Angewandte
Zuschriften

Supramolecular Chemistry

DOI: 10.1002/ange.201106632

Self-Assembly of Amphiphilic Molecules in Droplet Compartments: An Approach Toward Discrete Submicrometer-Sized One-Dimensional Structures**

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Great progress has been made in the construction of well-defined nanoarchitectures through the self-assembly of programmed molecules with intermolecular interactions controlled through a sophisticated molecular design.[1] In particular, the unique self-assembling ability of amphiphilic molecules finds application in the creation of diverse nanostructures for use as nanomaterials or as building units for further hierarchical nanoarchitectures.[2,3] Despite rapid advances in this area, no effective strategies have been developed to date to control the sizes of one-dimensional (1D) self-assembled structures (e.g., rods and tubes) in the submicrometer regime because the component amphiphilic molecules tend to aggregate in a noncontrollable manner, resulting in the formation of dispersive structures. In contrast, spherically closed structures (e.g., micelles and vesicles) feature a limited number of amphiphilic molecules packed in a restricted space. An interesting challenge in supramolecular chemistry is the construction of versatile self-assembling systems in which more than a thousand component molecules interact simultaneously beyond molecular programs to form uniform 1D architectures in the submicrometer region. One solution would be to use noncovalent bonding to self-organize a limited number of molecules onto a 1D template possessing a well-regulated length (e.g., oligomeric molecular templates synthesized in a bottom-up manner).[3g] To construct further comprehensive systems that govern self-assembly events hierarchically from the nano- to the micrometer range might require a combination of top-down and bottom-up strategies.

Oil-in-water (O/W) emulsions (i.e., oil droplets) have a broad range of applications in, for example, drug delivery, high-throughput analysis, and microreactors.[4] Each droplet offers a spherical interface between two immiscible liquids; this interface can act as a scaffold of submicrometer dimensions for the self-assembly of amphiphilic molecules. In addition, because the droplet size is controllable in a top-down manner (e.g., through sonication or the use of microfluidic devices), a controlled number of amphiphilic molecules can be compartmentalized into the droplet by changing its size and/or the concentration of the dissolved molecules. If the trapped amphiphilic molecules could also form 1D assembled structures on the surfaces of the isolated droplets, these 1D structures would be monodisperse—a potentially expeditious route towards discrete submicrometer-sized 1D structures. To test this intriguing hypothesis, we designed amphiphilic molecules having the potential to undergo 1D self-assembly after entrapping on the droplet surface. Remarkably, we found that moderate heating of an O/W emulsion (i.e., dynamic shrinking of droplets) containing appropriately designed amphiphilic molecules induced a dramatic structural change from spherical droplets to discrete tubular structures having dimensions in the submicrometer regime.

We prepared Zn–chlorophyll-based amphiphilic molecules featuring dendritic tetra(ethylene glycol) (TEG) units at position 17 and isonicotinic acid (Chl-4Py, Figure 1a) and nicotinic acid (Chl-3Py, see Figure S1 in the Supporting Information) moieties at position 3 of the chlorophyll ring.[5] These amphiphilic molecules, which feature a perpendicular orientation between the TEG units and the coordination moieties, are suitable for 1D self-assembly on the surfaces of dynamically shrinking droplets, stabilized mainly through Zn–pyridine coordination (Figures 1b and c). In addition, we also prepared Chl-OMe and Chl-OH, amphiphilic molecules that can form stiffer 1D assembled structures through π–π stacking in addition to Zn–oxygen coordination (see Figure S1 in the Supporting Information).

