Artificial Vesicles

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Nanometer-Sized Fluorous Fullerene Vesicles in Water and on Solid Surfaces**

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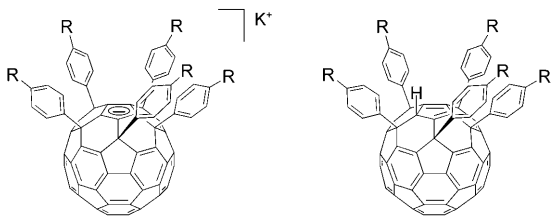
A lipid molecule has a polar head/nonpolar aliphatic tail structural motif, and, in water, forms a bilayer vesicle, in which the polar heads are exposed to the aqueous environment and the aliphatic tails cluster together to form the core of the bilayer membrane.[1] The lipid vesicle is mechanically labile because of the polymorphic behavior of the aliphatic chains. Although the polar head/nonpolar tail motif is universally accepted, the question may arise as to whether such a binary motif is mandatory for vesicle formation in aqueous media. We report herein that fluorous fullerene anion **1a**, which features a nonpolar/polar/nonpolar ternary motif (Scheme 1 and Figure 1 a), spontaneously forms vesicles with an average diameter of 36 nm in water that expose its nonpolar fluorous chains to the aqueous environment. These



1a: R=*n*-C_8F_{17}, **2a**: R=H **1b**: R=*n*-C_8F_{17}, **2b**: R=H

Scheme 1. Potassium salts of water-soluble fullerene anions **1a** and **2a** and their neutral precursors **1b** and **2b**.

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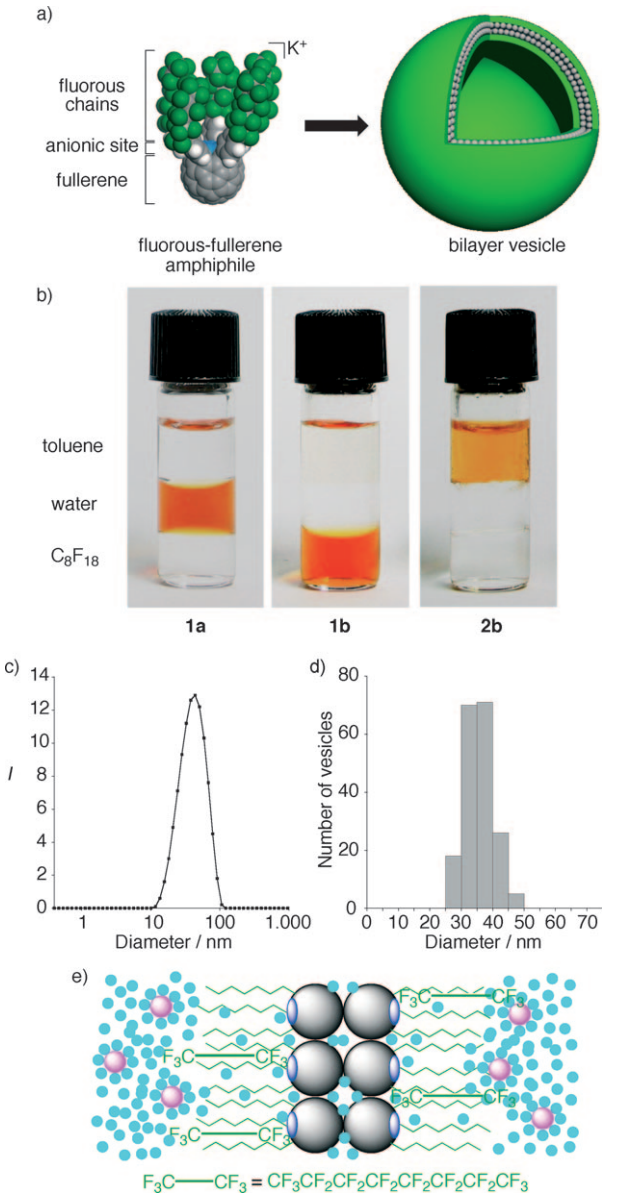

