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Reverse Vesicles from a Salt-Free Catanionic Surfactant System: A Confocal Fluorescence Microscopy Study

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We give a detailed confocal fluorescence microscopy study on reverse vesicles from a salt-free catanionic surfactant system. When tetradecyltrimethylammonium laurate (TTAL) and lauric acid (LA) are mixed in cyclohexane at the presence of a small amount of water, stable reverse vesicular phases form spontaneously. The reverse vesicular phases can be easily labeled with dyes of varying molecular size and hydrophobicity while the dyes are nearly insoluble in cyclohexane without reverse vesicles. This indicates the reverse vesicular phases can be good candidates to host guest molecules. With the help of a fluorescence microscope combined a confocal method, the features of these interesting reverse supramolecular self-assemblies were revealed for the first time. Because of the absence of electrostatic repulsions and hydration forces between adjacent vesicles, the reverse vesicles have a strong propensity to aggregate with each other and form three-dimensional clusters. The size distributions of both individual reverse vesicles and clusters are polydisperse. Huge multilamellar reverse vesicles with closely stacked thick walls (giant reverse onions) were observed. Besides the spherical reverse vesicles and onions, other supramolecular structures such as tubes have also been detected and structural evolutions between different structures were noticed. These interesting supramolecular self-assemblies form in a nonpolar organic solvent may serve as ideal micro- or nanoreaction centers for biological reactions and synthesis of inorganic nanomaterials.

Introduction

Vesicles, which are curved molecular bilayers formed by amphiphilic molecules in water, have received increasing research interests since their discovery.[1,2] Consisting of both hydrophobic and hydrophilic microdomains, vesicles have been regarded as ideal candidates for drug delivery and mimicking biological membranes.[3−5] In recent years, novel vesicles have been developed from cationic/anionic (catanionic) surfactant mixtures.[6−16] Like micelles and lytropic liquid crystals which have conterparts in nonpolar environments, reverse vesicles were also found to exist as pioneered by Kunieda et al. in a nonionic surfactant system.[17] After that, more proofs were found by the research group of Kunieda,[18−29] our group,[30,31] and others.[32,33] With a nonpolar organic solvent being the continuous medium, amphiphilic molecules in reverse vesicles self-assemble in an opposite way compared to their counterparts in water. That is, within the molecular bilayer the hydrophobic parts of the molecules stay outside while their hydrophilic parts are hidden inside the bilayer (see Scheme 1). The study of reverse vesicles is of great fundamental interest since knowledge about molecular bilayers, which play an important role in living cells, can be obtained through a thoroughly new viewpoint. They also have many potential

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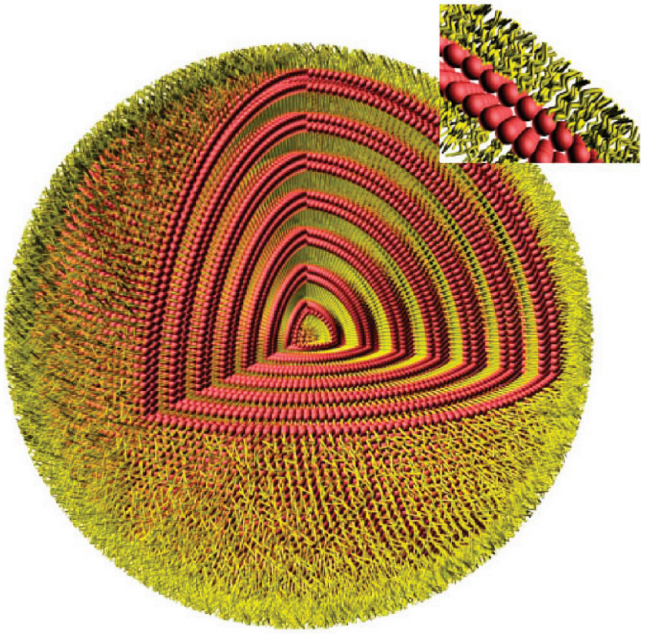
Scheme 1. A Proposal Model of Multilamellar Giant Reverse Onions[a]



[a] Seven bilyers comprise the multilamellar giant reserve onions. An enlargement part of reverse bilayers has been shown to enhance the visibility.

applications worth for further investigations. For example, the highly restricted two-dimensional water channels within the bilayers of reverse vesicles can trap guest molecules such as enzymes[33] and inorganic ions,[31] providing ideal micro- or nanoreaction centers for biological tests and synthesis of inorganic nanomaterials.

To fully realize the promising aspects mentioned above, a detailed understanding of the structural features of reverse vesicles is required. Compared to the abundant studies related to vesicles formed in water, however, investigations on reverse vesicles formed in nonpolar organic solvents are far from satisfactory and have not met such a requirement. This can be mainly ascribed to two challenges. The first one comes from structural characterizations. Although imaging studies based on freezing techniques such as freeze-fracture TEM (FF-TEM) and cryo-TEM are proved to be powerful to reveal structural features of vesicles formed in aqueous solutions, severe difficulties are encountered when utilizing them to reverse vesicular phases due to the high volatility and low freezing points of most organic solvents. Light scattering is also known as a promising technique to probe the supramolecular structures in aqueous solutions. However, the polydisperse, micrometer-sized reverse vesicles are lying out of the detection limits of most instruments, thus preventing the possibility of applying this technique on reverse vesicular systems as well. Until now, most of the structural features of reverse vesicles have been obtained mainly by optical microscopy studies utilizing either a polarized light or a fluorescent dye. By these methods the structural features of reverse vesicles can be only understood to a limited level. The second challenge is the stability of reverse vesicles. So far investigations on reverse vesicles have been mainly focused on nonionic surfactant systems,[17,18,20−29,32,33] where the reverse vesicles only have limited stability. After constructed by hand-shaking a two-phase sample (a bottom reverse lamellar phase plus an upper excess oil phase), thus-formed reverse vesicular phases will normally rebound to a two-phase sample within a few days. This greatly limits the possibility of detailed characterization of these interesting supramolecular self-assemblies. For deeper investigations on reverse vesicles, stable reverse vesicular phases are highly required.