First, we investigated the fundamental self-assembly of Chl-4Py in a homogeneous water/THF mixture. We mixed THF solutions containing 2.0 mM Chl-4Py with water at various water/THF compositions. The Soret and Q bands of Chl-4Py gradually shifted from 424 to 447 nm and from 651 to 668 nm, respectively, upon increasing the composition of water in THF (Figure 2a); accordingly, the circular dichroism (CD) intensity also increased dramatically (Figure 2b). In

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[**] We thank the "Kyoto-Advanced Nanotechnology Network" from MEXT (Japan) for TEM observations. We also thank Mr. K. Kiguchi and Prof. A. Ikeda for kindly supporting our TEM observations. This study was partially supported by Grants-in-Aid for Scientific Research (B) (grant number 22310069) and Exploratory Research (grant number 23651118).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106632.

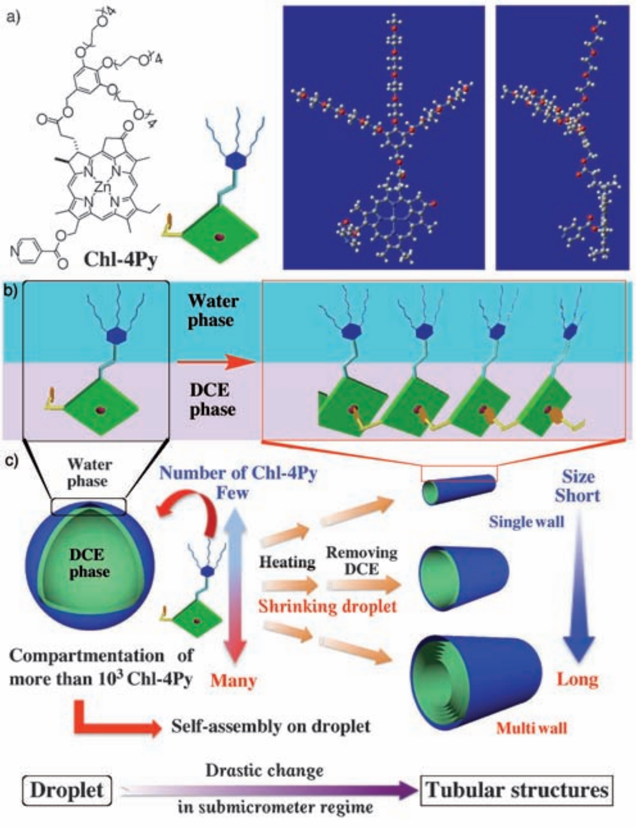

Figure 1. a) Structure of Chl-4Py. b) 1D self-assembly of Chl-4Py at the DCE–water interface. c) Chl-4Py compartmentalized on a DCE droplet and manifesting its inherent 1D assembling ability at the water–DCE interface through a dramatic change in the shape of the DCE droplet from spherical to tubular upon evaporating the DCE.

