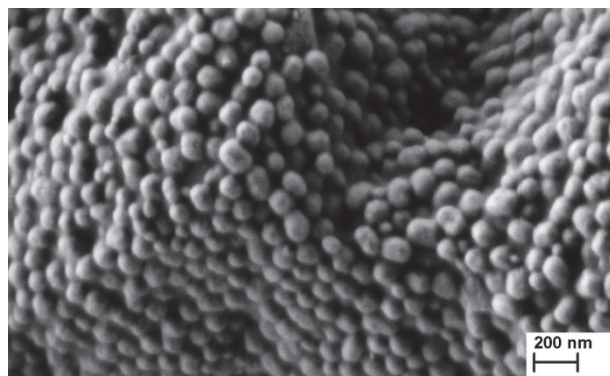


Fig. S1. Scanning electron micrograph of solvent-extracted MSNs.

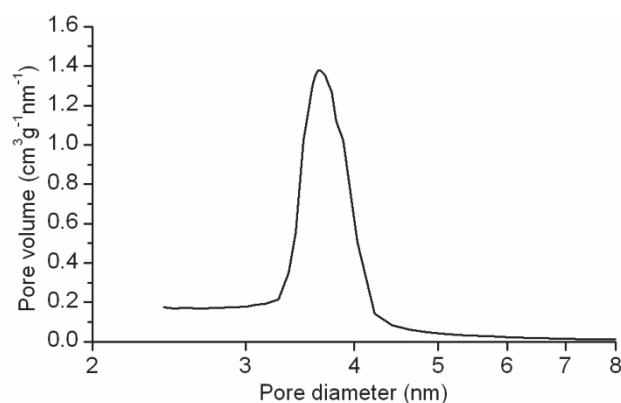


Fig. S2. Narrow pore diameter distribution of solvent-extracted MSNs with peak at 3.7 nm.

Dendrimer derivatization

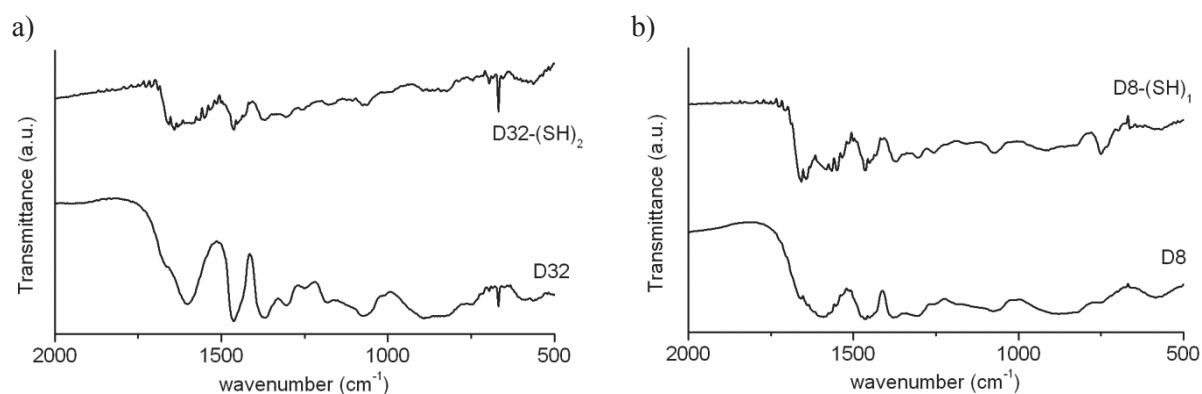


Fig. S3. FTIR of a) D32 and SMP-derivative D32-(SH)₂ and b) D8 and SMP derivative D8-(SH)₁. D32-(SH)₂ and D8-(SH)₁ show a characteristic peak at 1653 cm⁻¹ in amide I band.

Dendrimer content on MSNs

Dendrimer content on functionalized nanoparticles was determined by elemental analysis.

