

Supplementary Information

Size-tunable supramolecular nanoparticles mediated by ternary cucurbit[8]uril host-guest interactions

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1. Materials and Equipment

Starting materials for organic synthesis were obtained from Sigma-Aldrich and used as received. Highly branched poly(ethylene imine) (PEI, MW = 10kD) was purchased from Polysciences Inc. and contained primary, secondary, and tertiary amine groups in a ratio of 25/50/25. 1-(1-Undecyl-11-thiol)-1-methyl-4,4-bipyridinium and 2-naphthoxy- terminated poly(ethylene glycol) monomethyl ether were prepared according to literature procedures.^{S1, 2} The supramolecular host CB[8] was purchased from Acros and Strem Chemicals and its purity determined via microcalorimetry against paraquat. For dialysis and preparation of SNPs, distilled water was purified by MilliQ Advantage A10, Millipore R=18.2 MΩ before usage. The synthesized products were analyzed by ¹H-NMR on a Bruker 400 MHz and 600 MHz system. The samples were dissolved in deuterated solvent purchased from Cambridge Isotope Laboratories Inc. Mass analysis was done using the matrix-assisted laser desorption ionization (MALDI) and electrospray ionization using a Voyager DE-RP and a micromass LCT from Waters, respectively. Dynamic light scattering analysis was carried out on a Zetatrac and Nanotrac by Anaspec operating with a Microtrac FLEX Operating Software. Furthermore, the supramolecular NPs were analyzed by a Carl-Zeiss high 1500 resolution scanning electron microscope (hrSEM). Calorimetry was performed on a Microcal VP-ITC. UV/Vis and fluorescence measurements were carried out respectively on a Perkin Elmer UV/Vis spectrometer Lambda 850 and on a Perkin Elmer fluorescence spectrometer LS55.

2. Synthesis and characterization of guest-functionalized building blocks

Synthesis of naphthol-poly(amido amine) (PAMAM)

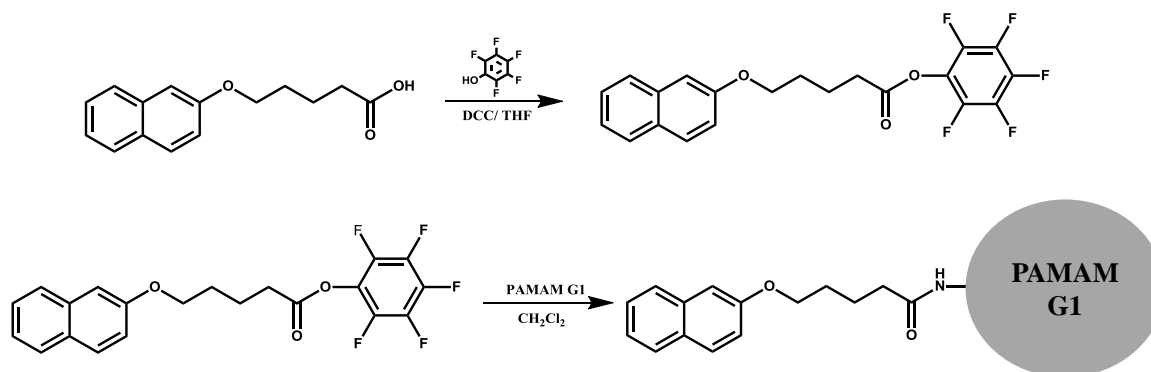


Figure S 1: a) Synthesis of 2-naphthol-1-hexanoic acid-pentafluorophenylester and b) Schematic synthesis of Np₈-PAMAM

