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Electronic Supplementary Information

Self-assembly of size-tunable supramolecular nanoparticle clusters in a microfluidic channel

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1. Materials and Equipment

Starting materials for organic synthesis were obtained from Sigma-Aldrich and used as received. The bifunctional linker NHS-$(PEG)_6$-maleimide was purchased from Fisher Scientific. The supramolecular host CB[8] was purchased from Strem Chemicals and its purity was determined by microcalorimetry against paraquat. 1-(1-Undecyl-11-thiol)-1-methyl-4,4-bipyridinium dibromide, naphthol-terminated poly(ethylene glycol) monomethyl ether and naphthol-terminated poly(amido amine) dendrimer were prepared according to literature procedures.[S1-3] The synthesized products were analyzed by ^{1}H-NMR on a Bruker 400 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. UV/Vis measurements were carried out on a Perkin Elmer UV/Vis spectrometer Lambda 850. HEPES buffer and the solutions required for SNPC formation were prepared in distilled water purified by MilliQ Advantage A10, Millipore R=18.2 MΩcm^{-1} before usage. Silicon wafers with (100) orientation and single side polished was purchased from OKMETIC. The microfluidic-assisted preparation of the SNPCs was carried out in a microreactor with a residual volume of 8 µL, and was purchased from Miconit Microfluidics. It has dimensions of 645.6 mm length, 52 µm depth, 254 µm channel top width, and 150 µm channel bottom width. In all the microreactor experiments, the sample solutions were mobilized by means of a PHD 22/2000 series syringe pump (Harvard Apparatus, United Kingdom) equipped with 1 mL flat tip syringes (Hamilton). Syringes were connected to fused silica capillaries (100 µm i.d., 362 µm o.d., Polymicro Technologies) by means of Upchurch NanoportTM assembly parts (i.e., Nano-TightTM unions and fittings, Upchurch Scientific Inc. USA). During the experiments the microreactor was placed in a home-built chip holder designed for fitting fused silica fibers into the inlet/outlet chip reservoirs by means of commercially available Upchurch NanoportTM assembly parts. Dynamic light scattering (DLS) analysis was performed

on a Nanotrac from Anaspec operating with a Microtrac FLEX Operating Software. The SNPCs were analyzed by a Carl-Zeiss 1500 high-resolution scanning electron microscope (SEM).

2. Synthesis and characterization of methyl viologen-functionalized silica nanoparticles

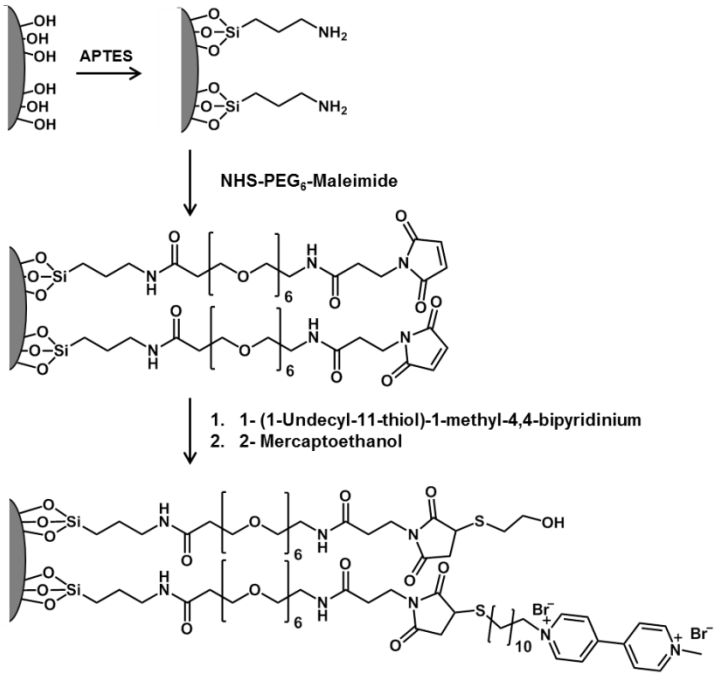

Figure S1: Synthesis of methyl viologen-conjugated silica NPs (MV-SiO_2).

a) Synthetic procedure

Bare silica nanoparticles SiO_2-OH: In accordance to a literature procedure,[S4, 5] bare silicon oxide nanoparticles were prepared by adding 3.8 mL tetraethyl orthosilicate to a mixture of 5.7 mL NH_4OH in 114 mL ethanol under stirring.

Amino-functionalized silica nanoparticles SiO_2-NH_2: Under argon, 2.3 mL (3-aminopropyl)triethoxysilane (APTES) was added under vigorous stirring to 120 mL of bare silica nanoparticles in ethanol. The dispersion was stirred overnight and the excess of APTES removed by repeated NP centrifugation and redispersion in 60 mL ethanol. The particles were dried under reduced pressure and stored at 4 °C as white solid.