Table S1. Quantity of C, H and N atoms on S-2 and dendrimer-functionalized MSNs in weight percentage.

sample	C (%)	H (%)	N (%)
S-2	9.50	2.18	1.57
S-D4-(SH) ₂	10.60	2.52	2.17
S-D8-(SH) ₂	17.13	3.55	3.99
S-D16-(SH) ₂	16.78	3.65	4.12
S-D32-(SH) ₂	14.58	3.39	3.41
S-D64-(SH) ₂	16.22	3.47	3.76

Table S2. Quantity of C, H and N atoms on S-2 and dendrimer-functionalized MSNs in mmolg⁻¹.

sample	C (mmolg ⁻¹)	H (mmolg ⁻¹)	N (mmolg ⁻¹)
S-2	7.91	21.63	1.12
S-D4-(SH) ₂	8.83	25.00	1.55
S-D8-(SH) ₂	14.26	35.22	2.85
S-D16-(SH) ₂	13.97	36.21	2.94
S-D32-(SH) ₂	12.14	33.63	2.43
S-D64-(SH) ₂	13.50	34.42	2.68

Quantification of the dye and release tests with pH increase of S-D4-(SH)₂ through S-D64-(SH)₂

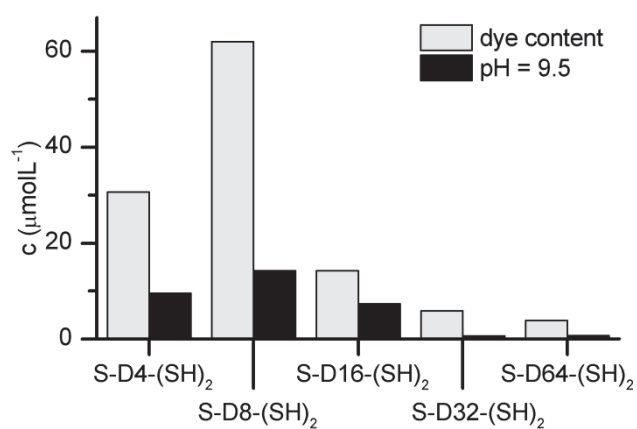


Fig. S4. Quantity of dye in the final materials and dye release with pH increase from systems S-D4-(SH)₂ through S-D64-(SH)₂.

