Porous Nanostructures

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Supramolecular Capsules with Gated Pores from an Amphiphilic Rod Assembly**

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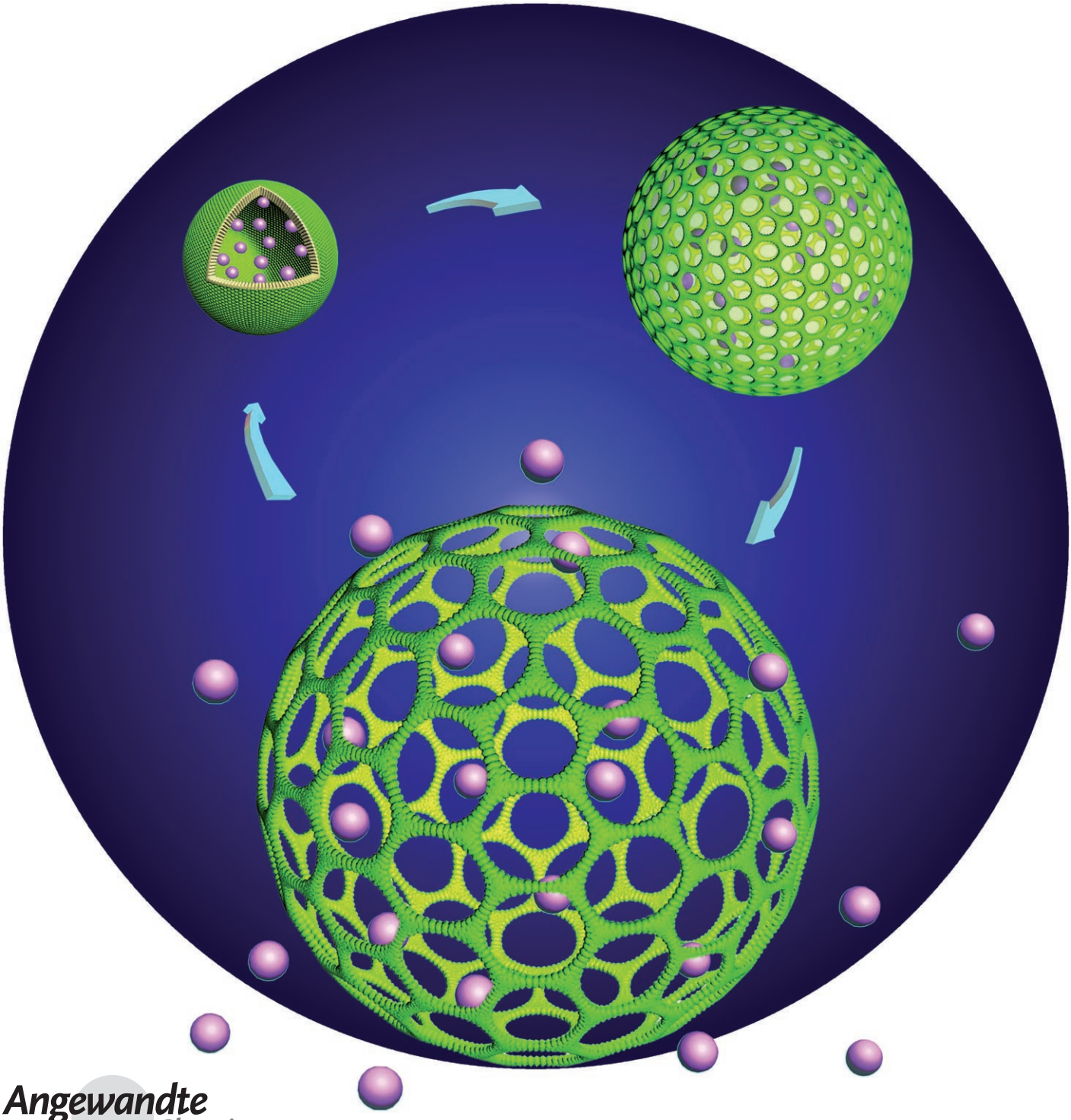

Self-organization of lipids into plasma membranes and various internal organelle membranes provides one of the most significant structural features in living systems.[1] These membranes prevent molecules generated inside the cell from leaking out and unwanted molecules from diffusing in. Inspired by this organization in nature, self-assembly of synthetic molecules into hollow spheres has received considerable attention owing to their encapsulation capability of guest molecules into their internal cavity, which is essential for delivery vehicles and nanoreactors.[2,3] Artificial systems exhibiting hollow structures are formed from self-assembly of a wide variety of synthetic scaffolds, including block copolymers consisting of hydrophilic and hydrophobic segments.[3,4]

