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Highly Stable Giant Supramolecular Vesicles Composed of 2D Hydrogen-Bonded Sheet Structures of Guanosine Derivatives**

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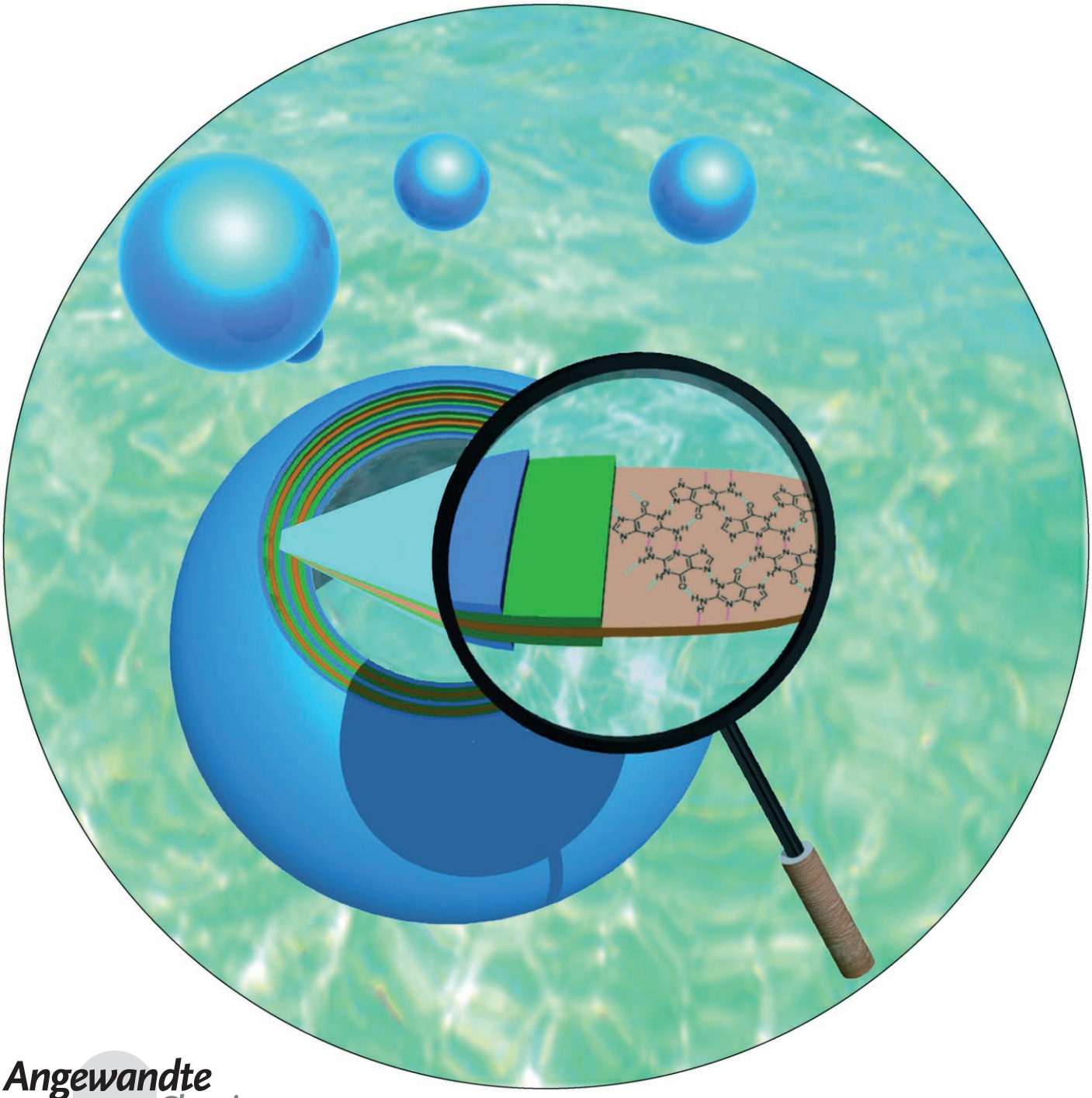

