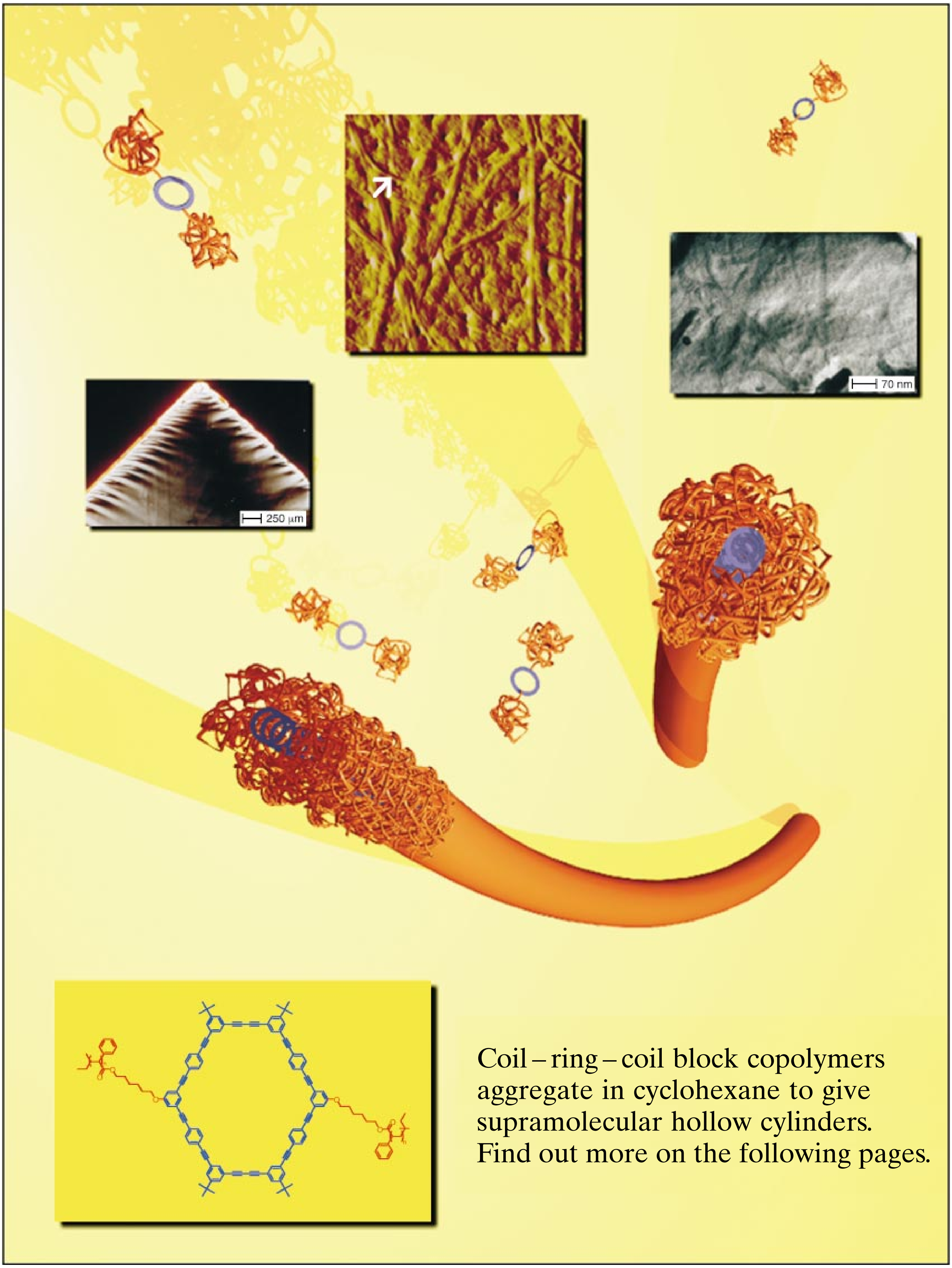


Coil–ring–coil block copolymers aggregate in cyclohexane to give supramolecular hollow cylinders. Find out more on the following pages.