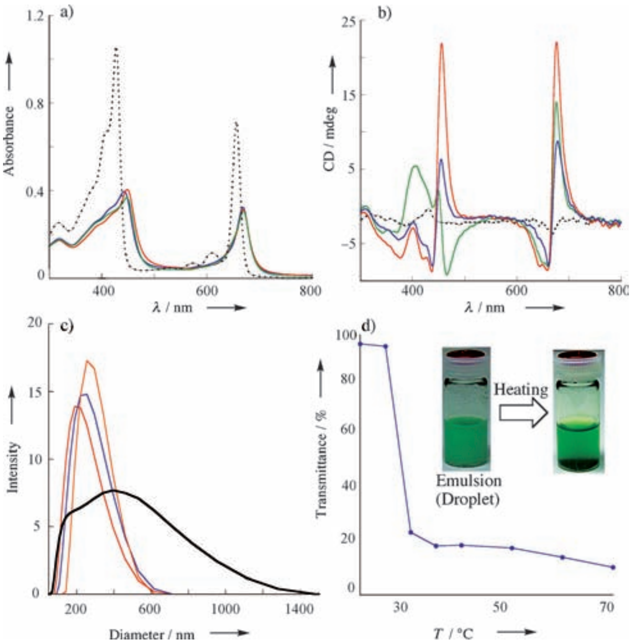

Figure 3. a) UV/Vis and b) CD spectra (0.5 cm cell, RT) of the emulsion prepared from 2.5 mM Chl-4Py in DCE (blue lines: O/W emulsion; red lines: after heating of the emulsion) and the corresponding homogeneous water/THF (99:1, v/v) solution (green lines) and the DCE solution (black dotted lines). c) Size distributions of the droplets formed from 2.5 mM (red line), 7.5 mM (blue line), and 12.5 mM (orange line) solutions of Chl-4Py in DCE; black bold line: size distribution of rodlike structures obtained from homogeneous water/THF (99:1, v/v) solution. d) Transmittance at 800 nm plotted with respect to temperature. Inset: Photographs of the emulsion sample (left) and clear solution obtained after heating (right).

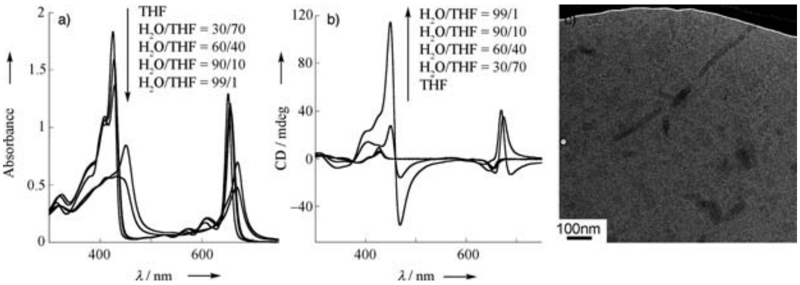

Figure 2. a) UV/Vis and b) CD spectra (1.0 cm cell, RT) of Chl-4Py solutions at various water/THF compositions. c) Cryo-TEM image of the obtained rodlike structures.

contrast, we observed no such spectral changes in either the UV/Vis or CD spectra of Chl-3Py, presumably because of an unfavorable Zn–pyridine orientation (see Figures S2 and S3 in the Supporting Information). These results suggest that Chl-4Py has a 1D self-assembling ability mediated through effective Zn–pyridine coordination. Cryogenic transmission electron microscopy (cryo-TEM) of the obtained aqueous solution [water/THF, 99:1 (v/v)] confirmed that Chl-4Py self-assembled to form rodlike structures (Figure 2c). The diameter of each rodlike structure was quite consistent at approximately 30 nm, whereas the length extended to several hundreds of nanometers with a rather wide distribution. Dynamic light scattering (DLS) provided further quantitative data regarding the size distribution (Figure 3c).

Based on these results, we focused our attention on the self-assembling behavior of Chl-4Py on 1,2-dichloroethane (DCE)/water emulsion (droplet) surfaces. We prepared DCE solutions of Chl-4Py at three different concentrations (2.5, 7.5, and 12.5 mM) and used a probe-type sonicator to disperse each of them into an excess of distilled water.[6] DLS of the obtained emulsion samples revealed that the average diameter of the DCE droplets was approximately 250 nm with narrow distributions (Figure 3c).[7] TEM analysis of the emulsion sample revealed vesiclelike inner structures of the DCE droplets (Figures 4a and b), suggesting that the amphiphilic Chl-4Py was entrapped at the DCE–water interface. UV/Vis and CD spectra supported this hypothesis. Figure 3a presents the UV/Vis spectra of the emulsion sample prepared from 2.5 mM Chl-4Py in DCE and the rodlike structure obtained from a homogeneous water/THF (99:1, v/v) solution. The Soret and Q bands appear at 446 and 668 nm, respectively, in each spectrum; these features are almost identical, but they are red-shifted relative to those of monomeric Chl-4Py, indicating

