Supramolecular Nanoclusters

Host–Guest and Electrostatic Interactions in Supramolecular Nanoparticle Clusters


Abstract: The influence of the charge, structure, and host-guest interactions of different soft polymeric components on their assembly with negatively charged inorganic gold and up-converting nanoparticles (NPs) functionalized with cyclodextrins (CD-AuNPs and CD-UCNPs) was studied. The inter-/intra-molecular interplay was evaluated by changing the morphology and the charge of the polymeric component. Use of a linear, anionic guest-functionalized polymer resulted in the formation of thin coating layers around the CD-AuNPs and CD-UCNPs without further NP aggregation. When the branched cationic polymer poly(ethylene imine) (PEI) was used instead, small clusters were obtained before the electrostatic neutralization point, whereas large NP aggregates were obtained above the neutralization point. When branched cationic guest-functionalized dendrimers were used, clusters were obtained at a 1:1 host/guest stoichiometry. In this case, self-assembly occurred when still far away from the neutrality point, indicating that this cluster formation is driven by supramolecular host–guest interactions. The luminescence of the CD-UCNPs with the linear polymer was quenched due to C–H vibrational modes of the guest polymer, whereas in the case of electrostatic cluster formation by PEI the luminescence of CD-UCNPs was enhanced after the neutralization point, due to the formation of densely packed NP assemblies. These findings should be useful for the design of hybrid polymeric–inorganic assemblies for sensing and biomedical applications.

Introduction

Inorganic nanoparticles (NPs) have cores in the size range of 1–100 nm and display new properties different from those of the bulk material, arising from their nm size.[1] The cores of these nanoparticles can be made from a large variety of materials, such as iron oxide, gold (AuNPs), silver, semiconductors (quantum dots), and lanthanide oxides or fluorides (up-converting and down-converting NPs, UCNPs and DCNPs). Of these types of inorganic nanoparticles, AuNPs and UCNPs are particularly interesting for nanomedicine, in particular for photothermal therapy (AuNPs) and for bioimaging applications (UCNPs).[2]

Inorganic nanoparticles are generally synthesized with a stabilizing coating layer, which can vary from different charged ligands[3] to host or guest supramolecular components.[4] The presence of this coating allows the interaction of the inorganic nanoparticles with soft materials and at the same time provides water solubility, which is essential for biomedical applications. Noncovalent interactions with soft materials such as polymers and dendrimers results in the organization, through controlled aggregation, of the inorganic nanoparticles into hybrid supramolecular assemblies, creating network aggregates.[5] Depending on the chosen soft component, it is possible to control these assemblies with regard to size, shape, and morphology, and thereby to tune the distance between the inorganic nanoparticles within the assembly.[5d] This is important for the targeted applications, especially for sensing and imaging.[5d,6] In cases in which supramolecular host–guest interactions are used as the way to direct the self-assembly process, there have been studies on the influence of the valency, the morphology, and the flexibility of the used guest on the formation of intra- or intermolecular assemblies.[6,7] However, a systematic study of these parameters in combination with the influence of electrostatic interactions on the formation of hybrid network aggregates, especially of UCNPs, is lacking.

Recently, we have studied the preparation and characterization of supramolecular nanoparticles (SNPs), composed of soft anionic polymeric host and guest components, and the influence of electrostatic interactions on the particle formation and stability.[8] Inorganic lanthanide-doped UCNPs, surface-modified with cyclodextrins (CDs), were also synthesized and characterized.[9] Here, our aim was to study the formation of hybrid inorganic-organic, supramolecular nanoparticle clusters (SNPCs), composed of inorganic NPs functionalized with CDs (CD-AuNPs and CD-UCNPs) and different types of soft polymers. In the choice of the polymeric component of the SNPCs, we took account of three parameters: host–guest interactions, elec-
Results and Discussion