Maleimide-functionalized silica nanoparticles SiO_2-mal: The amine functionalized SiO_2-NPs were dispersed in degassed HEPES 20 mM buffer (pH 7.4) to prepare a dispersion with a concentration of 10 mg/mL. 10 mL of the particle solution was added to an equal volume of NHS-PEG_6- maleimide 23 mM in 20 mM HEPES buffer (pH 7.4). The dispersion was sonicated for 5 min and further shaken overnight. The milky dispersion was purified by centrifugation and subsequent redispersion of the NPs, twice in HEPES buffer (pH 7.4) and twice in HEPES buffer (pH 6.8) to prepare the NP dispersion for the reaction with 1-(1-undecyl-11-thiol)-1-methyl-4,4-bipyridinium dibromide.

Methyl viologen-functionalized silica nanoparticles SiO_2-MV: SiO_2-mal was dispersed in degassed HEPES buffer (pH 6.8) to obtain a concentration of 5 mg/mL. 1-(1-Undecyl-11-thiol)-1-methyl-4,4-bipyridinium dibromide (1.7 mg/ 3.1 µmol) was dissolved in 3 mL degassed HEPES buffer (pH 6.8) and added to 15 mL of the SiO_2-mal dispersion under stirring. After shaking the dispersion for 2 h, the unfunctionalized terminal maleimide groups were capped by reacting them with 10 µL of 2-mercaptoethanol for 5 min. The resulting SiO_2-MV NPs were purified by repeated centrifugation, once in HEPES buffer (pH 6.8), twice in ethanol and twice in water. Finally, the nanoparticles were freeze-dried and kept as a white solid in the fridge.

b) Characterization of SiO_2-MV

DLS

Characterization of the SiO_2-MV in water by dynamic light scattering (Fig. S2a) showed that the NPs are relative monodisperse with an average number diameter of 61.5 ± 2.7 nm and an average intensity diameter of 66.2 ± 3.8 nm.

SEM

The particles were dispersed in MilliQ water (0.4 mg/ mL) and drop-cast on a pre-cleaned surface of silica. The droplet was gently removed by filter paper 10 s after addition and analyzed without further treatment of the sample. Size evaluation (Fig. S2b) showed that the NPs have an average diameter of 52.6 ± 6.9 nm.

UV-Vis spectroscopy

The content of methyl viologen on the SiO_2-MV NPs was determined using the characteristic UV adsorption band of MV at 259 nm. Therefore a dispersion of SiO_2-MV in water (0.5 mg/mL) was prepared and analyzed by UV spectroscopy (Fig. S2c). The content of MV was determined using a calibration curve of 1-(1-undecyl-11-thiol)-1-methyl-4,4-bipyridinium dibromide in water. After subtracting the enhanced background signal induced by NP scattering,[S6] the results show a grafting density of 5.3 µmol MV per g of NP.

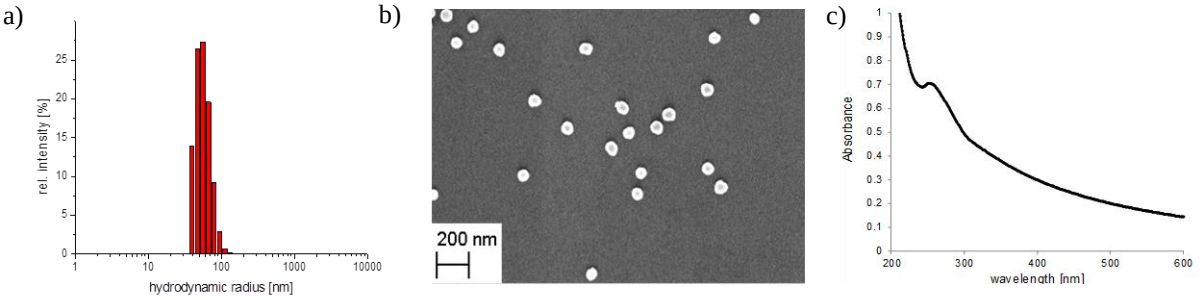

Figure S2: Characterization of SiO_2-MV: a) DLS analysis b) SEM analysis c) UV/Vis spectroscopy