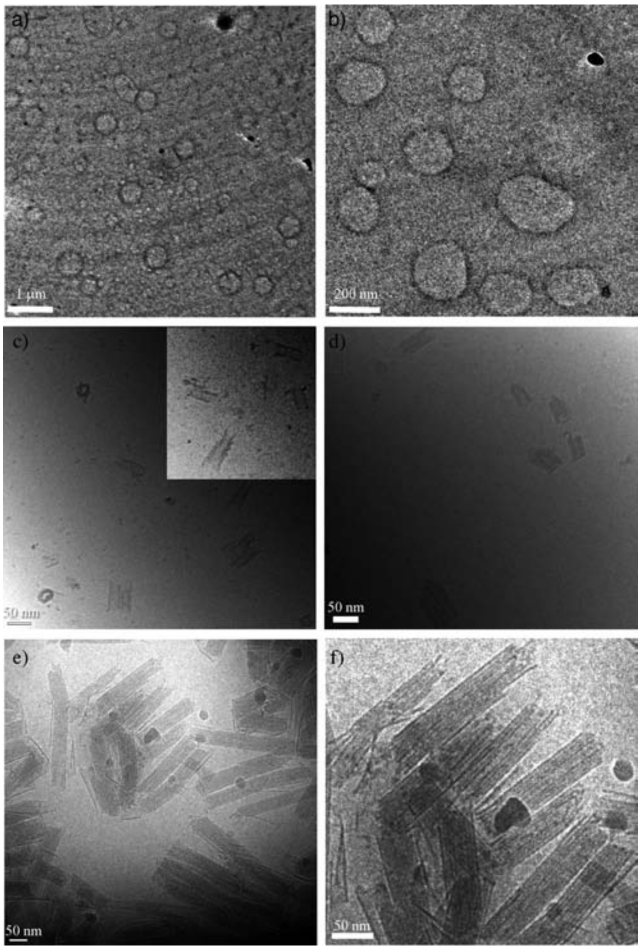

Figure 4. a) TEM image of DCE droplets prepared from a 12.5 mM DCE solution and b) magnified image. c–f) Cryo-TEM images of the tubular structures obtained from DCE solutions of Chl-4Py at c) 2.5 mM (inset: magnified image), d) 7.5 mM, and e) 12.5 mM. f) Magnified image of (e). For other TEM images, see Figure S9–11 in the Supporting Information.

that Chl-4Py units were present at the DCE–water interface, where they exerted their inherent 1D self-assembling ability. In contrast, the CD spectrum of the emulsion sample was different from that of the water/THF solution, suggesting that the Chl-4Py units self-assembled on the droplet surface in a molecular arrangement unlike that in the rodlike structure (Figure 3b). These spectral discrepancies are characteristic and common with those of other samples containing different concentrations of Chl-4Py (see Figures S6 and S7 in the Supporting Information). Overall, as supported by TEM and UV/Vis and CD spectra, we conclude that the amphiphilic Chl-4Py aligns at the DCE–water interface, with its hydrophilic TEG units solvated by water.

The aqueous solutions containing Chl-4Py showed reversible transformations from transparent to translucent states at temperatures above 30°C, which is the lower critical solution temperature (LCST) originating from dehydration of the TEG units (Figure 3d). Therefore, we heated the emulsion sample moderately above the LCST, expecting that the self-assembly of Chl-4Py would be further accelerated on the droplet surface. When we heated the obtained emulsion sample at 65°C for 4 min, the turbid emulsion transformed into a clear green solution (Figure 3d, inset), suggesting that most of the DCE molecules gradually evaporated from the droplets. This energy-consuming process induced the shrinking of the droplets and enhanced the concentration of Chl-4Py on the droplet surfaces, further facilitating their self-assembly.[8] As expected, the CD intensity of the resulting solution was greater than that of the initial emulsion (Figure 3b), suggesting tighter molecular packing of Chl-4Py units leading to stiffer self-assembled structures formed on the droplets' surfaces.