Synthesis and Characterization of the Components

AuNPs surface-functionalized with β-CD (CD-AuNPs) were synthesized by a previously reported procedure\(^\text{[4a,b]}\) based on the reduction of HAuCl\(_4\) with NaBH\(_4\) in DMSO containing perthiolated β-CD. The CD-AuNPs were purified by precipitation in acetonitrile and ethanol, collected by freeze-drying, and characterized by TEM, DLS, and UV/Vis spectroscopy (Figure 2). The
sizes of their cores were $(3 \pm 1)$ nm by TEM (Figure 2a). The hydrodynamic diameters were $(10.5 \pm 1.8)$ nm as measured by DLS (Figure 2b). This size difference stems from the presence of the CDs, which are not visible by TEM, on the surfaces of the particles and from the hydrated nature of the NPs during DLS. UV/Vis spectroscopy (Figure 2c) exhibited the characteristic surface plasmon absorption band,[10] with the maximum absorbance wavelength ($\lambda_{\text{max}}$) at 516 nm. There are on average 14 CD units per particle. This number was calculated by assuming that each particle is fully covered with a CD shell, that the diameter of the core of the particle is 3 nm, and that the center–center distance between densely packed CDs in a monolayer is ca. 2 nm.[11]

The $\beta$-CD-grafted poly(isobutyl-alt-maleic acid)-capped, Yb$^{3+}$(15 %)/Tm$^{3+}$(0.5 %)-doped Sr$_2$GdF$_7$ nanoparticles (CD-UCNPs) were prepared and characterized as recently described.[9] The crystallite size of the NPs was 11.7 nm as determined by powder X-ray diffraction (XRD), and the amount of CD was estimated by thermogravimetric analysis (TGA) to be 5.6 wt.-%, which means that a solution of CD-UCNPs of 0.5 mg mL$^{-1}$ provides a CD concentration of 15 μM. These particles were characterized further by DLS and $\zeta$-potential measurements. To study the cluster formation, the concentration of CD-UCNPs used was 3 μM of CD, because at higher concentrations the CD-UCNPs were not fully stable in water and started to flocculate, likely due to hydrophobic interactions between CDs. Because this CD concentration is too low to allow detection of cluster formation by DLS,[8] free CD-PiBMA (9 μM CD) was also added to the solution to reach a sufficiently high CD concentration (12 μM CD). This addition of free CD-PiBMA also assisted in the water solubilization of the UCNPs, probably due to the presence of carboxylate groups on the periphery of the nanoparticle surface. By DLS (Figure 3), a hydrodynamic diameter ($d_h$) of $(60 \pm 9)$ nm was obtained. The $d_h$ differs significantly from the crystallite size as determined by XRD and the particle core size as determined by TEM. We attribute this difference to: (i) the presence of the polymer coating, which is not detected by XRD and TEM, around the NPs, (ii) the hydration of the NPs during DLS measurements, and (iii) some degree of particle aggregation. The $\zeta$-potential [($–39 \pm 2.5$) mV] indicates the presence of negatively charged moieties due to the negatively charged carboxylate groups of the capping ligand.

Cluster Formation with the Linear Polymer TBP-PiBMA through Host–Guest Interactions

The negatively charged linear polymer TBP-PiBMA was added to the negatively charged CD-AuNPs at CD/TBP ratios of 1:1 and 1:4, at a CD concentration of 13.1 μM in water. The hydrodynamic diameter was monitored by DLS over time (Figure 4a). The results show that, although initially there was a slight increase in particle size from 10.5 nm of the individual particles to 15 nm (at a CD/TBP ratio of 1:4), $d_h$ rapidly dropped to the starting value. Therefore, it is concluded that no cluster formation occurred, irrespective of the host/guest ratio and of the time of addition. These results suggest instead that the guest polymer forms a thin coating around the CD-AuNPs. Another indication was given by the (although small) difference in the $\zeta$-potential values. The CD-AuNPs have $\zeta$-potentials of ($–42 \pm 2$) mV, which change to ($–53 \pm 4$) mV upon addition of 1 equiv. of TBP-PiBMA. Addition of another equivalent hardly changes the $\zeta$-potential ($–51 \pm 3$ mV), indicating that 1 equiv. of guest polymer is sufficient to form a layer. The formation of a thin coating without particle aggregation indicates efficient binding of all or most guest moieties to the same particle. This is attributed to the linear structure of the guest polymer and the effective molarity ($EM$) of the guest TBP in that polymer.[12] The $EM$ can be defined as the probability that two different binding sites (for example, a host and a guest group) meet, forming an intramolecular complex, and its value depends on the length and flexibility of the components. It thus provides a measure of the local concentration of the guest binding sites. Here, the $EM$ of TBP in TBP-PiBMA is likely to be higher (mM range) than the concentration of TBP in solution (μM range) and, therefore, intramolecular binding forming a capping layer around the CD-AuNP, instead of intermolecular binding between different particles, is favored. Consequently, cluster formation is suppressed. Earlier work on the adsorption of very
similar polymers on CD monolayers on flat gold, in which very strong, practically irreversible but self-limited, adsorption of the polymers was observed, is fully in line with the behavior observed here.[13] In contrast, steric hindrance occurring in branched molecules, such as dendrimers, prevents full use of all their guest moieties, thus exposing free guest sites for intermolecular, crosslinking interactions.[14]