3. Supramolecular cluster formation in batch reaction

For the preparation of size-tunable supramolecular nanoparticle clusters (SNPCs), the SiO_2-MV nanoparticles were dispersed in MilliQ water to obtain a concentration of 0.8 mg NP/mL. The solution was sonicated until only individual NPs were observed by DLS. In addition, 8 µM solution of CB[8] and various ratios of aqueous solutions of Np-PEG (5000 g/mol) and Np_8-PAMAM in DMSO were prepared, maintaining the MV:CB[8]:Np ratio at 1:1:1. For example, to form the SNPCs containing 50% Np derived from dendrimer, 500 µL SiO_2-MV was added to a previously prepared solution of 250 µL Np-PEG (4 µM), 10 µL Np_8-PAMAM (12.5 µM) and 250 µL CB[8] (8 µM). The sample was mixed using a vortex mixer and analyzed by DLS at 10 min and 14 h after mixing. As visible in the DLS results shown below, the size of the SiO_2-MV increased drastically directly after addition of CB[8] and the Np bearing components. Furthermore, the observed SNPC size depends on the concentration of Np from the multivalent dendrimer, but the sizes of the individual samples within one sample batch are not reproducible. In addition, no SNPCs were observed after measuring the mixed solution 14 h after SNPC preparation. This might be attributed to uncontrolled aggregation and precipitation.

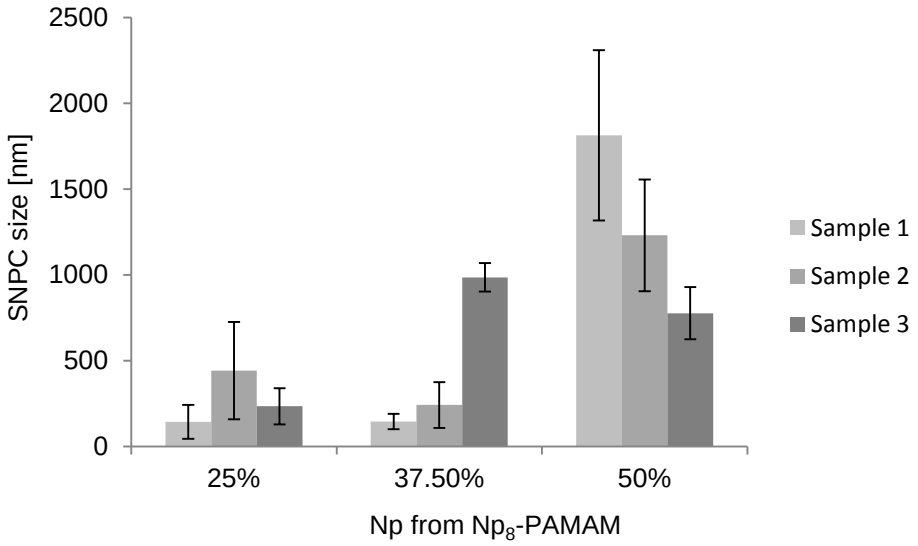

Figure S 3: DLS sizes of in batch reaction prepared SNPCs at a constant ratio MV:CB[8]:Np of 1:1:1, as a function of Np derived from Np Np_8-PAMAM dendrimers used during SNPC self-assembly.

4. Diffusive mixing inside the microfluidic channel

The diffusion coefficients of the SiO_2-MV and CB[8] were calculated based on the Stokes-Einstein-equation $D = \frac{kT}{6\pi\eta R}$ with k as Boltzmann constant, T the temperature, η the viscosity and R the radius of the particle or molecule. The hydrodynamic radii for PAMAM G1 and the different Np-PEG derivatives were taken from values reported in literature[S7-9] to calculate the diffusion coefficients. It can be assumed, that the real diffusion coefficient of Np_8-PAMAM is slightly smaller than the value given in the table, because the dendrimer is getting a little more bulky upon functionalization.

These values indicate clearly that the particles diffuse much slower than the smaller supramolecular building blocks. To calculate the required time of the different components at the interface to reach the other side of the microfluidic reactor, the Einstein-Smoluchowski-equation for planar diffusion was used $t = \frac{d^2}{2D}$ in which d indicates half of the microchannel width (d = 125 µm) and D is equal to the diffusion coefficient.

Supramolecular building block	D (m^2/s)	t [s]
SiO_2-MV	$8.6 * 10^{-12}$	911
CB[8]	$2.5 * 10^{-10}$	32
PAMAM G1	$2.3 * 10^{-10}$ (Ref [S7])	34
Np-PEG (1000 g/mol)	$2.2 * 10^{-10}$ (Ref [S8])	35
Np-PEG (5000 g/mol)	$9.4 * 10^{-11}$ (Ref [S9])	83

Table S1: Diffusion coefficients and diffusion times of the supramolecular building blocks used in SNPC formation in a microfluidic device

The Peclet number (Pe) describes the transport phenomenon of a molecular species, notably the ratio between adjective and diffusive flow, which is calculated by: Pe = *UL*/*D*. Here, *U* describes the mean flow velocity, *L* is the critical length, and *D* is the diffusion coefficient. Because we need to evaluate the importance of flow vs. diffusion in the relative transport of particles in and orthogonal to the flow, the (half of the) width of the microchannel is the critical length here (125 µm). Additionally, the two incoming flows have the same flowrate, which also does not lead to shear effects at the interface. Diffusion constants are given in Table S1. Based on the flow rates used here, the Peclet number for the SiO_2-MV NPs used in this study is in the range of 10^5-10^6. This confirms that the mixing of the two inlet streams is not determined by the diffusion of the SiO_2-MV.