Coil–Ring–Coil Block Copolymers as Building Blocks for Supramolecular Hollow Cylindrical Brushes

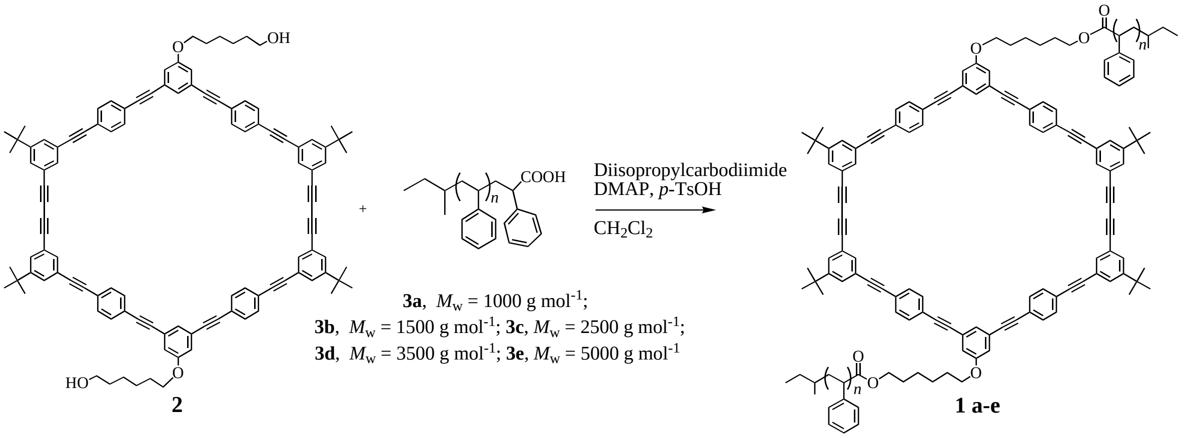
Silvia Rosselli, Anne-Désirée Ramminger, Thomas Wagner, Beate Silier, Simone Wiegand, Wolfgang Häußler, Günter Lieser, Volker Scheumann, and Sigurd Höger*

Dedicated to Professor Fred Wudl
on occasion of his 60th birthday

Molecular self-assembly of relatively small, well-defined molecules to complex structures by noncovalent interactions is well known and often found in nature.[1] Considerable effort has been given not only in the investigation and understanding of the basic principles and driving forces in self-organizing systems but also in the design of structures which imitate the complexity of these systems.[2] One of the most intensively studied natural supramolecular systems is the hollow cylindrical-shaped tobacco mosaic virus (TMV) formed by self-assembly of the constituent protein subunits and the viral RNA.[3] Synthetic supramolecular architectures based on gallic acid derivatives have been prepared and indeed show superstructures which are very similar to the natural model.[4] In addition, cylinders having a lumen, as the virus, have also been described. Based on cyclopeptides or cyclosaccharides, the driving force for the aggregation are specific interactions, such as hydrogen bonding donors and acceptors.[5]

Recently, we synthesized functionalized shape-persistent macrocycles, that should also be suitable building blocks for the formation of nanoscale hollow cylinders. An objective of this study is to facilitate aggregate formation through nonspecific interactions, opposite to the macrocycles mentioned above (for example, solvophobic effects and shape complementarity). For this purpose, side groups that are incompatible with the cyclic backbone of the ring are attached to the macrocycle to induce (in a suitable solvent) microphase segregation and aggregation into cylindrical objects. Previous work in our group[6] has shown that the aggregation behavior of oligoalkyl-substituted shape-persistent rings is strongly solvent dependent.[7] For example, in solvents which act as good solvents for the rigid core and the flexible corona (such as CH_2Cl_2), no aggregation was observed. By adding a solvent which can solubilize only the alkyl corona but not the rigid core (for example *n*-hexane), a solvophobic aggregation of the structures ($K_{dim} = 130\,\text{M}^{-1}$ in hexane/CH_2Cl_2 (3/1); $790\,\text{M}^{-1}$ in hexane/CH_2Cl_2 (6/1)) can be induced. However, with this approach we cannot observe larger aggregation constants by further decreasing the solvent polarity of the solvent mixture, since the connection of twelve dodecyl groups is still not sufficient to keep the materials in solution. Herein, a simple method is described to overcome this solubility problem by attaching short, non-crystallizable oligomeric side groups to the ring.