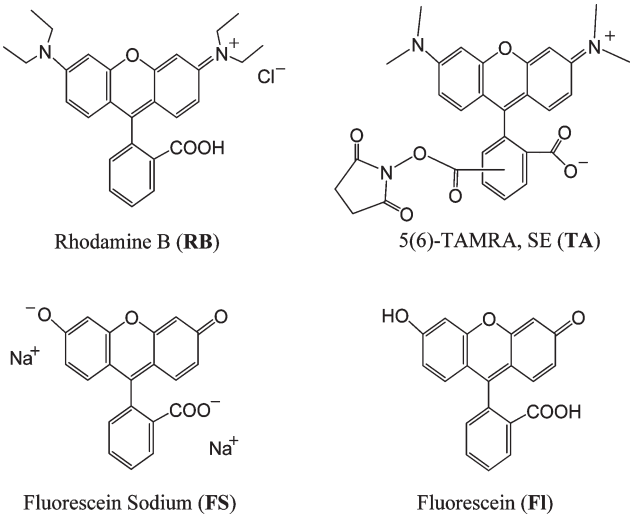


Figure 1. Molecular structures of the dyes used in this study.

Recently we have shown stable reverse vesicular phases can be constructed spontaneously by mixing a salt-free catanionic surfactant (tetradecyltrimethylammonium laurate, TTAL) and a fatty acid (lauric acid, LA) in nonpolar organic solvents like cyclohexane.[31] This opens the door for further and deeper inverstigations on these interesting supramolecular self-assemblies. In this work we give the first and detailed confocal fluorescence microscopy observations on these reverse vesicular structures. We hope this work will lead to a deeper understanding on reverse vesicles and can extend the possibility of their practical applications in biological tests and materials science.

Experimental Section

Chemicals and Materials. Tetradecyltrimethylammonium bromide (TTABr) was purchased from Merck and used without further purification. Lauric acid (LA) and cyclohexane were purchased from Fluka and used as received. Fluorescent dyes including rhodamine B (RB), 5- (and 6-)-carboxytetramethyl rhodamine, succinidyl ester (5(6)-TAMRA, SE, abbreviated to be TA hereafter), fluorescein sodium (FS), and fluorescein (Fl) were purchased from Sigma-Aldrich and used as received. Molecular structures of different dyes are shown in Figure 1. Tetradecyltrimethylammonium hydroxide (TTAOH) was prepared from TTABr by anion exchange following previous procedures.[15] The catanionic surfactant ion-pair TTAL was prepared by reacting equimolar TTAOH and LA in aqueous solution followed by removal of water.

Preparation of Reverse Vesicles. For fabrication of reverse vesicles, desired amounts of solid TTAL and LA were weighed to glass bottles, to which cyclohexane and a small amount of water were added. The weight fraction of TTAL in total solid ($W_{TTAL}/W_{TTAL+LA}$) is fixed at 0.88. The weight fraction of surfactant solids (TTAL plus LA) as respect to the whole sample (surfactant solids plus solvents) is calculated to be 0.02. The volume ratio of water to cyclohexane is fixed at 1:10.

Sample Preparation Method for Microscopy Observations. Samples for fluorescence microscopy observations and confocal fluorescence microscopy observations were prepared by dropping 6−8 μL reverse vesicular solution onto a bottom glass, followed by covering the solution with a cover glass (24 × 60 mm). We have also carried out fluorescence microscopy observations on sealed samples with a much higher sample

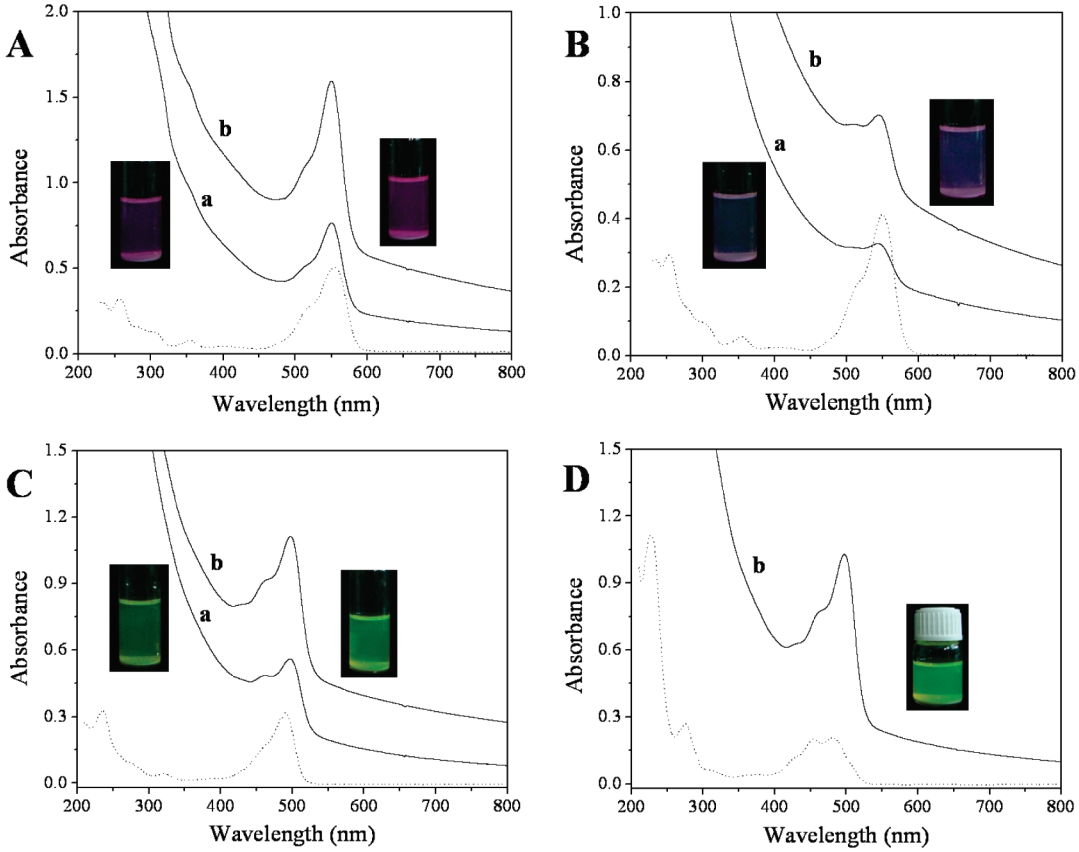


Figure 2. UV−vis absorptions of reverse vesicular phases labeled with 1×10^{-5} (curve a) and 2×10^{-5} mol L^{-1} (curve b) RB (A), TA (B), FS (C) and Fl (D). Absorptions of each dye recorded in water (A−C, 1×10^{-5} mol L^{-1}) or ethanol (D, 4×10^{-5} mol L^{-1}) are also given as dotted lines for comparison. Insets are photos of samples labeled with corresponding dyes.

thickness. The sample was prepared as follows: desired amount of reverse vesicular solution was dropped onto a bottom glass, on which two copper wires whose diameter is ~100 μm were placed as spacers. A cover glass with 20 × 20 mm in size was then placed on the spacers, and the edges of the sample were sealed immediately using epoxy resin glue.