5. SNPC formation in a microfluidic device

For the preparation of SNPCs inside the microfluidic device, a dispersion of SiO_2-MV in water (0.8 mg/mL, 4 µM MV) and different mixtures of CB[8], Np-PEG (5000 or 1000 g/mol) and Np_8-PAMAM in water were prepared before mixing. The concentration of the different components were set such that all 3 binding motifs CB[8], MV and Np were present in equimolar amounts and at a 2 µM concentration after mixing. For different residence times, the flow rates were adjusted accordingly.

5.1 Variation of the ratio of the Np-functionalized components inside the microfluidic channel

To evaluate the effect of the content of multivalent dendrimer, different concentrations of Np-PEG and Np_8-PAMAM were prepared before mixing. The dispersion solution of SiO_2-MV was injected in one of the two inlets of the microchannel, while the premixed solution of CB[8] and Np-bearing components was injected in the second inlet, and the two fluid streams were flowed in parallel with an equal flow rate of 4 µL/min, which corresponds to the residence time of 60 s.

5.2 Variation of flowrate during microfluidic assisted self-assembly

The experiments carried out with variable residence times of the mixing solutions inside the microreactor, were carried out using a steady-state origin of the Np bearing components. Therefore the SiO_2-MV dispersion and the mixture of CB[8], Np-PEG (5000 g/mol and 1000 g/mol) and Np_8-PAMAM were prepared and mixed within the microfluidic reactor using different flowrates to obtain variable residence times of the mixing solutions. i.e. The flow rates of two inlets were equal and were varied from 16.0, 8.0, 5.2 to 4.0 µL/min, which resulted in residence times of 15, 30, 45 and 60 s, respectively. The solution was collected until the volume of 330 µL required for analysis was obtained. SEM samples were prepared by collecting the sample directly from the outlet of the microreactor.

5.3 Variation of the stoichiometry during microfluidic assisted self-assembly

The experiments with different ratios of CB[8]/MV/Np were carried out using a constant content of MV while increasing the amount of CB[8] and Np stoichiometrically. Np-PEG (1000 g/mol) was used as the stopper, and 50% of Np was derived from the multivalent dendrimer at all CB[8]/MV/Np concentrations. E.g. a 0.8 mg/mL dispersion of SiO_2-MV in water was used in one inlet, and a premixed solution of CB[8] (8 µM), Np-PEG (1000 g/mol, 4 µM) and Np_8-PAMAM (0.5 µM) in the other inlet to obtain an overall CB[8]/MV/Np ratio of 2:1:2. The other ratios (3:1:3 and 5:1:5) were made similarly, using accordingly higher concentrations of CB[8] and the Np components. All experiments were carried out with the same flowrate of 16 µL/min, which corresponds to the residence time of 30 s.

6. Analysis of microfluidic assisted supramolecular cluster formation

6.1 DLS

The size of the formed SNPCs was analyzed by dynamic light scattering measurement after collecting sufficient sample (350 µL) from the outlet of the microreactor. The observed sizes and standard derivations of the supramolecular SNPs were based on the average number distributions of minimum 5 individual measurements per sample. Three samples were measured for each reported NP formulation.

6.2 SEM

SEM samples were prepared by drop-casting an approximate 10 times diluted SNPC dispersion onto a cleaned silicon wafer. This was done by adding 2 µL of water followed by collecting a small droplet directly from the outlet of the microreactor. The dispersion was completely removed 10 s after addition and analyzed without further treatment. The sizes of at least 150 individual clusters were used to determine the average size of the SNPCs. Additionally another average SNPC size was determined by excluding all unbound NPs.

7. Variation of the ratio of the Np-functionalized components inside the microfluidic channel

7.1 SNPC polydispersity

SNPCs were prepared with different contents of monovalent and multivalent Np bearing guest molecules as described in section 5. Next to the SNPC size also the polydispersity (PDI) of the samples were reported by DLS. The following tables shows the average observed PDI values for the individual measured samples.

