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Electronic Supplementary Information

Effects of the Molecular Weight and the Valency of Guest-modified Poly(ethylene glycol)s on the Stability, Size and Dynamics of Supramolecular Nanoparticles **

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1. Experimental section

1.1 Materials and Equipment

Starting materials for organic synthesis were obtained from Sigma-Aldrich and Nanocs and used as received. Octa-(*E*)-5-naphthol-pentanoic-acid poly(amido amine) dendrimer of generation 1 (8 end groups, Np_8-PAMAM), methyl-4,4-bipyridinium (methyl viologen, MV)-substituted poly(ethylene imine) (MV-PEI, degree of substitution: 4.5 MV units per polymer chain),and naphthalene poly(ethylene glycol) methyl ether (Np-PEG_{111}) were prepared as described previously.{Stoffelen, 2013 #126} Cucurbit[8]uril (CB[8]) was purchased from Strem Chemicals and the concentration of a stock solution was assessed by microcalorimetric titration (Microcal VP-ITC) against paraquat. Deuterated solvents used for NMR spectroscopy were purchased from Cambridge Isotope and the water used for dialysis and SNP preparation was of MilliQ quality (Millipore, R= 18.2 MΩcm^{-1}), purified by MilliQ Advantage A10. The synthesized products were analyzed by ^{1}H-NMR and ^{13}C-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/Micromass, respectively. DLS experiments were performed with a Zetatrac and Nanotrac by Anaspec operating with a Microtrac FLEX Operating Software at 25◦C using a laser wavelength of 780 nm at a scattering angle of 90°. The observed sizes and standard derivations of the SNPs were calculated by taking an average of 4 or 5 measurements. In total, particles with sizes ranging from 0.8 to 6400 nm were detected. All SEM images were taken with a Carl-Zeiss high 1500 resolution scanning electron microscope. Therefore the aqueous SNP solutions were drop-cast on a Formvar coated copper TEM grid. The solution was dried and analyzed without further treatment of the samples.

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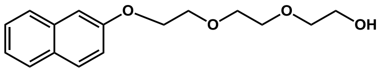
1. 2 Synthetic procedures

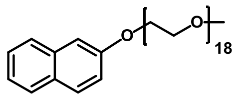
Synthesis of 2-(2-(2-(naphthalen-2-yloxy)ethoxy)ethoxy)ethanol (Np-PEG$_3$) (**1**)



Under argon, 2-naphthol (0.52 g, 3.6 mmol) was dissolved in anhydrous THF (10 mL). At 0°C, NaH (60% in mineral oil, 140 mg, 3.6 mmol) was added and the solution was stirred 1 h, while warming to room temperature. Subsequently, the solution was transferred under argon to a second flask charged with chloro-ethoxy-ethoxy-ethanol (1.5 g, 8.95 mmol) in THF (40 mL). A trace of KI was added to promote Finkelstein halogen exchange and the solution was stirred at room temperature for 12 h under argon. The solvent was removed under reduce pressure and the residue dissolved in 30 mL CH_2Cl_2. Unreacted chloro-ethoxy-ethoxy-ethanol was removed by extraction of the organic solution with (6 × 50 mL) 2 M HCl followed by brine (30 mL). The organic phase was dried with $MgSO_4$ and the solvent removed under reduced pressure. Flash chromatography (SiO_2, gradient elution CH_2Cl_2 (100%) to CH_2Cl_2/MeOH (98:2)) gave Np-PEG$_3$ as a slightly yellow oil. Yield: 550 mg, 55%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.75-7.69 (q, 3H, 3ArH); 7.43-7.39 (t, 1H, 1ArH); 7.33-7.29 (t, 1H, 1ArH); 7.18-7.12 (m, 2H, 2ArH); 4.23 (t, 2H, CH_2); 3.91 (t, 2H, CH_2); 3.73-3.67 (m, 6H, 3CH_2); 3.6 (t, 2H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$): δ= 156.65, 134.49, 129.46, 129.08, 127.66, 126.78, 126.39, 123.72, 118.96, 106.77, 72.56, 70.85, 70.36, 69.73, 67.35, 61.76. ESI–MS (m/z): calculated for $[C_{16}H_{20}O_4]^+$: 276.14, found: 277.10$(M+H)^+$.