Instruments and Characterizations. UV−vis spectroscopy was carried out using a MultiSpec-1501, Shimadzu (Kyoto, Japan) apparatus. For measurements of dyes in water or ethanol, pure water or ethanol was used for baseline corrections. For measurements of dyes solubilized in reverse vesicular phases, blank reverse vesicular phases without dyes were used for baseline corrections. Fluorescence microscopy observations were carried out on a Nikon Eclipse 50i microscope equipped with a black-in-white fluorescence camera. A 100× objective was used for observation. For samples labeled with RB and TA, a filter which can cut off excitation light below 510 nm and above 560 nm was used. For samples labeled with FS and Fl, a filter which can cut off excitation light below 465 nm and above 495 nm was selected.

Confocal fluorescence microscopy observations were carried out using a Nikon D-Eclipse C1 controller combined with a EC-C1 software. The instrument has also been connected to a Nikon eclipse TE 2000-U fluorescence microscope with an inverted lens. This allows us to preview the sample before confocal measurements. For samples labeled with RB and TA, a He/Ne laser with a wavelength of 543 nm was used. For samples labeled with FS and Fl, an argon laser with a wavelength of 488 nm was selected. For samples labeled with mixed dyes, both lasers were applied and the intensity of individual laser was adjusted in a way that the signals of red color and green color are comparable. The EC-C1 software allows us to obtain micrographs with both mixed color and individual color by opening or closing each color channel.

Results

Phase Behavior and UV−vis Absorptions of Dye-Labeled Reverse Vesicles. At the presence of a small amount of water, salt-free catanionic surfactant ion-pair TTAL and fatty acid LA can spontaneously form stable reverse vesicular phase in cyclohexane which shows a bluish color.[31] To facilitate the observations using a fluorescence microscope, the reverse vesicular phases are labeled with four dyes with different molecular structures (Figure 1). After labeled with dyes, the bluish reverse vesicular phase changed to be red (in case of RB and TA) or yellow (in case of FS and Fl), and UV−vis measurements showed corresponding absorption peaks in the visible region for the four dyes (Figure 2), indicating the labeling is successful. In control experiments, solubility of the four dyes in pure solvent of cyclohexane (i.e., without reverse vesicles) was also examined. It was found that the saturated solutions of all the four dyes are colorless. UV−vis measurements only gave a very weak peak for the saturated solution of RB (Figure S1). For saturated solutions of other three dyes, no peak could be detected. This means the presence of reverse vesicles can greatly enhance the solubilities of dyes with various molecular structures.

After the reverse vesicular phases are fabricated, the excess water will deposit at the bottom and form an oil-in-water emulsion.[30,31] For samples labeled with RB, FS, and Fl, the bottom emusions are white, indicating most of the dyes are in the upper reverse vesicular phases. For the sample labeled with TA, however, both the upper reverse vesicular phase and the bottom emulsion are reddish, indicating participation of TA occurred between the two phases. Since TA partially enters the bottom

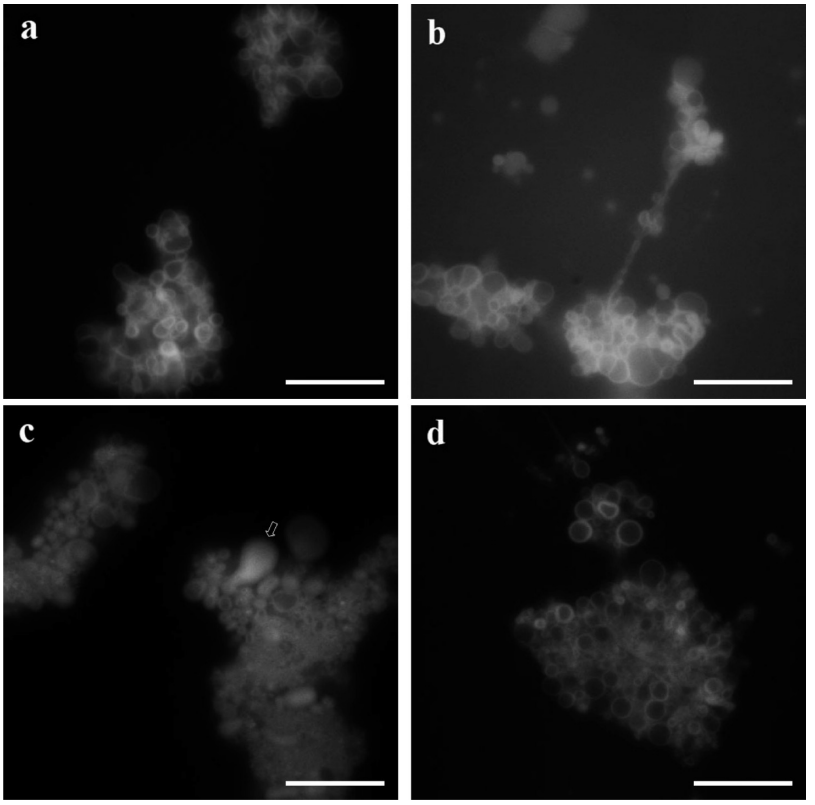


Figure 3. Typical fluorescence micrographs of reverse vesicles labeled with RB (a), TA (b), FS (c), and Fl (d). The scale bar corresponds to 20 μm.

emusion phase, the color of the upper reverse vesicular phase is weaker and the absorption peaks observed in UV−vis measurements are smaller compared to other dyes (Figure 2B).