Another important issue regarding the development of the hollow structures for specific applications is their ability to control the containment and release of the encapsulated species under the desired conditions without disruption of their original shape.[5,6] A typical example for hollow structures with these functions is provided by a natural self-assembling system, the capsid in cowpea chlorotic mottle virus (CCMV) consisting of identical protein subunits that self-assemble into a protein coat for transporting viral genome controlled by a gating mechanism.[7] However, self-assembled synthetic capsule structures with gated openings remain to be explored. In fact, with few exceptions,[8] the self-assembly of synthetic molecules including block copolymers,[3,4] surfactants,[5,9] and synthetic peptides[10] has only yielded closed vesicles.

Herein we present the spontaneous formation of a capsule structure with lateral nanopores, which is based on the two-dimensional self-assembly of dumbbell-shaped rod amphiphiles. In particular, the porous capsules undergo a structural transformation in which the nanopores in the shell are reversibly closed upon heating. The dumbbell-shaped rod amphiphiles that form these aggregates consist of a conjugated rod segment that is grafted by hydrophilic polyether dendrons at one end and hydrophobic branches at the other end (Figure 1a). The rod amphiphiles were prepared with the procedures described elsewhere.[11] Stiff rod segments, which are different from conformationally flexible chains, have a strong tendency to be packed with a parallel arrangement to form a locally two-dimensional structure in bulk solution.[12]

Aggregation behavior of the molecules was subsequently studied in aqueous solution, a selective solvent for polyether dendrons. Fluorescence microscopy investigations revealed that molecules of **1** self-assemble into sheet-like flat objects with a scale of as large as several micrometers (Figure 1b). The formation of the sheet-like objects in an aqueous solution

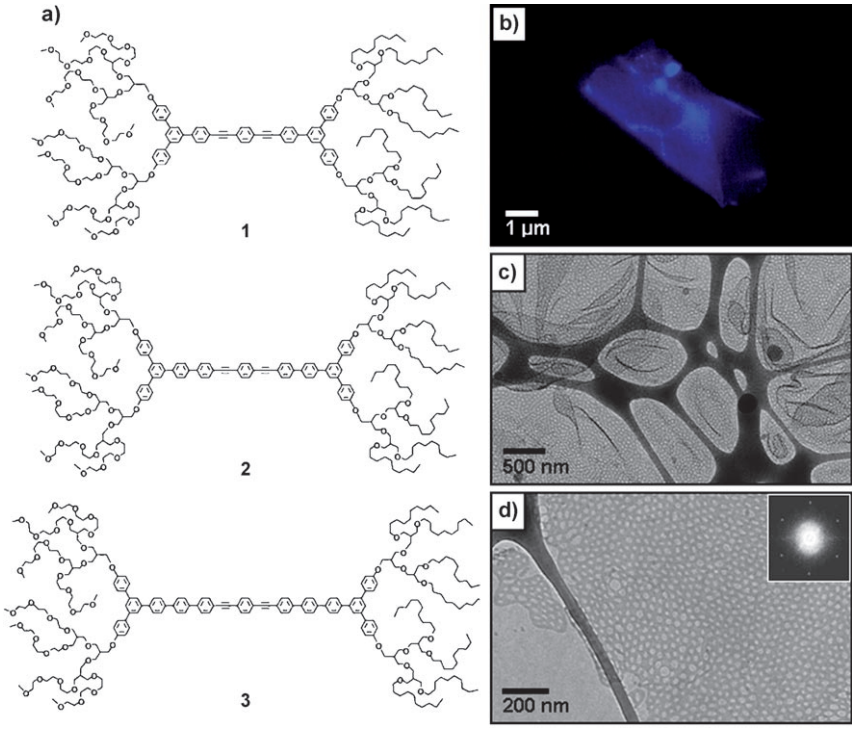

Figure 1. a) Molecular structures of asymmetric dumbbell-shaped rod amphiphiles **1**, **2**, and **3**. b) Fluorescence micrograph and c) a cryo-TEM image of planar network formed from aqueous solution of **1**. d) The high-magnification cryo-TEM image of **1**. Inset: A Fourier diffractogram of image revealing the two-dimensional hexagonal symmetry.