The central block of the target structures **1** is the macrocycle **2**, which has been obtained in good yield by oxidative Glaser coupling of the appropriate bisacetylene.[8] Onto the macrocycle, narrowly distributed ($M_w/M_n < 1.1$) polystyrene–carboxylic acid oligomers[9] (**3a–e**) of different molecular weights ($M_w = 1000$ (**3a**), 1500 (**3b**), 2500 (**3c**), 3500 (**3d**), 5000 g mol^{-1} (**3e**)) have been attached through carbodiimide coupling (Scheme 1) in good yields (70–90%).[10]



Scheme 1. Synthesis of coil–(rigid)ring–coil block copolymers **1a–e**. DMAP = 4-dimethylaminopyridine; *p*-TsOH = *p*-toluene sulfonic acid.

The resulting structures can be described as coil–(rigid)-ring–coil block copolymers, a subclass of rod–coil block copolymers, that are known to microphase separate to well-ordered superstructures, even at relatively small block sizes.[2, 11]

The block copolymers described here are readily soluble in chloroform, dichloromethane, THF, and toluene at room temperature, and **1b–e** in warm cyclohexane.[12] Upon cooling, **1b** forms a gel at concentrations above 0.5 wt%, **1c** rapidly forms a very viscous solution, and **1d** forms a similar viscous solution after some days. These solutions are also strongly birefringent (Figure 1).[13] The exceptions are

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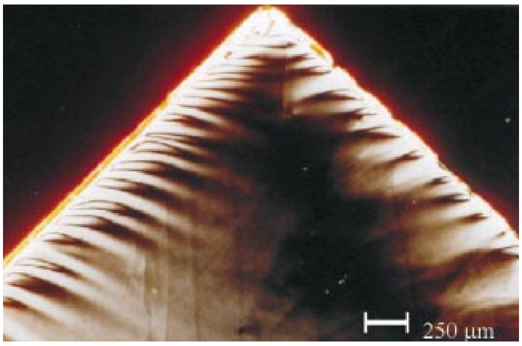

Figure 1. Texture of **1c** in cyclohexane between crossed polarizers (2 wt %; at the edges of the cover glass).

solutions of **1e**, which are neither very viscous nor birefringent.

These observations can be explained by the different solubility of the rigid cyclic block and the flexible polystyrene (PS) blocks of the copolymer. Cyclohexane is a solvent for PS but, in contrast to THF or toluene, a nonsolvent for the unsubstituted macrocycle **2**. The attachment of the polymeric side groups, even of relatively low molecular weight, is sufficient to solubilize the block copolymer in cyclohexane at elevated temperatures. However, upon cooling, the rigid parts of the block copolymer aggregate if the size of the coiled blocks is below a certain level. As expected, extension and aggregation kinetics strongly depend on the size of the PS block. Due to the structure of the block copolymers, the aggregation should lead to the formation of rodlike objects with large aggregate numbers, thereby reducing the contact surface of the rigid cyclic unit with the solvent.[14] In order to support this assumption, the aggregation behavior of **1c** was investigated in more detail. Dynamic light scattering (DLS) was performed on solutions of **1c** in toluene and cyclohexane. The distribution of the relaxation times in the autocorrelation functions were obtained by CONTIN analysis.[15] In toluene (0.11 wt %), only one maximum that corresponds to a hydrodynamic radius of approximately 2 nm is found, which correspond to the size of a simple block copolymer molecule **1c**. In contrast, the light scattering data in cyclohexane at the same concentration showed the formation of more complex structures. The elaboration of the measured autocorrelation function resulted in a distribution of relaxation times that contain two maxima, one at a hydrodynamic radius of approximately 2 nm, the other at approximately 60 nm (Figure 2a).

Concentration-dependent measurements showed that the fraction of the larger species in solution increases with increasing concentration.[16] The angular dependence of the scattered light intensity of the larger objects (Figure 2b) suggested the presence of rodlike objects (linear increase of the Kratky plot) with a "virtual" persistence length, a measure of the rigidity, of more than 100 nm.[17] The total length of the objects varies between 250 and 1300 nm, whereby a hydrodynamic radius of 60 nm corresponds to a rod length of approximately 500 nm.[18]

The diameter of these cylindrical objects was determined by ultra small angle X-ray scattering (USAXS) and small angle X-ray scattering (SAXS) in cyclohexane at 2 wt % (Figure 3).

