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

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The formation of size-tunable, supramolecular nanoparticles (SNPs), employing cucurbit[8]uril-assisted naphthol–viologen charge-transfer complexes, is strongly time and temperature dependent. Yet, the ternary complex formation is fast at all temperatures employed. This indicates that SNP formation requires dynamic disassembly and reassembly of the constituting ternary complex units.

Supramolecular chemistry promises the assembly of molecular units into well-defined architectures, *via* specific non-covalent interactions.[1–3] Nanoparticles (NPs) possess unique size-dependent properties that are not observable in bulk materials.[4] The combination of both fields is attractive, since stable, yet reversible hybrid assemblies hold great promise for biomedical applications.[5–7] External triggers can induce the selective disassembly of supramolecular systems, which leads to responsive drug delivery formulations.[8,9]

Supramolecular nanoparticles (SNPs) are particles in which multiple copies of different building blocks are brought together by specific noncovalent interactions, resulting in assemblies that are typically larger than the building blocks themselves. The most well studied SNP, a siRNA delivery system, has been developed by the group of Davis.[10,11] DNA delivery vectors were formed owing to electrostatic interactions between a β-cyclodextrin (CD)-containing polycation in the presence of negatively charged DNA. Stabilization of these relatively weakly bound particles was achieved by supramolecular host–guest interactions of the CD-containing polycation with monovalent adamantyl (Ad)-grafted poly(ethylene glycol).[12] In contrast, NP formation exclusively based on supramolecular CD–Ad interactions has been demonstrated by the group of Tseng, whereby the size of the formed NPs could be altered by varying the ratio of the monovalent and multivalent Ad derivatives.[13]

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Although multivalency plays an important role in the dynamic molecular assembly and disassembly processes targeting the formation of stable materials,[14–16] the mechanism of the formation and size control of the SNPs remains elusive. Beyond the Ad–CD binding motif, a variety of molecular recognition units are available in supramolecular chemistry, however, none of these has been used for the preparation of size-tuneable SNPs. Thus, a basic question arises whether SNPs can be formed using different interaction motifs. Additionally, the expected changes in interaction strength and exchange rate may have a profound influence on the assembly and disassembly processes of the SNPs.

Cucurbit[*n*]urils (CB[*n*]s) constitute a class of macrocyclic molecules that form inclusion complexes through hydrogen bonds and hydrophobic and ion–dipole interactions,[17,18] *e.g.* when using positively charged guests such as methyl viologen (MV). Moreover, MV and naphthol (Np) can form a charge-transfer (CT) complex which is included in the cavity of CB[8] to give a ternary complex with micromolar affinity.[19] Utilizing this or similar ternary supramolecular motifs, different nanoparticulates[20,21] and microstructures[22] have been reported by the groups of Scherman and Abell. In the NP studies,[20,21] however, the CBs have been used merely to crosslink intramolecularly connected guest moieties attached to a single polymer chain, thus smaller NPs are provided due to the collapse of the starting guest-modified polymer. The strong binding affinity of this interaction motif, but with a radically different kinetics compared to CD complexes, makes it an interesting candidate to explore its potential in the formation of SNPs and to study their assembly kinetics.

Here, we report a novel strategy for the preparation of size-tunable SNPs in water using CB[8] as an intermolecular host for MV and Np-containing building blocks, with the formation of a host–guest-assisted CT complex. As shown in Fig. 1, the host–guest recognition between MV-PEI, Np_8-PAMAM and Np-PEG (MW $\sim$ 5000 g mol^{-1}) in the presence of CB[8] leads to SNPs composed of four building blocks and held together by multiple ternary complexes. Characterization of the multivalent Np_8-PAMAM and the monovalent Np-PEG (Fig. 1b) showed that the reactive binding sites of the parent amino-terminated dendrimer and PEG were fully functionalized with Np guest moieties.

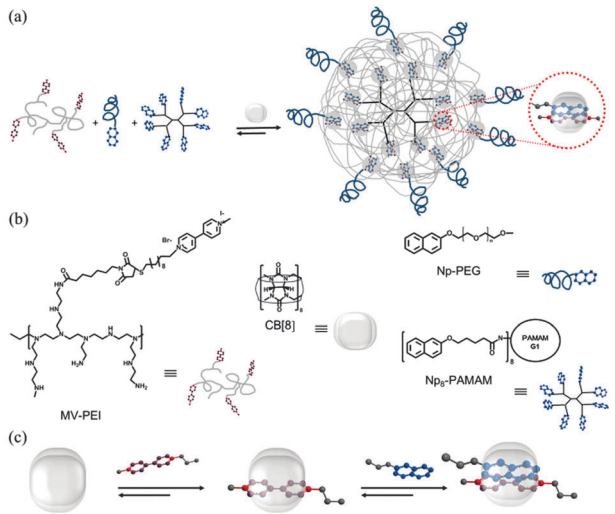

Fig. 1 (a) Supramolecular nanoparticle formation by ternary complex formation between cucurbit[8]uril (CB[8]), methyl viologen (MV) and naphthol (Np) moieties. (b) Supramolecular building blocks involved in particle formation: methyl viologen-poly(ethylene imine) (MV-PEI), naphthol-poly(ethylene glycol) (Np-PEG), cucurbit[8] uril and naphthol$_8$-poly(amidoamine) (Np$_8$-PAMAM). (c) Ternary complex formation by inclusion of MV in CB[8], followed by inclusion of Np.

