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Structurally Persistent Micelles: Theory and Experiment

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Abstract

We describe the progress made in understanding the factors that determine the size, structure and stability of structurally persistent micelles using a combination of designed synthesis, cryo-TEM imaging and molecular-dynamics simulations. The importance of specific counterion effects is revealed in detail. An unexpected effect of sodium counterions leads to attraction between the polycarboxylate head groups of the tailored dendrimers that make up the micelles. This effect even leads to the formation of "superlattices" of highly negatively charged micelles.

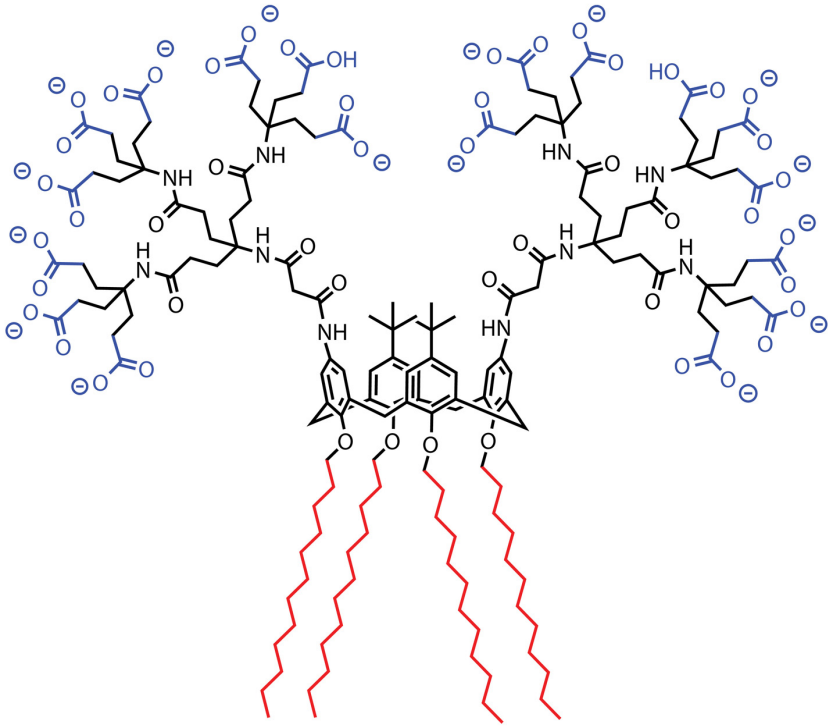
Introduction

Soft nanostructures can be considered to be the second class of nanomaterials after to ''hard'' nanoparticles and similar structurally defined and static nanoscale structures. Nanostructured soft matter represents a challenging research area, both for theory and experiment. This is because soft matter is inherently dynamic in its structure and cannot, therefore be treated as a single static object. Nonetheless, soft nanostructures can have significant advantages over hard nanoparticles. They are, for instance, formed in a dynamic equilibrium process, so that their self-assembly is governed by thermodynamics, rather than the less predictable process of kinetically controlled nucleation and precipitation. This advantage can, however, soon become a disadvantage because many factors may determine the delicate equilibrium that gives rise to soft nanostructures, so that they may be sensitive to their environment. Nature uses soft nanoparticles almost exclusively in living organisms, so that there can be no doubt that technological applications based on soft nanostructures are potentially extremely powerful. Soft nanostructures are most likely to self-assemble from organic precursor molecules in solution, probably aqueous. Conventional micelles [1] are perhaps the best known soft nanoparticles, but although a very large amount of data about, for instance critical micelle concentrations is available [2], relatively little is known about the detailed structure and dynamics of micelles and other soft nanostructures.

Structurally persistent micelles [3] therefore represent an important milestone in the science of soft nanostructures as they have consistent, well defined and persistent structures that can be observed in detail by techniques such as cryo-transmission electron microscopy (cryo-TEM). These characteristics not only make structurally persistent micelles intriguing experimental objects, but also make them ideal for testing and validating molecular-dynamics (MD) simulations and the force fields used for them. As we will describe below, simulations have played a major role in advancing our understanding of structurally persistent micelles and the factors that control their stability and structure.

Molecular Components

In 2004 Kellermann *et al.* [3] described the aggregation of seven amphiphilic dendrocalixarene molecules **1** to uniform and structurally persistent micelles (Scheme 1).