2-naphthol-1-hexanoic acid was synthesized as previously reported.^{S3} Carboxylic acid activation was carried out by dissolving 2-naphthol-1-hexanoic acid (0.5 g, 2.05 mmol) in 230 mL THF. Afterwards pentafluorophenol (0.509 g, 2.77 mmol) and DCC (0.570 g, 2.77 mmol) were added and the solution subsequently stirred for 2 days at RT. THF was removed in vacuum and the residue dissolved in CH₂Cl₂ and washed three times with saturated NaHCO₃ solution. The organic layer was dried with MgSO₄ and the solvent evaporated before the 2-naphthol-1-hexanoic acid-pentafluorophenylester was purified by flash column chromatography over silica gel with CH₂Cl₂ as eluent (R_F=0.83). Yield: 0.198 g (25%). ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 7.78-7.70 (m, 3H), 7.46-7.29 (m, 2H), 7.21-7.13 (m, 2H), 4.15 (t, 2H), 2.80 (t, 2H), 2.01 (m, 4H). Mass: (ESI) calculated: 410.1 (M), found: 411.3 (M+H)⁺.

To prepare the naphthol-terminated PAMAM dendrimer, 125 mg 20wt% solution of PAMAM dendrimer in methanol, ethylenediamine core, generation 1.0 solution (0.017 mmol) was weighed and the MeOH was evaporated. The residue was redissolved in 40 mL CH₂Cl₂ and 2-naphthol-1-hexanoic acid-pentafluorophenylester (0.074 g, 0.178 mmol) was added. The solution was stirred for 24 h at RT under nitrogen atmosphere. The solvent was evaporated and the product isolated by repeated precipitation with ether and hexane. Yield: 20 mg (35.3%). ¹H-NMR (400 MHz, DMSO): δ (ppm) 7.9-7.75 (m, 44H), 7.5-7.35 (m, 8), 7.35-7.25 (m, 16H), 7.2-7.1 (m, 8), 4.05 (t, 16H), 3.20-3.00 (m, 40H), 2.7-2.60 (m, 24H), 2.45-2.35 (m, 12H), 2.25- 2.05 (m, 38H), 1.8-1.65 (m, 32H). Mass: (ESI) calculated: 3239.8 (M), found: 3241.2 (M+H)⁺ and calculated: 1620.9 (M+2)²⁺ found: 1621.4 (M+2H)²⁺

Synthesis of MV-PEI

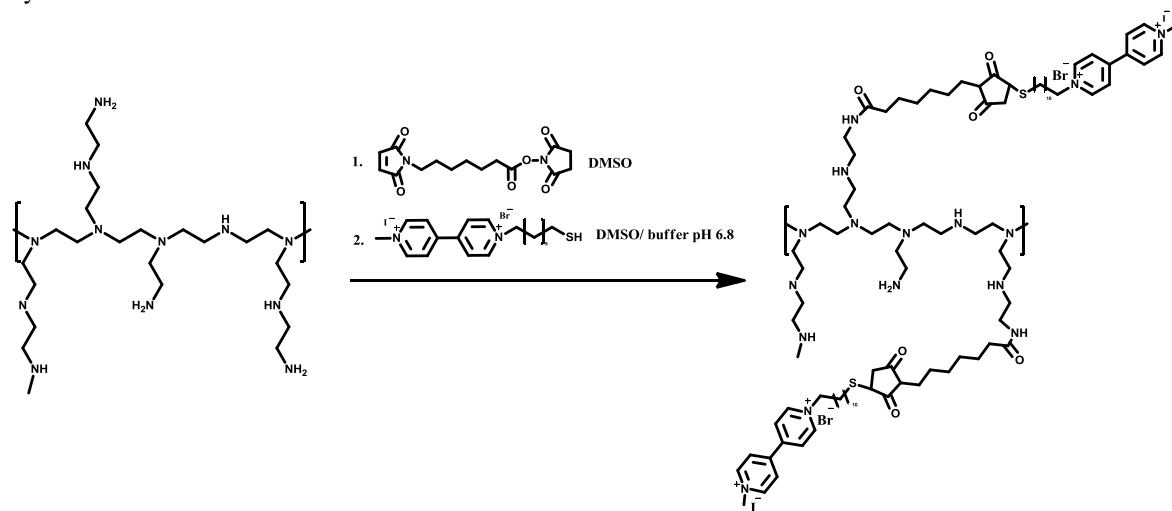


Figure S 2: Schematic illustration of the synthesis of methyl viologen- grafted poly (ethylene imine)

