Vesicles

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Self-Assembled Vesicles from an Amphiphilic *ortho*-Phenylene Ethynylene Macrocycle**

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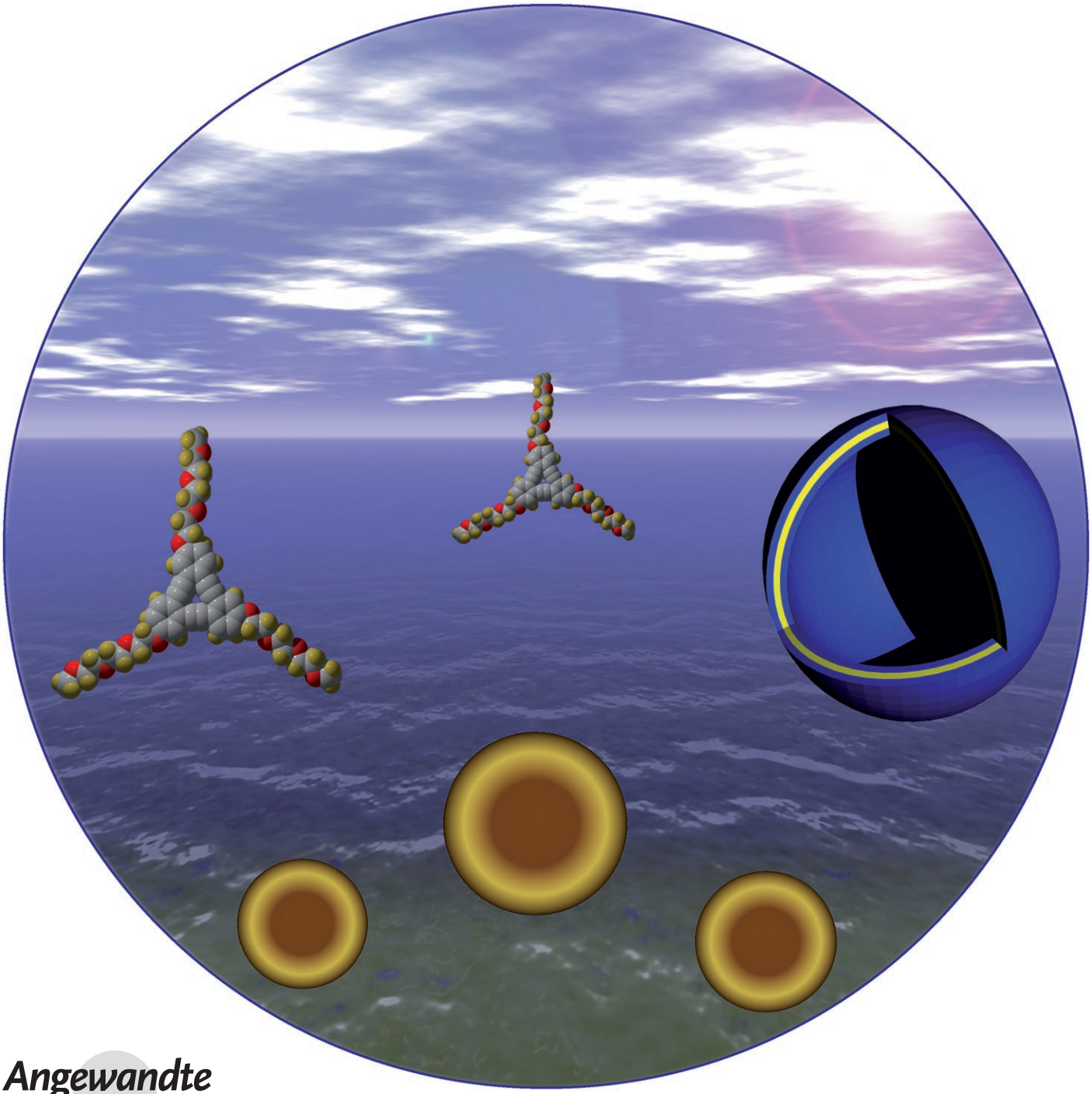
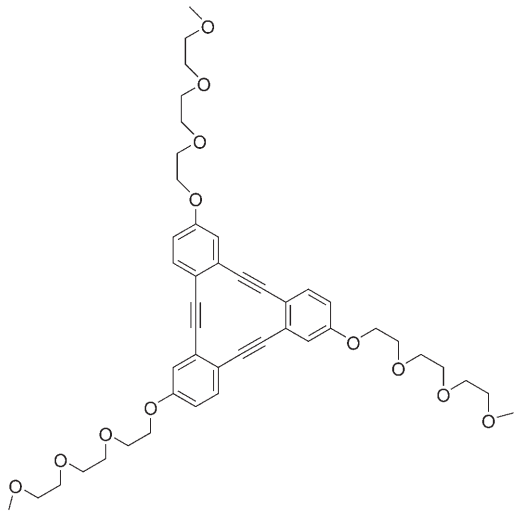