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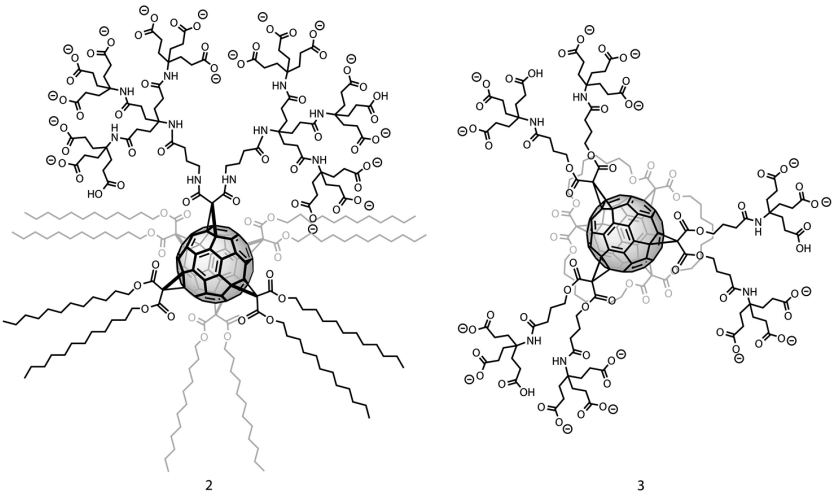
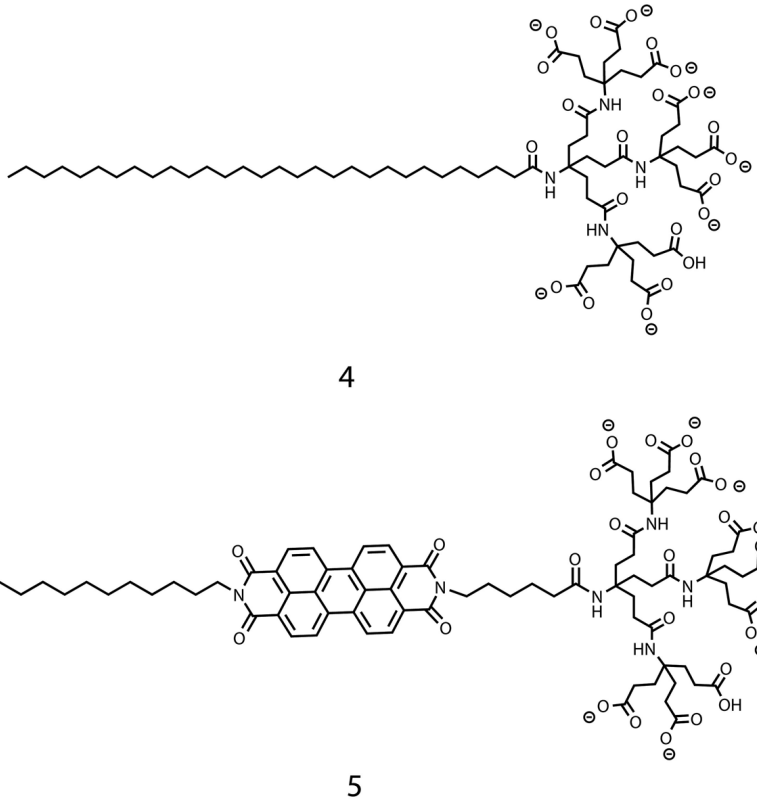
Scheme 1. Dendrocalixarene monomer.

The self aggregation behaviour of the molecules was investigated by pulse-gradient spin-echo (PGSE) NMR spectroscopy and cryo-TEM experiments. Remarkably, the experiments showed that a single distinct type of micelle was formed with no other aggregates of different sizes. The key feature of this first amphiphilic dendrimer investigated is its cone-shaped structure, which makes it possible to form small aggregates with high curvature. Each hydrophilic polycarboxylate head-group is linked to four hydrophobic alkane chains by a calyx[4]arene unit.

Hirsch *et al.* [4 – 7] later synthesized, characterized and investigated the aggregation behaviour of a series of new amphiphilic building blocks based on either calixarenes, fullerenes (Scheme 2: **2**, **3**) or later perylene [8, 9] as the central scaffold. These scaffolds allow a variety of hydrophilic and hydrophobic head and tail groups to be bound in a stereochemically controlled and tunable fashion. The polar head groups are Newkome-type oligocarboxylic acids in all cases. At neutral pH, they are predominantly deprotonated and guarantee excellent water solubility.



Scheme 2. Fullerene based amphiphilic building blocks.



Scheme 3. Single tailed **4** and perylene based **5** amphiphilic building blocks.

Structurally Persistent Micelles – cryo-TEM

As outlined, structurally persistent micelles were first observed [3] by a combination of spectroscopic and microscopic techniques, but the most striking results arise from cryo-TEM studies, which reveal thousands of essentially identical micellar structures, as shown in Figure 1a for the dendrimer **1**.