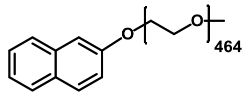
Synthesis of Np-PEG$_{18}$ (**2**)

Under nitrogen, naphthol (0.8 g, 5.6 mmol), tosyl poly(ethylene glycol) methyl ether (M_w 900 g/mol) (1 g, 1.11 mmol) and K_2CO_3 (1.7 g, 5.6 mmol) were dissolved in anhydrous acetonitrile (40 mL) and stirred at reflux for 5 days. The solvent was evaporated and the residue dissolved in 10 mL CH_2CL_2. The excess of naphthol was removed by repeated precipitation in ice cold diethyl ether. The collected solid was dissolved in water, filtered and dialyzed against water for 5 days (M_W cut-off 550 g/mol). The aqueous solution was freeze-dried to obtain a dark brown solid. Yield: 550 mg, 55%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ= 7.70-7.68 (q, 3H, 3ArH); 7.38 (td, 1H, 1ArH); 7.30 (td, 1H, 1ArH); 7.13 (m, 2H, 2ArH); 4.22 (t, 2H, CH_2); 3.90 (t, 2H, CH_2); 3.73 (m, 2H, CH_2); 3.64- 3.52 (m, 64H, 32 CH_2) 3.35 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ= 156.74, 134.49, 129.36, 129.02, 127.63, 126.76, 126.33, 123.64, 119.02, 106.73, 71.93, 70.86, 70.55, 69.74, 67.42, 59.04. ESI–MS (m/z): calculated for $[C_{47}H_{82}O_{19}]^+$: 950.55, found: 945.57.

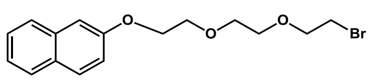


Synthesis of Np-PEG$_{484}$ (**3**)

Under argon, 2-naphthoylchloride (20 mg, 0.104 mmol), 130 mg amino-poly(ethylene glycol) methyl ether (M_n 20.000 g/mol) (130 mg, 0.013 mmol) and pyridine (8.3 µL, 0.104 mmol) were stirred in CH_2Cl_2 (15 mL) at room temperature for 48 h. The solvent was evaporated under reduced pressure and the obtained solid precipitated in diethyl ether repeated times. The obtained solid was dissolved in 40 mL MilliQ water, filtered and dialyzed against water (disposable membrane molecular weight cut-off 5 kDa). Afterwards, the aqueous solution was filtered and a white fluffy solid was obtained after freeze-drying. Yield: 129 mg, 98%. ^{1}H-NMR (400 MHz, D_2O): δ= 8.39 (m, 1H, 1ArH); 8.08-8.00 (m, 3H, ArCH); 7.88-7.82 (m, 1H, ArH); 7.62-7.70 (m, 2H, 2ArH); 4.0-3.0 (m, 940H, CH_2). ^{13}C-NMR (D_2O): δ= 69.56. Maldi-MS (m/z) calculated for $[C_{938}H_{1863}NO_{464}]^+$: 20581 g/mol, found: 20580 g/mol.

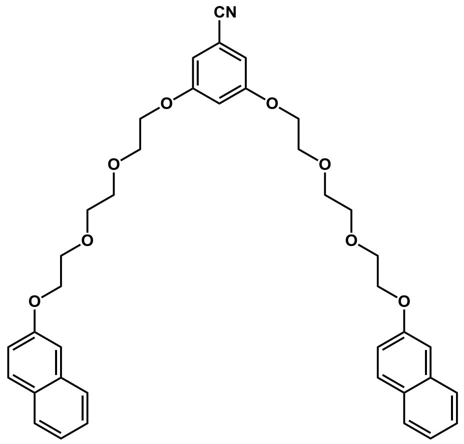


Synthesis of 2-(2-(2-(2-bromoethoxy)ethoxy)ethoxy)naphthalene (**4**)



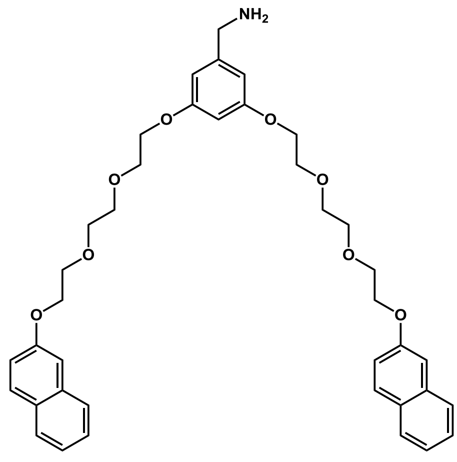
A solution of phosphorus tribromide (0.40 g, 1.5 mmol) in toluene (50 mL) was added dropwise to a cooled (0 °C) solution of compound **1** (1.02 g, 3.7 mmol) in toluene (50 mL). The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The organic layer was washed with water (3 × 50 mL) and brine (1 × 50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH = 99:1) to give 2-(2-(2-(2-bromoethoxy)ethoxy) ethoxy)naphthalene as a colorless oil. Yield: 920 mg, 73 %. ^{1}H-NMR ($CDCl_3$): δ= 7.75-7.69 (q, 3H, 3ArH); 7.44-7.39 (t, 1H, 1ArH); 7.33-7.29 (t, 1H, 1ArH); 7.18-7.12 (m, 2H, 2ArH); 4.25 (t, 2H, CH_2); 3.94 (t, 2H, CH_2); 3.77 (t, 2H, CH_2); 3.75 (m, 2H, CH_2); 3.70 (m, 2H, CH_2); 3.46 (t, 2H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$): δ= 156.73, 134.49, 129.40, 129.04, 127.65, 126.77, 126.37, 123.68, 119.02, 106.74, 71.28, 70.89, 70.63, 69.85, 67.43. ESI–MS (m/z): calculated for [$C_{16}H_{19}BrO_3$]: 338.05 found: 339.22 $(M+H)^+$.