From Figure 2 it can be seen that the molar absorption coefficients of dyes recorded in the reverse vesicular phases are much higher than those recorded in water or ethanol, and the peaks in the UV region could not be detected due to the too high absorptions. This may come from the presence of the large revese vesicles in the solution, which makes the sample slightly turbid. It has also been found that in the visible region the peak of RB recorded in reverse vesicular solutions is shifted by ~5 nm to the lower wavelengths compared to the peak recorded in water (Figure 2A). The same trend has been observed for TA (Figure 2 B). In the case of FS, however, the peak shift goes in the opposite way, i.e., a red shift by 6−7 nm compared to the peak in water (Figure 2C). For Fl, the shape of the absorption curve changes compared to that in ethanol (Figure 2D). This is probably caused by changes in the molecular structure of Fl. One possibility is the reaction between Fl and TTAOH dissociated from the catanionic surfactant ion-pair TTAL (see Supporting Information, Scheme S1). After reactions, Fl will be tranformed to the anion with TTA^+ as counterions. This is consistent with the observation that the absorption curve of Fl recorded in the reverse vesicular phase is quite similar to that of FS, which is also the anion of Fl with Na^+ as counterions.

Fluorescence Microscopy Observations. We first examined dye-labeled reverse vesicular phases under a fluorescence microscope. Despite the differences in molecular structures and amphiphilicity of the dyes, all the samples labeled with different dyes gave images with a satisfactory contrast. Four typical images are given in Figure 3. The reverse vesicles show a core−shell structure with bright shells, indicating the dyes are mainly distributed inside the shells. The size distribution of the reverse vesicles is broad, with diameters ranging from 2.5 to 7.5 μm. The wall thickness of the reverse vesicles is also polydisperse. In some cases, the walls are too thick to show the core−shell structure. Instead, the whole vesicle is bright under the microscope (Figure 3c, the empty arrow).

As revealed by fluorescence microscopy images shown in Figure 3, the reverse vesicles have a strong propensity to form clusters and the chance to detect individual vesicles is low. The clusters are also polydisperse and three clusters with increasing size are given in Figure 4. As can be seen in image c, the cluster can be so big that it even exceeds the screen window of the fluorescence microscope (over 80 μm). Observations were also carried out on sealed samples with a much higher sample thickness (~100 μm), and clusters of reverse vesicles were observed as well (Figure S2). This means the cluster formation has no obvious dependence on sample preparation method. Previously, we have shown the photographs of the reverse vesicles formed in cyclohexane under a polarized microscope.[31] In that case, the reverse vesicles appear more separated compared to current case. This difference, however, should be caused by the different microscopy techniques. Under a polarized microscope, mainly vesicles with very thick walls can be captured, and those with thin walls do not give enough signal. While the fluorescence microscope used in current study enables us to clearly detect the reverse vesicles with much thinner walls, it thus has more advantages to reveal the features of reverse vesicles.

Confocal Fluorescene Microscopy Observations. One limitation of fluorescence microscope is that measurements are carried out across a sample thickness of micrometers. Because of the interference of incident light and fluorescence within such a thick sample, important features of reverse vesicles may be lost. To remove this limitation, more observations were carried out by applying a confocal method. The high stability of the reverse vesicles allows such a measurement without any further sample treatment. Three typical micrographs of reverse vesicles obtained

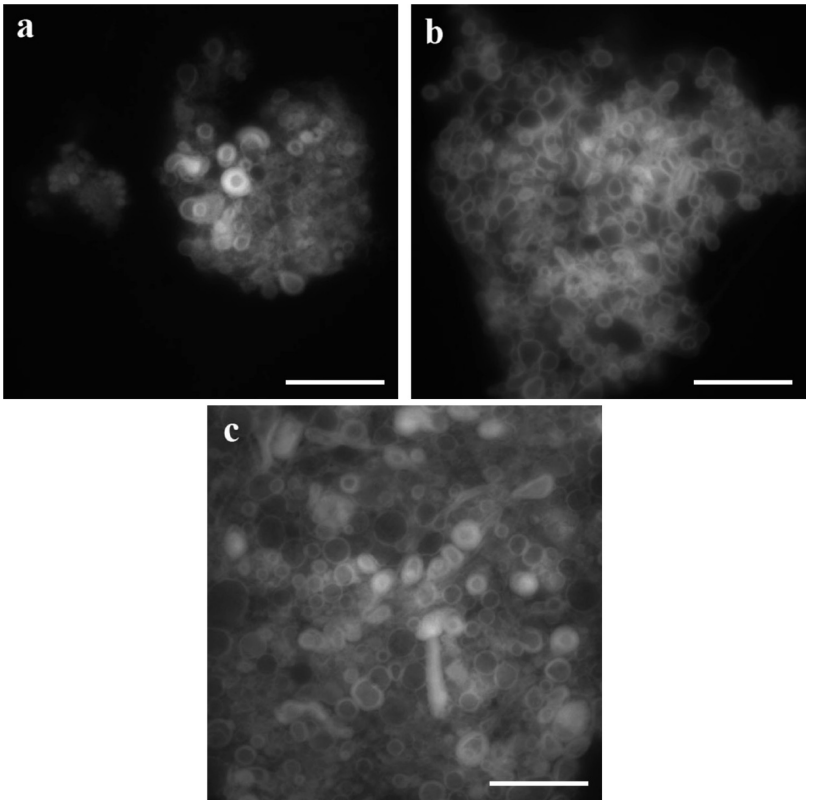


Figure 4. Three fluorescence micrographs showing clusters of reverse vesicles with increasing size. The sample is labeled with RB. The scale bar corresponds to 20 μm.