Np from Np_8-PAMAM	Sample	PDI	Np from Np_8-PAMAM	Sample	PDI
25%	1	0.4	50%	1	0.3
25%	2	0.38	50%	2	0.42
25%	3	0.3	50%	3	0.36
37.5 %	1	0.35	62.5 %	1	0.37
37.5 %	2	0.42	62.5 %	2	0.3
37.5 %	3	0.4	62.5 %	3	0.4

Table S2: PDI of the individual samples prepared with different concentrations of the Np bearing guest components.

8. Control experiments for supramolecular nanoparticle formation

8.1 SNPC formation in the absence of multivalent Np_8-PAMAM

To visualize the effect of the crosslinking multivalent dendrimer, SNPCs were prepared in the absence of Np_8-PAMAM. Therefore a SiO_2-MV dispersion (0.8 mg/ml) and a mixture of CB[8] (4 µM) and Np-PEG (4 µM) was prepared. These two solutions were flowed into the microreactor as described in section 5 with a residence time of 60 s. SNPC formation was analyzed by DLS and SEM.

As visible in the images below, microfluidic experiments carried out in the absence of Np_8-PAMAM did not show significant clustering of the SiO_2-MV.

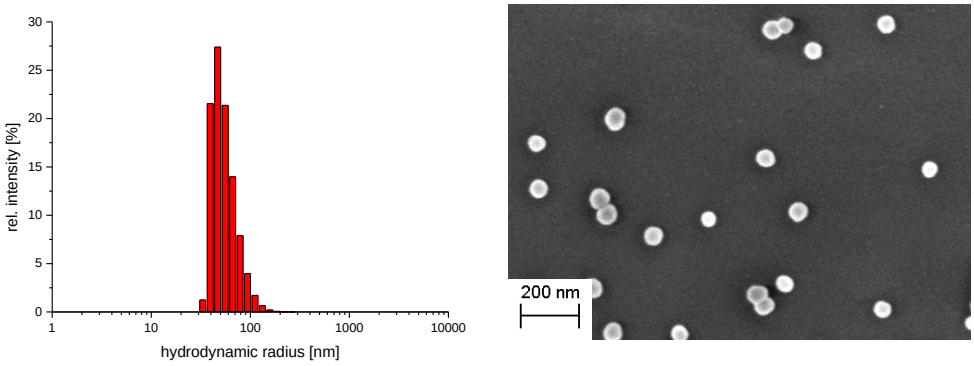

Figure S4: Average area DLS graph and SEM image of clustering experiment carried out with SiO_2-MV, CB[8] and Np-PEG.

8.2 SNPC formation in the absence of Np-PEG

Clustering of the SiO_2-MV was carried out in the absence of Np-PEG. Therefore the dispersion of SiO_2-MV (0.8 mg/mL) and a mixture of CB[8] (4 µM) and Np_8-PAMAM (0.5 µM) was prepared. These two solutions were flowed into the microreactor as described in section 5 above with a residence time of 60 s. The clustering sample was collected for DLS and the SEM samples directly collected from the outlet of the microreactor. By measuring the clusters by DLS, no hydrodynamic radius could be observed. It can be expected, that the absence of Np-PEG leads to uncontrolled aggregation of the NPs, which results in precipitation of the sample. This assumption is confirmed by the SEM images. As visible in the SEM images shown below, only very large NP aggregates were observed by SEM.

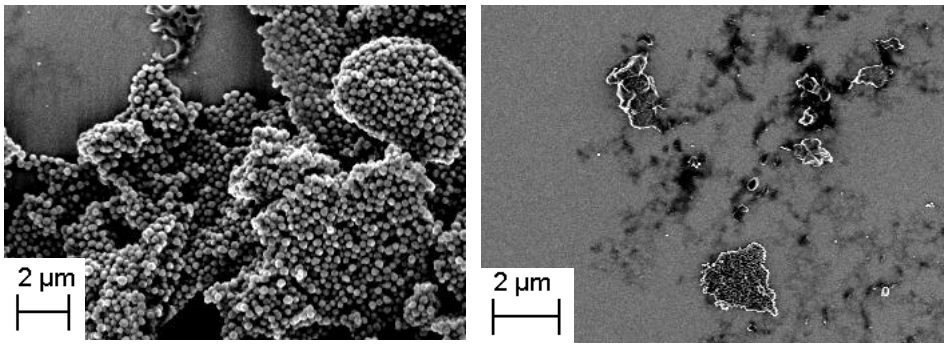

Figure S5: SEM images taken from SiO_2-MV clustering carried out with CB[8] and Np_8-PAMAM.

8.3 SEM as-assembled vs. SEM after 1 h sample collection

To evaluate whether the collection time affects the outcome of the DLS results, additional SEM microscopy was carried out. The obtained SEM images directly taken from the chip were compared with the SEM images obtained for samples kept standing for 1 h. As shown in the images below, no significant difference is visible between the two samples. The samples collected directly from the microreactor showed an average SNPC size of 144 ± 44 nm, whereas the sample taken 1 h after collection gave an average diameter of 149 ± 62 nm. Excluding the unbound NPs for determination of the average cluster size gave average diameters of 174 ± 41 nm and 170 ± 33 nm for direct and after 1 h measurement, respectively.