In the case of CD-UCNPs, the negatively charged linear polymer TBP-PiBMA was added to the CD-UCNPs at a CD/TBP ratio of 1:1 and 12 μM total CD concentration. The cluster formation was monitored by DLS over time (Figure 4b). Upon addition of the linear polymer, the particle size increased from 60 to 225 nm. However, with time, the particle size decreased again, and after 2 d it was the same as before the addition of the guest polymer. Because no precipitation (of larger clusters) occurred, we attribute the observations to breakdown of the clusters with time.

Therefore, we conclude that the same behavior occurs as with the CD-AuNPs: intramolecular bonding is favored over intermolecular interactions, and the guest polymer TBP-PiBMA arranges itself as a thin coating layer around the CD-UCNPs while directing all or most of its guest moieties to the CDs on the particle surface. The initial formation of clusters and their slower breakdown relative to the CD-AuNPs may be attributed to the larger size of the CD-UCNPs.

The up-converting luminescence properties of the CD-UCNPs after excitation at 980 nm were measured before and after (3 d) addition of the linear guest polymer TBP-PiBMA (Figure 5). After the addition (Figure 5b), the luminescence was much lower, which confirms the difference in the particle environment before and after addition of the linear polymer. This quenching of the luminescence is likely to be caused by C–H vibrational modes of the guest polymer.[15] Moreover, the blue luminescence band at 450 nm is quenched considerably more strongly than the 800 nm band, indicating a lower efficiency of the upconversion process than in the case of the uncomplexed UCNPs. This strong quenching might be caused by the formation of a densely packed coating layer around the CD-UCNPs due to multivalent host–guest interactions between CD (CD-PiBMA) and TBP (TBP-PiBMA).

Cluster Formation with the Cationic Polymer PEI through Electrostatic Interactions

In order to study the effect of attractive electrostatic interactions in induction of the aggregation of CD-UCNPs, the positively charged hyperbranched polymer PEI (MW 10 kDa) was added to the CD-UCNPs in a stepwise fashion in phosphate buffer (1 mM, pH 7.4). In this case, in the absence of complementary guest moieties, cluster formation can be induced only through attractive electrostatic forces between the negatively charged CD-UCNPs[9] and the protonated polymer backbone of the PEI. The size of the clusters as a function of the concentration of PEI was monitored by DLS (Figure 6a). Before a concentration of PEI of 0.7 μM was reached, relatively small clusters of well-defined size and small standard deviation were obtained.
Above 0.7 μM, large clusters started to appear. A similar trend was observed in the ζ-potential measurements (Figure 6b). Up to a concentration of 0.7 μM PEI, the clusters were negatively charged, whereas at that concentration, the neutralization point was reached. Above 0.7 μM PEI the clusters became positively charged, but the ζ-potential remained below 10 mV, indicating that no more PEI was adsorbing onto the, then positively charged, clusters. The theoretical neutralization point of the clusters was calculated to be at 0.65 μM PEI by comparing the amounts of positive charges of PEI and negative charges of the ligands on the UCNPs. This value agrees very well with the experimentally determined point of neutralization obtained through DLS and ζ-potential measurements. The process of electrostatically controlled self-assembly corresponds to that observed for anionic SNPs to which PEI was added.[8a]

Upon reaching the neutralization point the SNPs lost colloidal stability, and only large aggregates remained in solution (Figure 7).

