Pure Appl. Chem., Vol. 80, No. 3, pp. 571–587, 2008. doi:10.1351/pac200880030571 © 2008 IUPAC

Amphiphilic architectures based on fullerene and calixarene platforms: From buckysomes to shape-persistent micelles*

Andreas Hirsch[‡]

Department of Chemistry and Pharmacy and Interdisciplinary Center of Molecular Materials (ICMM), Friedrich-Alexander University Erlangen-Nürnberg, Henkestrasse 42, D-91054 Erlangen, Germany

Abstract: The supramolecular aggregation properties of new prototypes of artificial amphiphiles consisting of fullerene or calixarene building blocks serving as platforms for the connection of hydrophobic and dendritic hydrophilic groups are reported. Very water-soluble monoadducts of C_{60} carrying a defined number of negative charges at a given pH were hybridized with cationic porphyrins including cyctochrome C and investigated with respect to photoinduced electron transfer. Fullerene adducts with dendritic deprotected sugar addends self-assemble to supramolecular sugar balls in water with a narrow size distribution of the micelles of around 4 nm. Various examples of cone- or T-shaped amphiphiles involving calixarene and fullerene cores self-organize to the first examples of shape-persistent micelles whose supramolecular arrangement in water has been determined with unprecedented precision based on the analysis of cryo-transmission electron microscopy (TEM) investigations.

Keywords: fullerenes; calixarenes; micelles; amphiphiles; liposomes.

Within the last 15 years, molecular carbon allotropes such as the fullerenes have been established as valuable building blocks for the construction of new molecular and supramolecular architectures and as components for functional materials [1]. The most intensively studied representative is buckminsterfullerene C_{60} , which can be produced on the ton scale. The highly symmetrical π -conjugated carbon cage (Fig. 1) exhibits a variety of unprecedented structural, electronic, chemical, and biomedical properties [1]. Exohedral addition chemistry provides a means for further modification of the property profile and for its combination with those of other compound classes. A large number of exohedral functionalization methods such as additions of nucleophiles and radicals as well as cycloadditions to the [6,6]-double bonds of the fullerene framework have been discovered [1]. It is especially important to achieve control over the regioselectivity of multiple additions by applying, for example, template- or tether-controlled functionalization concepts [2–13]. As a consequence, a large number of fullerene derivatives with stereochemically defined architectures can be made available. Many of these systems have been used in various applications such as the development of neuroprotective drugs and as components for photovoltaic devices [1].

^{*}Paper based on a presentation at the 12th International Symposium on Novel Aromatic Compounds (ISNA-12), 22–27 July 2007, Awaji Island, Japan. Other presentations are published in this issue, pp. 411–667.

[‡]E-mail: andreas.hirsch@chemie.uni-erlangen.de





Fig. 1 Schematic representation of C_{60} tube model (top) and VB structure (bottom) with length of the two types of bonds ([6,6]-double bonds and [5,6]-single bonds).

One or several successive nucleophilic cyclopropanations of the [6,6]-double bonds represent an especially attractive method for the chemical functionalization of fullerenes (Scheme 1) [14]. Like in all addition reactions, the driving force is the reduction of strain energy introduced by the pyramidalization of the "sp²"-C-atoms of the parent fullerenes [2]. The advantage nucleophilic cyclopropanations have over many other exohedral functionalization methods is that mild reaction conditions tolerating many functional groups can be applied and in most cases the reaction products can easily be isolated and structurally characterized [1,14].



Scheme 1 Nucleophilic cyclopropanation of a [6,6]-double bond of C_{60} .

