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COMMUNICATION

Cyclodextrin-Based Supramolecular Nanoparticles Stabilized by Balancing Attractive Host-Guest and Repulsive Electrostatic Interactions

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Multicomponent, negatively charged supramolecular nanoparticles (SNPs) were formed by multivalent host-guest interactions without the need of a stopper in water and in PBS. Their size and stability are controlled owing to a balance of forces between attractive supramolecular and repulsive electrostatic interactions.

Supramolecular nanoparticles (SNPs) are formed by specific non-covalent multivalent supramolecular interactions in a multicomponent system.¹ These SNPs present several advantages such as size control, controlled assembly/disassembly, modular character, and easy incorporation of imaging agents or targeting ligands.¹ Three basic types of SNPs can be distinguished: a) SNPs for siRNA delivery by Davis,² in which the particles are based on electrostatic interactions between negatively charged siRNA and a positively charged cyclodextrin-containing polymer, b) a supramolecular system by Tseng,³ in which the particles are formed by multivalent host-guest interactions between a positively charged cyclodextrin-grafted polymer and positively charged PAMAM dendrimers containing adamantyl moieties and c) the dextran-based host-guest nanoparticles by Larsen,⁴ based on host-guest interactions between cyclodextrin and adamantyl-grafted neutral dextran polymers. These systems have been used for different biomedical applications, such as imaging,⁵ photothermal therapy,⁶ drug delivery,^{3, 7, 8} and gene delivery.^{2, 9}

The cyclodextrin-based SNPs make use of adamantyl-terminated poly(ethylene glycol) (AdPEG) as a monovalent capping agent (stopper), to terminate the multivalent supramolecular network, avoiding therefore the continuous growth of the particles and their aggregation due to the attractive multivalent interactions in the Tseng system and electrostatic forces in the Davis system.^{2, 3, 10} However, in the case of the dextran-based nanoparticles, the absence of a monovalent capping agent to compete with the multivalent interactions together with the neutral character of the two different building blocks, results in a near doubling of the size in less than five days.⁴ Despite the importance for SNPs, little is known about the interplay of electrostatics, host-guest interactions and steric influences in the control of particle size and stability. In the Davis system,² the electrostatic interactions are attractive; in the Tseng

system,³ they are repulsive; and in the Larsen system, electrostatics is assumed to play a negligible role because of the absence of formal charges on the building blocks.⁴ Here, we aim to study the influence of electrostatics in host-guest SNP systems, in which the host and guest components are two negatively charged polymers. The main focus points are size control, the role of the monovalent capping agent (stopper), and the SNP stability. These SNPs are formed by supramolecular multivalent interactions between β -cyclodextrin (CD) and the *p-tert*-butylphenyl group (TBP),¹¹ using a multicomponent system based on the linear, negatively charged polymer poly(isobutyl-*alt*-maleic acid) (PiBMA) and the monovalent capping agent AdPEG, as shown in Fig. 1. PiBMA is a biocompatible anionic polymer,^{12, 13} being widely used as a coating polymer to make inorganic particles water soluble, and for the formation of polymeric nanoparticles for drug and gene delivery.¹²⁻¹⁵

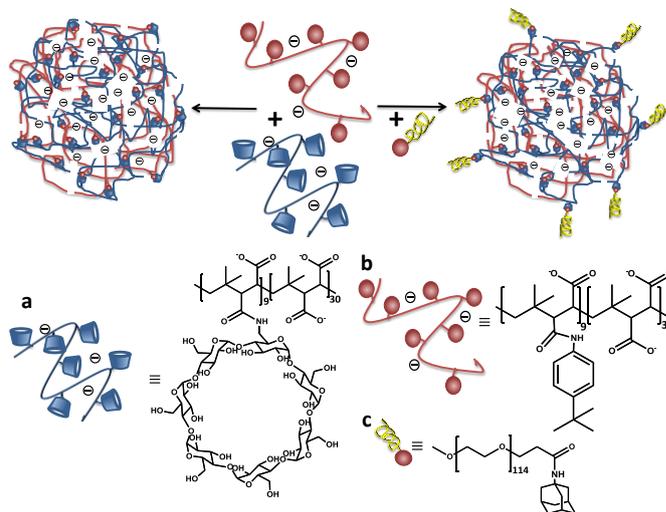


Fig. 1. Schematic representation of the assembly of the SNPs from the host polymer PiBMA grafted with β -cyclodextrins (PiBMA-CD, a), the guest polymer PiBMA grafted with *p-tert*-butylphenyl groups (PiBMA-TBP, b), and AdPEG (c).