of **1** was further confirmed by transmission electron microscopy (TEM). Figure 1c. A cryogenic TEM (cryo-TEM) micrograph obtained from the 0.01 wt% aqueous solution of **1** shows a single layer of wrinkled flat networks against the vitrified solution background. The image at higher magnification shows that the two-dimensional networks consist of interconnected cylindrical components with a uniform cross-section of 15 nm and in-plane, roughly hexagonal packing of pores with a diameter of circa 25 nm (Figure 1d), which are more ordered than the pores observed in a nonconjugated rod system.[11]

The formation of a sheet-like structure led us to investigate whether an increase in both hydrophobicity and rigidity of a rod segment through an increase in rod length enforces

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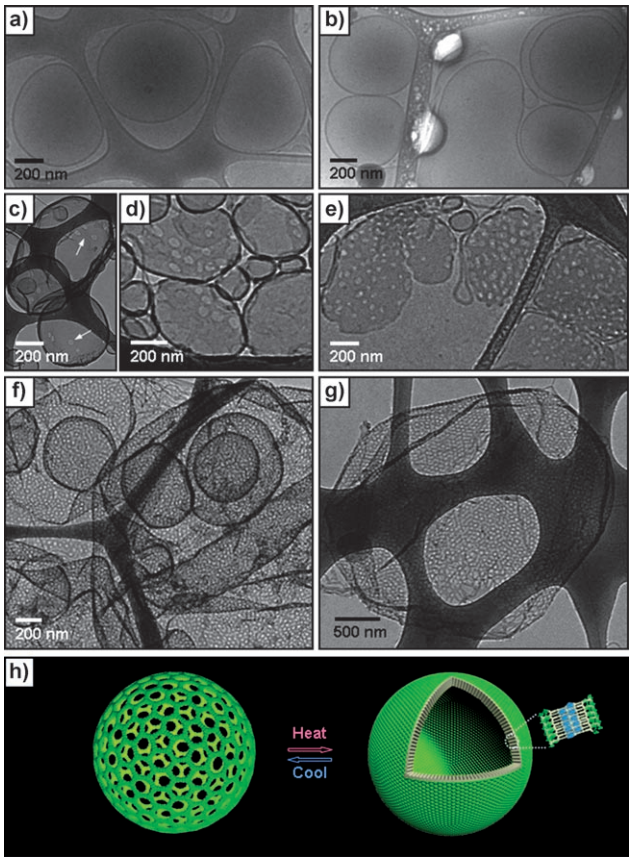

Figure 3. a) A cryo-TEM image of **3** revealing closed capsule structures in aqueous solution. b) A cryo-TEM image of **2** after heating to 65 °C. c) A cryo-TEM image obtained after 12 hrs of annealing at room temperature. Arrows indicate the small openings of the shell. d,e) After further annealing, the number of the pores gradually increases. f,g) Completely recovered into the original nanoporous capsules. h) Schematic representation of a reversible open/closed gating motion in the lateral nanopores of capsule **2** (green, polyether dendrons; yellow, aromatic segments; blue, hydrophobic branches).

the system toward closed spheres to reduce the surface edges exposed to the water molecules.[13] With this in mind, we have prepared dumbbell-shaped rod amphiphile **2** based on a longer conjugated rod block. In significant contrast to **1**, the fluorescence microscopy image of **2** (0.01 wt% aqueous solution) shows spherical aggregates as large as several micrometers in diameter (Figure 2a). The formation of the spherical aggregates was also confirmed by cryo-TEM. As shown in Figure 2b,c, the cryo-TEM micrographs show spherical objects with a porous shell together with a few emanating cylindrical micelles. The shell thickness of about 16 nm indicates that the rods are arranged in a bilayer packing in which the hydrophobic alkyl chains are intercalated between the rod segments. The pores in the shell have a narrow size distribution with a typical diameter of circa 25 nm, which is similar to those of the two-dimensional networks from **1**. Closer examination reveals that the lateral pores located at the central part of the spheres show to be circular in shape. Moving toward the edges of the objects, however, the shape of the pores gradually changes to be ellipsoidal. In addition, the frameworks on the opposite side can be discernable through the front pores. These results clearly demonstrate that the spherical objects are hollow in nature with porous shells, which was further confirmed by confocal microscopy (see the Supporting Information, Figure S3).