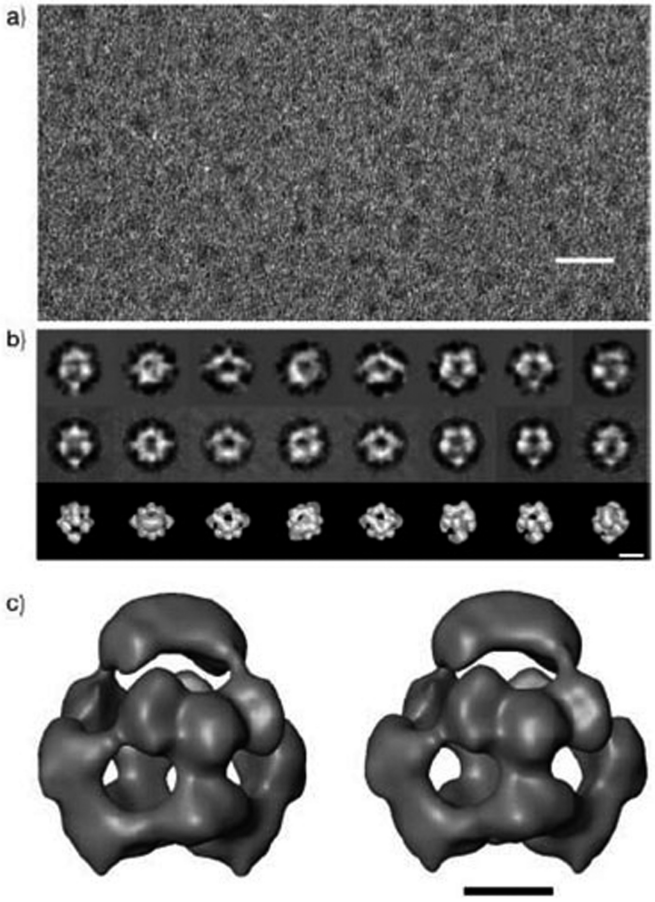


Figure 1. (a) Representative electron micrograph of calixarene micelles (the bar is 100 Å). **(b)** Row 1 shows class averages representing different spatial views of the micelles. Based on the assignment of corresponding Euler angles. 3D structure information can be retrieved at a resolution of 12 Å. Reprojections (row 2) into the 3D volume (row 3) the fit with the experimental data (bar is 50 Å). **(c)** Stereo view of the isosurface rendered 3D structure (bar is 25 Å). Reprinted with permission from ref 3. Copyright Wiley-VCH Verlag GmbH & Co. KGaA 2004.

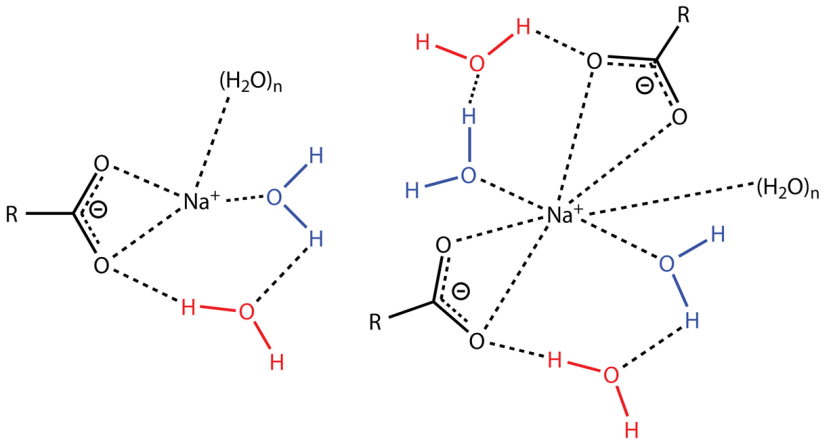
Because the micelles are oriented randomly, the TEM-picture contains views from literally thousands of different angles. This ensemble of views can be used to calculate a 3D-structure for the micelles [10 – 12], as shown in Figure 1c for the micelles depicted in Figure 1.

The reconstruction shows high-density areas that were interpreted as corresponding to the carboxylate head-groups of the dendrimer and a hollow core. The low density and high mobility of the alkane chains of the dendrimer explains that the core of the micelle does not show up in the TEM picture. However, the dimensions of the micelles led to the conclusion that a relatively large concentration of water must be present in the hydrophobic core of the micelles. The first MD simulations were therefore designed to test this hypothesis and to investigate the structure of this water in a hydrophobic environment.

Molecular Dynamics Simulations – Heptameric Micelles

Finding a suitable starting geometry for MD simulations is always critical to their success. In this case, the 3D reconstruction of the positions of the head-groups was used to construct a putative complete micelle structure by adding the alkane chains and flooding the resulting structure with water and adding sodium ions to neutralize the ensemble. The resulting geometry was then first geometry-optimized and then equilibrated very slowly by performing successive MD simulations in which the geometrical restraints that held the micelle together were removed very slowly and carefully. A micelle resulted that was stable for 100 ns simulation time. This probably indicates that it is a stable and observable entity as unstable micelles dissociate within just a few nanoseconds in the simulations. The relaxation and equilibration led to the expulsion of the water molecules from the hydrophobic core, which became very ''dry'', and a concomitant shrinking of the micelle until it was approximately 5 Å smaller than suggested by the cryo-TEM images [13]. This discrepancy is larger than would normally be expected and raised the question as to exactly what the cryo-TEM images were showing.

Unusually, staining with heavy-metal derivatives was not necessary in order to be able to ''see'' the micelles in the TEM images. TEM is usually considered to visualize differences in density [14], so that we analyzed the density of our simulation box divided into small voxels. The results revealed areas of higher than average density associated with the sodium ions close to the anionic head-groups of the micelle. Detailed examination showed these areas of high density to correspond to contact ions pairs or ion triplets with the general structures shown in Scheme 4.



