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Micelles

Supramolecular Structure of 5-nm Spherical Micelles with D_3 Symmetry Assembled from Amphiphilic [3:3]-Hexakis Adducts of C_{60}**

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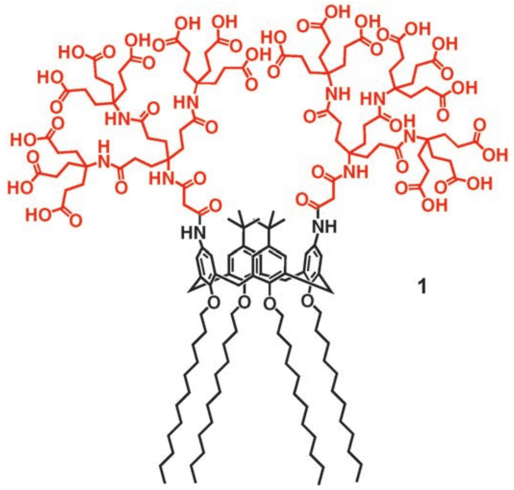
We recently reported on the formation of the first structurally persistent micelles and their three-dimensional characterization by cryo-transmission electron microscopy (cryo-TEM).[1,2] The amphiphilic calixarene derivative **1** forms stable aggregates in water each consisting of exactly seven molecules. It became evident that owing to the polar dendritic head groups and the overall shape and rigidity of the molecules, the close parallel packing arrangement typical for amphiphiles is not possible for **1**. In this intriguing spherical arrangement that **1** assumes, the hydrophobic parts of the molecules are effectively shielded from the polar aqueous phase. Interestingly, not only the steric bulk of the dendritic head groups but also the nature of the chemical linkage to the hydrophobic backbone have significant influence on the ultrastructure. For example, in the amphiphilic fullerene hexakis adduct **2**, the dendritic head groups are connected by ester bonds to the fullerene cage.[3] In this case a variety of aggregation motifs result, most notably extensive bilayered liposomal structures.



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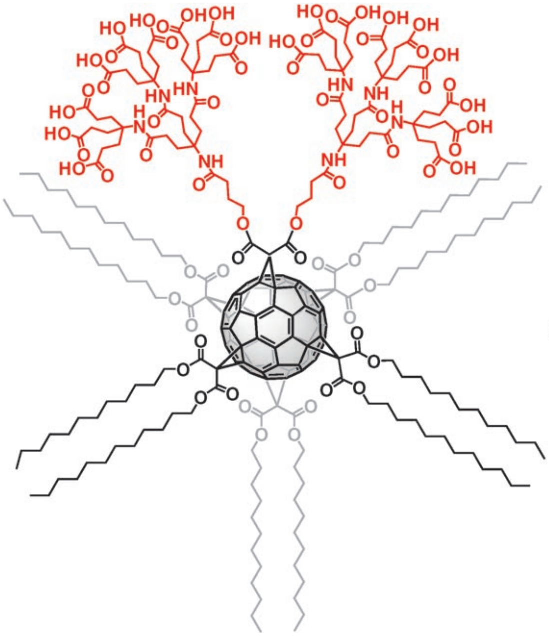

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If, however, the hydrophilic dendrons are linked by amide bonds, as in amphiphile **3**,[2] the formation of diverse molecular architectures is definitely more restricted and solely small micelles are formed. This behavior is attributed to the more pronounced rigidity of the dendritic amide substituents, which disfavors a planar alignment of the amphiphiles as in bilayer sheets or liposomes. Additionally, this effect can be controlled by the pH value. Whereas at neutral pH a mixture of cylindrical and spherical aggregates can be obtained, switching to pH 9.2 leads to the exclusive formation of structurally defined spherical micelles with a diameter of about 8.5 nm. They are composed of exactly eight individual molecules of **3** in an almost cubic arrangement. The degree of protonation apparently determines the extent of dendron