Poly (ethylene imine) [MW~10000 g/mol] (132 mg, 13.2 μmol) was dissolved in 35 mL dry DMSO and a solution of maleimido-hexanoic acid-N-hydroxy succinimide ester (61 mg, 197.86 μmol , 15 equiv) in 3 mL DMSO added under inert conditions. The obtained solution was stirred for 1 day at 50 °C. The second reaction step was carried out after cooling down the reaction mixture to RT. Phosphate buffer (30 mL) was added and the pH of the reaction mixture decreased to 6.9 (controlled via pH-meter). 1-(1-Undecyl-11-thiol)-1-methyl-4,4-bipyridinium (110 mg, 199.98 μmol , 15.16 equiv) was dissolved and added to the reaction mixture. The solution was stirred overnight at RT and purification was carried out by dialysis (molecular cut-off 10.000 g/mol). The product was lyophilized and analyzed by $^1\text{H-NMR}$, UV and micro-calorimetry.

As visible in the figure 6, the UV-absorption of the MV-grafted polymer had a baseline shift at 268nm, which could be attributed to the characteristic UV absorbance peak of 1-(1-undecyl-11-thiol)-1-methyl-4,4-bipyridinium at this wavelength. To determine, how many methyl viologen were grafted onto the polymer, a calibration curve of 1-(1-undecyl-11-thiol)-1-methyl-4,4-bipyridinium in water was prepared. Using this technique it could be determined, that on average 4 methyl viologen entities were grafted to one polymer chain.

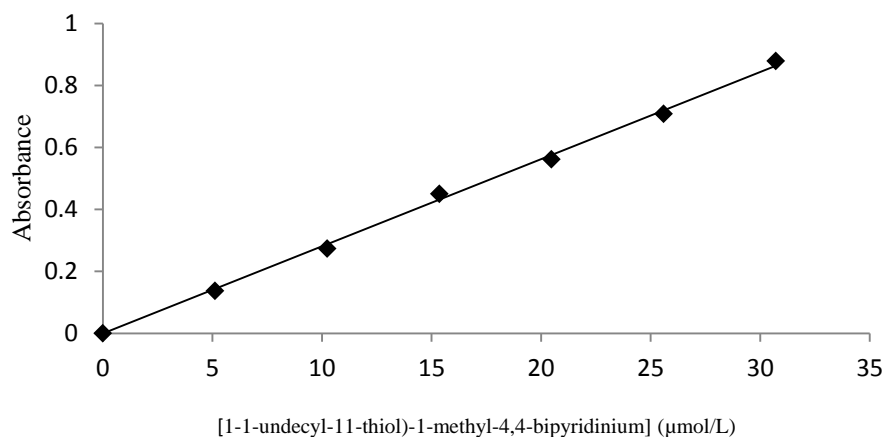


Figure S 3: UV calibration curve of 1-(1-undecyl-11-thiol)-1-methyl-4,4-bipyridinium in water at $\lambda=259\text{nm}$