Water-soluble adducts of C60 are of interest because of their interesting biomedical properties such as high reactivity against toxic reactive oxygen species (ROS) and anti-HIV activity [15-18]. In order to achieve water-solubility of mono-cyclopropanated fullerenes, very polar hydrophilic sidechains have to be introduced [19–20]. An example is dendrofullerene 1, which is the most water-soluble (254 mg/mL at pH = 10) fullerene adduct synthesized so far [21]. It contains two Newkome-type 2G-dendrons in the side-chains bearing altogether 18 carboxy termini. Upon changing the pH value, the degree of deprotonation can be tuned. Titration experiments reveal that at pH = 7.4 a total of 16 negative charges are present. Dendrofullerene 1 as well as related water-soluble adducts like 2–12 exhibit pronounced neuroprotective properties both in vitro and in vivo as exemplified by extensive investigations using zebrafish embryo models [22]. Next to biomedical properties of water-soluble fullerene derivatives, photophysical and materials properties are of interest, since a derivative such as 1 can be considered as an amphiphilic redox-active oligo-electrolyte. The redox-activity is provided by the C_{60} core, whose monoaddition pattern still resembles the remarkable electronic properties of the parent fullerene. At the same time, the fullerene framework serves as the hydrophobic part of the amphiphilic architecture. The hydrophilic dendritic part provides a defined number of negative charges at given pH. Demonstration of the photophysical properties of **1** was provided by the formation of electrostatically assembled donor-acceptor dyads, where cytochrome C, an eightfold positively charged redox-protein involving a heme-group, served as the electron donor (Fig. 2). The association constant $K_s = 3.1 \times 10^5$ M^{-1} of the electrostatic binding to the oppositely charged redox-systems was determined by fluorescence titration experiments [23]. After irradiation, photoinduced electron transfer from the cytochrome C to the fullerene takes place. The lifetime of the charge-separated state $\tau = 1.8 \,\mu s$ was determined by time-resolved absorption spectroscopy. This concept of electrostatically assembled donor-acceptor hybrids was extended for the hierarchical build-up of redox-active oligoelectrolytes by the layer-by-layer (LBL) assembly method [24–25]. Using cytochrome C or charged tetraphenyl porphirins as electron donors and 1 or related fullerenes as electron acceptors, it was possible to generate photovoltaic devices, where both a redox- and photonic gradient was established for efficient light-harvesting and subsequent generation of charge-separated states.



1



© 2008 IUPAC, Pure and Applied Chemistry 80, 571–587





Fig. 2 Schematic representation of a photoinduced electron transfer in an electrostatic complex between 1 and cytochrome C formed at pH = 7.4.

The first example for a very water-soluble, fullerene-based amphiphile carrying non-ionic addends is the C_{60} glycoconjugate **13** [26]. This fullerene sugar contains two dendritic mannopyranosides that are connected through two adjacent imino bridges to the all-carbon framework. In this adduct type of C_{60} , which represents a 1,9-dihydro-1a-aza-1(2)a-homo(C_{60} - I_h) [5,6]fullerene derivative, the entire 60- π -electron system of the fullerene core is retained. The amphiphilic nature of **13** with its coneshaped structure forces the formation of small supramolecular aggregates in aqueous solutions to shield the hydrophobic fullerene units from the water subphase. Diffusion-ordered NMR spectroscopy (DOSY) and transmission electron microscopy (TEM) investigations reveal micellar sugar balls with an extremely narrow size distribution of around 4 nm.



The concept of amphiphilic fullerenes can considerably be extended, taking advantage of multiple additions in a geometrically defined way. An appealing motif is represented by hexakis adducts of C_{60} involving a T_h -symmetrical octahedral addition pattern (Fig. 3) [27]. Considering that numerous constitutional isomers could in principle be formed during the sixfold addition, the one-pot synthesis of such highly symmetric C_{60} derivatives in remarkably high yields was a major breakthrough in exohedral fullerene chemistry. The key point was the pretreatment of C_{60} with dimethylanthracene, which undergoes reversible [4+2]-cycloadditions to the fullerene core favoring the thermodynamically preferred octahedral addition patterns [3]. Binding of malonates to the preferred unoccupied octahedral sites and replacement of dimethylanthracene addends is the second step in the reaction sequence. In this way, many examples of hexakisadducts, including those containing a combination of different kinds of malonates, have been prepared in high yields and large quantities.