Scheme 4. Schematic structures of sodium carboxylate contact ion pairs (left) and triplets (right). Note the importance of the waters (red) that form strongly hydrogen-bonded bridges between waters (blue) coordinated directly to the sodium ion and oxygen atoms of the carboxylates.

Since the simulations were carried out with quite simple force fields that might not reproduce the behaviour of ions in aqueous solution correctly, we tested their stability by using snapshots from PM3 [15, 16] MD simulations of sodium formate in water as starting structures for geometry optimizations using density-functional theory (DFT). These calculations confirmed that structures of the types shown in Figure 2 are both stable and persistent in simulations and on geometry optimization.

This observation resolves the apparent difference between the stable micelle structures found in the simulations and the 3D-reconstructions from the cryo-TEM images. The carboxylate head-groups and their associated sodium ions together provide the high-density regions that appear in the unstained cryo-TEM images. This observation has since been confirmed for several systems in which the micelles are not observable in the TEM without staining if potassium, rather than sodium counterions are present. Figure 2 shows the time-averaged regions of high density, which should correspond to the 3D-reconstruction obtained from the cryo-TEM images from a simulation with sodium ions compared to a simulation with potassium ions only.

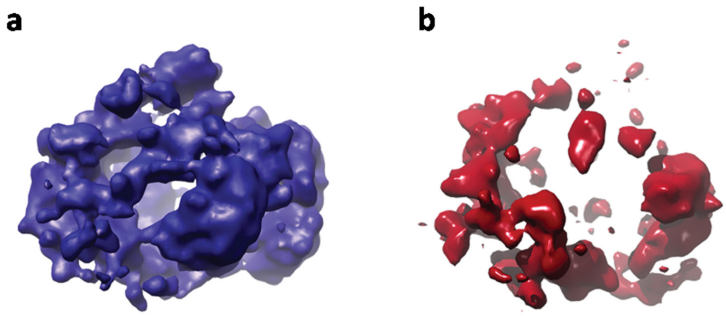


Figure 2. Snapshots taken from micelle simulations with sodium **(a)** and potassium **(b)** counterions. The figures show isodensity surfaces at a density value 20% higher than the mean for the snapshot. Reprinted with permission from ref 13. Copyright Wiley-VCH Verlag GmbH & Co. KGaA 2009.

One further aspect of the simulations [13] was also noteworthy; it proved far more difficult to obtain a stable micelle structure using the procedure described above if potassium, rather than sodium ions were used. This effect was traced to a far larger concentration of sodium ions than potassium in the immediate environment of the polycarboxylate head-groups of the dendrimers. Figure 3 shows an analysis of the time-averaged concentration of alkali-metal ions in simulations of the micelle with sodium and potassium counterions. The sodium ions associate far more tightly with the dendrimer head-groups and remain associated for far longer than their potassium counterparts [13]. This specific counterion effect was found to be responsible for the higher stability of micelles in 5:1 sodium:potassium buffer than with only potassium ions.

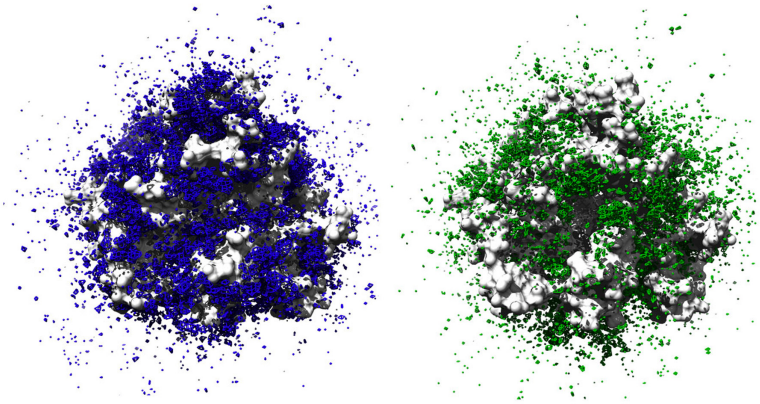


Figure 3. Areas of high sodium (blue) and potassium (green) ion density around the surface of the micelle (white).

Specific Ion Effects and the Hofmeister Series

Franz Hofmeister was born in Prague in 1850 and died in Würzburg in 1922 [17]. He studied medicine in Prague and became Professor of Pharmacology there in 1885. After Czech became the only language at the Charles University in Prague, he moved to Strasbourg in 1896, but was eventually forced to move to Würzburg when Strasbourg became French. He enjoyed a remarkable career and was the first to suggest that peptides and proteins consist of amino-acid residues connected by amide bonds. This honour is often accorded Emil Fischer, but Hofmeister spoke before Fischer at the conference in which both announced their discovery. Hofmeister is best known for what is now known as the Hofmeister series [18 – 21]. This series now describes the effects of ions on the solubility of proteins in water, although Hofmeister never formulated it in terms of individual ions, but rather for salts. Although phenomenological rationalizations for the Hofmeister series abound [22 – 26], no really convincing microscopic explanation exists. Early ideas that ions could provoke (or destroy) long-range order in water proved not to be correct [18, 27 – 29]. However, the effects described by the Hofmeister series clearly affect self-aggregation by polyelectrolytes and may therefore even determine the shape, size and stability of structurally persistent micelles.