Figure 1. Bilayer vesicle from fluorous fullerene amphiphile **1a** in water. a) Drawing of **1a** and a model of its vesicle. F green, C gray, H white, cyclopentadienide moiety blue. b) Fluorous anion **1a** dissolves in water (middle phase). Neutral fluorous fullerene **1b** dissolves well in C_8F_{18} (bottom phase) and sparingly in toluene (top phase). Neutral phenyl fullerene **2b** dissolves in toluene. c, d) Size distributions of vesicles of **1a** as determined by DLS (c) and by SEM (d); the two methods agree well with each other. e) Schematic model of the bilayer showing a solvent-separated ion-pair structure and binding of C_8F_{18} molecules on the surface. Pentakis(*p*-perfluorooctyl)phenyl moiety in green on fullerene in gray, together with the potassium cation in pink separated from the cyclopentadienide anion on fullerene in blue and water in light blue.

vesicles tightly yet noncovalently bind fluorous molecules on their surfaces. Unlike lipid vesicles that easily loose their structural integrity when removed from aqueous solution,[2] the present vesicles are very robust and retain their spherical shape even on a solid substrate under high vacuum, and hence they look like nanometer-sized hollow Teflon balls. When the vesicle solution is coated and dried on a hydrophilic surface, it becomes water-insoluble and makes the surface as water-repelling as a Teflon surface. These properties suggest the utility of the vesicle surface as a scaffold for molecular display, the interior for molecular delivery, and the solution for macroscopic surface modification.

Perfluoroalkanes are not only hydrophobic but are also miscible only with fluorous compounds.[3] Similarly, fullerene hardly dissolves in water, but it dissolves in aromatic solvents and shows high cohesive power in the solid form.[4] Thus, pentakis[*p*-(perfluorooctyl)phenyl]fullerene **1b** is insoluble in water, sparingly soluble in toluene, and soluble in perfluorooctane (C_8F_{18}; Figure 1b). However, the anion of **1b** (**1a**)[5] is highly soluble in water (at concentrations of up to 10 $g\,L^{-1}$ at 25 °C) and forms a vesicle (see below), while it is insoluble in toluene and C_8F_{18}. Note, however, that the potassium salt **1a** is water-soluble, while the corresponding lithium and sodium complexes are not, thus suggesting that the potassium cation contributes to the solubility of **1a** in water.[6] The pentaphenylfullerene **2b**, a reference compound lacking the fluorous groups, dissolves in toluene but not in C_8F_{18} (Figure 1b), and its anion **2a** dissolves in water to form vesicles, as previously reported.[7] Having a dipolar structure, **2a** shows some resemblance to a lipid molecule, but **1a** is unique for its ternary structure and the rigidity of the fluorous chains.

The potassium salt of the fluorous anion **1a** dissolves in THF as a monomer (determined by dynamic light scattering (DLS), see the Supporting Information, Figure S1). When a THF solution of **1a** was injected into water (using a syringe), it afforded a homogeneous orange solution of spherical vesicles (see below) with an average radius of (18.1 ± 0.1) nm (Figure 1c). The size distribution of the perfluoroalkylfullerene vesicles (called fluorous vesicles hereafter) was narrow and unimodal. The size of these vesicles is comparable to that of the vesicles made of **2a** (called phenyl vesicles hereafter) and does not change much between 10 and 90 °C (see the Supporting Information, Figure S2) and after being stored for more than a year at room temperature.

A solution of the fluorous vesicles with a hydrodynamic radius (R_h) of 26.7 nm was analyzed by static light scattering (SLS) experiments[7a] to obtain the radius of gyration (R_g) of 26.7 nm. The R_g/R_h value is thus 1.00, indicating the hollow vesicular structure in water.[7a] The fluorous vesicle contains an average of 5.98×10^3 molecules of **1a**, calculated on the basis of the molecular weight of 1.94×10^7 Da determined by the SLS measurements. From these data, we calculate the area occupied by one fullerene molecule to be 1.39 nm^2 assuming a monolayer vesicle and 2.57 nm^2 assuming a bilayer one. Considering the minimum cross section of **1a** to be larger than 1.55 nm^2, which is a simple sum of the cross sections of five perfluorooctyl chains (0.31 nm^2),[8] we conclude that the fluorous vesicle can be regarded as a bilayer vesicle, and also that the molecules are aligned perpendicular to the membrane surface, keeping either the fullerene moiety[7] or the fluorous chains in the interior of the membrane.