Figure 6. Characterization of the clusters between PEI and CD-UCNPs through (a) DLS, and (b) ζ-potential.

Figure 7. TEM images of the clusters between PEI and CD-UCNPs (a) before (0.34 μM PEI), and (b) after (0.85 μM PEI) the neutralization point.

Figure 8. Photoluminescence spectra upon excitation (980 nm) of CD-UCNPs (3 μM CD) + CD-PiBMA (9 μM) (a) before addition of PEI, and after addition of PEI (b) before (0.34 μM PEI), and (c) after (0.85 μM PEI) the neutralization point.
The up-converting luminescence properties of the CD-UCNPs/PEI clusters were studied by measuring their photoluminescence (PL). Figure 8 shows the PL spectra (a) of the CD-UCNPs alone, (b) after addition of PEI before the neutralization point, and (c) after the neutralization point. The luminescence characteristics did not change significantly after addition of PEI before the neutralization point, which suggests that the environment around the particles in the clusters formed through electrostatic interactions did not change. On the other hand, the luminescence increased significantly once the neutralization point was reached. Interestingly, the intensity of the blue band at 450 nm increased proportionally more than that at 800 nm (1.5- vs. 1.2-fold), which is most probably caused by the increase in size of the clusters once neutralization is reached.\(^{[16]}\) The increase in luminescence is most probably due to a combination of two factors: firstly, the presence of a smaller amount of water in the clusters, because they are more densely packed and solvent is removed upon clustering, and secondly, a reduction in the CD-UCNP interparticle distance upon cluster formation.\(^{[17]}\) One of the main current problems with UCNPs is the loss of luminescence intensity after their transfer into water, due to the OH vibrations of the solvent. Therefore, the strategy followed here, with the formation of dense structures through electrostatic interactions, is an interesting and straightforward way to increase the luminescence of these particles in aqueous solvents.

### Cluster Formation with the Branched Dendrimer Ad\(_d\)-PAMAM through Host–Guest and Electrostatic Interactions

The positively charged, branched, guest-functionalized dendrimer Ad\(_d\)-PAMAM was added to the CD-UCNPs, at a 1:1 host/guest (CD/Ad) ratio (12 μM CD, with 3 μM from CD-UCNPs and 9 μM from CD-PiBMA) in phosphate buffer (1 mM, pH 7.4). The branched guest dendrimer Ad\(_d\)-PAMAM was used instead of the linear TBP polymer in order to promote interparticle binding. In this case not only electrostatic, but also host–guest (CD–Ad) interactions are involved. Within minutes after addition, the \(d_h\) had increased from 60 to 225 nm with a polydispersity index (PDI) of 0.2, which indicates the formation of clusters (Figure 9a). Clusters of similar size were still present after 24 h, indicating that the cluster formation was not reversible as in the case of the linear polymer. However, the PDI had at the same time increased almost threefold (to 0.6), which indicates that the clusters were not homogeneous in size. The addition of Ad\(_d\)-PAMAM caused the formation of clusters, due to attractive host–guest and electrostatic interactions. The clusters increased almost four times in size, although the (calculated) neutralization point was still far away: the concentration of positive charges (from the dendrimer) is ca. 23 μM, whereas the concentration of negative charges (“free” carboxylate groups in solution) is ca. 75 μM; thus, there are approximately three times more negative charges present in solution. Therefore, we conclude that the SNPC formation, as witnessed in the increase of \(d_h\), is primarily caused by the attractive host–guest interactions maintaining the 1:1 host/guest ratio. These results correspond to those obtained with CD-AuNPs, in which large aggregates of CD-AuNPs with an Ad-functionalized poly(propylene imine) dendrimer were obtained in the absence of a monovalent capping agent.\(^{[14]}\) The formation of clusters was also visualized by TEM (Figure 9b), which shows large and compacted structures after the addition of Ad\(_d\)-PAMAM.