This sensitivity of structurally persistent micelles to counterion effects makes them ideal research objects for investigating Hofmeister-like effects on polyelectrolytes as the micelles are uniformly structured and react strongly to changes in their ionic environment. These sensitive but nonetheless well defined systems provide an unprecedented level of information about specific ions effects in general and also about the factors that affect micelle structure and stability.

Experimental (cryo-TEM) tests of the differences between sodium and potassium counterions on the structures and stability of the micelles revealed strong effects. Replacing the original 5:1 Na:K buffer with a pure potassium one at the same concentration resulted in larger micelles than those observed originally. Remarkably, the original heptameric micelles were obtained by titrating the solution with a five-fold excess of pure sodium buffer [13]. At higher concentrations, the Na:K buffer gave the original heptameric micelles once more, but the cryo-TEM images revealed fewer than at lower concentrations. At the same high concentration, a pure potassium buffer gave no micelles at all. Figure 4 shows the relevant cryo-TEM images.

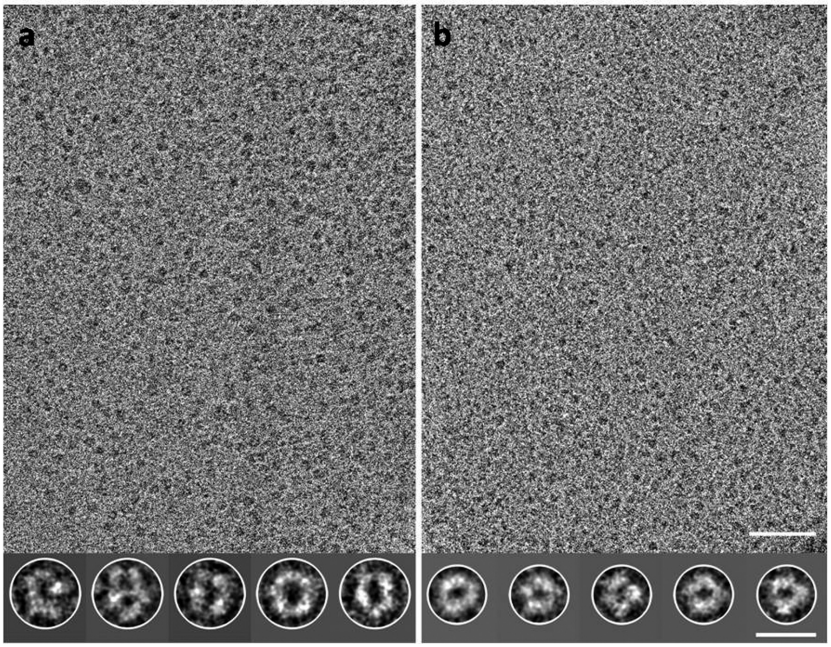


Figure 4. Cryo–TEM images of **1** in the presence of 0.027 M potassium **(a)** and sodium/potassium (5:1) **(b)** phosphate buffer. The images reveal significant differences in the micelle diameter (~10 vs. ~7 nm). Each of the above images is combined with a row of selected class sum images calculated from corresponding data sets of 2,300 images of individual assemblies. The general difference in the diameter between the two preparations is obvious and highlighted by white circles. Scale bars are 500 Å **(above)** and 100 Å **(below).** Reprinted with permission from ref 13. Copyright Wiley-VCH Verlag GmbH & Co. KGaA 2009.

These results not only emphasize the importance of specific counterion effects, but also suggest a possible rationalization for the experimental observations. The heptameric micelles are marginally stable in potassium solution because the binding provided by the interaction of the hydrophobic chains in their core is just large enough to hold them together. The additional stabilization provided by sodium counterions stabilizes the small micelles. This interpretation is consistent with the observation [30] that ultrasonification of the original solution of **1** (with Na:K buffer) under a layer of hexane for 24 hours results in larger (dodecameric) micelles, once again with a well defined and persistent structure. The original heptameric micelles remained unchanged when treated with ultrasound for 24 hours without a hexane layer, showing that the change is caused by the hexane.

Once again, MD simulations were used to test the stability of micelles constructed on the basis of the TEM images. The fact that ‘‘unstable’’ micelle structures dissociate within a few nanoseconds in the simulations allowed us to study many possible starting structures with varying amounts of hexane in the core of the micelle. All simulations except those with 36

hexane molecules led to fast dissociation of the micelles into smaller aggregates, whereas that with 36 remained stable over 100 ns, both with sodium and potassium counterions [30]. The structure of the micelles in the simulations consisted of an equatorial ring of seven dendrimers with two different caps of two and three, whereas the reconstructed cryo-TEM 3D structures suggest two equivalent caps, each consisting of three dendrimer monomers, and a central ring of six dendrimers. This discrepancy is small and may either be caused by force-field deficiencies or by the fact that the MD simulations sampled a slightly less stable structure than that found in the experimental studies. However, the extremely well resolved cryo-TEM images pointed to a further indication of the importance of the alkali-metal counterions.