The cryo-TEM images of the clear aqueous solutions showed that the DCE droplets underwent dramatic morphological changes, from spherical to tubular structures having dimensions in the submicrometer regime. The TEM images in Figure 4c–f show that the lengths and diameters of the tubular structures were almost uniform, in contrast to the rodlike structures formed in the homogeneous solution. The narrow size distributions imply that the number of assembling Chl-4Py units was controlled by the compartment effect of each DCE droplet. We confirmed this effect by changing the concentration of Chl-4Py and the pipetted volume of the DCE solutions, both of which should directly affect the number of Chl-4Py molecules compartmentalized within each droplet. In the case of the DCE droplets prepared from 50 μL of the 2.5 mM Chl-4Py solution, the average length and outer diameter of the tubular structure were approximately 45 and 20 nm, respectively (Figure 4c). The hydrophobic chlorophyll units appeared as a dark layer having a thickness of approximately 1.0 nm, consistent with the calculated molecular length of a chlorophyll moiety; in contrast, the solvated TEG units were not directly visible in the cryo-TEM image. In the case of the DCE droplets prepared from 50 μL of the 7.5 mM Chl-4Py solution, the average length and outer diameter both increased to approximately 60 and 25 nm, respectively. The wall thickness also increased to 4–10 nm (Figure 4d). Furthermore, in the case of the DCE droplets prepared from 50 μL of the 12.5 mM Chl-4Py solution, the length and outer diameter of the tubular structures increased further to approximately 200 and 45 nm, respectively. Notably, in contrast to the structures formed from the lower-concentration (i.e., 2.5 and 7.5 mM) samples, the tubular structures obtained from the 12.5 mM Chl-4Py solution featured multiple layers (Figures 4e and f), with a wall thickness of approximately 15 nm with very regular intervals. We estimated the interval between the dark layers to be approximately 4 nm, in good agreement with the calculated molecular length of Chl-4Py. Similarly, when we decreased the pipetted volume of 12.5 mM Chl-4Py to 10 μL, the droplet size decreased to approximately 180 nm, as shown by DLS analysis (see Figure S8 in the Supporting Information). This result implies that the number of molecules entrapped in each droplet also decreased. Upon heating the emulsion sample, the tubule size decreased (see Figure S8 in the Supporting Information) and we could not observe any of the multilayer structures inside the tubes as we had seen in Figure 4e. Consequently, a small droplet resulted in small tubes. These results clearly indicate that a controlled number of amphiphilic molecules can be compartmentalized into each droplet by changing its size and/or the concentration of the dissolved molecules and that the self-assembly of Chl-4Py occurred within each droplet. We also confirmed that heating above the LCST was necessary to create the tubular structures; when we evaporated DCE under reduced pressure without heating, in a reference experiment, the droplet merely shrank to form spherical assembled structures similar to micelles (see Figure S15 in the Supporting Information).[9] The increase in hydrophobicity of the TEG units upon heating above the LCST encouraged the Chl-4Py units to aggregate on the droplet surface to avoid unfavorable contact with the water phase. Furthermore, when the entire surface of the droplet was covered with Chl-4Py units prior to shrinking, the dehydration of the TEG units decreased the effective volume of each Chl-4Py moiety, leading to their aggregation in DCE layers and the creation of multilayer structures.

Next, we correlated the number of compartmentalized molecules with the surface area of the prepared DCE droplets. When we employed 50 μL of the 2.5 mM Chl-4Py solution in DCE, we estimated the number of compartmentalized molecules and the surface area to be 6300 and 1.26×10^5 nm^2, respectively. Assuming that the area occupied by a single Chl-4Py molecule is approximately 3.7 nm^2 (from a CPK model), we calculated the total area occupied by all the Chl-4Py molecules to be 2.33×10^2 nm^2. As a result, the ratio of the surface area of the droplet to the total occupied area was 5.4, indicating that sufficient space was available on the droplet surface for the self-assembly of the Chl-4Py units. In the case where we added of 50 μL of the 7.5 mM Chl-4Py solution, we estimated this ratio to be 1.6. This value decreased further to 0.8 in the case of the 12.5 mM Chl-4Py solution (50 μL), implying that not all of the Chl-4Py molecules could be organized on the droplet surface, leading to the creation of multilayered tubular structures upon shrinking of the droplet.