Fig. 3 Schematic representation of the $T_{\rm h}$ -symmetrical addition pattern realized in an octahedral hexakisadduct of C_{60} .

One example is the amphiphilic mixed [5:1]-hexakisadduct **14** with C_{2v} -symmetry (Fig. 4), where one hydrophilic malonate addend is bound to the north-pole and five apolar malonates containing C12-chains are connected to the equator and the south-pole [29]. The aggregation behavior of these artificial lipids in water was studied by freeze-fracture- and cryo-electron microscopy revealing a wide variety of objects, the fine structure of which is invariably based upon the same structural principle, namely, the formation of double-layer membranes. The dominant structures are vesicles ("buckysomes") of dimensions ranging between 50 and 400 nm. The overall thickness of the corresponding double-layers was determined as about 7 nm.



Fig. 4 Addition motif in a mixed $C_{2\nu}$ -symmetrical [5:1]-hexakisadduct of C_{60} .





Compared to normal vesicles composed of glycerol-based lipids, the buckysomes are characterized by the presence of a large number of peripheral carboxylic groups, which can be further functionalized without losing the aggregation properties. Moreover, the assembly and disassembly can be influenced by the pH value. As shown, for example, with monolayers of **14** assembly to membranes is favored at low pH and disfavored at high pH, where electrostatic repulsion between the deprotonated carboxylic groups and incorporation of solvated counterions into the dendritic branches is getting important [29]. Both features, facile membrane functionalization (e.g., biocompatibilization) and pH-dependent assembly and disassembly, make these buckysomes interesting candidates for new drug-delivery vehicles.

As pointed out above, when dissolved in water the fullerene sugar **13** leads to the formation of small micelles with a narrow size distribution, whereas the amphifullerene **14** predominantly afforded double-layer liposomes. The major geometrical difference between these two amphiphiles is the overall shape. Whereas **13** has a conical shape, the hexakisadduct **14** exhibits a much more balanced structure. Here, the hydrophobic and -philic parts occupy similar space, which allows for an even alignment of the molecules in extended double-layer membrane arrangements. The conical shape of **13**, however, forces the amphiphile to self-organize into small micelles. Only in this way can a dense packing optimizing all kinds of contributing noncovalent interaction (in particular, solvophobic and van der Waals interactions) be realized. Indeed, it has been predicted that very small aggregates of high curvature require cone-shaped amphiphiles that involve sterically demanding and charged head-groups and a considerably smaller hydrophobic part [30]. All micelles investigated so far are loose, mostly spherical ag-

gregates. They are short-lived dynamic species, which rapidly disassemble and reassemble. Only the average shape and aggregation number (*n*) could have been determined in classical micelles. The design of new artificial amphiphiles that give rise to precisely defined and stable micelles is a challenge. The information determining the specific assembly must be encoded in the molecular structure of the amphiphiles. Our idea was to develop an amphiphile with a rather unusual T-shaped structure, which at the same time is comparatively rigid. A rigid T-shaped amphiphile would not have the possibility to form extended double-layer membranes, because no dense packing of the molecules optimizing, for example, van der Waals interactions can be achieved. Instead, the formation of rather curved micellar object is expected. Moreover, these objects should be comparatively rigid since the uneven shape of the constituting amphiphiles will not allow free and unrestricted motion within and in-between micelles.

These structural requirements are combined in the amphiphilic dendro-calixarene **15**, which was obtained in a multistep synthesis from readily available starting materials [31]. It readily dissolves in water (23 mg/mL at pH 7.2) at room temperature. The aggregation behavior of **15** was investigated by pulse gradient spin-echo (PGSE) NMR spectroscopy, by encapsulation experiments, and by cryo-TEM. Most importantly, cryo-TEM micrographs of **15** (Fig. 5) revealed a very defined internal structural pattern, which was even amenable for advanced image-processing procedures (alignment, classification), as well as 3D reconstruction (Fig. 6). This is an established method for the 3D structure determination, for example, of proteins [32]. Extracted images of individual single aggregates corresponding to identical spatial orientations (identical Euler angles) could thus be identified, extracted, aligned, and summed up to eventually yield class-sum images with an enhanced signal-to-noise-ratio.