When the sample was cast from the 0.01 wt% aqueous solution and thereafter negatively stained with uranyl acetate, the images show highly deformed porous capsules that are similar to deflated soccer-ball frameworks in shape (Figure 2d). The combination of microscopy images leads to the conclusion that **2** self-assembles into a capsule structure ranging from several hundreds to a few micrometers in diameter, with a typical pore size of about 25 nm. The self-closure of the planar networks into hollow spheres with an increase in the length of the rod building block could be partly attributed to the strong association within the micellar core, and the strengthened intermolecular interactions.

A further increase in the rod length could be envisioned to direct the porous system to form closed capsules. The cryo-TEM image of **3** (0.01 wt% aqueous solution) shows hollow spherical objects with diameters ranging from several hundred nanometers to a few micrometers and a uniform bilayer thickness of circa 18 nm (Figure 3a). Consequently, the results described herein demonstrate that the porous capsules exist as an intermediate phase between planar networks and closed capsules.

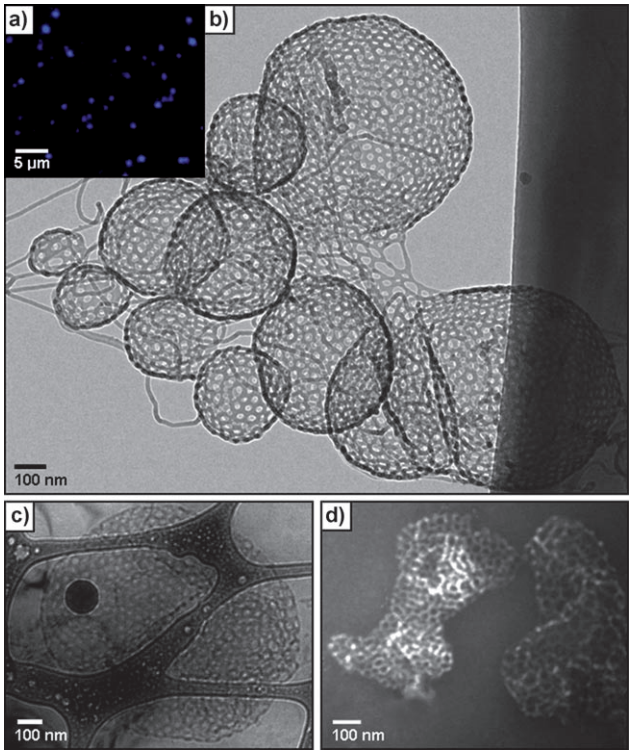

Figure 2. Hollow spheres with a lateral nanoporous shell formed by self-assembly of **2** in aqueous solution (0.01 wt%). a) Fluorescence micrograph, and b,c) cryo-TEM images of an aqueous solution of **2**. d) A negatively stained TEM image of **2**.

Remarkably, the solution of **2** exhibits a thermoreversible phase transition at 60 °C (see the Supporting Information, Figure S8). Upon heating to 65 °C, cryo-TEM of the solution revealed a hollow spherical structure with diameters ranging from several hundreds nanometers to a few micrometers with a layer thickness of about 16 nm (Figure 3 b). However, the image showed that the lateral pores in the shell are completely closed, demonstrating that the porous capsules transform spontaneously into closed ones upon heating without any noticeable changes in spherical shape. After 12 hours of annealing at room temperature, the shells started to form small openings (Figure 3 c). With further increases of annealing time, the number of the openings gradually increases and the pore sizes become more uniform (Figure 3 d,e). Complete recovery to the original porous capsules were observed over a period of approximately 7 days resting at room temperature (Figure 3 f,g), indicating that the structural transformation between open and closed states is accompanied with considerable hysteresis. This hysteretic behavior in open/closed motion of the pores seems to arise from the kinetic effect related to slow break-up of strong π–π stacking interactions between the rod segments when opening the pores. These results demonstrate that the lateral pores in the hollow sphere are reversibly gated in response to temperature without affecting the overall spherical shape (Figure 3 h).