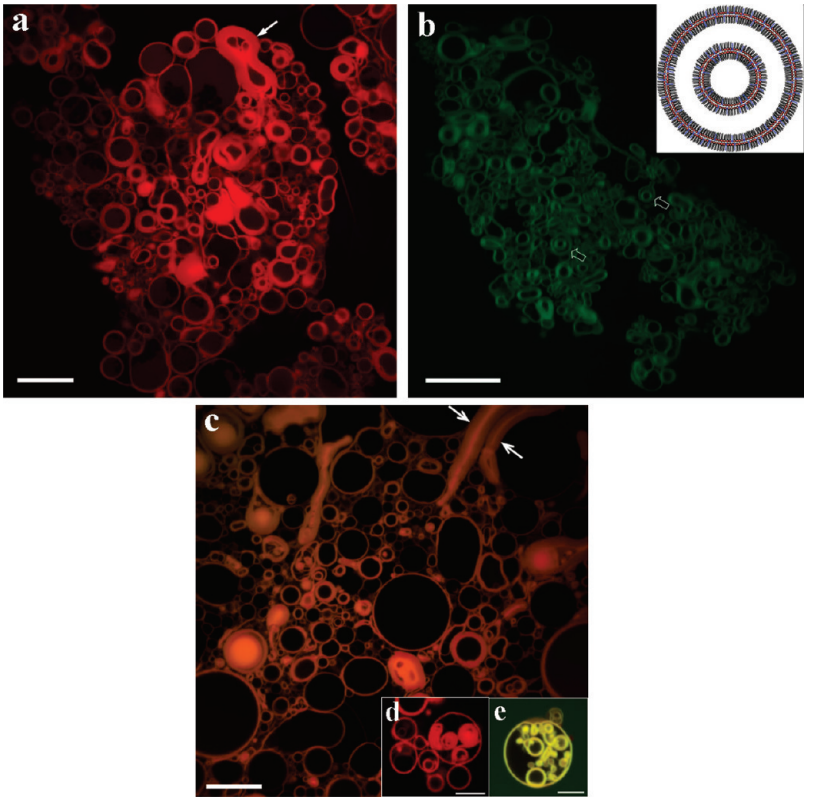


Figure 5. Typical confocal fluorescence micrographs of reverse vesicles labeled with (a, d) RB, (b) Fl, (c) RB and Fl, and (e) RB, TA, FS, and Fl. The inset of image b shows schematic illustration of a reverse vesicle with large separation between bilayers. The scale bar corresponds to 20 μm.

from confocal fluorescence microscopy observations are given in Figure 5. With a higher contrast, the structural features of reverse vesicles become more obvious. Reverse vesicles with closely packed thick walls, i.e., giant reverse onions, were detected

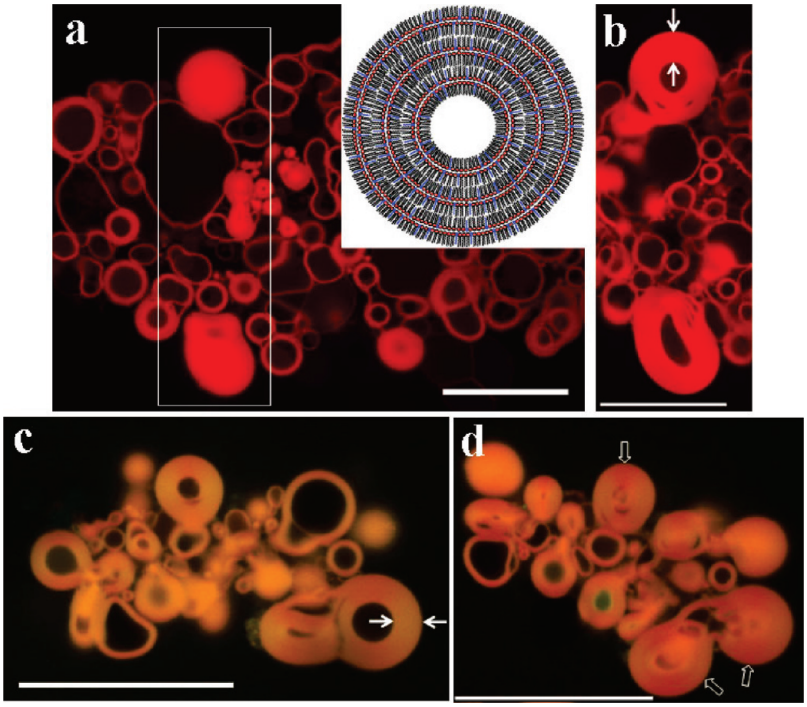


Figure 6. Typical micrographs showing giant reverse onions. The samples were labeled with (a, b) RB and (c, d) RB and Fl. Image b is an expansion of image a, with the laser focused on the cross section of the two giant onions. A schematic illustration of giant reverse onion containing three bilayers is shown in the inset of image a. The scale bar corresponds to 20 μm.

(solid arrow in image a). In some cases, the bilayers in reverse vesicles may also have a separation which is large enough to be captured by the fluorescence microscope (empty arrows in image b).

More examinations revealed that the reverse vesicular phase is a highly inhomogenous system with different types of supramolecular self-assemblies coexist. Besides spherical ones, reverse vesicles with irregular shapes as well as open bilayers were also found. In fact, it is quite common to find open bilayers which connect adjacent vesicles within a cluster. Moreover, vesicle clusters which are embedded in a larger vesicle (images d, e) and tubular structures (between sharp arrows in image c) have been observed. The coexistance of different supramolecular assemblies indicates the diversity of supramolecular structures formed by catanionic surfactant mixtures in a nonpolar organic solvent. It should be mentioned that similar phenomenon has also been observed for surfactant supramolecular self-assemblies fabricated in aqueous solutions. For example, Hoffmann et al. pointed out that the L_α phase can coexist with vesicular or L_3 phase in the alkyldimethylamine oxide/heptanol/water system.[34] For another example, coexistance of lamellar structure and disk-like micelles has been clarified by Dubois et al. in the system of cetyltrimethylammonium hydroxide/myristic acid/water.[35] In the following, we will further highlight the features of interested structures as well as transitions between different morphologies.

Giant Reverse Onions. Among the detected supramolecular self-assemblies with different morphologies, of special interest is the giant reverse onions. This can be seen from image a in Figure 5 where a giant onion with a peanut shape is embedded in a cluster of reverse vesicles. More examples are gathered in Figure 6. The average diameter of the onions is above 5 μm, with the largest one over 10 μm. The wall thickness of the onion is polydisperse, with a typical value of 3−4 μm (between solid arrows in images b and c). The interiors of some onions are fully occupied by the bilayers with almost no empty core (empty arrows in image d). Consistent with the observations mentioned above, most of the onions are not intact. Instead, a part of the bilayers of an onion are usually open and connected to the neighboring onions. It is known that catanionic surfactant ion pairs can form giant onions in aqueous solutions.[14] Here, we show for the first time the presence and structural features of the giant reverse onions formed by the catanionic surfactant ion pairs in a nonpolar organic solvent.