### Conclusions

The influence of the charge, structure, and host–guest interactions of different soft polymeric components on supramolecular assembly with hard, negatively charged inorganic nanoparticles (CD-AuNPs and CD-UCNPs) was studied. Whereas linear and flexible anionic guest polymers favored intramolecular assemblies forming soft, thin coatings around the particles, branched cationic guest dendrimers favored interparticle interactions and therefore cluster formation, producing clusters at a 1:1 host/guest ratio, still far from the electrostatic point of neutrality. In the case in which only electrostatic forces were used, with the branched, densely positively charged PEI, cluster formation was induced by attractive electrostatic interactions. The size of the aggregates increased upon increasing the number of positive charges. However, above the neutralization point, large aggregates were obtained. The luminescence properties of the CD-UCNPs were also evaluated: with the linear polymer, the luminescence was quenched considerably due to \(\text{C–H}\) vibrational modes of the guest polymer. In the case of cluster formation with PEI, the luminescence after the neutrality point increased considerably due to the formation of dense structures, which bring the particles closer together and also reduce the amount of water around them.

This study explains and clarifies how to control and tune the network aggregate formation of inorganic nanoparticles by changing the nature and structure of the guest component, inducing intra- or intermolecular assemblies through host–guest and/or electrostatic interactions. Moreover, an easy and straightforward way to increase the luminescence of UCNPs in water, through the formation of dense assemblies based on attractive electrostatic interactions, is presented. These results could be useful for developing new inorganic nanoparticle-based systems for bioimaging applications.
Experimental Section

Materials: Chemicals and solvents were obtained from Sigma–Aldrich and used as received. TBP-PiBMA and CD-PiBMA were prepared as described.[8a,b] Ad₈-PAMAM was prepared as described by Tseng and co-workers.[14] MilliQ water with a resistivity of 18.2 MΩ cm at 25 °C was used when indicated. The CD-UCNPs were prepared as recently reported.[9]

Preparations

Preparation of the CD-AuNP Assemblies with TBP-PiBMA for DLS Experiments: The CD-AuNP assemblies with TBP-PiBMA were prepared in MilliQ water at 1:1 and 1:4 host/guest ratios at 13.1 μM CD, from stock solutions of CD-AuNPs (13.1 μM CD) and TBP-PiBMA (13.1 μM TBP). The particle size was measured at pre-established size intervals.

Preparation of the CD-UCNP Assemblies with TBP-PiBMA for DLS, ζ-Potential, TEM, and PL Experiments: The CD-UCNP assemblies with TBP-PiBMA were prepared in phosphate buffer (1 mM, pH 7.4) at a 1:1 host/guest ratio at 12 μM CD (3 μM CD from UCNPs and 9 μM CD from CD-PiBMA), from stock solutions of CD-UCNPs (16 μM CD from CD-UCNPs and 48 μM CD from CD-PiBMA), and TBP-PiBMA (3.87 μM TBP) by mixing the components with a vortex. The final composition of these SNPs was CD-AuNPs (13.1 μM CD) and TBP-PiBMA (13.1 and 52.4 μM TBP). The particle size was measured at pre-established size intervals.

Preparation of the CD-UCNP Assemblies with Ad₈-PAMAM for DLS and TEM Experiments: The CD-UCNP assemblies with Ad₈-PAMAM were prepared in phosphate buffer (1 mM, pH 7.4) at a 1:1 host/guest ratio at 12 μM CD (3 μM CD from UCNPs and 9 μM CD from CD-PiBMA), from stock solutions of CD-UCNPs (16 μM CD from CD-UCNPs and 48 μM CD from CD-PiBMA), and PEI (25 μM) by mixing the components with a vortex. The final composition of these SNPs was PEI (0.34 μM PEI) and is calculated in the same way as before.[8a] The number of positive charges is from the PEI and is calculated in the same way as before.[8a] The results are summarized in Table 1.

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