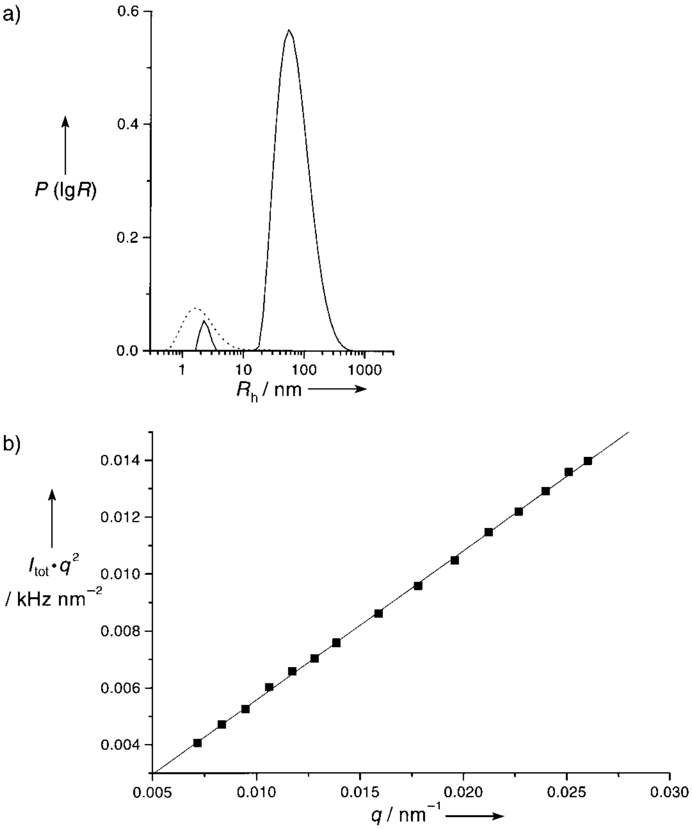

Figure 2. a) CONTIN fit of DLS-derived distributions of **1c** in toluene (- - - -) and in cyclohexane (——) at a concentration of 0.11 wt %; b) Kratky plot for the slow mode of **1c** in cyclohexane (■) at a concentration of 0.09 wt % and linear data fit (——). R_h = hydrodynamic radius; I_{tot} = total intensity; q = scattering vector.

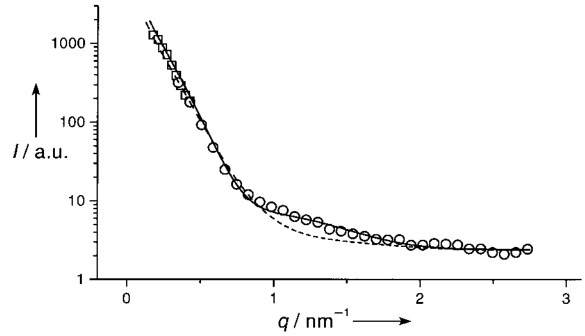

Figure 3. USAXS (□) and SAXS (○) data of a 2.0 wt % cyclohexane solution of **1c**. The lines represent the form factor for a solid cylinder (- - - -) with an external diameter of 10 ± 3.8 nm, and for a hollow cylinder (——) with an external diameter of 10 ± 3.8 nm and an internal diameter of 1.8 ± 0.5 nm. I = total intensity; q = scattering vector.