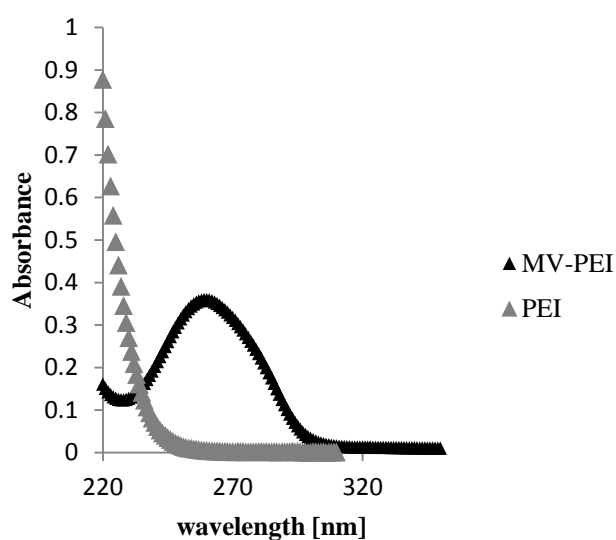


Figure S 4: UV absorbance of MV-PEI and PEI in aqueous solution

To show the exact amount of attached MV, calorimetry was carried out additionally. For studying the complexation of MV in the supramolecular host, 9.06 μM solution (10 μL) of the MV-PEI in the burette were added to a 18.3 μM solution of CB[8] in the calorimetric cell, monitoring the heat change after each addition. The concentration of aqueous CB[8] solution had previously been determined by calorimetric analysis with a known aqueous solution of 1,1'-dimethyl-4,4'-bipyridinium dichloride. The analysis revealed that, on average, each polymer chain is functionalized with 4.2 MV entities, confirming the UV/Vis and $^1\text{H-NMR}$ analyses.

3. Supramolecular nanoparticle assembly

For the preparation of size-tunable supramolecular NPs utilizing CB[8] as supramolecular host, various aqueous solutions of Np-PEG and Np-PAMAM in DMSO were prepared before mixing. Concentration of MV-PEI and CB[8] were kept the same regardless which size of NPs were formed. E.g. for preparing a solution having 20% Np entities derived from the aliphatic dendrimer, 1000 μ L MV-PEI (0.336 μ M) was added to a previously prepared solution of 500 μ L Np-PEG (2.15 μ M), 20 μ L Np₈-PAMAM (5.25 μ M) and 500 μ L CB[8] (2.688 μ M). The sample was mixed, combined and kept at RT for 2 days before DLS and SEM analysis.

3.1 Analysis of supramolecular nanoparticles

Dynamic Light Scattering (DLS)

Size evaluation of the SNPs was carried out by dynamic light scattering measurement using the Particle Analyzer Nanotrak and Zetatrac from Anaspec. The observed sizes and standard derivations of the supramolecular NPs were calculated by taking an average of 5 measurements. In total, particles with sizes ranging from 0.8 to 6400 nm were detected.

High resolution Scanning Electron Microscopy (SEM)

The appearance and sizes of the prepared supramolecular NPs size was analyzed by high-resolution scanning electron microscopy. Therefore, aqueous solutions of the aqueous NP samples were drop-cast on a Formvar coated copper TEM grid. The solution was dried and analyzed without further treatment of the samples.

UV/Vis analysis

NP formation was carried out as described previously. The formation of particles was controlled using DLS. For UV/Vis analysis, the sample was concentrated 13.5 times to observe sufficient UV absorbance on the Perkin Elmer UV/Vis spectrometer Lambda 850. As a control, an equivalent concentration of CB[8] and a NP formulation prepared in the absence of CB[8] were measured as well.

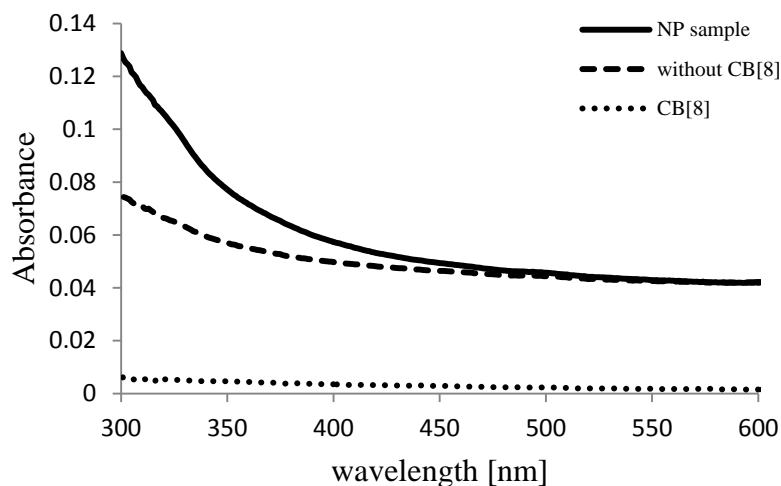


Figure S 5: UV/Vis absorption spectra of a NP solution in the presence of CB[8] and of an analogous sample in the absence of CB[8] (dashed line)

Fluorescence spectroscopy

To evaluate the formation of the supramolecular NPs, the NP formulation was prepared in presence and absence of CB[8]. In presence of the supramolecular host, naphthol can accumulate in the host molecules and a fluorescence decay is observable. This decay was observed directly after adding the solutions, whereby stable NP formation have been just observed 2 days after preparation at RT.