The vesicle surface is negatively charged (zeta potential = (−33.4 ± 1.8) mV, pH 8.4; (−38.0 ± 0.4) mV, pH 8.4 for the phenyl vesicle), thus suggesting that **1a** forms a solvent-separated ion pair in water (Figure 1e). Upon acidification, the neutral compound **1b** precipitated. Neither ^1H, ^{13}C, nor ^{19}F NMR spectra of the aqueous vesicle solution showed any signals at 25 and 80 °C, as was found for the phenyl vesicles.[7b]

The fluorous vesicles of **1a** show unique properties not found for the phenyl vesicles. For example, the vesicle solution dissolves C_8F_{18}, which is completely insoluble in water[3] and does not dissolve fullerene, as opposed to the phenyl vesicle solution, which does not dissolve C_8F_{18} at all. When we stirred a mixture of C_8F_{18} and the fluorous vesicle solution (1.37 mM in D_2O) at 25 °C for 1 h, the ^{19}F NMR spectrum of the resulting aqueous layer showed one broad peak at $\delta = -86.2$ ppm arising from the terminal CF_3 group (signal 1, Figure 2a). At 80 °C, broad signals arising from the three CF_2 groups appeared (signals 2, 3, and 4, Figure 2b). The chemical shift values match those of neat C_8F_{18} (Figure 2c) but differ from those of C_8F_{18} in $CDCl_3$ (Figure 2d). On the basis of these data and the absence of ^{19}F signals arising from the vesicle itself (see above), we consider that the most of the rod-like C_8F_{18} molecule is noncovalently bound to the surface of the vesicles, while a CF_3 terminus still maintains some degree of freedom, as illustrated in Figure 1e. From the integrated area of the CF_3 signal, we calculated the concentration of C_8F_{18} in the vesicle solution to be approximately 1.9 $g\,L^{-1}$ (i.e., 3.2 molecules of C_8F_{18} per molecule of **1a**). The noncovalent binding of C_8F_{18} to the vesicle surface is so strong that we could not extract the C_8F_{18} molecule from the vesicle solution into chloroform. In addition to C_8F_{18}, the fluorous vesicles can solubilize the aromatic fluorocarbon C_6F_6 (5.9 molecules per molecule of **1a** on surface) more efficiently than phenyl vesicles (0.72 molecules per molecule of **1b** on

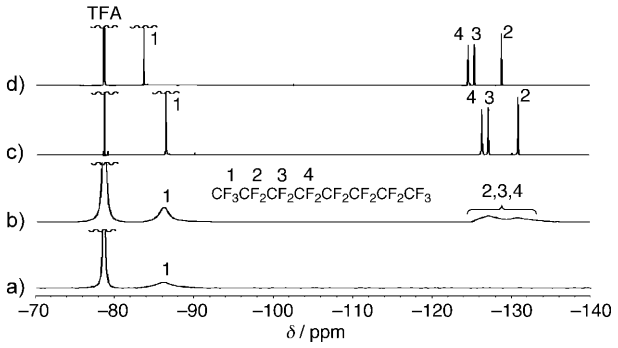

Figure 2. ^{19}F NMR spectra of C_8F_{18} under various conditions. a) C_8F_{18} in a D_2O solution of **1a** at 25 °C. b) The same sample at 80 °C, showing the structure of C_8F_{18} and atom labels. c) Neat C_8F_{18} at 25 °C. d) C_8F_{18} in $CDCl_3$ at 25 °C. Spectrum (a) recorded at 20 °C shows a broad signal of the terminal CF_3 group of C_8F_{18} at $\delta = -86$ ppm, and spectrum (b) recorded at 80 °C shows broad signals arising from the CF_2 moieties between $\delta = -120$ and −130 ppm. The signal of trifluoroacetic acid (TFA) in an internal capillary was used as a reference standard ($\delta = -78.5$ ppm).