Fig. 5 Representative electron micrograph of micelles of 15 in vitreous ice (bar is 50 nm). M. Kellermann et al. *Angew. Chem., Int. Ed.* 43, 2959 (2004). Copyright Wiley-VCH Verlag GmBH & Co. KGaA, reproduced with permission.



Figure 6 (top) shows a gallery of selected class-sum images out of a total of 180, which were used for the final 3D reconstruction. It turned out indeed that a micellar structure of unprecedented definition has been found. The reconstruction appears as a hollow spherical cage of about 7.5 nm diameter with a specific pattern of surface penetrations (Fig. 6). The head-groups are homogeneously distributed over the micelle surface as a result of nonlinear repulsive forces. It has been proven that the hydrophobic chains of amphiphiles tend to be disordered in supramolecular aggregates [33]. This fact might explain why density information in the reconstruction, corresponding to the interior hydrophobic core, is averaged out by the image-processing procedure. Structural information from flexible nonisomorphic apolar hydrocarbon chains is, therefore, lacking in the reconstruction. The reconstructed structure information is assumed to exclusively correspond to the spatially defined comparatively rigid head-groups of the calixarenes. Visual 3D fitting by using the low-energy conformer of the calixarene head-group (Fig. 6) showed that exactly seven molecules can be precisely accommodated in the reconstructed density map. The best fit revealed a surprisingly unusual packing of seven molecules with C_2 symmetry: Two molecules are intercalated in such a way that one polar wing of the T-shaped molecule is located between the two polar wings of a second molecule. Two such "dimers" are almost exactly oriented opposite to each other. The distance corresponds to a typical bi-layered arrangement (6.7 nm). Three more molecules form additional clip-like connections in such a manner that the hydrophobic edges of the dimers are shielded. The structure is so robust and stable that if the solvent is entirely removed from the sample before imaging, the structural pattern can still be reconstructed in exactly the same manner as described above. Next to the T-shaped structure of the amphiphiles which induces pronounced backfolding, the specific arrangement of the partially intercalated charged head-groups reduces flexibility within the aggregate, for example, by hydrogen bonding, which is probably solvent-mediated. Specific directional interactions of the head-groups thus stabilize a defined structural pattern with typical surface



Fig. 6 (Top) Row 1 shows class averages representing different spatial views of the micelles of **15**. Reprojections (row 2) into the 3D volume (row 3) illustrate the fit with the experimental data (bar is 5 nm); (middle) stereo view of the iso-surface rendered 3D structure (bar is 2.5 nm); (bottom) C_2 -symmetrical arrangement of seven molecules **15** (head-groups) fitted into the EM density map. M. Kellermann et al. *Angew. Chem., Int. Ed.* **43**, 2959 (2004). Copyright Wiley-VCH Verlag GmBH & Co. KGaA, reproduced with permission.

penetrations, in contrast to classical detergent micelles, in which nonlinear repulsion forces determine a fluid-like molecular organization.

In order to develop a second example of a comparatively rigid amphiphile, which is suitable for the formation of shape-persistent micelles, we modified the molecular architecture of the amphifullerene **14**, which prefers to form buckysomes instead of micelles. It turned out that just a minor modification caused significantly different self-assembly properties in water. Instead of connecting the polar dendritic head-groups via ester linkages, we synthesized the related amphifullerene **16** involving the corresponding amide connections, resulting in enhanced rigidity [34].