Amphiphilic molecules, namely those that carry both hydrophilic and hydrophobic parts within one structure, self-assemble into an amazing variety of structures:[1] for example, micellar structures (usually globular), ellipsoids, disks, cylinders, vesicles, and lamellae.[2] This assembly usually depends strongly on the molecular architecture, concentration, and solvent environment. Of these geometric forms, vesicles (enclosed spherical bilayer assemblies) have attracted much attention from a fundamental perspective as well as for their potential applications in drug or gene delivery, nanotechnology, and as model systems of biomembranes.[3,4a] Block copolymers that assemble into vesicles have attracted considerable attention.[4] In contrast, other than classical head-group surfactants and phospholipids, relatively few small synthetic molecules are known that spontaneously self-assemble into vesicles. Recent exceptions include facially amphiphilic segmented dendrimers as well as functionalized calixarenes, cyclodexdrins, and cucurbit[6]urils.[5] The calixarenes, cyclodexdrins, and cucurbit[6]urils all have built-in curvature that promotes vesicle formation. Growing the number of molecular architectures that spontaneously form vesicular structures remains an important goal that will increase understanding and widen applications. Herein we report vesicle formation from a new molecular architecture, an amphiphilic, discotic *ortho*-phenylene ethynylene (*o*-PE) macrocycle.

Phenylene ethynylene and other shape-persistent cyclic structures remain of great interest in the fields of supramolecular chemistry and materials science because of their unique properties.[6] Recently, we reported the synthesis and columnar hexagonal liquid-crystalline properties of this novel triethylene glycol (TEG) substituted triangular-shaped macrocycle.[7] As well as these bulk properties, the rigid, hydrophobic core and polar, flexible side chains suggested that this molecule might display interesting self-assembly behavior in aqueous solutions. This amphiphilic macrocycle self-assembles into vesicles with average diameter of approximately 500 nm from solutions in chloroform/H_2O (1:1).[8] The vesicular morphologies of this amphiphilic macrocycle were studied by atomic force microscopy (AFM) and transmission electron microscopy (TEM). The assembled vesicles are robust enough to be dried onto a carbon surface and examined by AFM without collapse. The vesicles collapsed following exposure to the high vacuum of TEM, as confirmed by AFM, but otherwise the morphology remains unchanged. To our knowledge, this is the first example of a vesicle formed from an amphiphilic discotic macrocycle.



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The morphological properties of these self-assembled vesicles were initially examined by AFM. Height and phase AFM images of the structures obtained from aqueous solutions of macrocycle **1** at two different magnifications and from two different samples are shown in Figure 1 a–d. These AFM images show spherical aggregates with diameters in the range of 200 nm to 1 μm. AFM cross-sectional analysis (Figure 1 f) of a typical structure shows that the diameters of the vesicles (650 nm) are generally seven- or eight-times larger than the heights of the vesicles (83 nm). This observation is consistent with deformation of a spherical vesicle following adsorption onto the carbon surface of the copper grid and indicates that these structures are robust yet soft enough to deform upon drying.