surface). The large difference in the affinity of C_6F_6 to **1a** than to **1b** suggests that C_6F_6 solubilization is largely due to fluorophilic interactions rather than to anion–π interactions.[9]

Note that the addition of the perfluorocarbons had little effect on both the size of the vesicles of **1a** (from 36.4 to 36.0 nm upon addition of C_8F_{18}, and from 38.4 to 38.5 nm upon addition of C_6F_6) and the turbidity of the vesicle solution (no change of UV/Vis absorption).

An intriguing bulk property of the fluorous vesicle solution is its ability to form a hydrophobic coating on a solid substrate.[10] When we spin coated the vesicle solution in water ([**1a**] = 2.0 mM) on indium tin oxide (ITO) and dried it at room temperature, the hydrophilic surface of ITO became as water-repellent as a poly(tetrafluoroethylene) (PTFE, Teflon) surface. Thus, the water contact angle of ITO ((4.4 ± 0.4)°) after treatment with the fluorous vesicle solution became (111.7 ± 1.3)° (Figure 3a), which is comparable

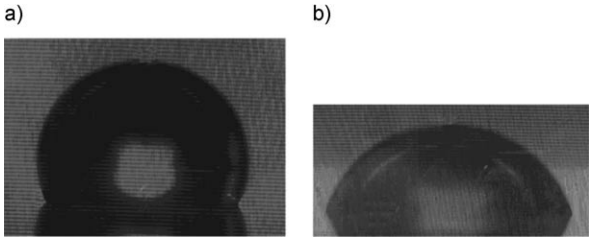

Figure 3. Contact-angle measurements of an ITO surface. a) ITO surface covered with the fluorous vesicle made of **1a** ((111.7 ± 1.3)°). b) ITO surface covered with the phenyl vesicle made of **2a** ((73.9 ± 0.6)°).

to that of Teflon ((108–114)°)[11] and is much larger than that of a fullerene-modified self-assembled monolayer ((100 ± 3)°)[12] and the phenyl-vesicle-covered ITO surface ((73.9 ± 0.6)°; Figure 3b). The water-repellent property was maintained even after repeated rinsing with water (i.e., the vesicles became insoluble in water after drying) and after storing in air at room temperature for several months. We subsequently became curious about the surface morphology that caused such a dramatic change of the surface properties.

We examined the vesicle-covered ITO surface by field-emission scanning electron microscopy (FE-SEM) at a low acceleration voltage of 0.1–1.5 kV, which allows observation without the metal coating of an insulating substrate (Figure 4), and made several notable observations. First, as shown in Figure 4a, the surface is only sparsely covered by the vesicles ((228 ± 14) vesicles μm^{-2} as determined for two independently prepared samples) in spite of the use of a rather concentrated (2 mM) spin-coating solution, and such thin coverage is enough to make the ITO surface as water-repelling as a Teflon surface. Second, the vesicles are uniformly dispersed on the substrate, which suggests that the vesicles repel each other. In the SEM image, 94% of the 545 vesicles were located without sticking to each other. This morphology stands in contrast to that of the far more sticky phenyl vesicles, which tend to form a flat mass on ITO (data not shown). These observations indicate that the vesicle