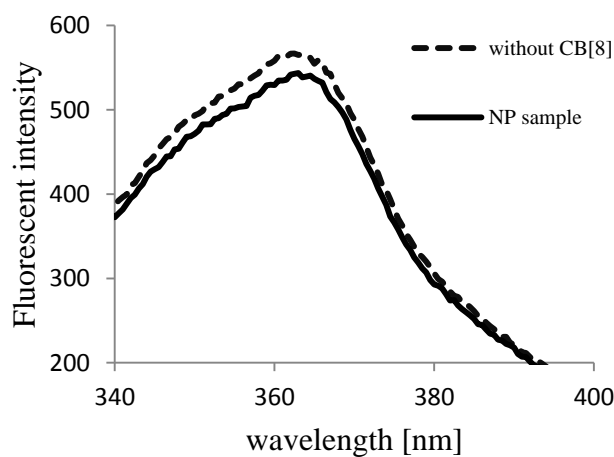


Figure S 6: Fluorescence spectra of the NP formulation before (dashed line) and after addition of CB[8] (solid line).

3.2 Control experiments for supramolecular nanoparticle formation

As control experiments, the different supramolecular components were mixed in the absence of CB[8] or MV-PEI. Therefore the initial solutions were exchanged with an equivalent amount of MilliQ water to establish the same concentrations as used for NP assembly. In addition particle formation was carried out in the presence of CB[7] instead of CB[8], the former of which is not able to form a ternary complex. For samples prepared with CB[7], DLS measurement could not be carried out, since an appropriate loading index was not reached. Furthermore, in SEM polymeric material was seen but no clear particles were observed.

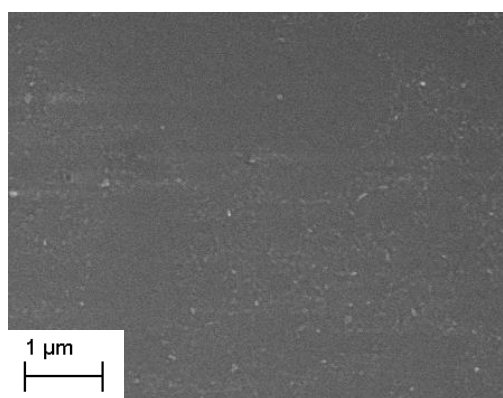


Figure S 7: SEM observed for samples prepared with CB[7] instead of CB[8]

3.3 Reductive NP disassembly

The reduction of the MV species should lead to disassembly of formed NPs. To evaluate if that concept is applicable for our NP formulation, particles having an average hydrodynamic radius of 93 nm were prepared. The samples was prepared as previously described and the size evaluated via DLS measurements. NP disassembly was carried out by adding 10 μL aqueous $\text{Na}_2\text{S}_2\text{O}_4$ (5 mmol) to the NP sample under inert conditions. Particle size analysis was carried out via DLS at the following day. As visible in the DLS results shown below, the previously observed particle signal disappears and only a peak at 1 nm was observable, which can be attributed to CB[8] in solution.