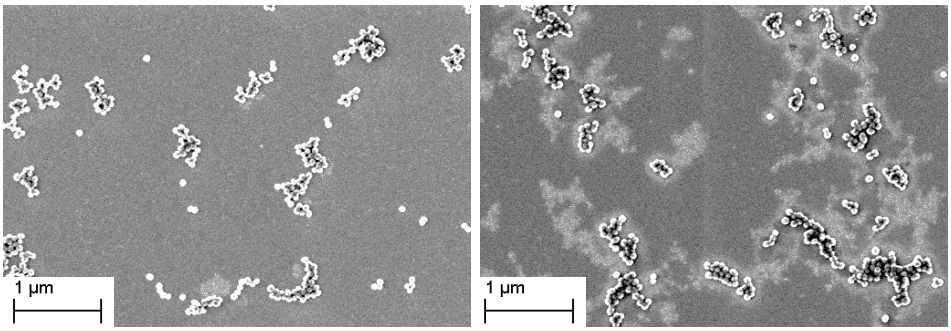

Figure S6: SEM images of SNPC sample collected directly from microfluidic reactor vs. SEM images of sample prepared 1h after starting the supramolecular self-assembly.

8.4 SNPC formation in the presence of CB[7] instead of CB[8]

As a control, cluster formation was attempted using the smaller cucurbit[n]uril homolog CB[7]. The SiO_2-MV NPs were dispersed in water (0.8 mg/ml) and a mixture of CB[7] (4 µM), Np-PEG (1.5 µM) and Np_8-PAMAM (0.313 µM) was prepared. SNPC formation was performed in a microreactor as described in section 5 with a residence time of 60 s. As visible in Fig. S9, individual particles were observed with both analyzing techniques. DLS gave an average number diameter of 63 ± 3 nm and an average area diameter of 72 ± 4 nm.

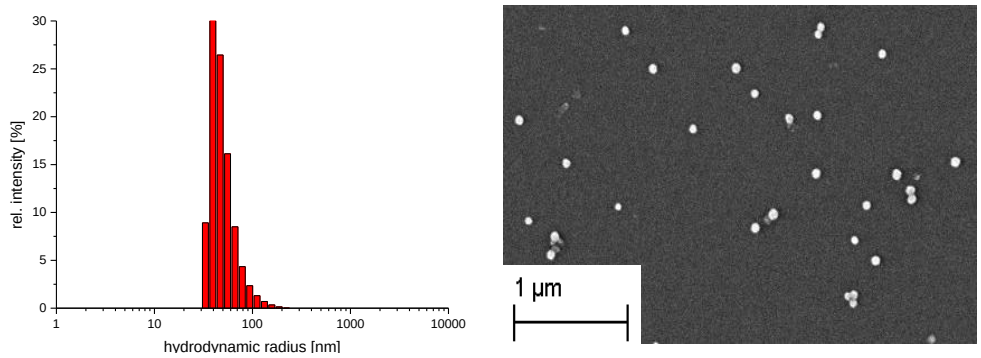

Figure S7: Average area DLS graph and SEM image of clustering experiment carried out with SiO_2-MV, CB[7] (instead of CB[8]), Np-PEG and Np_8-PAMAM (62.5% Np from Np_8-PAMAM).

8.5 SNPC formation in presence of SiO_2-OH instead of SiO_2-MV

To additionally control if the SNPC formation is triggered by selective host-guest interaction bare silica particles were used for clustering experiments instead of SiO_2-MV. Clustering experiments were carried out by mixing the particles dispersion (0.8 mg/mL SiO_2 NPs in water) with a pre-mixed solution of Np-PEG, CB[8] and Np_8-PAMAM containing a 4 µM concentration of Np and CB[8] binding sites as well as 62.5% Np derived from the multivalent dendrimer. The two solutions were flowed into the microreactor as described in section 5 with a residence time of 60 s. DLS was carried out after collecting sufficient sample, whereas SEM was prepared by collecting the clustering solution directly from the outlet of the microreactor. As is visible in Fig. S12, no clustering can be observed for the non-functionalized silica particles in presence of CB[8], Np-PEG and Np_8-PAMAM.