Tubular Structures. In the cluster of reverse vesicles, tubular structures can be detected as indicated in Figure 5c. New examples are given in Figure 7 where the tubes have different length and diameter. Similar with giant reverse onions, the walls of the tubular structures are also thick and closely packed. We speculate that the formation of these tubular structures results from the deformation of some giant reverse onions after losing the oil in the core. Indeed, this trend is already seen from Figure 5a where a giant reverse onion deforms into a peanut-shaped supramolecular structure. From Figure 7 it can be concluded that if the oil in the core is fully driven out, the bilayers on the opposite walls of onions will totally contact each other, and the resulting tubes will be fully occupied by bilayers (image a, solid arrow). If the oil is only partially driven out, the spherical onions will deform into tubes with various shapes with remaining oil locating in the center (image a, empty arrows and image c) or the ends (image b).

To get more information about the onions and tubular structures, layer-by-layer measurements were carried out. Selected layers of such a measurement on a cluster containing five representative onions are illustrated in Figure 8. Over the course of the confocal observations, two dynamic processes were observed. First, there is some movement in the position of peripheral onions (especially note onion "1"). This may be caused by the evaporation of solvent from the edge of the cover glass or by

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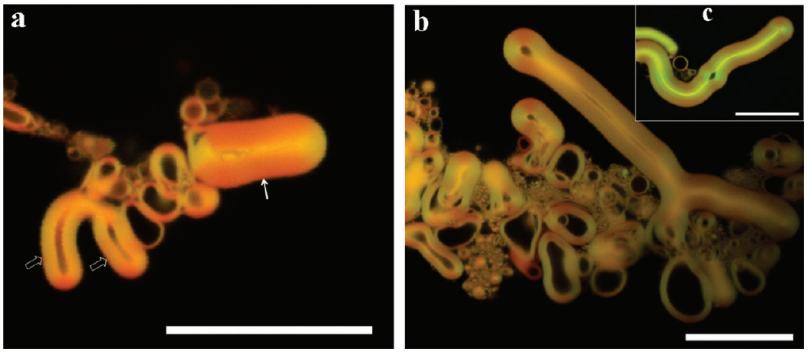

Figure 7. Typical confocal fluorescence micrographs showing the presence of tubular structures. The samples are labeled with mixed dyes. The scale bar corresponds to 20 μm.

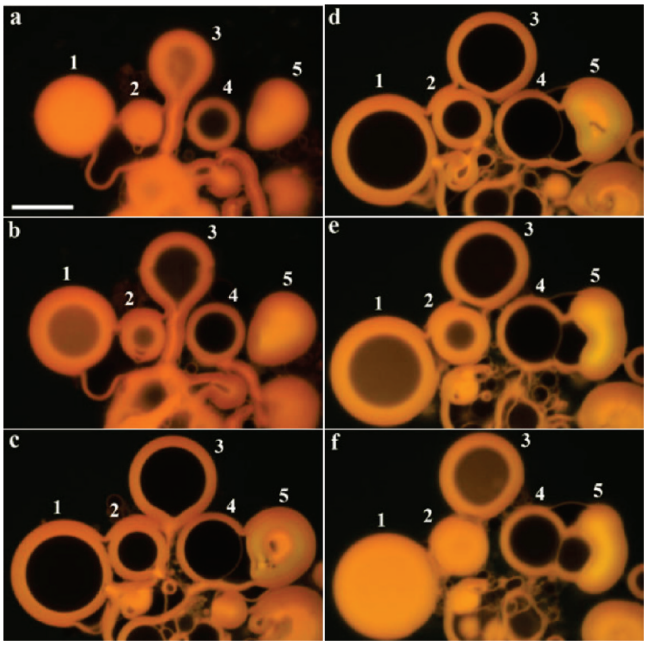

Figure 8. Typical results of a layer-by-layer confocal fluorescence microscopy observations. The sample is labeled with RB and Fl. We did measurements across a sample thickness of 9.6 μm, and the thickness between adjacent layers is 0.3 μm. A total of 33 layers were obtained. The six layers shown in this figure from (a) to (f) correspond to the 10th, 13th, 21st, 27th, 31st, and 33rd layer, respectively. The scale bar corresponds to 10 μm.

thermal motion caused by temperature fluctuations around the laser. Second, the structural evolution of some vesicles can be observed. For example, onion 5 holds an egglike shape at the beginning. However, it becomes flat and finally forms a deformed cylinder. This observation provides a proof for our assumption mentioned above; i.e., the tubular structures are the result of the deformation of some giant reverse onions (another proof of the onion−tube transition is given in Figure S3).

Pseudo-Two-Dimensional Network of Reverse Vesicles. The reverse vesicular cluster has a three-dimensional character. If the hydrodynamic radius of a cluster is smaller than one-half of the sample thickness (~20 μm), its three-dimensional character will not be disturbed and the cluster may move or rotate slowly. In this case, the reverse vesicles within the cluster will distribute at different depth. This means during confocal observations the laser may across the center of a vesicle while only goes through the head or the end of the other. This is exactly what we have observed for smaller clusters. If the hydrodynamic radius of a cluster is much bigger than one-half of the sample thickness, however, the cluster will be compacted by the bottom and cover glasses. In such a case the reverse vesicles within the cluster are forced to pack tightly to minimize the gap between adjacent vesicles. In the vicinity of the glass surface, the vesicles can arrange themselves at a similar depth, forming a pseudo-two-dimensional network. This can be seen from the image shown in Figure 5c. When the sizes of vesicles are similar, they will adopt a pseudohexagonal arrangement to minimize the gap (Figure 9, indicated by the hexagons). This observation provides us a facile way to regulate the arrangement of the reverse vesicles by applying external forces.

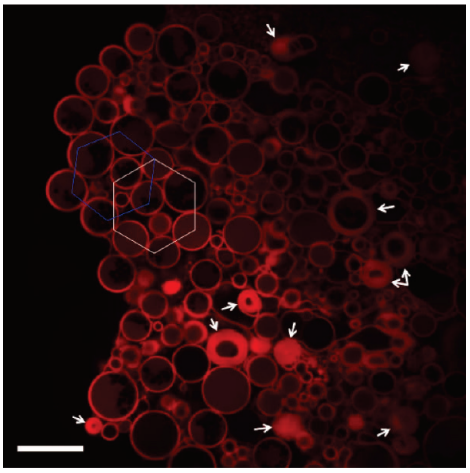

Figure 9. Confocal fluorescence micrograph showing a pseudo-two-dimensional network of reverse vesicles. The solid arrows indicate the presence of giant reverse onions. The sample is labeled with RB, and the scale bar corresponds to 20 μm.