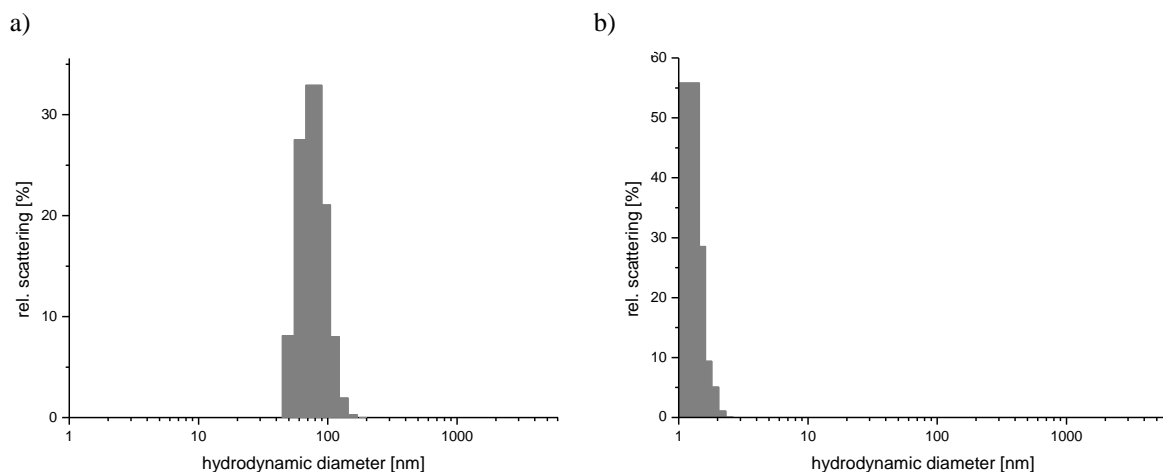


Figure S 8: DLS analysis a) before and b) after addition of $\text{Na}_2\text{S}_2\text{O}_4$

3.4 NP formation in time

The kinetics of the formation of supramolecular NPs was analyzed by carrying out continuous DLS measurements over 24 h at different temperatures. Therefore the required building block solutions were mixed as previously described and the NP size was evaluated directly after mixing. The samples measured at 30°C and 40°C were warmed up utilizing an oil bath. DLS data were observed directly after mixing, which implies, that stabilization of the NP temperature was not yet reached for measurements carried out at elevated temperatures in the starting 30 min. In contrast to regular DLS measurements, single measurements were done for each reported data point. For samples prepared at 21°C and 30°C, distinct particle formation was confirmed by measuring the size of the NP sample 48 h after sample preparation.

3.5 Size-tuning of supramolecular NPs

Size-tuning of the supramolecular NPs was observed by changing the concentrations of Np-PEG (2.419 μM - 0.900 μM) and Np₈-PAMAM (2.63 μM - 17.51 μM) in such a way, that the overall ratio between the supramolecular building blocks remained constant at 1:1:1 (CB[8]/ MV/ Np). Sizes of the prepared NPs were analyzed using DLS and SEM, as previously described. The graph shown in Figure S9 as well as the SEM images displayed in Figure S10 show that controlled NP formation was observed using up to 35% Np from Np₈-PAMAM. In contrast, uncontrolled aggregation was observed for samples prepared with an enlarged content of Np₈-PAMAM. Both, individual and aggregated particles were observed for an SNP sample prepared with 50% Np (Fig S10 f) derived from the Np₈-dendrimer. As stated previously, the particles tend to aggregate since insufficient capping nap-PEG is available in the NP solution. Since larger particles have an extended influence in DLS scattering, average sizes around 300 nm were observed for these NP samples.