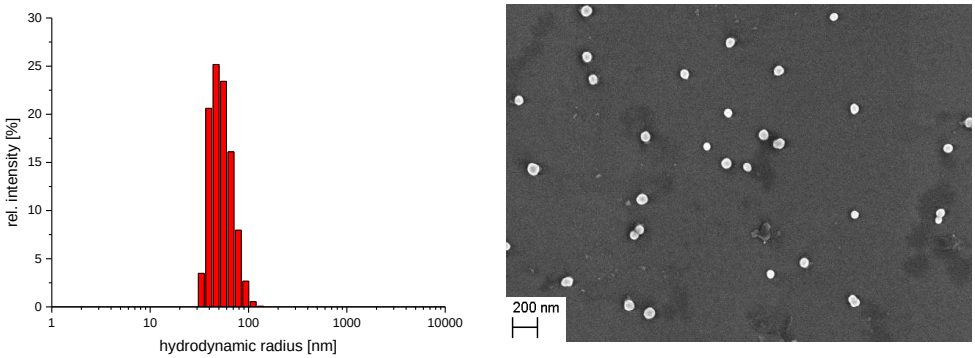

Figure S8: Average area DLS graph and SEM image of clustering experiment carried out with SiO_2-OH, CB[8], Np-PEG and Np_8-PAMAM (62.5% Np from Np_8-PAMAM).

9. Variation of flowrate during microfluidic assisted self-assembly

9.1 SNPC size analysis

SNPCs were made at different flow rates as described in section 5. DLS was measured immediately after collecting sufficient volume as well as 1 h after starting the microfluidic assembly. This 1 h represents the time required to collect enough sample for the sets prepared with 60 s residence time. The following DLS graphs show, that only slight variation is observable between the two different measurement sets. As for the clustering experiments carried out with variable Np origin, SEM samples for experiments with varying flowrates and varying stoichiometry were prepared by diluting a small droplet directly collected from the outlet of the microreactor. At least 150 individual clusters were measured to determine the average size of the SNPCs. Additionally another average SNPC size was determined by excluding all unbound NPs.