Effect of Sonication. To probe their dynamic properties, the reverse vesicles have been subjected to sonication and the recovering process has been monitored by fluorescence microscopy observations. Typical fluorescence micrographs recorded during the recovering process of a sonicated sample are summarized in Figure 10. It is found the reverse vesicles are destroyed and some flat objects are noticed within the first 2 min (image a). When this sample is aged, the flat objects gather themselves into many blocks and at the same time, reverse vesicles with a cylindrical shape and core−shell structure begin to form (images b, c). This experiment emphasizes the spontaneous formation of the reverse vesicles. Even after they are destroyed by external forces like sonication, they can recover by themselves. This is consistent with our previous report where the reverse vesicles were found to recover themselves after they were extruded throw a membrane filter.[31]

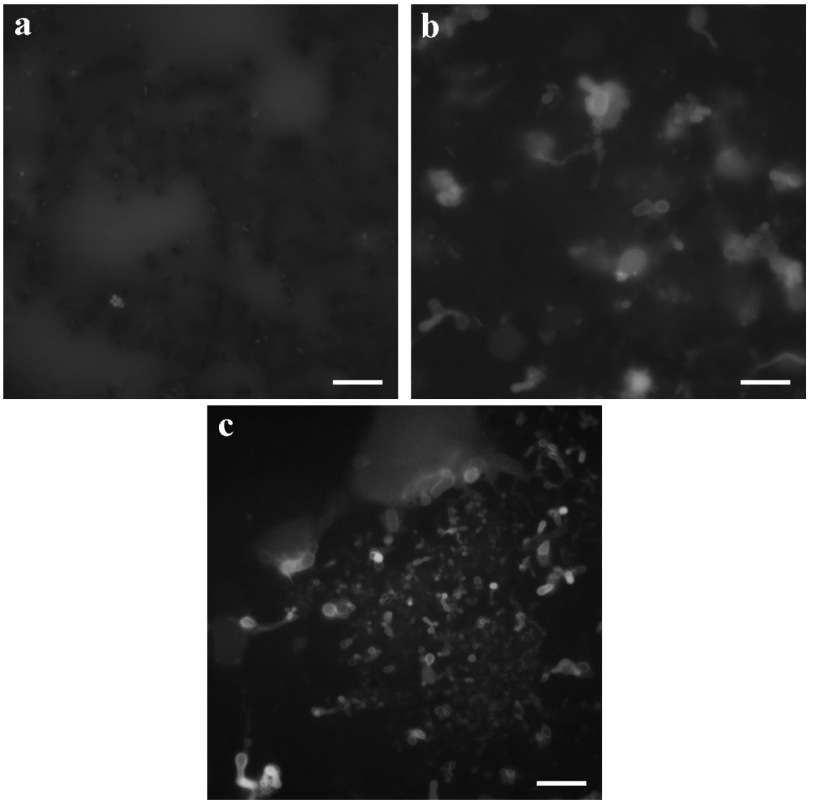

Figure 10. Typical fluorescence micrographs recorded for a sonicated sample labeled with RB. (a, b) Images recorded 2 (a) and 10 min (b) after sonication at the same region. (c) Image recorded at a different region 20 min after sonication. The scale bar corresponds to 20 μm.

Discussion

Spontaneous Formation of Stable Reverse Vesicles. In our previous study, we have shown the spontaneous formation of stable reverse vesicles by TTAL and LA in cyclohexane by a detailed phase behavior study and polarized microscopy observations.[31] Current study has further strengthed these points. As shown in the main text, the reverse vesicles are stable enough for confocal fluorescence microscopy observations without any further sample treatment. Once the samples are sonicated, reverse vesicles can recover by themselves. This is quite different from what have been found for reverse vesicles fabricated by nonionic surfactant systems where phase separation occurs with time. We speculate that this high stability and spontaneous formation feature originate from the unique properties of catanionic surfactant mixtures. With a distribution freedom of the two components in the outer and inner layer of a molecular bilayer, the energy cost for forming a reverse vesicle will be lower. Thus, the reverse vesicle formation would be easier in case of catanionic surfactant mixtures compared to single nonionic surfactant. It should be mentioned that similar rule is also believed to work for vesicles formed in water.[36] This can be seen from the fact that catanionic surfactant mixtures can form spontaneously in water, and the vesicles are much more stable than those formed by phosphalipids or double-chained amphiphiles like didodecyldimethylammonium bromide (DDAB).[6]

It should be noted that there is another factor contributing to the high stability of the reverse vesicles from catanionic surfactant mixtures. Unlike nonionic surfactant whose hydrophilic part is a flexible and bulky poly(ethylene oxide) functional group, the hydrophilic parts of catanionic surfactant mixtures are charged heads with a much less steric hindrance. Thus, within a reverse molecular bilayer formed by catanionic surfactant mixtures, the thickness of the water channel can be much narrower. This will lead to a lower curvature difference between the outer and inner layer within the molecular bilayers of reverse vesicles. In other words, the bilayers can be packed more efficiently and the formation of reverse vesicles can be easier. Within the highly restricted water layers, the property of water molecules might be different from that of free water molecules. Hydrogen bonds may also form between carboxy groups of LA and carboxylate groups of TTAL, which further adds the stability of reverse vesicles.

Cluster Formation of Reverse Vesicles. One feature of reverse vesicles observed from fluorescence microscopy observations is their high tendency to form three-dimensional clusters. The cluster formation has also been observed for their couterparts in water where closely packed vesicles have been evidenced by FF-TEM or fluorescence observations as well as rheological measurements.[11,15,37] Compared to the case in water, however, the extent of cluster formation for reverse vesicles is more obvious. This can be explained by taking into account the unique structure of reverse vesicles. For vesicles formed in water, electrostatic repulsion (for ionic surfactants) and hydration force (for both ionic and nonionic surfactants) exist between adjacent vesicles. Thus, there is some energy barrier to be overcome for vesicles to aggregate together. In case of reverse vesicles, however, the outer layer of vesicles are alkyl chains (thus no charge) and the medium between adjacent vesicles is nonpolar organic solvent with a much lower dielectric constant. So both the electrostatic repulsion and hydration force are absent between adjacent vesicles. Thus, the energy barrier for clustering of reverse vesicles, if there is some, should be much smaller, accounting for the more

(36) Safran, S. A.; Pincus, P.; Andelman, D. *Science* **1990**, *248*, 354.