When 16 was examined by cryo-TEM at neutral pH in water (phosphate buffer, pH 7.2), predominantly rod-shaped aggregates with a double-layer ultra-structure were found. The diameter of these rods is 6.5 nm, whereas the length is strongly variable and exceeds the diameter considerably. In addition, a few globular aggregates with a diameter of about 8.5 nm were found. Significantly, when the pH of the neutral solution was increased to roughly 9 (borate buffer, pH 9.2), those globular micelles with a diameter of about 8.5 nm were found exclusively. Now, these globular micelles also show a distinct internal structure, and it was possible to determine the 3D organization from the image data. Based on a simulated conformation of 16 in its completely deprotonated form (Fig. 7), the globular micellar structure was constructed from eight molecules in a C_2 -symmetrical arrangement. The molecular organization of this aggregate can be described best by considering two planar U-shaped structures consisting of four molecules each. This molecular organization ensures a nearly spherical shape overall and thus very efficient shielding of all hydrophobic molecule parts from the aqueous environment. As was the case for the structurally persistent micelles derived from 15, the innermost core area of the micelle formed by the hydrophobic alkyl chains is not reproduced in the reconstruction. Only the head-groups, which are located easily due to the high-contrast fullerene entity, prove to be sterically persistent, thus yielding the found structural pattern. The significant structural change when the pH is increased is explained by electrostatic repulsion of the completely deprotonated carboxy groups. Enhanced solvation of the dendritic branches is a secondary effect.



Fig. 7 Arrangement of eight molecules of **16** in the micelle (a,b). (c) Schematic representation of the head-group assembly: Two planar U-shaped assemblies consisting of four molecules each are displayed by 90° along their longitudinal axes and are inserted into each other at their open ends. (d) View of (c) tilted by 90° toward the front side. S. Burghardt et al. *Angew. Chem., Int. Ed.* **44**, 2976 (2005). Copyright Wiley-VCH Verlag GmBH & Co. KGaA, reproduced with permission.

In order to receive further insight into the factors that determine structural persistence, we developed the [3:3]-hexakis adduct **17** [35,36], which in contrast to the amphiphiles **15** and **16** contains three instead of one pair of dendritic carboxylate branches. Moreover, also in contrast to **15** and **16**, G1-dendrons were employed, which results in only three instead of nine carboxylates per dendritic branch. The



17

A. HIRSCH

total number of charges, however, is still equal to that in compounds **15** and **16**. The hydrophobic part of **17** is represented by the trifunctional cyclo-[3]-octylmalonate addend, thus occupying only very little space. When studying the aggregation properties of **17** by TEM, uniform spherical objects with a diameter of about 5 nm were found (Fig. 8), which is significantly smaller than the diameter of aggregates of the fullerene derivative **16** (about 8.5 nm).



Fig. 8 3D structure determination of micelles of **17**. (Top) Negative staining preparation of **17** (scale bar: 50 nm). (First row) Typical spatial views to the micelles. (Second row) The surface views show the corresponding orientation in space as determined from class-sum images in the above representations. (Third row) Backprojection images. B. Schade et al. *Angew. Chem., Int. Ed.* **46**, 4393 (2007). Copyright Wiley-VCH Verlag GmBH & Co. KGaA, reproduced with permission.

The reconstructed structure of a stained sample of **17** 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). Analysis of the data revealed the existence of an inherent ultra-structure. Volume calculations based on molecular modeling investigations indicated that relatively small aggregates were formed. The 3D reconstruction, which was determined from 3973 single particles, revealed D_3 symmetry. These spherical micelles consist of three identical S-shaped motifs, in keeping with the inherent symmetry (Fig. 9). 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 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, an overall spherical structure is generated, whose supramolecular arrangement allows for the optimal efficient shielding of the hydrophobic core from the aqueous surrounding. The six



Fig. 9 Two perpendicular orientations of the electron density maps of 3D structure determination of the micelles of 17 (top). CPK representations of the arrangement of six molecules of 17 in the micelles.

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 (Fig. 9).