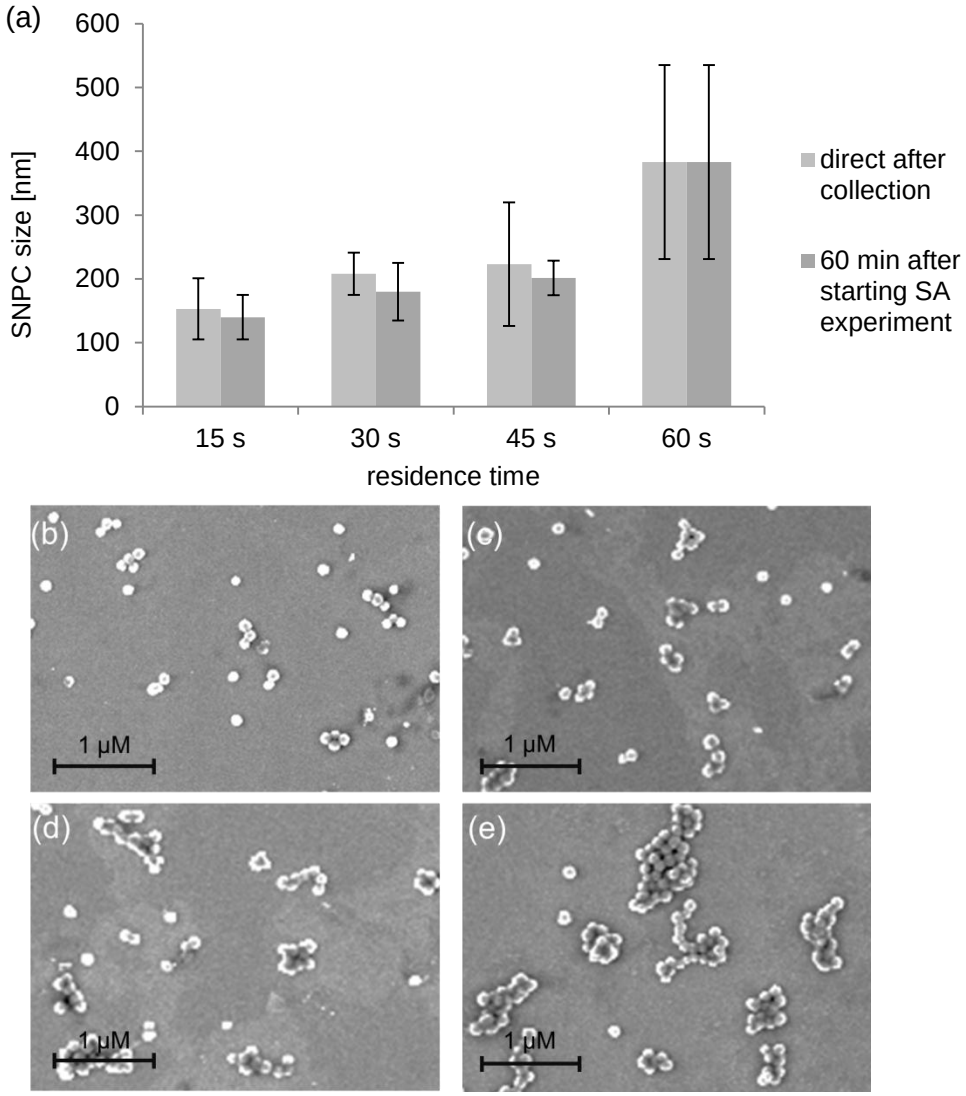

Figure S9: Average NP cluster diameter via DLS of clustering experiments carried out with SiO_2-MV, CB[8], Np-PEG (1000 g/mol) and Np_8-PAMAM (50% Np derived by the dendrimer) directly after collecting sufficient sample (light grey bars) and 60 min after starting the self-assembly experiments (dark grey bars). SEM images (b-e) of the resulting SNPCs prepared within a microfluidic device at different residence times of the two interaction streams (b: 15 s c: 30 s d: 45s e: 60 s).

9.2 SNPC polydispersity

Next to the SNPC size also the polydispersity (PDI) of the samples were reported by DLS. The following tables shows the average observed PDI values for the individual measured samples directly after sample collection.

Residence time	Sample	PDI	Residence time	Sample	PDI
15 s	1	0.58	45 s	1	0.31
15 s	2	0.72	45 s	2	0.23
15 s	3	0.65	45 s	3	0.36
30 s	1	0.51	60 s	1	0.30
30 s	2	0.61	60 s	2	0.28
30 s	3	0.72	60 s	3	0.35

Table S3: PDI of the individual samples prepared with different microfluidic residence times.

10. Variation of stoichiometry during microfluidic assisted self-assembly

10. 1 SNPC size analysis

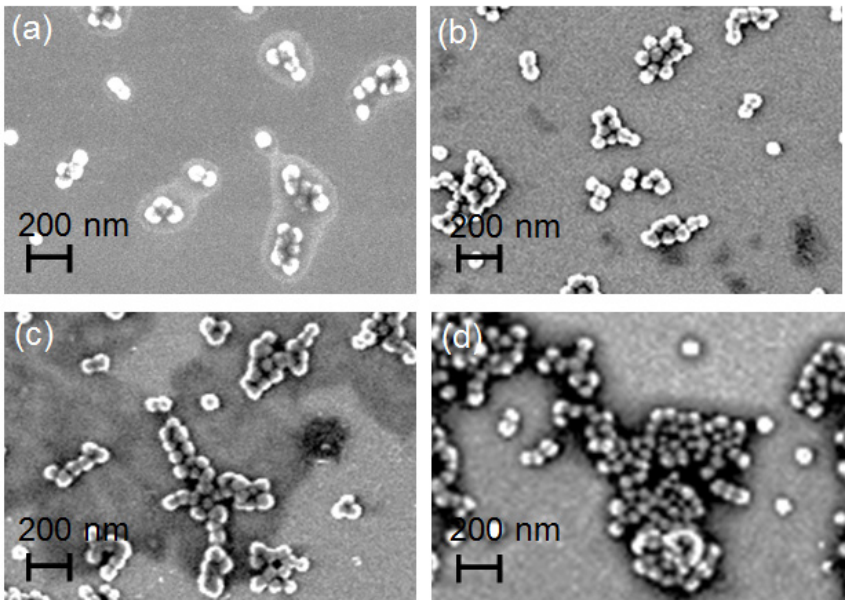

Figure S10: SEM images of the resulting SNPCs prepared within a microfluidic device with different CB[8]/MV/Np ratios (a: 1:1:1; b: 2:1:2; c: 3:1:3; d: 5:1:5).

10.2 SNPC polydispersity

Next to the SNPC size also the polydispersity (PDI) of the samples were reported by DLS. The following tables shows the average observed PDI values for the individual measured samples after sample collection. It has to be noted, that the PDI observed for samples prepared with the highest concentration of CB[8] are doubtful as DLS analysis were barely possible because of sample aggregation.

CB[8]/ MV/ Np	Sample	PDI	CB[8]/ MV/ Np	Sample	PDI
1:1:1	1	0.68	3:1:3	1	0.80
1:1:1	2	0.53	3:1:3	2	1.26
1:1:1	3	0.57	3:1:3	3	0.97
2:1:2	1	0.89	5:1:5	1	1.25
2:1:2	2	0.54	5:1:5	2	1.40
2:1:2	3	0.77	5:1:5	3	1.54

Table S4: PDI of the individual samples prepared with different host guest partner stoichiometrys.

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