A histogram of 270 structures collected from several samples gives an average diameter for the vesicles of 493 nm (Figure 2). Light scattering measurements confirm the presence of vesicles in solution and provide an average diameter of 516 nm, which is in excellent agreement with the AFM results. TEM images (Figure 3) reveal structures with diameters of around 500 nm as well as a few larger structures (for example, only 2 of 57 structures in Figure 3 e, or 3.5%, are larger than 1 μm).

Figure 3 shows a series of six TEM images (some stained with RuO_4 for different time periods) and confirms that the structures previously observed by AFM are vesicles. The sizes and diameters of these spherical vesicles observed by TEM are similar to those visualized by AFM. Samples observed without staining (Figure 3 a,b) revealed circular forms with dark outlines, which is consistent with vesicle formation. Images of stained samples (Figure 3 c–f) confirm the presence

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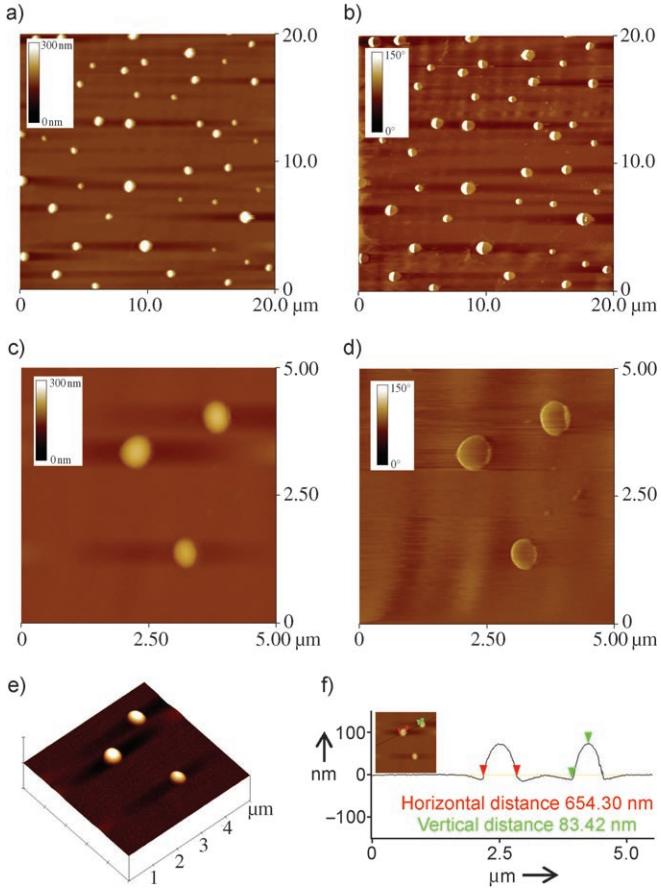

Figure 1. Tapping-mode AFM images of vesicles formed by **1** on a carbon-coated copper grid: a,c) height images; b,d) phase images; e) 3D image; and f) cross-sectional analysis.

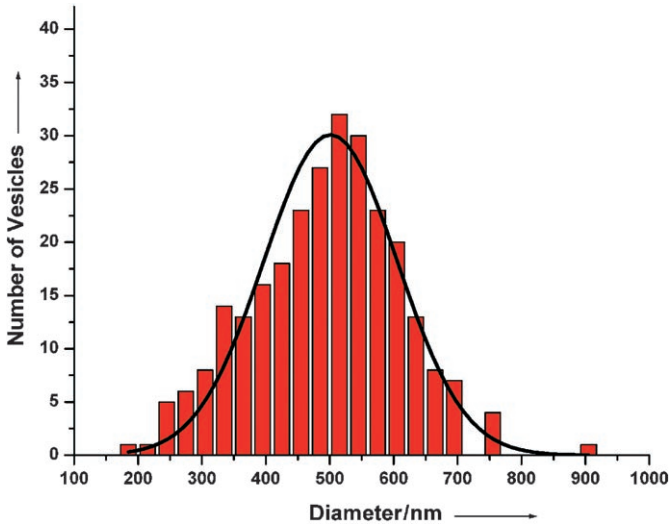

Figure 2. Diameter histogram obtained from 270 vesicles observed by AFM (the maximum of the fitted distribution is at 493 nm).