Attraction Between Polycarboxylates

The 3D-reconstructions of the cryo-TEM images suggest orientations for the polycarboxylate head groups that indicate them to be attracting each other, as shown in Figure 5.

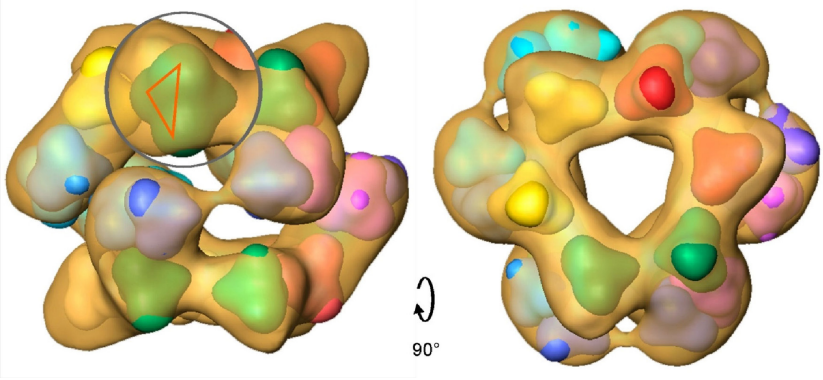


Figure 5. 3D-reconstruction of the micelles of **1** after 24 hours ultrasonification with n-hexane. Reconstructed volume with visually fitted dendron fragments of **1** simplified to tetrahedra to represent their overall t-butyl-like shape (tetrahedra of the same color belong to the same molecule, the calixarene ring and alkyl chains are not shown for clarity). The circled area on the left shows a magnification of the three branches of a single dendron (highlighted by the red triangle). Reprinted in part with permission from ref 30. Copyright 2010 American Chemical Society.

Closer examination of the results of the MD simulations [30] revealed that the micelles formed by the sodium salt have a more compact and less dynamic structure than their potassium equivalent. Closer examination revealed that the orientation of the polycarboxylate head groups in the former case does indicate attraction between head groups of different dendrimer monomers and that this attraction is caused by bridging sodium ions in $RCO_2^-...Na^+...^-O_2CR$ ion triplets and quadruplets. A snapshot of the interstitial area between two head groups is shown in Figure 6.

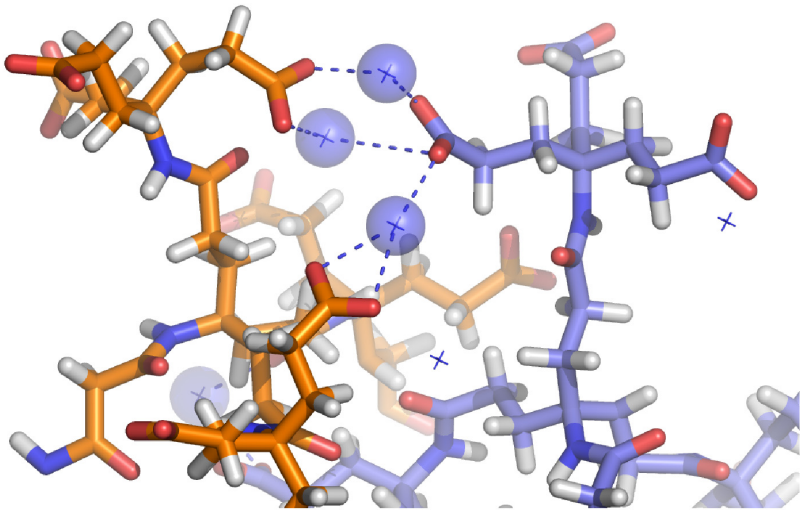


Figure 6. Snapshot of the interstitial area between two headgroups of the micelle. The dendrimer head-groups are from two different dendrimers, which are colored blue and orange. Three bridging sodium ions (blue spheres) are shown that bind three carboxylate groups together. Two non-bridging sodium ions are shown as blue crosses. Reprinted with permission from ref 30. Copyright 2010 American Chemical Society.

These results suggest a counterintuitive attraction between the polyanionic head groups. This effect was confirmed by further cryo-TEM images, in which the micelles were observed to form hexagonal ''superlattices'' in solution with sodium buffer, but not with potassium [31]. These structures represent a further important step in the controlled self-organization of soft particles as they provide an additional level of organization over and above that represented by the micelles themselves. Further experimental studies were based on a new single-tailed Newkome-dendritic surfactant **4** that lacks a central linking unit and varies in the length of the hydrophobic alkane chain. In this study [31], the effect of counterions on the formation of such structurally persistent micelles was investigated systematically for the first time. The critical micelle concentrations (CMCs) were found to decrease with increasing alkyl chain length, but most importantly were lower for the pure sodium salts than either for lithium or potassium, confirming the special role of sodium counterions in stabilizing polycarboxylate micelles.