The new amphiphilic architectures **15–17** give rise to the formation of the first shape-persistent micelles, whose supramolecular arrangement could be determined with unprecedented precision. We are currently investigating the basic requirements for achieving shape persistence by synthesizing further model amphiphiles related to **15–17** but having simpler structures. These studies will be complemented by theoretical investigations. A very important task is to provide insight into the organization of the water molecules and solvated counterions located around the micelles. All micelles contain very large pores on the surface, but no water is located inside the micelles. This means that water itself forms probably a very ordered structure around the pores serving as a barrier for solvent penetration. The structural ordering might be facilitated by the solvated Na⁺ counterions of the carboxylic groups. The results of these systematic investigations will be reported in due course.

ACKNOWLEDGMENT

This work was supported by the Deutsche Forschungsgemeinschaft (DFG).

REFERENCES

- 1. A. Hirsch, M. Brettreich. Fullerenes-Chemistry and Reactions, Wiley-VCH, Weinheim (2004).
- 2. A. Hirsch. Top. Curr. Chem. 199, 1 (1999).
- (a) I. Lamparth, C. Maichle-Mössmer, A. Hirsch. Angew. Chem. 107, 1755 (1995); (b)
 I. Lamparth, C. Maichle-Mössmer, A. Hirsch. Angew. Chem., Int. Ed. Engl. 34, 1607 (1995).
- 4. A. Hirsch, O. Vostrowsky. Eur. J. Org. Chem. 829 (2001).