of spherical assemblies with a dark outer ring expected from the 2D projection of vesicular structures typically observed by TEM.[9] Figure 3d contains one large (ca. 1 µm) vesicle; the image was enlarged approximately 100 times (not shown) so

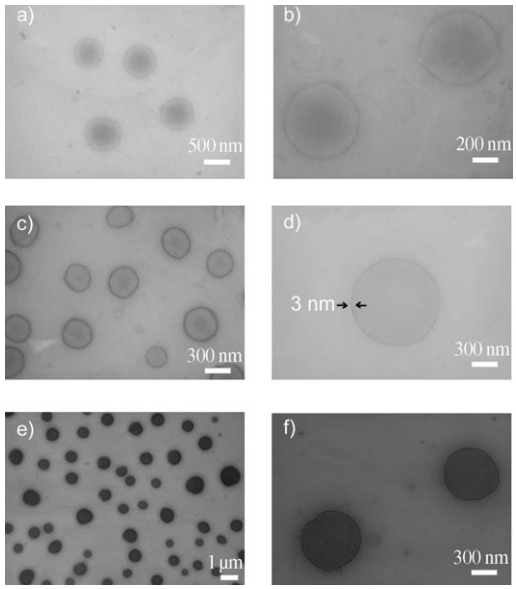

Figure 3. TEM images of vesicles of **1**: a,b) without staining; c,d) stained for 15 minutes; and e,f) stained for 45 minutes [with RuO_4 (0.05 wt% solution)]. d) The bilayer thickness is 3 nm.

that the shell thickness could be measured. The width of the dark line around the exterior is approximately 3 nm. This image provides the clearest measurement of shell thickness of all TEM images collected. Similar shell thicknesses are observed in Figure 3a,b but the shells are lighter because of the lack of staining whereas heavier staining (Figure 3e,f) increases the thickness of the dark line as staining penetrates the collapsed structure. Therefore, this measurement of approximately 3 nm collected from Figure 3d is the most accurate and represents an upper limit of shell dimensions. It is an upper limit because the TEM is a 2D projection of a 3D object in which the maximum contrast corresponds to the thickest part of the structure. As the sample is stained for longer, the contrast is lost as the entire object becomes darker. In view of the wall thickness of approximately 3 nm and the molecular dimensions of macrocycle **1**, we postulate that the macrocycle packs into a bilayer structure with the phenylene ethynylene core toward the interior of the bilayer and the more polar TEG side chains exposed to solvent. This is illustrated schematically in Figure 4 and supported by the TEM, AFM, and light-scattering data.

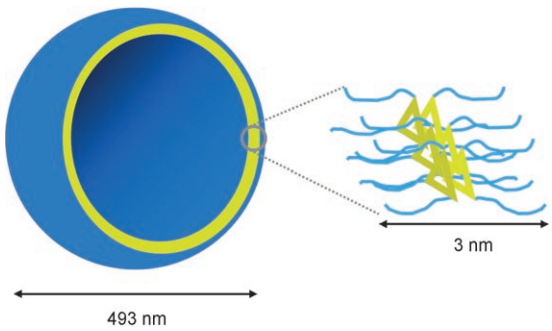

Figure 4. A schematic illustration of the bilayered unilamellar vesicle characterized by AFM and TEM images.

To collect further evidence for the hollow spherical vesicle, the same samples used previously for AFM and TEM were reinvestigated by AFM. Unlike AFM images obtained before TEM, the 2D and 3D AFM images in Figure 5 show spherical aggregates with collapsed centers.