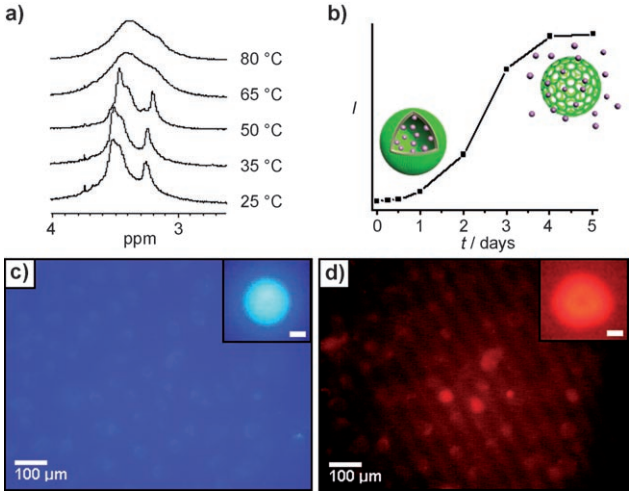

Figure 4. a) ^{1}H NMR spectra of ethylene oxide regions of **2**. b) Time course of calcein release from closed capsules of **2**. Fluorescence microscopy images of HeLa cells c) after intracellular delivery of the capsules and d) rhodamine-labeled DNA oligomer. Images of the capsules after DNA encapsulation showing that the blue fluorescence from the capsules (inset in c) overlaps with the red fluorescence from the DNA (inset in d). Scale bars in the insets: 4 µm.

This gating behavior of the pores can be explained by the fact that the oligo(ethylene oxide) dendritic exterior exhibit a lower critical solution temperature (LCST) behavior in aqueous media.[14] Above the LCST, the ethylene oxide segments are dehydrated to collapse into molecular globules, which leads to a decrease in the effective hydrophilic volume as the hydrodynamic volume of the polyether dendrons decreases. As a result, the porous structure with a highly curved local interface transforms into a closed structure with flat interface to reduce interfacial energy associated with unfavorable segmental contacts. This dehydration was confirmed by ^{1}H NMR experiments. Upon heating above the LCST, the resonances associated with the ethylene oxide chains are noticeably broadened together with a decrease in intensity (Figure 4 a), demonstrating the loss of hydrogen bonding interactions between ether oxygen atoms and water molecules.[15]

A reversible open/closed gating motion of the pores in the shell, triggered by external stimuli, suggests that the porous capsules may selectively encapsulate guest molecules and then release them in a controlled manner. To substantiate thermoresponsive gating behavior of the lateral pores, encapsulation experiments were performed with hydrophilic guest molecules. Calcein (100 mM), as a guest, was added to the pre-equilibrated solution of **2** at room temperature where the capsule exists in its open form. The resulting solution was then heated to 65 °C, at which temperature the pores in the shell close. Release of encapsulated calcein was accompanied by an increase in fluorescence emission as the free calcein in solution was dequenched.[16] As shown in Figure 4 b, essentially no leakage of the entrapped calcein was observed over a period of 12 hours at room temperature. After 12 hours, however, the entrapped calcein was released gradually over a period of 5 days, indicating that, when cooled down to room temperature and then annealed, the capsules undergo a transition from closed to open states, releasing the entrapped guests from the internal cavity.

To address the potential utility of the porous capsules as a virus-like delivery vehicle, intracellular delivery experiments were performed in mammalian cells. Toward this direction, fluorescently labeled DNA oligomer (TAMRA-DNA) as a cargo was encapsulated within **2** as described above. The intracellular fluorescence distribution after the treatment of DNA-encapsulated capsules showed that nearly all of the cells were stained, showing blue (the capsules) and red fluorescence (TAMRA-DNA) simultaneously (Figure 4 c,d). This result indicates that the capsules can encapsulate the relatively large DNA molecules (molecular weight ca. 6700 Da), deliver the encapsulated cargo into the inside of the cell.

The notable feature of the dumbbell-shaped rod amphiphiles investigated herein is their ability to self-assemble into a capsule structure with gated nanopores in the shell. These lateral nanopores undergo a transition from the open state to the closed state upon heating, which is capable of blocking cargo transport. Accordingly, the responsive nanopores endow the spherical objects with a reversible encapsulation capability of cargos under a controlled manner, with preservation of their hollow spherical structure. Considering that the capsules with reversibly gated lateral pores are internalized in their closed form to deliver the entrapped cargos into the inside of cells, our capsules can be considered as synthetic analogues to viral capsids.[7] Such a fascinating function of the pores may provide a new strategy for the design of synthetic systems with virus-like functions. Furthermore, the capability of the capsules to encapsulate large molecules suggests that

such a system has the potential to be developed as the gated plasma membrane of an artificial cell.

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