A. HIRSCH

- 5. I. Lamparth, A. Herzog, A. Hirsch. Tetrahedron 52, 5065 (1996).
- 6. X. Camps, A. Hirsch. J. Chem. Soc., Perkin Trans. 1 1595 (1997).
- (a) B. Kräutler, T. Mueller, J. Maynollo, K. Gruber, C. Kratky, P. Ochsenbein, D. Schwarzenbach, H.-B. Buergi. Angew. Chem. 108, 1294 (1996); (b) B. Kräutler, T. Mueller, J. Maynollo, K. Gruber, C. Kratky, P. Ochsenbein, D. Schwarzenbach, H.-B. Buergi. Angew. Chem., Int. Ed. Engl. 35, 1204 (1996).
- 8. R. Schwenninger, T. Müller, B. Kräutler. J. Am. Chem. Soc. 119, 9317 (1997).
- 9. A. Duarte-Ruiz, K. Wurst, B. Kräutler. Helv. Chim. Acta 84, 2167 (2001).
- (a) L. Isaacs, R. F. Haldimann, F. Diederich. Angew. Chem. 106, 2434 (1994); (b) L. Isaacs, R. F. Haldimann, F. Diederich. Angew. Chem., Int. Ed. Engl. 33, 2339 (1994).
- 11. F. Diederich, R. Kessinger. In *Templated Organic Synthesis*, p. 189, Wiley-VCH, Weinheim (2000).
- 12. F. Diederich, R. Kessinger. Acc. Chem. Res. 32, 537 (1999).
- 13. U. Reuther, T. Brandmüller, W. Donaubauer, F. Hampel, A. Hirsch. Chem.-Eur. J. 8, 2833 (2002).
- 14. C. Bingel. Chem. Ber. 126, 1957 (1993).
- R. Sijbesma, G. Srdanov, F. Wudl, J. A. Castoro, C. Wilkins, S. H. Friedman, D. L. DeCamp, G. L. Kenyon. J. Am. Chem. Soc. 115, 6510 (1993).
- S. H. Friedman, D. L. DeCamp, R. P. Sijbesma, G. Srdanov, F. Wudl, G. L. Kenyon. J. Am. Chem. Soc. 115, 6506 (1993).
- 17. L. L. Dugan, D. M. Turetsky, C. Du, D. Lobner, M. Wheeler, C. R. Almli, C. K. F. Shen, T.-Y. Luh, D. W. Choi, T.-S. Lin. *Proc. Natl. Acad. Sci. USA* **94**, 9434 (1997).
- 18. T. Da Ros, M. Prato. Chem. Commun. 663 (1999).
- 19. I. Lamparth, A. Hirsch. Chem. Commun. 1727 (1994).
- P. Witte, F. Beuerle, U. Hartnagel, R. Lebovitz, A. Savouchkina, S. Sali, D. Guldi, N. Chronakis, A. Hirsch. Org. Biomol. Chem. 5, 3599 (2007).
- 21. M. Brettreich, A. Hirsch. Tetrahedron Lett. 39, 2731 (1998).
- 22. F. Beuerle, P. Witte, U. Hartnagel, R. Lebovitz, Ch. Parng, A. Hirsch. J. Exp. Nanosci. (2007). In press.
- 23. M. Braun, S. Atalick, D. M. Guldi, H. Lanig, M. Brettreich, S. Burghardt, M. Hatzimarinaki, E. Ravanelli, M. Prato, R. v. Eldik, A. Hirsch. *Chem.—Eur. J.* **9**, 3867 (2003).
- 24. I. Zilbermann, A. Lin, M. Hatzimarinaki, A. Hirsch, D. M. Guldi. Chem. Commun. 96 (2004).
- 25. D. M. Guldi, I. Zilbermann, G. Anderson, A. Li, D. Balbinot, N. Jux, M. Hatzimarinaki, A. Hirsch, M. Prato. *Chem. Commun.* 726 (2004).
- 26. H. Kato, C. Böttcher, A. Hirsch. Eur. J. Org. Chem. 2659 (2007).
- 27. A. Hirsch, O. Vostrowsky. Eur. J. Org. Chem. 829 (2001).
- (a) M. Brettreich, S. Burghardt, C. Böttcher, T. Bayerl, S. Bayerl, A. Hirsch. Angew. Chem. 112, 1915 (2000); (b) M. Brettreich, S. Burghardt, C. Böttcher, T. Bayerl, S. Bayerl, A. Hirsch. Angew. Chem., Int. Ed. 39, 1845 (2000).
- 29. A. P. Maierhofer, M. Brettreich, S. Burghardt, O. Vostrowsky, A. Hirsch, S. Langridge, T. M. Bayerl. *Langmuir* 16, 8884 (2000).
- 30. J. N. Israelachvilli. Intermolecular and Surface Forces, Academic Press, New York (1985).
- (a) M. Kellermann, W. Bauer, A. Hirsch, B. Schade, K. Ludwig, C. Böttcher. Angew. Chem. 116, 3019 (2004);
 (b) M. Kellermann, W. Bauer, A. Hirsch, B. Schade, K. Ludwig, C. Böttcher. Angew. Chem., Int. Ed. 43, 2959 (2004).
- For electron cryo-microscopy preparation, see (a) K. Ludwig, B. Baljinnyam, A. Herrmann, C. Böttcher. *EMBO J.* 22, 3761 (2003); (b) M. M. Golas, B. Sander, C. L. Will, R. Lührmann, H. Stark. *Science* 300, 980 (2003); (c) A. Sali, R. Glasere, R. Earnest, W. Baumeister. *Nature* 422, 216 (2003).
- 33. D. W. R. Gruen. J. Colloid Interface Sci. 84, 281 (1981).

- 34. (a) S. Burghardt, A. Hirsch, B. Schade, K. Ludwig, C. Böttcher. *Angew. Chem.* 117, 3036 (2005);
 (b) S. Burghardt, A. Hirsch, B. Schade, K. Ludwig, C. Böttcher. *Angew. Chem.*, *Int. Ed.* 44, 2976 (2005).
- 35. U. Hartnagel, D. Balbinot, N. Jux, A. Hirsch. Org. Biomol. Chem. 4, 1785 (2006).
- 36. (a) B. Schade, K. Ludwig, C. Böttcher, U. Hartnagel, A. Hirsch. *Angew. Chem.* 119, 4482 (2007);
 (b) B. Schade, K. Ludwig, C. Böttcher, U. Hartnagel, A. Hirsch. *Angew. Chem., Int. Ed.* 46, 4393 (2007).