MD simulations designed specifically to investigate the ''head to head'' aggregation of polycarboxylate dendrimers confirmed that sodium counterions can mediate an attractive interaction between deprotonated polycarboxylates by taking up bridging positions between carboxylate anions. The pH-dependence of micelle formation [31] suggests that these Na^+ bridges stabilize more strongly than conventional hydrogen bonds between protonated carboxylic acids.

One remarkable feature of all these results is how well the simple force-field based simulations are able to reproduce the quite subtle effects observed experimentally. There has been considerable discussion of the lack of accuracy of force fields for metal ions, or more accurately of combinations of force fields for metal ions and for water [32 – 34]. Surprisingly, the interactions between ions of opposite charge seem to be far less critical than the hydration of ions. Both direct MD simulations using the PM3 semi-empirical molecular orbital Hamiltonian and subsequent geometry optimizations with density-functional theory density reproduce the structures [31] observed in snapshots from the classical MD simulations. The strongest argument for the reliability of the simulations, however, is that they have been able to reproduce and in many cases even predict the unusual effects observed experimentally.

Summary and Outlook

Structurally persistent micelles remain fascinating research objects, both from the point of view of potential technological applications and because they reveal effects that are hidden in more complex or less well defined systems. Above all, the combination of synthesis, cryo-TEM and simulations has proven to be extraordinarily powerful and to lead to significant progress. It is important in this respect that impulses for new research directions and specific experiments or simulations may come from both experiment and simulation.

Acknowledgments

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References

[1] Shah, D.O. (Ed.) *Micelles, Microemeulsions and Monolayers*, Marcel Dekker, New York, 1998.

[2] IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by McNaught, A.D. and Wilkinson, A. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006) created by Nic, M., Jirat, J., Kosata, B. updates compiled by Jenkins, A. ISBN 0 – 9678550 – 9-8.
doi: 10.1351/goldbook.

[3] Kellermann, M., Bauer, W., Hirsch, A., Schade, B., Ludwig, K., Böttcher, C. (2004) The First Account of a Structurally Persistent Micelle. *Angew. Chem. Int. Ed.* **43**:2959 – 2962.
doi: 10.1002/anie.200353510

[4] Burghardt, S., Hirsch, A., Schade, B., Ludwig, K., Böttcher, C. (2005) Switchable Supramolecular Organization of Structurally Defined Micelles Based on an Amphiphilic Fullerene. *Angew. Chem. Int. Ed.* **44**:2976 – 2979.
doi: 10.1002/anie.200462465

[5] Schade, B., Ludwig, K., Böttcher, C., Hartnagel, U., Hirsch, A. (2007) Supramolecular Structure of 5-nm Spherical Micelles with D_3 Symmetry Assembled from Amphiphilic [3:3]-Hexakis Adducts. *Angew. Chem. Int. Ed.* **46**:4393 – 4396.

[6] Hirsch, A. (2008) Amphiphilic architectures based on fullerene and calixarene platforms: From buckysomes toshape-persistent micelles. *Pure Appl. Chem.* **80**:571 – 587.
doi: 10.1351/pac200880030571

[7] Becherer, M., Schade, B., Böttcher, C., Hirsch, A. (2009) Supramolecular Assembly of Self-Labeled Amphicalixarenes. *Chem. Eur. J.* **15**:1637 – 1648.
doi: 10.1002/chem.200802008

[8] Schmidt, C.D., Böttcher, C., Hirsch, A. (2007) Synthesis and aggregation properties of water-soluble Newkome-dendronized perylenetetracarboxdiimines. *Eur. J. Org. Chem.* 5497 – 5505.

[9] Schmidt, C.D., Böttcher, C., Hirsch, A. (2009) Chiral Water-Soluble Perylenediimides. *Eur. J. Org. Chem.* 5337 – 5349.
doi: 10.1002/ejoc.200900777

[10] van Heel, M., Harauz, G., Orlova, E.V., Schmidt, R., Schatz, M. (1996) A new generation of the IMAGIC image processing system. *J. Struct. Biol.* **116**:17 – 24.
doi: 10.1006/jsbi.1996.0004

[11] van Heel, M. (1987) Angular reconstitution: a posteriori assignment of projection directions for 3D reconstruction. *Ultramicroscopy* **21**:111 – 123.
doi: 10.1016/0304-3991(87)90078-7

[12] Orlova, E.V., Dube, P., Harris, J.R., Beckman, E., Zemlin, F., Markl, J., van Heel, M. (1997) Structure of keyhole limpet hemocyanin type 1 (KLH1) at 15 A resolution by electron cryomicroscopy and angular reconstitution. *J. Mol. Biol.* **271**:417 – 437.
doi: 10.1006/jmbi.1997.1182

[13] Jäger, C.M., Hirsch, A., Schade, B., Böttcher, C., Clark, T. (2009) Counterions Control the Self-Assembly of Structurally Persistent Micelles; Theoretical Prediction and Experimental Observation of Stabilization by Sodium Ions. *Chem. Eur. J.* **15**:8586 – 8592.
doi: 10.1002/chem.200900885

[14] Williams, D.B., Carter, C.B. *Transmission Electron Microscopy: A Textbook for Materials Science*, Plenum Press, New York, 1996.