The rapid progress in nanoscale technology has resulted in the design and fabrication of nano- to micrometer-scale materials composed of highly organized structures attracting much attention.[1] Although covalently bonded polymers have been used predominantly as macroscale materials, attempts to control their chain arrangement and/or alignment has encountered considerable difficulty because of the robustness of the covalent bonds. In contrast, supramolecular materials which are assembled by relatively soft intermolecular interactions are suitable for this purpose. In aqueous media, lipids and amphiphiles self-assemble into nano- to mesoscopic-scale membranes and vesicles mainly through hydrophobic interactions.[2] Although these structures have dynamic properties, as a result of the reversible nature of the intermolecular interactions, the relatively poor stability of the structures, especially giant micrometer-sized vesicles, is a major drawback for practical applications.[3] Polymerized vesicles,[4] diblock copolymer systems,[5] and other types of vesicles[3a,6] have been studied extensively to circumvent this problem. Supramolecular vesicles prepared from specially designed nonlipidic molecules are being increasingly reported.[7] Although the use of relatively strong and directional hydrogen bonds seems to be a promising approach to this end, hydrogen-bonding interactions only operate effectively in nonpolar environments[8] and not in highly polar aqueous media.[9] Herein, we show that a rational molecular design to sandwich two-dimensional (2D) hydrogen-bonding networks between nonpolar protective layers and proper control of the polar surfaces of the resultant sheet structure allow for the fabrication of nano- and microcapsules with high stability, even in aqueous media.

We previously showed that alkylsilylated deoxyguanosine derivative **1a** can be fabricated into a flexible macroscale supramolecular film by a simple solvent-cast method (Figure 1).[10] In this macroscale film, 1D tapes of guanine molecules with N^1–H···N^7 and 2-NH_2···O=C^6 hydrogen bonds are further connected by two 2-NH_2···N^3 hydrogen bonds to form a 2D hydrogen-bonded layer. This hydrogen-bonded guanine layer is sandwiched between nonpolar and flexible alkylsilyl side-chain layers, thereby forming a 2D sheetlike structure. The oxyethylene groups located at the surface of the sheet structures enhance the intersheet interactions and results in the formation of the lamellar-like film structure. However, **1a** did not dissolve or disperse in water at all. To increase the hydrophilicity, **1b** and **1c**, which have extended oxyethylene units, were prepared. An attempt to prepare a solvent-cast film from their tetrahydrofuran (THF) solutions gave only a highly viscous liquid (**1b**) or a gumlike solid (**1c**); destruction of the 2D hydrogen-bonding networks was suggested from X-ray diffraction (XRD) analysis (see Figure S1 in the Supporting Information). Since a simple increase in the hydrophobic alkyl chain from propyl (**1b**) to hexyl (**1c**) was not effective, the size and rigidity of the alkylsilyl unit had to be re-designed to protect the hydrogen-bonding networks from the hydrophilic oxyethylene moieties.

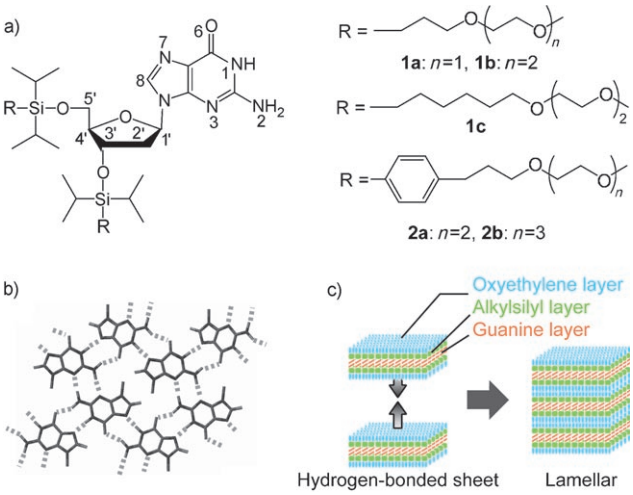

Figure 1. a) Structures of the alkylsilylated derivatives of guanosine, b) their 2D hydrogen-bonding pattern, and c) the film structure.

The newly designed guanosine derivatives, **2a** and **2b**, have a rigid phenyl unit within the alkyl chain of the extended oxyethylene units. Macroscale flexible supramolecular films of **2a** and **2b** were successfully obtained by casting their THF solutions (5 wt%) on to a teflon plate. The absence of a free NH stretching band of 2-NH_2 at $\tilde{\nu}=3495\ cm^{-1}$ in the IR spectrum of the film of **2a** (Figure 2a) suggested the formation of 2D hydrogen-bonded sheet structures (see Figure S2 in the Supporting Information).[11] The presence of a sharp diffraction peak at 3.80° (2.32 nm), which corresponds to the thickness of the sheet structure in the X-ray diffraction pattern (Figure 2b), confirmed the layered structure. Spreading the solution of **2a** or **2b** in THF gently on to water gave the same flexible films at the surface after diffusion of the THF, which suggests that the hydrogen-