The synthetic route of the different components is described in the ESI†. The host polymer PiBMA-CD (Fig. 1a) and the guest polymer PiBMA-TBP (Fig. 1b) were synthesized by amidation of poly(isobutyl-*alt*-maleic anhydride) (MW 6 kDa) with 6-monodeoxy-6-monoamino- β -cyclodextrin¹⁶⁻¹⁸ or *p*-*tert*-butylaniline, respectively, and subsequent ring-opening of the unreacted anhydrides with a sodium hydroxide solution.¹⁹ The crude products were purified by dialysis and neutralized before freeze-drying. ¹H-NMR (ESI) analysis revealed that the polymers contained nine CD or nine TBP moieties per polymer backbone by comparing the methyl signals of the polymer with the H1-CD signal of the CD moieties (PiBMA-CD) or the backbone signals of the polymer with the *tert*-butyl signal (PiBMA-TBP). AdPEG was synthesized as described in the literature.⁵

The particles were easily prepared by mixing the components maintaining a 1 : 1 ratio between host (CD) and guest (TBP and Ad) moieties at 15 μ M CD and varying the multivalent/monovalent (TBP/Ad) ratio between 0% and 100% AdPEG. Fig. 1 shows a schematic representation of the assembly of these SNPs from the host (a, PiBMA-CD) and the guest (b, PiBMA-TBP) polymers with/without AdPEG (c).

DLS measurements of the particles obtained without AdPEG (142 nm; Fig. 2a) and with 10% AdPEG (145 nm; ESI) in water showed nanoparticles of comparable hydrodynamic diameters. SEM images (Fig. 2b without AdPEG, and ESI with 10% AdPEG) show amorphous nanoparticles with a size of 70 nm. The particle size by SEM is smaller than that measured by DLS in aqueous solution due to (i) shrinking of the particles upon drying, and (ii) DLS reporting the hydrodynamic radius of the hydrated particles. SEM images of the host or guest polymers individually, and of the mixtures of host or guest polymer together with unfunctionalized PiBMA (controls, ESI) only revealed some big polymer aggregates due to hydrophobic-hydrophobic interactions between the polymers²⁰ but no nanoparticle formation. This confirms that specific multivalent supramolecular interactions occur between the components of the SNPs in case PiBMA-CD and PiBMA-TBP are used together. The particles were also prepared in phosphate buffered saline (PBS, 138 mM NaCl, pH 7.4) as a mimic of the ionic strength under physiological conditions, resulting in particles with comparable size and morphology as the particles in water as determined by DLS and SEM (see Fig. 2c and ESI).

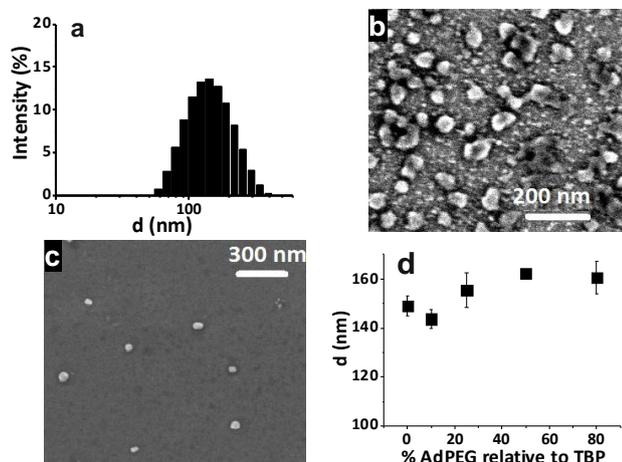


Fig. 2. DLS graph (a) and SEM images of the particles prepared without AdPEG in water (b) and in PBS (138 mM NaCl, pH 7.4) (c). (d) Particle size as a function of the percentage of AdPEG at host : guest = 1 : 1.

In order to investigate further the function of AdPEG in this system, SNPs were formed at different AdPEG : TBP ratios while maintaining the host : guest 1 : 1 stoichiometry. Fig. 2d shows that the particle size is comparable over the whole range of 0-100% of AdPEG. This means that the competition between multivalent (PiBMA-TBP) and monovalent (AdPEG) guest moieties does not have an influence on the particle formation and its size. This is in contrast to other supramolecular nanoparticles systems, where size control is achieved by changing the ratio between monovalent and multivalent components.^{3, 10, 21} This is even more remarkable when one recognizes that the Ad-CD interaction is stronger than the TBP-CD interaction (by approx. a factor 5).¹¹ Overall, these results indicate that multivalent CD-TBP interactions, which are stronger than monovalent CD-Ad interactions, cause SNP formation but that electrostatic repulsion prevents further PiBMA building blocks to add to the outer layer of the SNP upon a certain particle size has been reached.

The stability of the nanoparticles in pure water over time at 25 °C and 36 °C without AdPEG (Fig. 3a) and with AdPEG (ESI) at 15 μ M CD was studied by DLS. The results show that these particles are very stable in water: the size of the NPs is maintained for at least one month at 25 °C and for at least 20 days at 36 °C. This also demonstrates that these particles are stable at physiologically relevant temperatures. Furthermore, it is striking that in the absence of AdPEG, the particle stability is not affected over time.^{2, 3, 21} The particle stability was also studied in PBS (138 mM NaCl, pH 7.4), at room temperature and at 36 °C without AdPEG (Fig. 3b), with 10% and with 80% AdPEG (ESI). The graphs show that the particle size is maintained for almost 8 hours, after which it starts to slowly increase leading to particle aggregation. The results obtained are comparable with those in water and not influenced by the presence of AdPEG: the particle stability at 36 °C without (Fig. 3) and with 80% AdPEG (ESI) is alike. This 8 hours stability under these conditions make these SNPs suitable for biomedical applications.