Small-angle X-ray scattering (SAXS) analysis of the multilayered tubular structure obtained from the 12.5 mM Chl-4Py solution (50 μL) showed a broad reflection peak assignable to a *d* spacing of 4.4 nm (see Figure S12 in the Supporting Information), consistent with the extended molecular length calculated for Chl-4Py (4.2 nm). This result suggests that Chl-4Py self-assembled into single-layer structures, which further aggregated to form multilayered structures. The calculated molecular model for the 1D self-assembled structure displayed (see Figure S13 in the Supporting Information) that the Chl-4Py units aligned one-dimensionally merely through Zn–pyridine coordination, without direct π–π stacking between adjacent chlorophyll units. The periodical alignment of chlorophyll units bestows the created 1D structures with regular intervals for interdigitation through π–π stacking, leading to the formation of robust assembled structures through 2D assembly on each droplet's surface and, subsequently, to the spontaneous shape transition from spherical to tubular structures. Notably, among our tested amphiphilic chlorophyll derivatives, including Chl-OH and Chl-OMe, the creation of this unique 1D structure with molecular clefts was possible only for Chl-4Py (see Figure S14 in the Supporting Information). Although, spectroscopic measurements suggested that Chl-OH and Chl-OMe had potential to form 1D assemblies on the droplets' surfaces, cryo-TEM did not show any such tubular structures after heating their droplets (see Figures S4 and S5 in the Supporting Information).

Unlike the conventional tubular structures created from various amphiphilic molecules in homogeneous solvents, we expected the tubular structures obtained from the DCE droplets to contain DCE within the tubes. Indeed, the cryo-TEM images often provided evidence for the presence of traces of volatilized DCE within the tubes, appearing as local heating under electron beam irradiation (see Figure S11 in the Supporting Information). The unique features of the tubular DCE droplets stimulated us to investigate whether hydrophobic functional molecules could be extracted into the DCE phase from the uncapped edges. To test this hypothesis, we mixed a hexane solution of cyanine dye (1,1-didodecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, 0.2 mM, 400 μL) with the same volume of aqueous solution containing the tubular structure prepared from 2.5 mM Chl-4Py in DCE (50 μL). After stirring the two immiscible layers for 30 min with a magnetic stirrer, the pink color of the cyanine dye disappeared from the hexane layer (Figure 5a). The UV/Vis spectrum of the resulting aqueous solution showed that the extraction process did not affect the tubular structure. In addition, a new signal representing the characteristic absorption band of the cyanine dye appeared near 520 nm, bridging the green gap (see Figure S16 in the Supporting Information). Because the cyanine dye itself cannot be extracted into the aqueous phase, this result implies that it had been extracted from hexane into the DCE layer directly. Fluorescence spectra of the resulting aqueous solution provided further evidence for the extracted cyanine dye existing within the DCE phase. Upon excitation at 520 nm, a strong emission peak appeared at 572 nm, consistent with that of a cyanine

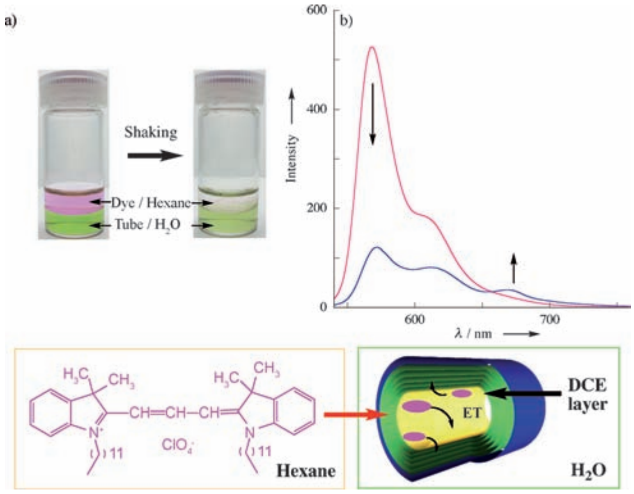

Figure 5. a) Photographs of two immiscible solutions. Left: an aqueous solution containing Chl-4Py and a hexane solution containing the cyanine dye. Right: the mixture of (a) after shaking. b) Fluorescence spectra of the cyanine dye after extraction into the tubular structure (blue line) and the sample containing the same concentration of the cyanine dye dissolved in DCE/hexane (99:1, v/v; red line). All samples were excited at 520 nm (ET = energy transfer).