The description of the measured data was not possible by using a simple solid cylinder model (showing an abrupt radial electron density change) of any length or thickness.[19] Therefore, a model was used that describes the electron density profile on the outside of the cylinder more accurately. The different length of the oligomeric PS chains and the radial decrease of the cylinder density was taken into account by giving the cylinder a polydispersity in the diameter. Since variations in the cylinder length do not change the form factor at all, it was set to 500 nm in accordance with the average object length determined by the light scattering. The calculated form factor of a solid cylinder of mean polydisperse diameter 10 nm ($d_{\mathrm{out}} = 10$ nm, polydispersity 3.8 nm, half width at half maximum of a Gaussian distribution) agrees with the observed data at low q values. However, the observed scattering intensity is remarkably higher than the theoretical prediction in the q range between 1 and 2 nm^{-1} and it was not possible to adjust the theoretical form factor to the experimental data, neither by variation of the cylinder diameter nor its polydispersity. Only the use of a radial density profile with a reduced electron density inside the cylinder resolved this discrepancy. The calculated form factor of a cylinder with the same electron density profile on the outside as described before and with an additional reduced electron density on the inside (hollow diameter $d_{\mathrm{inner}} = 1.8$ nm, polydispersity 0.5 nm, half width at half maximum of a Gaussian distribution) fits with the observed data over the whole q range.[20] The scattering data undoubtedly show that the coil–ring–coil block copolymers aggregate in solution into hollow cylinder-shaped objects with a high virtual persistence length.

Further evidence of the existence of these supramolecular cylinders was obtained by imaging methods applied to solid samples prepared under nonequilibrium conditions.[21] The transmission electron micrograph (TEM) of a sample obtained by freeze drying a cyclohexane solution (Pt/C shadowed film) shows ribbons of different width at the sample surface, the narrowest in the range of approximately 15 nm (Figure 4 A).

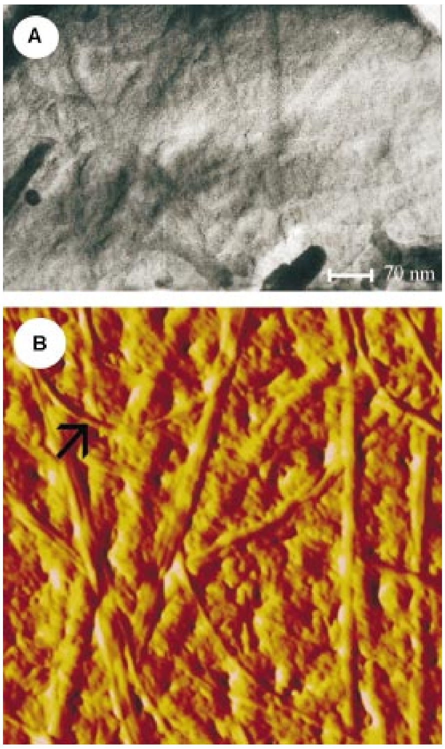

Figure 4. A) TEM: C/Pt shadowed film obtained by freeze drying a 0.15 wt% cyclohexane solution of **1c**; B) AFM (amplitude picture, 1.5 × 1.5 μm^2): Film obtained by dipping mica into a 0.15 wt% cyclohexane solution of **1c**.

Atomic force microscopy (AFM) images of a block copolymer film obtained by dipping mica into a cyclohexane solution of **1c** show long bundles of two or three cylindrical aggregates, together with individual aggregates (Figure 4 B).[22] Taking in consideration the width of the AFM tip, the cylindrical units have a diameter of approximately 10–20 nm. The dimensions of the aggregates obtained by TEM and AFM correspond well with the molecular dimensions obtained by the scattering methods and are in accordance with the molecular dimensions of the molecular building blocks.[23] Even the most curved cylindrical objects found in the AFM images (Figure 4 B, black arrow) show a large radius of curvature indicating their rigid nature.

The correspondence of our aggregates with covalently linked polymer brushes[24] suggests a description of them as supramolecular hollow cylindrical brushes (Figure 5).

The results shown here demonstrate that coil–ring–coil block copolymers can aggregate into hollow cylinderlike objects by a combination of shape complementarity and demixing of rigid and flexible polymer parts (namely by nonspecific interactions). This is opposite to most of the previously reported tubular superstructures, where attractive forces induce the self-assembly. Since this approach bears the possibility to organize functionalized macrocycles while leaving the functional groups of the ring "free", it opens a new entry to intra-annular functionalized rods. In other words, the functional groups do not have to be used to organize the macrocycles, but the formation of the superstructure is used to organize the functional groups.

Figure 5. Schematic representation of the aggregation of the block copolymers into hollow cylindrical brushes. The PS block is the corona (orange) and the rigid ring is located in the core (blue) (not in scale).