Synthesis of 3,5-bis(2-(2-(2-(naphthalen-2-yloxy)ethoxy)ethoxy)ethoxy)benzonitrile (**5**)



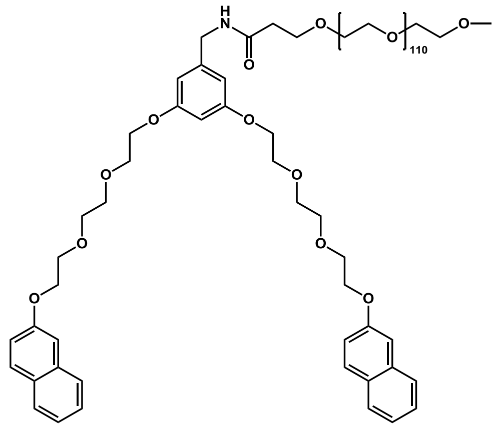
A suspension of **4** (350 mg, 1.26 mmol), 3,5-dihydroxy benzonitrile (59 mg, 0.57 mmol), dried potassium carbonate (210 mg, 1.52 mmol) and 18-crown-6 (70 mg, 0.27 mmol) in acetone (14 mL) was refluxed for 78 h. The solvent was evaporated and the residue was partitioned between water (30 mL) and diethyl ether (30 mL). The aqueous layer was extracted with diethyl ether (3 × 25 mL) and the combined extracts were dried over $MgSO_4$. The solvent was evaporated and the residue was purified by column chromatography (CH_2Cl_2/ MeOH=98:3) to give 3,5-bis(2-(2-(2-(naphthalen-2-yloxy)ethoxy)ethoxy)ethoxy) benzonitrile as a colorless oil. Yield: 190 mg, 50.3%. ^{1}H-NMR ($CDCl_3$): δ= 7.70-7.63 (m, 6H, 6ArH); 7.36-7.34 (t, 2H, 2ArH); 7.29-7.2 (t, 2H, 2ArH); 7.10-7.06 (m, 4H, 4ArH); 6.67 (sd, 2H, 2ArH); 6.60 (sd, 1H, 1ArH); 4.19-4.17 (t, 4H, $2CH_2$); 3.99-3.97 (t, 4H, $2CH_2$); 3.87-3.85 (t, 4H, $2CH_2$); 3.77-3.75 (m, 4H, $2CH_2$); 3.73-3.68 (m, 8H, $4CH_2$). ^{13}C-NMR ($CDCl_3$): δ= 160.11, 156.71, 134.47, 129.40, 129.02, 127.65, 126.75, 126.38, 123.70, 118.98, 118.72, 113.76, 110.71, 106.80, 106.71, 70.95, 70.92, 69.84, 69.50, 67.91, 67.41. ESI–MS (m/z): calculated for [$C_{39}H_{41}NO_8$]: 651.28 found: 652.26 $(M+H)^+$.

Synthesis of 3,5-bis(2-(2-(2-(naphthalen-2-yloxy)ethoxy)ethoxy)ethoxy)benzylamine