The degree of functionalization of the multivalent MV-PEI polymer (~10 kDa) was evaluated using UV/Vis spectroscopy, microcalorimetry and ^{1}H-NMR, and all techniques showed each polymer chain to be functionalized with, on average, four MV guest moieties.

Upon addition of aqueous MV-PEI to a previously prepared aqueous solution of CB[8], Np-PEG and Np$_8$-PAMAM, SNPs were formed using a 0.67 μM concentration of each of the three molecular recognition groups (CB[8], MV and Np). The concentrations of Np-PEG and Np$_8$-PAMAM were varied in different ratios, while keeping the total Np concentration constant, in order to study the influence of the multivalent–monovalent competition on the SNP size. Thereby, the resulting intrinsic ratio of the three functional host–guest recognition units CB[8]/MV/Np was kept constant at 1 : 1 : 1 in accordance with the stoichiometry of the basic ternary complex motif. After 2 days, particle formation was confirmed using DLS and SEM. As shown in Fig. 2 (right panel), utilizing 20% Np derived from Np$_8$-PAMAM, SNPs with an average hydrodynamic diameter (d_h) of 85 ± 15 nm were obtained as observed using DLS. In good agreement, SEM (Fig. 2, left panel) showed particles with an average diameter of 74 ± 13 nm.

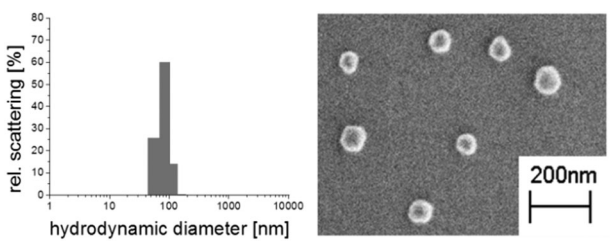

Fig. 2 Characterization of CB-mediated SNPs prepared using Np : MV : CB[8] = 1 : 1 : 1, with 20% Np derived from Np$_8$-PAMAM : DLS data (left panel); SEM image (right panel).

To evaluate whether the SNP formation is based on the selective host–guest interaction of the different supramolecular building blocks, the formation of the CT complex was studied. An increase in UV/Vis absorbance (Fig. S5, ESI†) as well as a decrease in fluorescence intensity (Fig. S6, ESI†) indicate that the SNPs are assembled in aqueous solution as a result of formation of a ternary supramolecular complex. Congruently, no SNPs were formed in the absence of MV-PEI or CB[8], or upon addition of CB[7] instead of CB[8] (Fig. S7, ESI†). The dicationic MV moiety is required to enable inclusion of the neutral Np inside the cavity of the CB[8] host, hence no CT complex is formed in the absence of MV. Additionally, intermolecular crosslinking between the MV and Np derivatives cannot occur in the presence of CB[7]; its cavity is too small to host both guest moieties.

Remarkably, a decrease in fluorescence was observed directly after mixing the different supramolecular building blocks at room temperature, whereas consistent SNP formation is only detectable after 48 h. This leads to the presumption that the supramolecular host–guest interactions, forming the CB-assisted ternary complexes, are established immediately after mixing the components. Initially, undefined, kinetically trapped structures are formed which slowly reassemble into stable SNPs by exchange of the guest moieties *via* (slow) dissociation and (fast) reassociation. In order to monitor the assembly behaviour of the supramolecular building blocks in more detail, time-dependent DLS measurements were carried out at different temperatures. As seen in Fig. 3, the observed sizes using SNP formulations containing 25% Np derived from DLS data varies with time, and the extent of variations in the SNP size depends on the temperature. At RT the observed amplitude of the SNP size variation was very high and the measurements were not consistent. In contrast, SNP assembly occurred faster at elevated temperatures. Samples kept at 40 °C showed distinct particle formation and consistent DLS measurements already 2 h after sample preparation, and a stable SNP size (96 ± 14 nm) was evident after 10 h.