Received: March 19, 2001 [Z16805]

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[16] The concentrations of the samples analyzed were 0.05, 0.093, and 0.113 wt.%.

[17] The persistence length l is a measure for the stiffness of the polymer, and is defined as $\langle\cos\theta(s)\rangle = \exp(-s/l)$, with contour length s. In the case of a supramolecular aggregate, the calculation using the formula above leads to the "virtual" persistence length. For comparison, the persistence length of simple synthetic polymers is less than 1 nm, whereas the persistence length of DNA is in the range of 100 nm; A. Y. Grosberg, A. R. Khokhlov, *Giant Molecules*, Academic Press, London, **1997**.

[18] For the estimation of the rod length L from R_h, the relation $1/\sqrt{3}(\ln(L/d)+0.38)$ was used (valid for a rodlike object of length L and diameter d). In our case we assume that $d \ll L$ because of the high value of R_h. If the ratio L/d is about 40, the ratio $R_g/R_h = 2.34$ can be used. With $R_g^2 = L^2/12$ follows $L \approx [12(2R_h)^2]^{1/2}$; a) M. Schmidt, W. H. Stockmayer, *Macromolecules* **1984**, *17*, 509; b) H.-G. Elias, *Makromoleküle, Bd. 1*, Hüthig & Wepf, Basel, **1990**.

[19] All form factors of cylindrical objects without polydispersity in the diameter exhibit pronounced oscillations in the investigated q range.

[20] The fit function is given by the expression (1).

$$\int_0^{\frac{\pi}{2}}\int_0^{\frac{R_a+R_i}{2}}\int_{\frac{R_a+R_i}{2}}^{\infty} [\{4r_a j_1(qr_a\sin(\alpha)) - 4r_i j_1(qr_i\sin(\alpha))\}\sin(ql\cos(\alpha/2))/ \\ \{q\sin(\alpha)ql\cos(\alpha)(r_a^2 - r_i^2)\}]^2\sin(\alpha) \\ \exp[-(r_a-R_a)^2/(2\Delta r_a^2) - (r_i-R_i)^2/(2\Delta r_i^2)]\,\mathrm{d}r_a\,\mathrm{d}r_i\,\mathrm{d}\alpha \tag{1}$$

A better fit is obtained by a more sophisticated electron density profile in the range $2 < d < 4$ nm. However, a precise analysis of the electron density requires more accurate scattering data.

[21] Preliminary investigations show that solid samples obtained from solution by very slow solvent evaporation ("equilibrium conditions") form different superstructures, indicating that the solution studies cannot be transferred to the morphology in the solid state.

[22] At the same time some of the block copolymer forms an undulating background structure on the mica.

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From Structure to Function: A New Approach to Detect Functional Similarity among Proteins Independent from Sequence and Fold Homology

Stefan Schmitt, Manfred Hendlich, and
Gerhard Klebe*

The explosion in information regarding protein sequence and structure demands new computational tools for the direct inference of protein function. The molecular function of proteins is almost invariably linked with the specific recognition of substrates and endogenous ligands in given binding pockets; proteins of related function should therefore share comparable recognition pockets. We have developed a new approach that is based on the placement of physicochemical descriptors assigned to the exposed binding-site residues. The deduced property descriptors can be used to retrieve common sub-structures and, thereby, related binding pockets. The solutions obtained are scored by comparing similarly exposed surface patches assigned to the same property, thus allowing detection of functional relationships among proteins independent of a particular sequence or fold homology.

The sequencing of the human genome represents only the first step toward understanding the functional and structural interplay of proteins in biological systems. Through methodological developments in "structural genomics", such as high-throughput X-ray crystallography,[1] we will be increasingly confronted with 3D structures of proteins whose actual biochemical function has yet to be assigned. In addition, structure prediction and homology modeling techniques might mature to a state where the overall protein geometry can be predicted correctly from sequence.[2] Thus, methods to infer protein function from 3D structure are desperately required. Since protein function is not necessarily confined to a particular fold or vice versa,[3] this assignment is by no means straightforward.

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1433-7851/01/4017-3141 $ 17.50+.50/0