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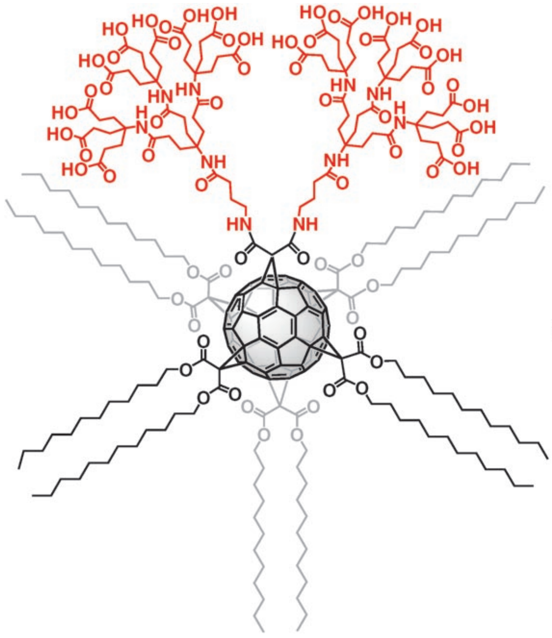

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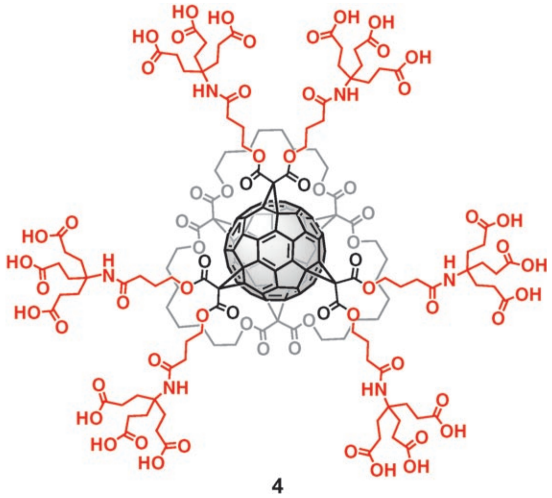
repulsion and therefore the size of the hydration shell, which results in different degrees of curvature in the aggregates.

The fact that the formation of these ultrastructures can be monitored in three dimensions makes it possible to study and understand in more detail the fundamental principles and interdependence between molecular design and supramolecular architecture. Since the variation of the substituents demonstrably leads to different molecular organization (and can be monitored by 3D reconstruction), all prerequisites are fulfilled to further study the factors that determine structural persistence. These studies include aspects of the balance of hydrophilic and hydrophobic substituents in terms of their individual spatial demand required for aggregation. Moreover, the type of chemical bonding as well as the charge distribution must be considered in terms of the degrees of conformational freedom.

We report herein on the aggregation of the [3:3]-hexakis adduct **4**,[4] which in contrast to the amphiphiles **2** and **3** contains three instead of one pair of dendritic carboxylate branches. Moreover, also in contrast to **2** and **3**, we used G1 dendrons, which results in only three instead of nine carboxylates per dendritic branch. However, it should be noted that the total number of charges in **4** is still equal to that in compounds **2** and **3**.



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The hydrophobic part of **4** was also significantly reduced in size by use of the trifunctional *cyclo*-[3]-octylmalonate[5] as the addend, which occupies three equatorial sites in the hemisphere opposite to the head groups. The hydrophobic macrocycle covers one hemisphere of the fullerene, whereas the three pairs of Newkome dendrons are located on the opposite side. The negative charge density located on the carboxylates should cause them to be located very far from each other. In contrast to common amphiphiles,[6,7] the hydrophobic part of **4** represented by the trismalonate macrocycle occupies relatively little space. The diameter of the amphiphile is about 2.5–3 nm.

The molecular topology of the octadecaanion of **4** without counterions was determined by molecular dynamics simulation (Figure 1). First, geometry optimization was carried out using the MM+ force field, followed by a semiempirical PM3 calculation. This structure was then imbedded in a water box ($40\times40\times40$ Å^3) containing 4100 water molecules; this was the starting situation for the MD simulation (see the Supporting Information for details). An almost C_3-symmetrical structure was obtained.