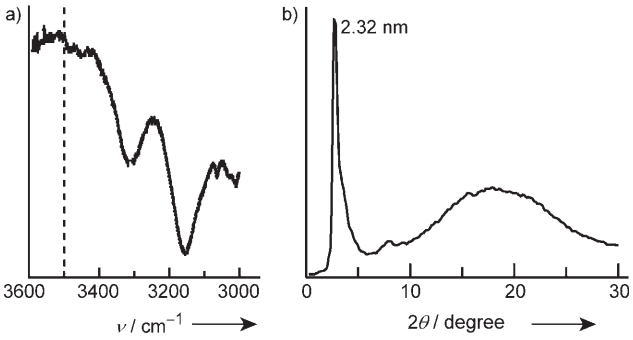

Figure 2. a) IR spectrum and b) X-ray diffraction pattern of a flexible supramolecular film of **2a**.

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bonding network sandwiched by the alkylsilyl-protecting layers was retained even after contact with water.

For the preparation of the vesicles, a small amount of the solution of **2a** or **2b** in THF was injected into deionized water and the solution was subjected to sonication for 5 minutes at 80 °C (see the Supporting Information). The resultant translucent aqueous solution was stable for more than a day without the formation of precipitates or oily droplets. Although slow sedimentation was noticed for the solution of the less hydrophilic **2a** after a prolonged period, the translucent aqueous solution of **2b** remained unchanged for more than a month. The differential interference contrast microscopy (DICM) image showed the formation of small particles, and the size distribution measured by dynamic light scattering (DLS) indicated the diameter of the particles to be mostly within 190 to 335 nm (Figure 3a and b). A transmission electron microscope (TEM) image (Figure 3c) of the particles showed vesicular structures with diameters of 150 nm.

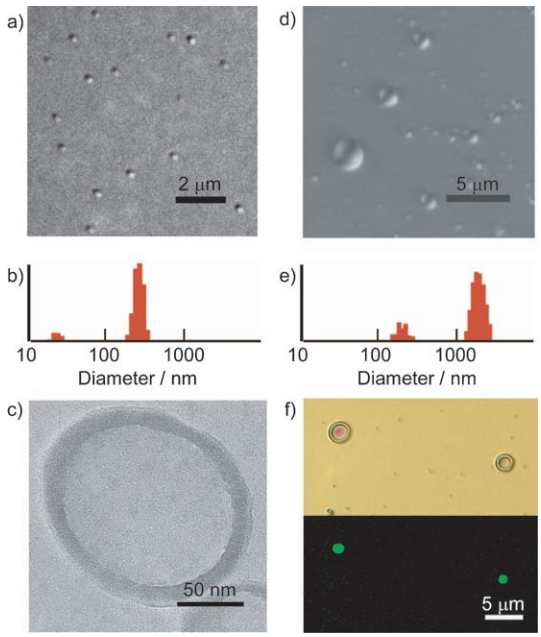

Figure 3. The vesicles of **2b** prepared by the injection method (a–c) and the thin-film method (d–f). a) DIC image, b) size distribution determined by DLS, and c) TEM image (stained with sodium phosphotungstate) of the vesicle prepared by the injection method. d) DIC image, e) size distribution determined by DLS, and f) microscopy (upper half) and fluorescence (lower half) images of the vesicles containing eosin Y ($\lambda_{abs} = 524$ nm, $\lambda_{fl} = 544$ nm) and prepared by the thin-film method.

Stable micrometer-sized giant vesicles were obtained by the thin-film method. After preparing a thin film from the solution of **2b** in benzene inside a glass tube and subsequent drying at 50 °C in vacuo for 3 h, water was added and the aqueous solution was subjected to sonication at 80 °C for 10 minutes. The DICM image of the resultant translucent solution clearly showed the presence of the micrometer-sized giant vesicles as the major fraction, and the DLS profile confirmed this observation (Figure 3d and e). Vesicle formation was further confirmed by fluorescence microscopy after the incorporation of a water-soluble fluorescent marker (eosin Y) inside the vesicle. The vesicle, after replacing the outer aqueous phase with fresh water, clearly showed an orange color and green fluorescence as a result of the trapped eosin Y inside the vesicle (Figure 3f).

The vesicle solution retained the same turbidity and gave the same DICM image when it was heated at 80 °C, after addition of methanol (16.7 v/v %), or after being kept under ambient conditions for more than a month. Centrifugation of the vesicle solution (10000 G) for 10 minutes caused sedimentation of the vesicles at the bottom of the tube, which could be re-dispersed in fresh water by gentle shaking. The re-dispersed vesicles were confirmed to be intact (see Figure S3 in the Supporting Information). These results demonstrated that the vesicles have sufficiently high stability in water to be processed or handled in a variety of ways.

