VIP Self-Assembly

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Preparation of Nanostructures by Orthogonal Self-Assembly of Hydrogelators and Surfactants**

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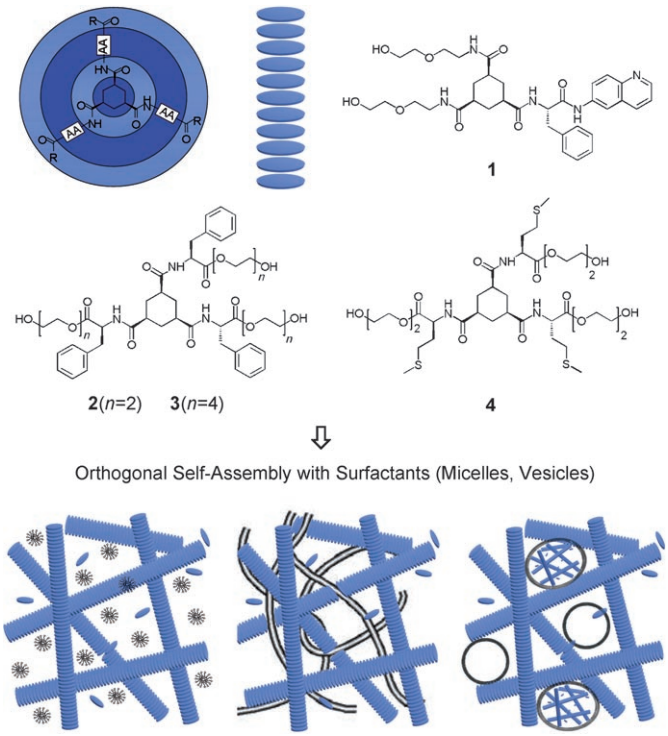
Nature, through its ubiquitous examples of complex nano-objects constructed by the self-assembly of molecular components, in particular lipids and proteins, can deliver the topologically controlled compartmentalized architectures that are essential to high-end physiological functions.[1] The formation of natural systems is driven not only by complementary intermolecular interactions, but also emerges from the inherent incompatibility of components that leads to (micro)phase separation, as manifested in protein folding and biological membranes. Microphase separation has been extensively exploited in the self-assembly of block copolymers,[2,3] however, there are only a few examples in which the controlled phase separation of distinct components has been utilized to fabricate artificial nanostructured assemblies. These examples include, phase-separated polymer systems,[4] bilayers of hydrocarbon and perfluorinated phospholipids,[5] low-molecular-weight hydrogelators in liquid-crystalline phases[6] or with surfactant micelles[7] as well as nanocapsules[8] Despite the progress in supramolecular chemistry,[9] man-made self-assembled structures can not yet compete with the level of complexity and functionality of natural systems.[10] Herein we show that the orthogonal self-assembly of multiple components, that is, the independent formation within a single system of different supramolecular structures, each with their own characteristics, is a versatile and powerful approach towards the formation of novel and more complex architectures. These architectures include self-assembled interpenetrating networks and vesicle configurations that coexist with hydrogel fibers (Scheme 1).



Scheme 1. Schematic representation of hydrogelators, which self-assemble preferentially in one dimension. Light blue regions correspond to hydrophilic groups and dark blue areas to hydrophobic entities (AA = amino acids). Chemical structures of hydrogelators **1**–**4** based on 1,3,5-cyclohexyltricarboxamide as well as a representation of the orthogonal self-assembly strategy using hydrogelators and surfactants to create more complex and compartmentalized structures are also shown.

Surfactants are typical small molecules that offer an interesting starting point for the fabrication of architectures through orthogonal self-assembly. They are essential components of cell membranes and have numerous technological applications. Despite their large structural diversity, surfactants lead to a limited range of supramolecular architectures, including spherical or rodlike micelles, bilayers, vesicles, and inverted micelles.[11] Another attractive class of self-assembling units are the low-molecular-weight gelators. Although they too lead to a similar range of supramolecular structures, gelators can also self-assemble into highly organized and responsive fibrillar architectures.[12] This property is the source of the considerable current interest in the development of smart materials.[13]

In this study, low-molecular-weight hydrogelator molecules based on 1,3,5-cyclohexyltricarboxamide were used.

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They were chosen because they can self-assemble into 1D arrays that are stabilized by both hydrogen-bonding and hydrophobic interactions (Scheme 1).[14] Similar interactions serve to stabilize protein secondary structures which are stable in the presence of weakly interacting surfactants.[15] These hydrogelators can be functionalized at the periphery with different solvophilic or pH-sensitive groups (Scheme 1), thus giving access to modular architectures and properties. Thermoreversible hydrogels of **1**–**4** can be prepared easily by cooling warm solutions, which typically contain 0.1–1 w/v % of the gelator, to below their gel-sol phase transition temperature (T_{gs}) thus leading to their spontaneous assembly into quasi-one-dimensional fibers, which in turn form a three-dimensional network.