Release tests of S-D4-(SH)₂ through S-D64-(SH)₂

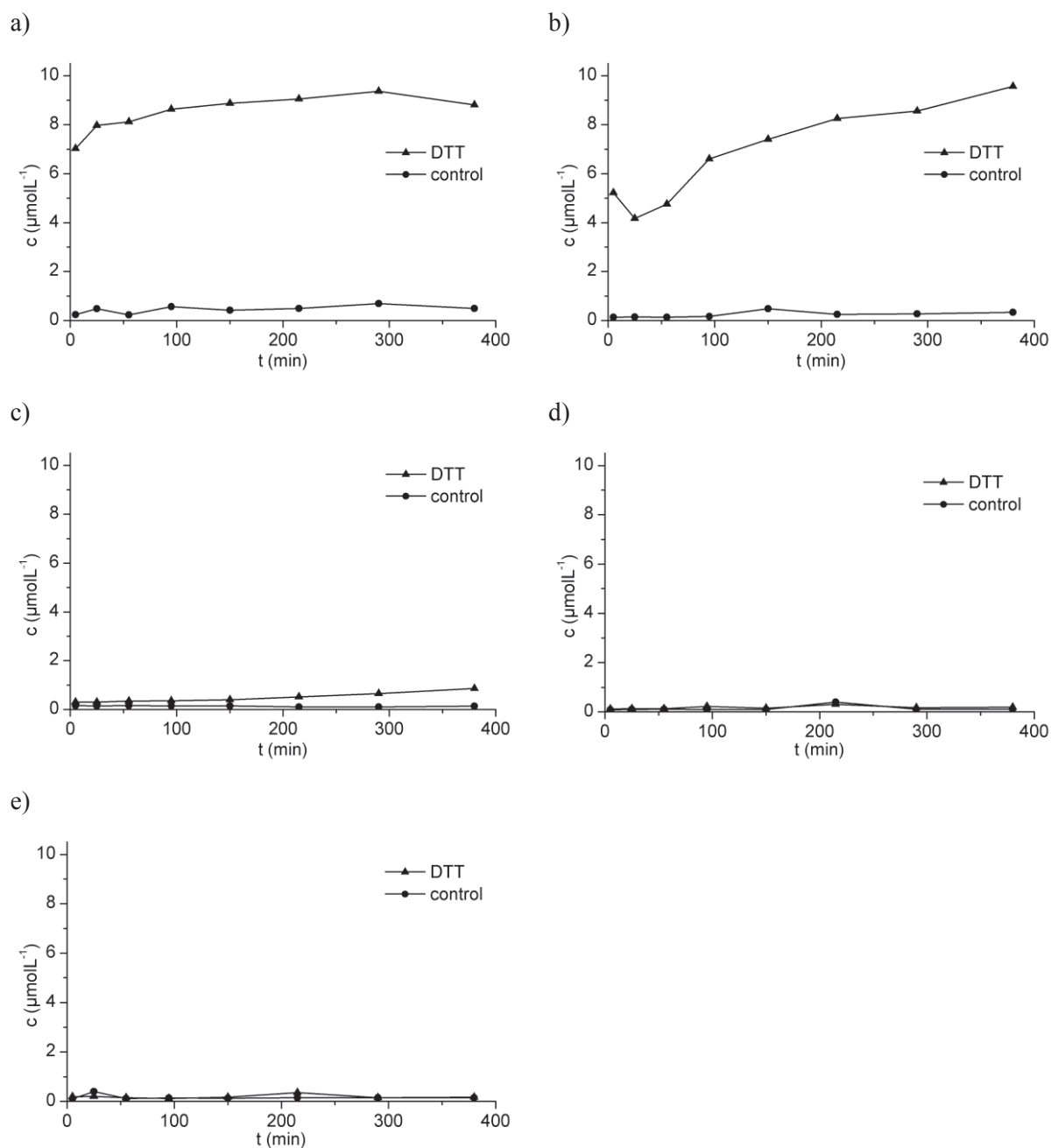


Fig. S5. Release test of the dye from S-D4-(SH)₂ systems through S-D64-(SH)₂ performed in presence and absence of DTT: a) S-D4-(SH)₂, b) S-D8-(SH)₂, c) S-D16-(SH)₂, d) S-D32-(SH)₂, e) S-D64-(SH)₂.