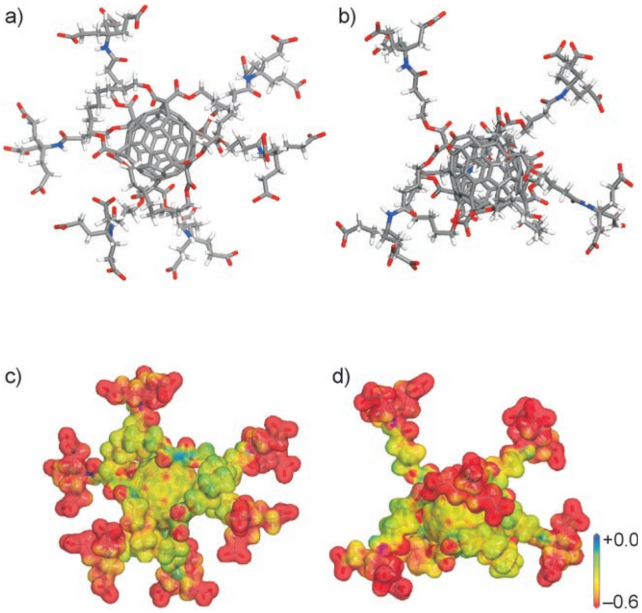

Figure 1. a), b) Molecular structure of the octadecaanion of **4** obtained form molecular dynamics simulation in water; a) bottom view, b) side view. c), d) Charge density distribution $\rho(r)$ of the calculated structure of the octadecaanion of **4**; the two perspectives are identical to those shown in (a) and (b). The MM+ and PM3 calculations as well as the MD simulations were performed with HyperChem.[9] Parameters for the MD simulation parameters: heating time: 15 ps, run time: 15 ps, cooling time: 15 ps, step size: 1 fs, starting temperature: 0 K, simulation temperature: 370 K, final temperature: 0 K, temperature step: 0.2 K.

When studying the aggregation properties of **4** by TEM, we were particularly interested to see if a) by compressing of the hydrophobic part a more densely packed aggregate would be formed and if b) less bulky head groups would have an effect on the aggregation number and the overall structure of the micelles. Indeed, we found uniform spherical objects with a diameter of about 5 nm, which is significantly smaller than the diameter of aggregates of the fullerene derivative **3** (≈8.5 nm).

Owing to the small size of the assemblies and consequently the low contrast in the TEM, we employed a negative staining technique to improve the contrast. This method was previously tested with compound **1** to determine the influence of the contrasting material (phosphotungstic acid) on the supramolecular structure (see the Supporting Information for reference [1]). The reconstructed structure of a stained sample of **1** showed no significant differences to that from vitrified samples (obtained by classical cryo-TEM) even upon completely removal of the water in the vacuum of the TEM (no cryofixation). It therefore appeared reasonable and also necessary to use contrast-enhancing materials for data collection.

In following the "single-particle technique" for studying single aggregates of **4**, image data were treated by multivariate statistical analysis.[8] This procedure determines the differences in a data set of statistically orientated particles and indicates whether there is an inherent ultrastructure. The subsequent analysis of the data allows for the three-dimensional reconstruction of the aggregates' structure; we have described this earlier for a different case.[1,2]

Simple volume calculations based on the above-mentioned simulations indicated that the relatively small aggregates should be composed of only five to six molecules. After a few iterative cycles, a threefold rotational symmetry was identified in the data. For the de novo determination of the structure and the following steps of refinements a C_3-symmetry restriction was therefore imposed. Figures 2 and 3 provide different representations of the final reconstruction, which was determined from 3973 single particles and revealed an even higher D_3 symmetry (for details see the Supporting Information).

These data supported the structure of a spherical micelle that consists of three identical S-shaped motifs, in keeping with the inherent symmetry. The core volume of the sphere, which is thought to accommodate the hydrophobic parts of the molecules (fullerene cage + malonic acid adduct) appears unstructured; this has been observed in all previous cases for the hydrophobic part and is most likely a result of structural indeterminacy. Averaging over thousands of objects therefore does not result in one distinct structure for this part of the assemblies. In the case of the dendronic parts, however, the structure can be determined precisely over the entire data set (this is a prerequisite for a successful reconstruction using the single-particle approach) and therefore allows for its precise allocation.

The interpretation of the structures turned out to be unambiguous owing to the combination of the assemblies' restricted size and distinct structural features. Based on the structural features obtained from the reconstruction, two molecules can be very well fitted into each of the three repeat S-shaped patterns. Interestingly, each dendritic branch interacts with only one nearest neighboring branch. This is facilitated by the slightly tilted arrangement of the three repeat motifs. In this way a overall spherical structure is generated, whose molecular arrangement allows for the optimal efficient shielding of the hydrophobic core from the aqueous surrounding. However, it turns out that the molecular conformation in the aggregates is slightly different from that of an individual molecule simulated in a water box (Figure 1). The specific interactions of the molecule apparently favor a more dense packing of the hydrophilic dendrons, which is achieved by stronger bending of the dendrons towards the water-exposed hemisphere.