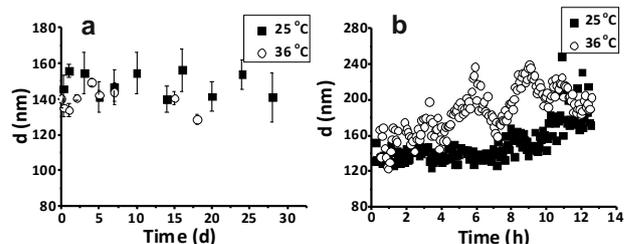


Fig. 3. Particle stability over time of the SNPs prepared without AdPEG at 25 °C and at 36 °C in a) water and in b) PBS (138 mM NaCl, pH 7.4).

We attribute the remarkable stability of the particles, even without the use of a stopper, to the existence of a balance of forces in these SNPs: attractive host-guest interactions and repulsive electrostatic forces between the negatively charged host and guest polymers co-exist, which apparently lead to a stable supramolecular nanoparticle system without the need for a monovalent stopper. Strikingly, in the Tseng system,³ repulsive interactions also exist between the positively charged poly(ethylene imine) polymer grafted with cyclodextrins and the PAMAM dendrimers functionalized with adamantyl groups, yet this system shows size control by AdPEG. Probably, the charge density in our system is larger, which results in a larger contribution of the electrostatic repulsive interactions. In the Davis system,² the electrostatic interactions are attractive, therefore, a monovalent capping agent is needed to stabilize the particles in biological media, avoiding their aggregation.

In acetic acid buffer at pH 4.2, the carboxylic groups of the PiBMA backbone are partly being protonated leading to a decrease of the overall charge of the host and guest polymers. Use of this buffer resulted in immediate aggregation and precipitation of the SNPs prepared without AdPEG. Analogously, decrease of the electrostatic repulsions between host and guest polymers was achieved by forming the SNPs at increasing ionic strengths varying from 100 to 1000 mM KCl^{22, 23} at a 1 : 1 host : guest ratio without AdPEG and 15 μ M CD at pH 7. Fig. 4a shows DLS results of these experiments which clearly demonstrate the destabilization of the particles with increasing ionic strength. While in the absence of KCl, the particles remained stable for over one month, at 100 mM KCl, the particle size was maintained for one day, after which it started to increase slowly. At 300 mM KCl, the SNPs began to grow already from the first day onward and reached almost twice their original size after 2 days. At 1000 mM KCl, the particles were continuously increasing in size and they reached four times their original size after 2 days. These results demonstrate that the balance between the attractive host-guest and the repulsive electrostatic forces between the host and the guest polymers can be disturbed by lowering the pH and/or increasing the ionic strength giving rise to the continuous growth of the supramolecularly cross-linked network.

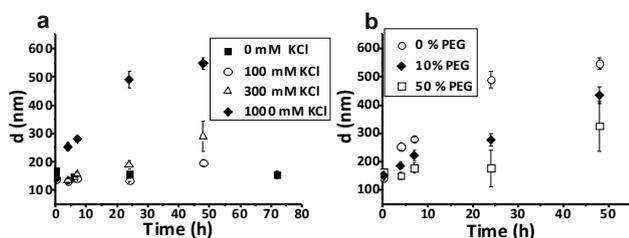


Fig. 4. a) Influence of ionic strength on particle stability in the absence of AdPEG. b) Influence of AdPEG on particle stability at 1000 mM KCl in water.

These results open up the possibility of particle stabilization induced by a monovalent capping agent (AdPEG) at high ionic strengths. To assess this, SNPs were prepared at 1000 mM KCl at a 1 : 1 host : guest ratio in the absence of AdPEG, with 10% AdPEG and 50% AdPEG and their stability was monitored over time. Fig. 4b clearly shows that, interestingly, AdPEG has a clear function in this case. Upon increasing the percentage of AdPEG in the SNPs, the particle stability improves: at 50% AdPEG the particle size was maintained for about 24 h, whereupon it started to increase. At 10% AdPEG, the particle stabilization is apparent but less pronounced. These results clearly prove that once the electrostatic repulsions between the host and guest polymers are disturbed, the stopper AdPEG starts to play a role in the particle stabilization and consequently stops the continuous growth of the supramolecular network.

In conclusion, we have developed a multicomponent supramolecular nanoparticle system using the negatively charged polymer PiBMA. The particles are formed by multivalent supramolecular interactions between the host CD and the guest TBP, and their size and stability are controlled owing to a balance of forces between attractive supramolecular host-guest interactions and repulsive electrostatic interactions between the host and the guest polymers. The understanding of the forces underlying SNP formation and stability will further the development of SNPs as carriers for biomedical applications such as imaging or drug delivery agents.

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†Electronic Supplementary Information (ESI) available: Synthesis and characterization of the polymers, ¹H-NMR spectra of the polymers, protocol of the preparation of the particles in different media, characterization of the particles (DLS, HRSEM), controls, particle stability with AdPEG. See DOI: 10.1039/c000000x/