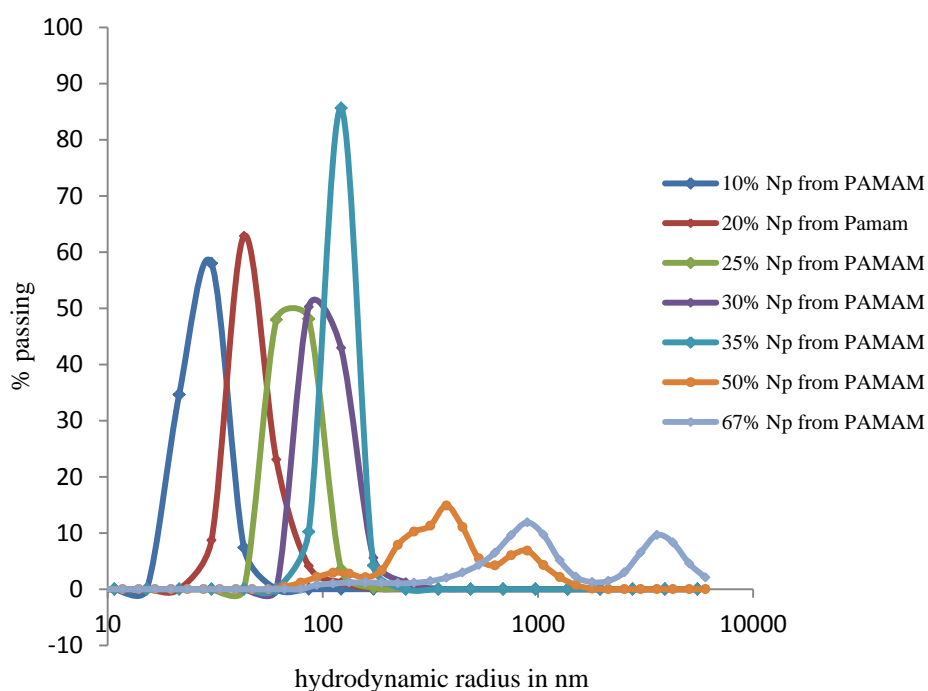


Figure S 9: DLS data observed for different NP formulations

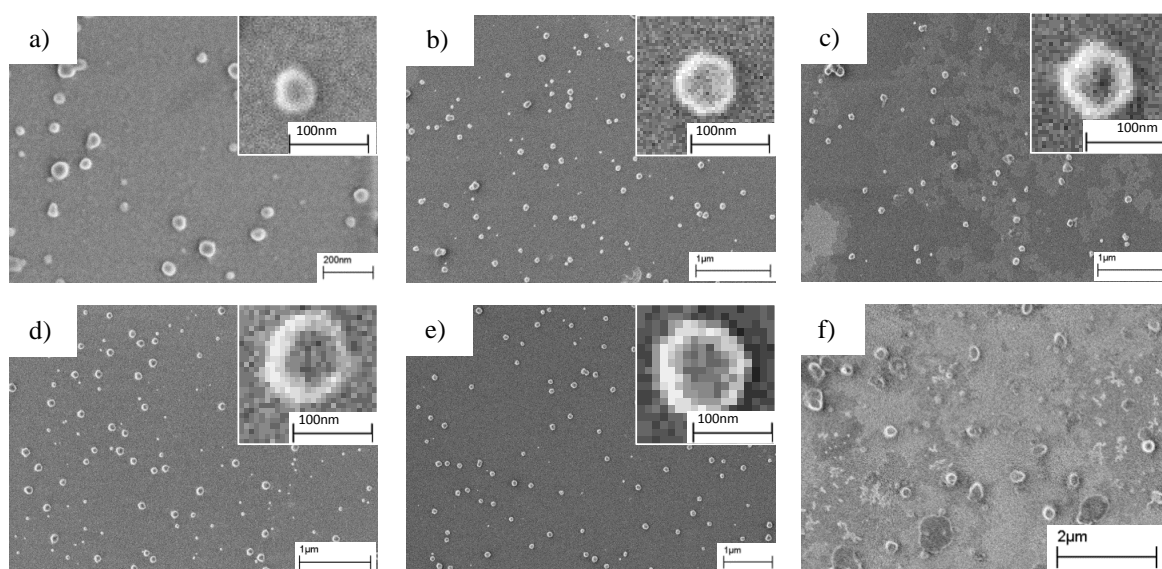


Figure S 10: Size determinations of supramolecular NPs prepared with different formulations: SEM images (a-f) of the resulting NPs, as a function of the Np content derived from Np₈-PAMAM dendrimers (a: 10% b: 20% c: 25% d: 30% e: 35% f: 50%) used during supramolecular assembly (using Np:MV:CB[8] = 1:1:1, with Np coming from Np₈-PAMAM and Np-PEG).

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- S1. H. Kotani, K. Ohkubo, Y. Takai and S. Fukuzumi, *J. Phys. Chem. B*, 2006, **110**, 24047.
S2. O. A. Scherman, *Polymer preprints*, 2010, **51** (1), 299.
S3. A. González-Campo, M. Brasch, D. A. Uhlenheuer, A. Gómez-Casado, L. Yang, L. Brunsveld, J. Huskens and P. Jonkheijm, *Langmuir*, 2012, **28**, 16364.