(37) Li, H.; Wieczorek, S. A.; Xin, X.; Kalwarczyk, T.; Ziebacz, N.; Szymborski, T.; Hozyst, R.; Hao, J.; Gorecka, E.; Pociecha, D. *Langmuir* **2010**, *26*, 34–40.

obvious aggregation behavior of reverse vesicles. This indicates that by changing the organization manner of amphiphiles in a molecular bilayer, significant property differences will be induced.

Because of the formation of clusters, movement of reverse vesicles is suppressed. This gives the advantage for examining the reverse vesicles by confocal fluorescence microscopy observations without any further sample treatment. Despite this advantage, however, the cluster formation of reverse vesicles seems more like a disadvantage for practical applications. Current study shows that the arrangement of reverse vesicles could be regulated by external forces, for example, by simply compressing them with parallel glass substrates. Further efforts should be devoted in near future to get monodispersed reverse vesicles for using these interesting supramolecular structures for micro- or nanoreaction centers of biological reactions and synthesis of inorganic nanomaterials.

Dynamic Properties of Reverse Vesicles. As seen from phase behavior study, the upper reverse vesicular phase coexists with an oil-in-water emulsion at bottom. Thus, dynamic equilibrium should exist between these two phases. In bulk solution, clusters will form when individual reverse vesicles meet each other. By gathering more vesicles, cluster growth continues. If the clusters are too big and reach a critical value, they will settle down to the bottom. At the same time, new reverse vesicles will form at the boundary of the upper and bottom phase and diffuse into the upper bulk solution. This hypothesis is proved by two facts: (i) although the boundary of the two phases can be made rough by hand-shaking, it will become smooth with time; (ii) when the upper reverse vesicular phase is transferred to a new bottle, a newly formed bottom phase will be developed with time.

Within the three-dimensional clusters, some dynamic processes can be also expected. Without repulsion forces among adjacent vesicles, they will contact each other. Because of the pressure given by others, some spherical reverse vesicles may deform to other supramolecular structures with various morphologies such as tubular structures. Fusing and recombination may also occur within the cluster. During these processes, the bilayers of reverse vesicles may partially open and become public ones connecting adjacent vesicles. Such trend has indeed been observed from confocal fluorescence microscopy observations where a giant reverse onion was destroyed into public bilayers by the pressure given by adjacent vesicles (Figure S4).

Location of Dyes in Reverse Vesicles. It is also of great interest to probe the location of dyes in reverse vesicles. From phase behavior study it can be concluded that RB, FS, and Fl mainly distribute in the upper reverse vesicular phases and are blind for the bottom water-rich phase. This conclusion is further confirmed by fluorescence microscopy observations where the water droplets in the sample (either labeled by single dye or the combination of these three dyes) are black, indicating almost no dyes inside. This is understandable for Fl since its hydrophilicity is not high. However, it is a bit difficult to understand for RB and FS since these two dyes are water-soluble and have been expected to stay in the water-rich region if there is no interaction between the dyes and surfactant molecules. This indicates that coupling occurrs between the dyes and surfactant molecules. We speculate that electrostatic interaction can exist between the dyes and surfactant cations (TTA^+) due to the anionic character of the dyes, pulling the dyes into the reverse vesices. Considering the narrow water channels within the bilayers of reverse vesicles, the hydrophobic parts of the dyes probably are embedded in the hydrophobic alkyl chain layer while the hydrophilic parts extruding into the water layer. Because of a less comformational freedom as well as a polarity change of the environment, the absortions of dyes change and peak shifts occur once they are solubilized into the reverse vesicles, as can be evidenced from UV−vis measurements.

For Fl, additional acid−base reaction may occur (Scheme S1). After reacting with TTAOH and being transformed into anionic salt, the location of this dye should be similar to that of its sodium salt, i.e., FS. In case of TA, arrangement of the dye molecule inside the bilayer of reverse vesicles can be difficult compared to other dyes due to its bulky hydrophobic part. This can explain why this dye can be found both in the upper reverse vesicular phase and the bottom water-rich phase and its solubility in the upper reverse vesicular phase is lower compared to that of other three dyes. Consistent with phase behavior study and UV−vis measurements, confocal fluorescence microscopy observations show the water droplets which coexist with reverse vesicles are red when TA is present in the mixed dyes (Figure S5).

Conclusions

In summary, stable reverse vesicular phases have been preparated from a catanionic surfactant ion pair TTAL and a fatty acid LA in cyclohexane, into which fluorescence dyes with different molecular structures can be solubilized. This indicates reverse vesicles can be good candidates for hosting guest molecules. The structural features and structural evolutions of the reverse vesicles have been characterized by fluorescence microscopy observations combined with a confocal method. It is found the reverse vesicles have a high tendency to form clusters, within which a variety of supramolecular structures including giant reverse onions and tubular structures have been detected. The bilayers are flexible, and in some cases open bilayers can be observed which connect adjacent vesicles. Confocal fluorescence microscopy observations showed that the presence of tubular structures and open bilayers could be the results of the structural evolution of some giant reverse onions. The three-dimensional feature of the reverse vesicular cluster has been investigated by layer-by-layer measurements. Dynamic properties of vesicle formation has also been probed with the help of sonication, revealing the spontaneous formation of reverse vesicles.

To the best of our knowledge, this is the first time to clearly reveal the structural features of these interesting supramolecular structures formed by catanionic surfactant mixtures in a nonpolar organic solvent. The ability to form stable reverse vesicles in nonpolar solvents should not be only applied on the catanionic surfactant mixtures used in this study, but rather should be a universal property of this class of amphiphiles. The beauty of reverse vesicles shown here also reminds us of the fascination of catanionic surfactant mixtures, which have already given us many surprises.

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Supporting Information Available: UV−vis absorption of RB in cyclohexane and more confocal microscopy micrographs.This material is available free of charge via the Internet at http://pubs.acs.org.