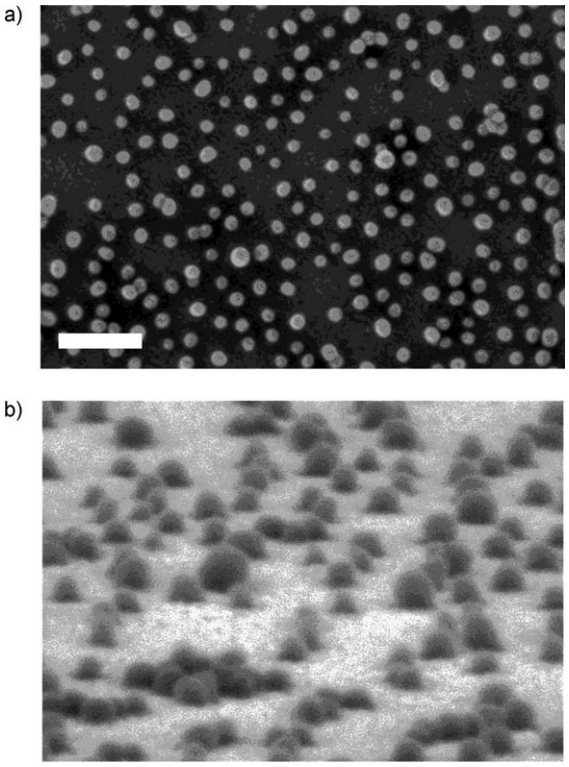

Figure 4. SEM images of the vesicle-covered ITO surface under approximately 10^{-5} Pa. a) The vesicle-**1a**-covered ITO surface. The scale bar represents 100 nm. The diameter of the vesicles (typically 20–40 nm) correlates well with the size determined by DLS (Figure 1c). The vesicles do not stick to each other—a behavior similar to that exhibited by Teflon balls. b) A different area of the same sample viewed with 80° tilting of the sample stage. The vesicles maintain their spherical shape even under vacuum on ITO.

surface is covered by the fluorous chains as illustrated in Figure 1a.

Finally, unlike lipid vesicles that lose their structural integrity under vacuum,[13] the vesicles are sufficiently robust to maintain their spherical shape on the solid surface, even at 10^{-5} Pa, as shown by the top and the 80°-tilted SEM images (Figure 4b). The very good agreement between the average radius of (17.8 ± 0.2) nm measured for 190 vesicles in the SEM image (Figure 1d) and the DLS radius ((18.1 nm ± 0.1) nm, Figure 1c) confirms the structural robustness of the vesicle shell.

We have described the distinctive properties of a unique amphiphile **1a** and a nanometer-size fluorous vesicle that forms in water. Having a nonpolar/polar/nonpolar ternary structure, the anion **1a** has little resemblance to lipid molecules (Figure 1a), yet it forms an aqueous solution of vesicles that exhibits unimodal size distribution. Several lines of experimental evidence suggest that the fullerene moiety of **1a** constitutes the core of the membrane and that the fluorous chains are exposed to the surface (Figure 1e). The presence of the fluorous chains does not hamper the vesicle formation in water; it might even increase the stability of the vesicles by clustering together on the vesicle surface. The potassium cation must be located in the aqueous phase to endow water-solubility to the vesicle, which, upon drying in vacuum on

ITO, may move into the membrane interior to maximize interaction with the anionic fullerene moiety. Such structural features offer a number of interesting future perspectives. For instance, the fluorous surface can serve as a nanoscopic scaffold for noncovalently anchoring fluorous organic molecules.[14] This property, coupled with the potent inclusion property[7b] and the surface-coating property of the vesicles, suggests applications to the targeted delivery of organic and inorganic materials in biological or man-made systems,[15] and to the nanoscale surface modification of a solid substrate.[16]

Experimental Section

Preparation of a fullerene bilayer vesicle solution: Potassium *tert*-butoxide in THF (0.98 M, 75.8 μL, 75 μmol) was added to a suspension of **1b** (50.0 μmol) in THF (3.93 mL), and the mixture was stirred under nitrogen. During the mixing, the suspension became a transparent dark orange solution. After 3 h, a portion of the solution of **1a** (12.5 mM, 1.60 mL, 20 μmol) was slowly injected into water (8.4 mL) using a syringe pump (ISIS Co.) over 1 min with stirring at 400 rpm to afford a vesicle solution of **1a** (2.0 mM) in 16% THF/water. THF and water were removed by evaporation at approximately 7 kPa, and the final concentration of **1a** was adjusted to 3.5 mM by addition of water. The radius of the vesicles always shows a unimodal distribution but varies in the range 10–40 nm (most often 10–20 nm) depending on the conditions of the preparation.

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