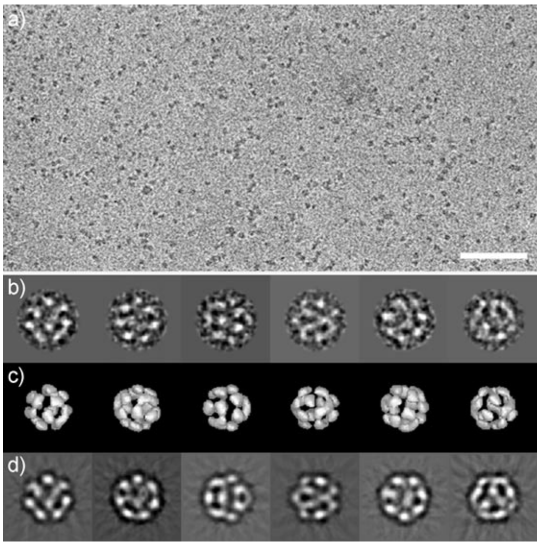

Figure 2. 3D structure determination of aggregates of **4**. a) Negative staining preparation of **4** showing several tens of individual aggregates (scale bar: 50 nm). Note that the contrast has not been inverted for improved visibility. b) Typical spatial views (representative class averages) of micelles (diameter: 5 nm) after filtering, multireference alignment, data compression by multivariate statistical analysis, and automatic classification (see the Supporting Information for details). c) The surface views show the corresponding orientation in space as determined from class sum images in (b). d) Back-projection images obtained by projecting through the 3D volume in exactly the same orientations as shown in (c). The similarity of projection ("3D error") and back projection indicate the fidelity of the structure calculated from the class-sum images.

The organization of the core volume can be understood indirectly by examination of molecular models (Figure 3). The six fullerene cages are organized in a compact arrangement, probably not very different from a dense sphere packing but also includes the interacting malonic acid adducts.

In summary, the aggregation process of the types of amphiphiles used in our recent investigations appears to be driven by the effective volume ratio of the hydrophilic and hydrophobic building blocks. Significantly, the considerable reduction in size of the hydrophobic part does not prevent micelle formation but instead leads to a very dense packing of the amphiphiles. The first-generation dendrons of **4** require

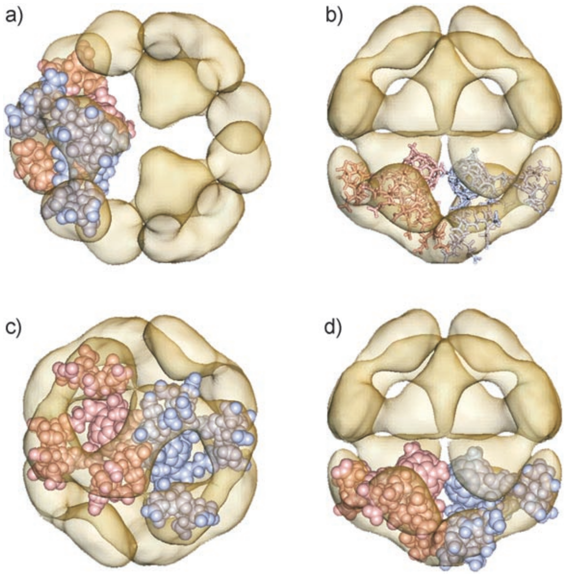

Figure 3. 3D reconstruction of the micelle and molecular fitting. For clarity and owing to the inherent D_3 symmetry, only two of the six molecular views of **4** are shown. The reconstructed structure is given as a surface representation together with visually fitted molecular structures of **4.** a) CPK and b) stick representations of two molecules (out of six) of **4** viewed from the side (perpendicular to the C_3 axis). The orientation of the fullerene cages towards the center of the micelle is clearly visible. c) Same as in (b) but viewed along the C_3 axis. d) Same as in (b) but rotated by 90° about the C_3 axis.

far less space than the second-generation dendrons in **1**–**3**, and consequently the corresponding micelles of **4** are much smaller. In fact they are the smallest persistent micelles detected so far. Although the dendrons' size is also reduced (first- rather than second-generation derivative), the formation of aggregates of **4** is still not prevented.

Systematic work that will lead to understanding the minimal structural requirements for the formation of persistent micelles is currently underway in our laboratories. This information should aid in the design of tunable supramolecular containers, which will have a considerable impact in the field of nanotechnology.

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