Release tests of systems with D4 and D64

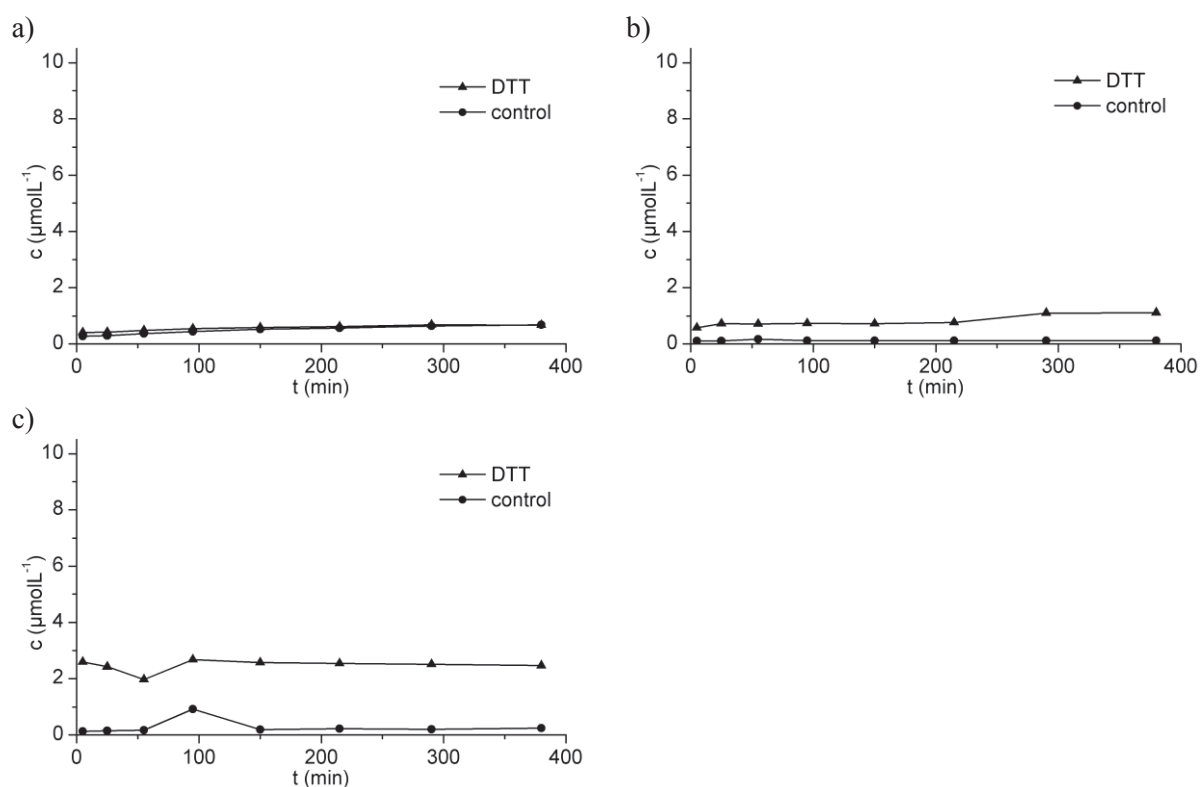


Fig. S6. Release test of the dye from systems based on D4 and D64 performed in presence and absence of DTT: a) S-D4-(SH)₀, b) S-D4-(SH)₁ and c) S-D64-(SH)₈.

Release tests of systems with D8

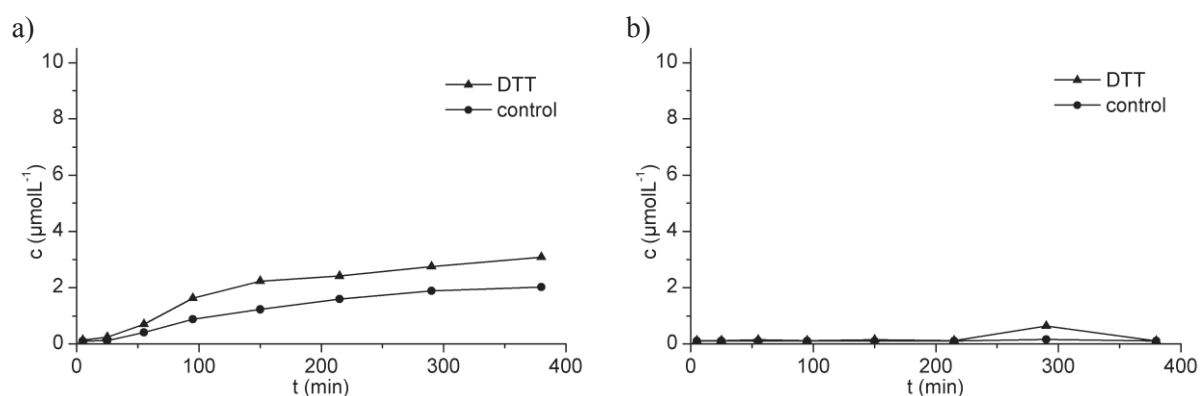


Fig. S7. Release of FSS from systems based on D8 performed in presence and absence of DTT: a) S-D8-(SH)₀ and b) NPS-D8-(SH)₁.

NPS characterization

NPS nanoparticles were spherical with diameter below 100 nm (Fig. S 5).

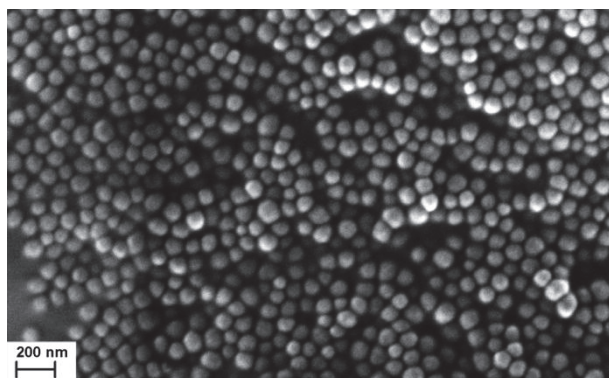


Fig. S8. Scanning electron micrograph of as-synthesized NPS nanoparticles.