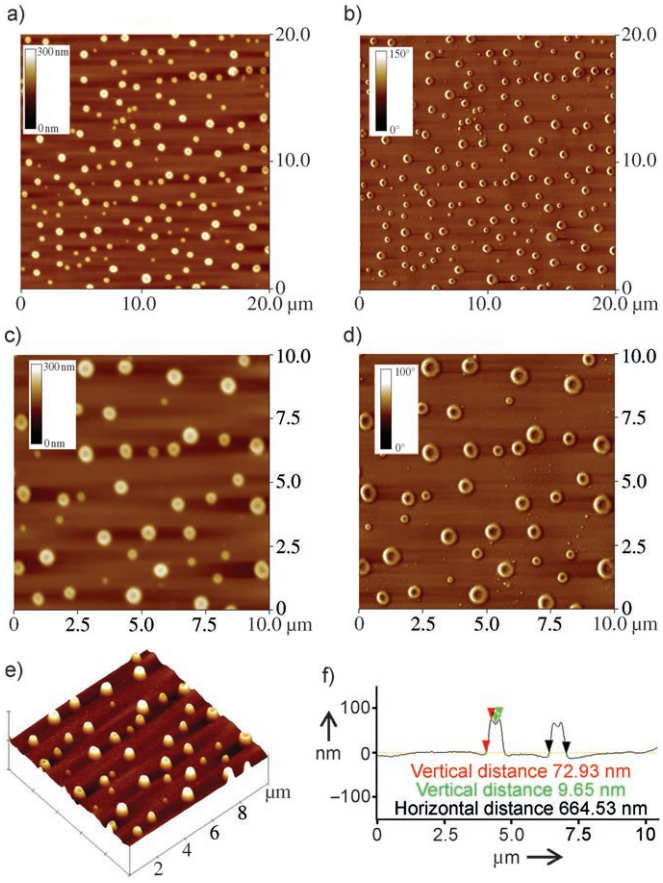

Figure 5. Tapping-mode AFM images of vesicles after TEM measurement: a,c) height images; b,d) phase images; e) 3D image; and f) cross-sectional analysis. These images show that the vesicles have collapsed after exposure to high vacuum.

The diameters of the vesicles are the same before and after exposure to the high vacuum of TEM, which indicates that no significant morphological changes occurred other than the collapse. Figures 1 f and 5 f show the cross-sectional analyses of vesicles with similar sizes (about 650 nm) obtained before and after TEM analysis, respectively. The vertical distance, or height, of the vesicle in Figure 1 f is about 83 nm compared with 73 nm in Figure 5 f, whereas Figure 5 f shows a depressed center consistent with a collapsed vesicle following exposure to high vacuum.[9] AFM was able to capture fractured vesicles before exposure to high vacuum, thus providing additional evidence that the initial structures observed by AFM are vesicles.[9]

In summary, the novel ethylene oxide substituted triangular macrocycle based on *ortho*-phenylene ethynylene was shown to self-assemble from aqueous chloroform into vesicles. AFM and TEM were used to characterize these assemblies with a bilayer wall thickness of about 3 nm and average diameter of around 500 nm. This is the first demonstration of vesicle self-assembly from discotic liquid-crystalline molecules and suggests the design parameters for vesicle formation may be broader than expected.

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[8] Vesicles were prepared by two separate methods that yield identical structures: 1) A solution of **1** in chloroform (1×10^{-3} M) was dropped on a carbon-coated copper grid and an equal volume of deionized water was subsequently placed on top of the solution. The copper grid was removed and dried under a nitrogen stream at room temperature. 2) A solution of **1** in chloroform (1×10^{-3} M) was mixed with water to yield a 1:1 mixture in a small vial. After manual shaking, the organic solution was taken from the vial and cast onto a carbon-coated copper grid placed on filter paper and dried under a nitrogen stream.

[9] See the Supporting Information for more details.