[15] Stewart, J.J.P. (1989) Optimization of parameters for semiempirical methods I. Method. *J. Comp. Chem.* **10**:209 – 220.
doi: 10.1002/jcc.540100208

[16] Stewart, J.J.P. (1989) Optimization of parameters for semiempirical methods II. Applications. *J. Comp. Chem.* **10**:221 – 246.
doi: 10.1002/jcc.540100209

[17] Abernethy, J.L. (1967) Franz Hofmeister – the Impact of his Life and Research on Chemistry. *J. Chem. Ed.* **44**:177 – 180.
doi: 10.1021/ed044p177

[18] See, for instance Zhang, Y., Cremer, P.S. (2006) Interactions between macromolecules and ions: The Hofmeister series. *Curr. Op. Chem. Biol*. **10**:658-663.

[19] Hofmeister, F. (1888) Zur Lehre von der Wirkung der Salze. Zweite Mittheilung *Arch. Exp. Pathol. Pharmakol.* **24**:247 – 260.
*doi:*10.1007/BF01918191

[20] Kunz, W., Henle, J., Ninham, B.W. (2004) Zur Lehre von der Wirkung der Salze (About the science of the effect of salts): Franz Hofmeister's historical papers. *Curr. Op. Colloid Interface Sci.* **9**:19 – 37.
doi: 10.1016/j.cocis.2004.05.005

[21] Kunz, W., LoNostro, P., Ninham, B.W. (2004) The Present State of Affairs with Hofmeister Effects. *Curr. Op. Colloid Interface Sci.* **9**:1 – 18.
doi: 10.1016/j.cocis.2004.05.004

[22] Collins, K.D, Washabaugh, M.W. (1985) The Hofmeister effect and the behaviour of water at interfaces. *Q. Rev. Biophys.* **18**:323 – 422.
doi: 10.1017/S0033583500005369

[23] Washabaugh, M.W., Collins, K.D. (1986) The systematic characterization by aqueous column chromatography of solutes which affect protein stability. *J. Biol. Chem.* **261**:12477 – 12485.

[24] Collins, K.D. (1995) Sticky ions in biological systems. *Proc. Natl. Acad. Sci. U.S.A.* **92**:5553 – 5557.
doi: 10.1073/pnas.92.12.5553

[25] Neilson, G.W., Enderby, J.E. (1996) Aqueous Solutions and Neutron Scattering. *J. Phys. Chem.* **100**:1317 – 1322.
doi: 10.1021/jp951490y

[26] Enderby, J.E. (1995) Ion solvation via neutron scattering. *Chem. Soc. Rev.* **24**:159 – 168.
doi: 10.1039/cs9952400159

[27] Collins, K.D., Neilson, G.W., Enderby, J.E. (2007) Ions in water: characterizing the forces that control chemical processes and biological structure. *Biophys. Chem.* **128**:95 – 104.
doi: 10.1016/j.bpc.2007.03.009

[28] Marcus, Y. (2009) Effect of ions on the structure of water: Structure making and breaking. *Chem. Rev.* **109**:1346 – 1370.
doi: 10.1021/cr8003828

[29] Collins, K.D. (2006) Ion hydration: Implications for cellular function, polyelectrolytes, and protein crystallization. *Biophys. Chem.* **119**:271 – 281.
doi: 10.1016/j.bpc.2005.08.010

[30] Jäger, C.M., Hirsch, A., Schade, B., Ludwig, K., Böttcher, C., Clark, T. (2010) Self-Assembly of Structurally Persistent Micelles is Controlled by Specific Ion Effects and Hydrophobic Guest. *Langmuir* **26**:10460 – 10466.

[31] Rosenlehner, K., Schade, B., Böttcher, C., Jäger, C.M., Clark, T., Hirsch A. (2010) Sodium-Effect on the Self-Organization of Amphiphilic Carboxylates: Formation of Structured Micelles and Superlattices. *Chem. Eur. J.* **16**:9544 – 9554.
doi: 10.1002/chem.201001150

[32] Joung, I.S., Cheatham, III, T.E. (2008) Determination of alkali and halide monovalent ion parameters for use in explicitly solvated biomolecular simulations. *J. Phys. Chem.* **112**:9020 – 9041.
doi: 10.1021/jp8001614

[33] Horinek, D., Mamatkulov, S.I., Netz, R.R., (2009) Rational design of ion force fields based on thermodynamic solvation properties. *J. Chem. Phys.* **130**:124507.
doi: 10.1063/1.3081142

[34] Hess, B., van der Vegt, N. (2009) Cation specific binding with protein surface charges. *Proc. Natl. Acad. Sci. U.S.A.* **109**:13296 – 13300.
doi: 10.1073/pnas.09029041061