dye dissolved in DCE/hexane (99:1, v/v; Figure 5b; λ_{em} = 568 nm). Notably, the fluorescence intensity of the cyanine dye decreased relative to that prior to entrapping within the tubes, and a new peak, which we ascribe to the emission from the chlorophyll unit, appeared at 668 nm. Upon excitation at 520 nm, chlorophyll provided no emission, because it lacks an absorption band in the green region (green gap). These results show that energy transfer occurred from the cyanine dye to the chlorophyll unit, and that the cyanine dye units were located within the tubes. Together, these findings suggest that the DCE solvent, which compartmentalized and pre-organized the Chl-4Py units in the initial stage, remained within the tubular structure. Because chlorophyll is very efficient in harvesting blue and red light, the present system, incorporating a functional dye bridging the green gap, would favor the efficient utilization of solar energy.

In conclusion, we have developed a novel supramolecular system for the formation of discrete 1D self-assembled architectures having dimensions in the submicrometer regime. This system combines a conventional supramolecular strategy with a dynamic liquid–liquid interface provided by shrinking droplets. In this system, the organic droplets act not only as dynamic templates but also as compartmentalizing solutions for amphiphilic molecules, allowing several thousands of molecules to spontaneously self-assemble on the isolated droplets' surfaces to form discrete 1D architectures beyond molecular programs. In other words, the amphiphilic molecules program the submicrometer-sized droplets. The size-regulated nanospace filled with organic solvent inside each tube might also be applicable as a container for a limited number of polymer strands or nanoparticles, or as a reaction nanovessel for preparing these materials in a bottom-up fashion. Recent developments in microfabrication techniques should enable us to further control the droplets—in a top-down manner—in terms of their size, concentration, and stability, allowing the number of self-assembling components to be regulated more precisely. We believe that this present system will open up new opportunities for supramolecular chemistry combining nano- and microscience.

Experimental Section

The emulsion samples were prepared according to the following general procedure: In a vial (internal diameter: 1.5 cm), a DCE solution (50 μL) containing Chl-4Py (2.5, 7.5, or 12.5 mM) was dispersed into distilled water (2950 μL) using a probe-type sonicator for 2 min, with the vial immersed in an ice water bath. Immediately after stopping the sonication, the obtained turbid O/W emulsion was heated moderately (65 °C, 4 min) in a water bath to give a clear green solution. The resultant aqueous solutions were stable for several months without forming any precipitates.

Received: September 19, 2011
Revised: December 9, 2011

Keywords: droplets · nanotubes · non-equilibrium conditions · self-assembly · supramolecular chemistry

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[6] In a control experiment, we confirmed that once Chl-4Py was solubilized in DCE, it could not be distributed into the aqueous phase upon vigorously shaking. All of the Chl-4Py molecules were, therefore, trapped in the DCE droplet after forming the emulsion.

[7] It was difficult to estimate the precise diameter of the droplets obtained immediately after sonication, because DCE gradually evaporated during the DLS measurements, even at room temperature.

[8] DLS analysis confirmed that fusion of DCE droplets did not occur during the heating process.

[9] To investigate the necessity of the O/W emulsion, we heated a DCE solution containing 12.5 mM Chl-4Py at 60 °C without forming an emulsion. Cryo-TEM analysis did not show any identifiable structures, indicating that self-assembly of the Chl-4Py units on the DCE–water interface was a prerequisite for the creation of tubular structures.