To test the stability of the vesicles further, a drop of the vesicle solution on a silicone substrate was air-dried and observed by atomic force microscopy (AFM) in the AC-mode under ambient conditions. The AFM image showed ellipsoidal micrometer-sized vesicles (Figure 4a). The vesicles before and after being kept in vacuo (2×10^{-3} Pa) for 12 h on the substrate retained essentially the same shape. The results indicated that the internal water was preserved even under vacuum, thus demonstrating that the vesicle membrane has high stability and low water permeability. When the AC-mode unidirectional scan was carried out with an increased maximum force,[12] typically from 2.3 to 4.7 nN, deformation, elongation, or lateral relocation of the vesicle along the scanning direction was observed, but without rupture

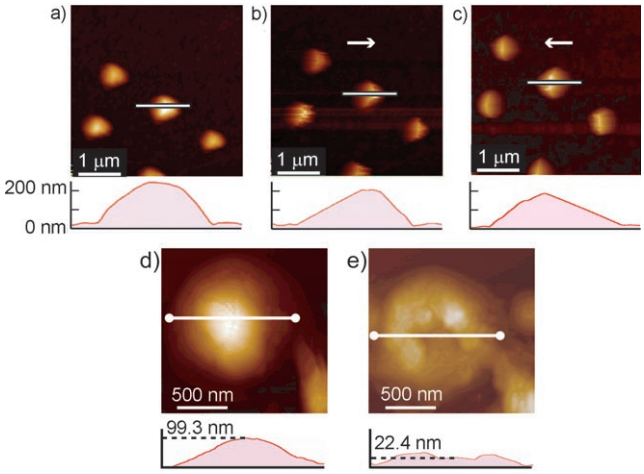

Figure 4. The AC-mode AFM images of the vesicles of **2b** prepared by the thin-film method. Height profiles along the lines within the images are shown under each image. a) Vesicles under ambient conditions observed with the default setting (maximum applied force: 2.3 nN). b) and c) Vesicles observed by unidirectional scanning with a maximum applied force of 4.7 nN. An arrow indicates the scanning direction of the tip. The image showed deformation of the vesicle in the scanning direction. d) The vesicle before rupture and e) 30 min after rupture during the repeated scan with an increased maximum applied force. The thickness of the membrane was estimated from the height of the inner flat part that was composed of the upper and lower membranes.

(Figure 4b), which suggests the vesicles have a high stability and flexibility.

Although it rarely happened, we encountered rupture of the vesicle membranes twice during the repeated AC-mode scan with a maximum force of approximately 6–17 nN (Figure 4b). From the heights of the inner flat parts of the residual solids after evaporation of the trapped water in these two cases, the thickness of the vesicular membranes was estimated to be approximately 11 and 15 nm, which correspond to 5 and 7 layers of the sheet assembly, respectively. A flat CaF_2 plate was pressed hard on to a second flat CaF_2 plate on which the vesicles were densely placed, and kept in vacuo overnight. The residual **2b** film on the CaF_2 plate showed essentially the same IR spectrum as that prepared from the THF solution (see Figure S2 in the Supporting Information), further confirming that the vesicle membrane was composed of the 2D hydrogen-bonded sheet structure of **2b**.

Thus, it has been shown that hydrogen-bond-directed micrometer-sized giant vesicles can be conveniently prepared, and these giant vesicles show sufficiently high stability in water. Furthermore, a cationic surfactant, hexadecyltrimethylammonium chloride (HTAC), induced an effective destruction of the vesicles. Upon adding a small amount of HTAC (5×10^{-4} mol dm^{-3}), the translucent vesicle dispersion immediately turned to a clear transparent solution, and no vesicular particles were observed in the DIC image. This dynamic response is the direct manifestation of the reversible nature of the intermolecular interactions and, therefore, is the major advantage of the self-assembled supramolecular materials.

It is well recognized that the 2D fibrous networks of cytoskeletal proteins play a critical role in maintaining membrane stability and integrity, and the high deformability and stability of cells under shear stress, as in circulating erythrocytes, are attributed to these networks.[13] In this current study, we have demonstrated by AFM that hydrogen-bond-directed micrometer-sized vesicles exhibit cell-like high stability, which is ascribed to the 2D hydrogen-bonding networks of the vesicular membrane. Thus, the strategy to protect the 2D hydrogen-bonded networks with nonpolar protective layers is an effective design principle for the fabrication of hydrogen-bond-directed supramolecular structures in highly polar aqueous media, further extending the scope of supramolecular materials.

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