Release tests of S-5 with D4 through D64 dendrimers

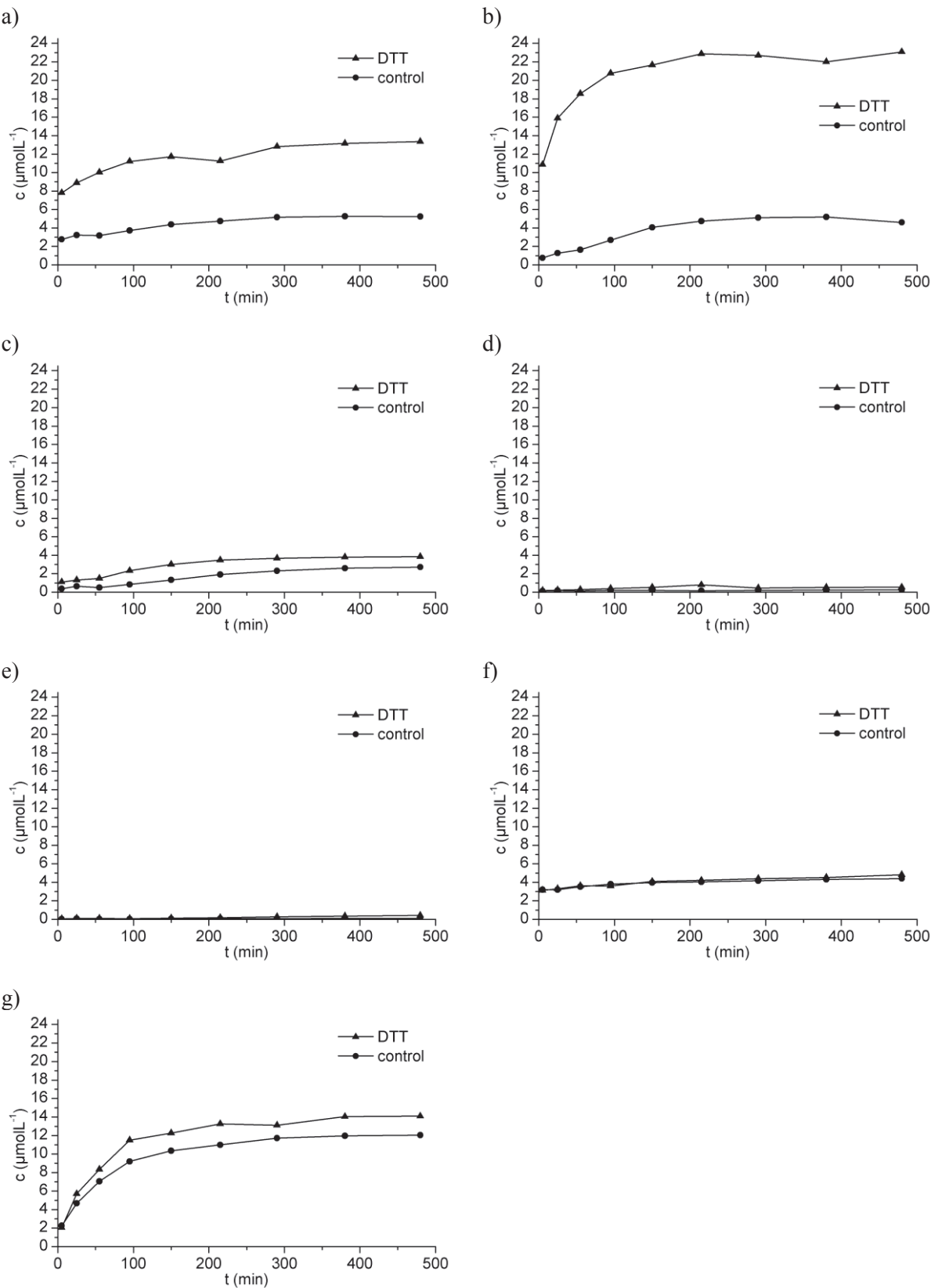


Fig. S9. Released CF from the S-5 family in the presence and absence of DTT, using the dendrimers: a) D4, b) D8, c) D16, d) D32, e) D64, f) D4 without SPDP and g) D8 without SPDP.

References

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