A suspension of compound **5** (136.2mg, 0.21mmol) and a catalytic amount of Raney-Ni was stirred under 10 bar H_2 (g) in 30 mL of 6 M NH_3 in ethanol for 48 h. The suspension was filtered over celite and washed with methanol. After evaporation of the solvent, the product was dissolved in chloroform. It was alkalize with 50 mL of NaOH 0.1 M. The water fraction was washed with chloroform (3 x 50 mL). The organic fractions were combined and solvent evaporated under reduced pressure to give compound slightly greenish colored oil. Yield: 113 mg, 81%, 80% pure. ^{1}H-NMR ($CDCl_3$): 7.67-7.63 (m, 6H, 6ArH); 7.36-7.34 (m, 2H, 2ArH); 7.29-7.22 (m, 2H, 2ArH); 7.10-7.06 (m, 4H, 4ArH); 6.50 (s, 2H, 2ArH); 6.37 (s, 1H, 1ArH); 6.26 (s, 1H, 1ArH); 4.17-4.12 (m, 5.6H, CH_2 $2CH_2$); 3.96-3.90 (m, 4H, $2CH_2$); 3.85-3.80 (m, 4H, $2CH_2$); 3.71-3.61 (m, 12H, $4CH_2$). ^{13}C-NMR ($CDCl_3$): δ= 162.59, 156.74, 134.47, 129.41, 129.02, 127.63, 126.79, 126.37, 123.69, 119.47, 109.42, 107.33, 106.78, 70.84, 70.70, 69.9, 69.50, 67.81, 67.39, 51.19. ESI –MS (m/z): calculated for [$C_{39}H_{45}NO_8$]: 655.31 found: 656.68(M+H)$^+$.

Synthesis of Np$_2$-PEG$_{113}$ (**6**)



3,5-bis(2-(2-(2-(naphthalen-2-yloxy)ethoxy)ethoxy)ethoxy) benzylamine (100 mg, 0.152 mmol), triethylamine (15 mg, 21 µL, 0.152 mmol) and methyl-poly(ethylene glycol) *N*-hydroxysuccinimide ester (M_w ~ 5000 g/mol) (253 mg, 0.051 mmol) were dissolved in CH_2Cl_2 (30 mL) under argon. The solution was stirred for 72 hours at room temperature. The solvent was removed under reduced pressure and the product purified by precipitation with diethyl ether and dialysis against water for 5 days. Yield: 265 mg, 93%. ^{1}H-NMR (D_2O): 7.72 (m, 6H, 6ArH); 6.99 (m, 2H, 2ArH); 6.87 (m, 2H, 2ArH); 6.74 (m, 2H, 2ArH); 6.61 (m, 2H, 2ArH); 6.22 (s, 2H, 2ArH), 5.98 (s, 1H, 1ArH), 3.98 (t, 4H, $2CH_2$), 3.78- 3.10 (m, 394 H, 197 CH_2). ^{13}C-NMR (D_2O): δ= 69.56. Maldi-MS (m/z) calculated for [$C_{265}H_{495}NO_{121}$]$^+$: 5631 g/mol, found: 5626 g/mol.

1.3 Supramolecular nanoparticle assembly

For the preparation of size-tunable supramolecular NPs utilizing CB[8] as supramolecular host, various aqueous solutions of the Np-PEGs (Np-PEG$_3$, Np-PEG$_{18}$, Np-PEG$_{111}$, Np-PEG$_{464}$ and Np$_2$-PEG$_{113}$) and different concentrations of Azo$_8$-PAMAM in DMSO were prepared. Additionally, aqueous solutions of MV-PEI (0.336 µM) and CB[8] (2.688 µM) were prepared and used for all SNP compositions. E.g. for preparing a solution having 20% Np entities derived from the aliphatic dendrimer and 80% Np entities derived from Np-PEG$_{18}$, 1000 µL MV-PEI was added to a previously prepared solution of 500 µL Np-PEG$_{18}$ (2.15 µM), 20 µL Np$_8$-PAMAM (1.65 µM) and 500 µL CB[8] (2.688 µM). These aliquots were mixed and kept at RT for 2 days before DLS and SEM analysis.

1.4 Supramolecular nanoparticle formation in time

To compare the kinetics of the formation of SNPs in the presence of Np-PEG$_{111}$ and Np$_2$-PEG$_{113}$, continuous DLS measurements were carried out for 40 h at RT and at 40°C. Therefore the supramolecular building blocks were mixed using a formulation containing 25% Np from Np$_8$-PAMAM, and DLS was started directly after mixing. The reported averaged SNP sizes and their standard deviations were obtained by averaging 4 data points measured over the course of 1 h.

1.5 Analysis of SNPs

Dynamic Light Scattering (DLS)

The hydrodynamic diameters of the self-assembled zwitterionic particles were investigated by dynamic light scattering. The observed sizes and standard deviations of the SNPs are based on the average number distributions of minimum 5 individual measurements per sample. Three samples were measured for each reported ZSNP formulation.

Scanning Electron Microscopy (SEM)

Sizes and shapes of SNPs were analyzed by SEM. Aqueous solutions of the prepared SNPs were dropcast on a Formvar coated copper TEM grid. The solution was dried and analyzed without further treatment.

2. References

1 Stoffelen, C.; Huskens, J. *Chem. Commun.* 2013, ***49***, 6740.