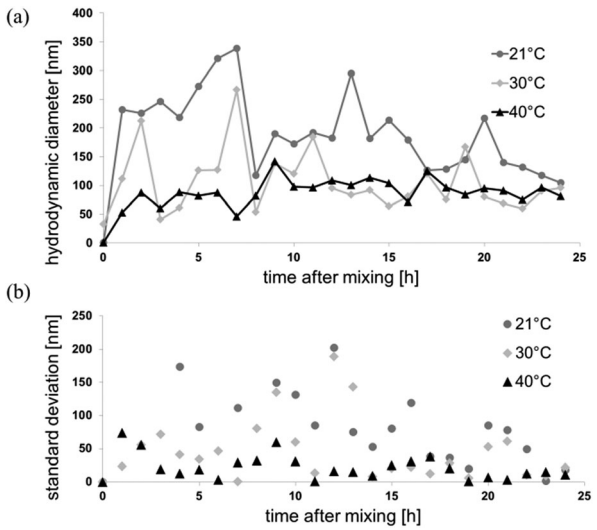

Fig. 3 (a) Hydrodynamic SNP diameters averaged over four measurements and (b) corresponding standard deviations as obtained by time-dependent DLS measurements as a function of temperature.

The temperature-dependent particle formation is ascribed to the dynamic character of the supramolecular host–guest binding. After initial rapid ternary complex formation, disassembly and reassembly of the four building blocks is required for well-defined particle formation. At elevated temperatures the required rearrangement is enhanced which leads to faster SNP formation. Such a time-dependent particle formation has not been reported so far for SNPs based on CD–Ad host–guest interactions. In all cases reported, SNP formation was observed directly after mixing the supramolecular components. We attribute the difference in dynamics to the stronger and more slowly exchanging complexes of CB *vs.* CD. An important advantage of the use of supramolecular interactions for the formation of SNPs is their responsive character. The triggered disassembly of the SNPs reported here has been induced by the reductant $Na_2S_2O_4$ (Fig. S8, ESI†). After addition of the reducing agent, initially observed NPs could not be detected anymore using DLS and SEM. Reduction of the dicationic MV species leads to the formation of MV + radical cations which undergo stable homo-ternary complex formation involving two MV radical cations in one CB[8] host.[23] Thereby, a different supramolecular complex is formed, and the Np entities are released from the CB[8] cavities and, consequently, the SNPs dissolve.

SNP size control was achieved by varying the ratio of monovalent Np-PEG to multivalent Np_8-PAMAM, while keeping the overall Np concentration constant and maintaining an equimolar CB[8]–MV–Np stoichiometry. SNPs were observed for all samples as shown by DLS (Fig. S9, ESI†) and SEM (Fig. S10(a)–(e), ESI†) and the size of the observed SNPs strongly depends on the origin of the Np derivative. In particular, by increasing the amount of Np derived from Np_8-PAMAM from 10% to 35%, an increase in particle size from 57 ± 11 nm to 115 ± 13 nm and from 51 ± 13 nm to 137 ± 16 nm was observed using SEM and DLS, respectively. As seen in Fig. 4, the observed SNP size exhibits an apparent linear relationship with the relative amount of Np derived from Np_8-PAMAM. For samples containing 50% or more of Np derived from Np_8-PAMAM, DLS showed d_h beyond 1000 nm (Fig. S9, ESI†), as well as precipitation. The formation of SNPs is established by multivalent interactions in the core and monovalent interactions of Np-PEG at the outer surface of the particles. Thereby an equilibrium between capping and crosslinking of Np building blocks is established with time, as indicated by the time-dependent measurements. Stable particle formation requires termination of the multivalent interactions that are established between Np_8-PAMAM and MV-PEI in the presence of CB[8]. The results obtained for the size dependence show that more than 50% monovalent Np-PEG is required to stabilize the NPs. Distinct supramolecular NP size control is achieved by fine-tuning the balance of multivalent *vs.* monovalent interactions. As expected, increasing the concentration of the multivalent dendrimer leads to the formation of larger SNPs.

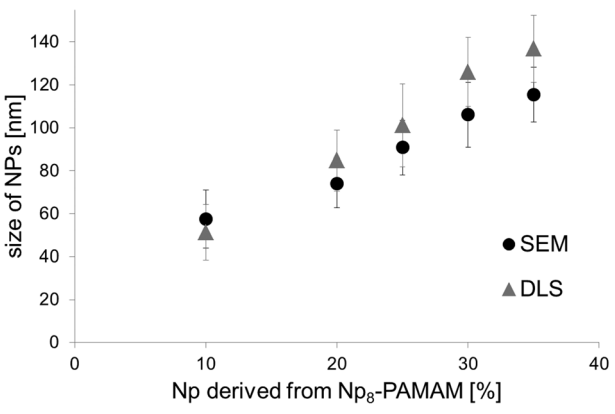

Fig. 4 SNP diameter obtained *via* DLS (▲) and hrSEM (●).

In conclusion, we have developed a novel, versatile strategy for the preparation of SNPs, in which CB[8] acts as an intermolecular connector between MV-PEI, Np_8-PAMAM and Np-PEG upon formation of a ternary supramolecular CT-complex. Of particular importance are the observed assembly kinetics and the size-focusing in time, which indicates progression towards thermodynamic equilibrium. The distinct size tunability as well as the stimulus-responsive particle disassembly make such NPs a potential candidate for biomedical applications in drug delivery, peptide therapeutics as well as *in vivo* sensing.

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