The compatibility of hydrogel formation by **1**–**4** with various types of surfactants was investigated by dissolving **1**–**4** at $T > T_{gs}$ in solutions of the surfactant and subsequently examining the samples for gelation after they had been cooled to room temperature. Transparent, thermoreversible gels of **1**–**4** were obtained in the presence of the spherical micelle forming surfactants alkyl trimethylammonium bromide (C_nTAB, $n = 12$, 14, 16) or sodium dodecyl sulfate (SDS), at concentrations below and above their critical micelle concentration (CMC). Precipitation occurred only when **1** was combined with SDS, presumably because of strong electrostatic interactions. Gel formation by **1**–**4** was also observed in combination with other surfactant aggregate morphologies. For example, cetyltrimethylammonium tosylate (CTAT) first forms spherical micelles just above its CMC (0.2 mM),[16] but progressively transforms into elongated entangled micelles upon increasing the surfactant concentration (ca. 20 mM).[17] Incorporation of CTAT at concentrations below and above the CMC, thus in the spherical and elongated micelle regimes, with **1**–**4** resulted in the formation of transparent, thermoreversible gels. Gelation by **1**–**4** took place even in the presence of zwitterionic lipids such as dioleoylphosphocholine (DOPC), dimyristoylphosphatidylcholine (DMPC), and dipalmitoylphosphatidylcholine (DPPC), that form bilayer vesicles.

These results show that gel formation by **1**–**4** is compatible with surfactants and lipids at the macroscopic level. The interdependence of the self-assembly of **1**–**4** and surfactants at the supramolecular level was examined by looking at the effect hydrogelators had on the self-assembly properties of the surfactants and vice versa. For micelle-forming surfactants, the CMC is a characteristic property to monitor because it is expected to change if interactions of the surfactants with other components occur.[18] Interestingly, the CMC value for $C_{14}TAB$ or SDS, as determined by conductimetry measurements, did not change in the presence of hydrogels of **1** and **2**, respectively. In the case of bilayer-forming lipids and surfactants, the transition of the lipid physical state from the ordered L_β phase to the disordered liquid-crystalline L_α phase is very sensitive to interaction with foreign molecules.[19] The differential scanning calorimetry (DSC) traces (Figure 1 a) show no significant differences in either the shape or position of the heating and cooling traces of DPPC, either with or without hydrogelator **1** at below and above the critical gelation concentration.[20] Evidently, the physical state of the lipid bilayers is not disturbed by the hydrogelator molecules, thus indicating that they are not incorporated into the bilayers.

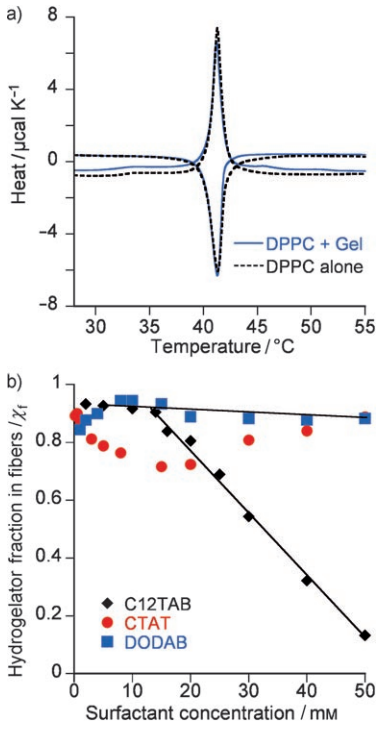

Figure 1. a) DSC heating and cooling traces of an aqueous dispersion of DPPC (4 mM) in the absence of **1** (black dotted line) or in the presence of **1** (blue solid line, 4 mM); b) Molar fraction of **1** incorporated in the fibers (X_f), at a fixed concentration ([**1**] = 4 mM) determined by ^{1}H NMR spectroscopy as a function of the concentration of $C_{12}TAB$ (spherical micelles), CTAT (cylindrical micelles), and DODAB (vesicles) concentrations.

Conversely, the behavior of the hydrogelators was examined for possible interactions with the amphiphiles. Gels of **1**–**4** are characterized by high T_{gs}, which is indicative of strong intermolecular interactions.[14b] Although the properties of the surfactant aggregates appear to remain unchanged upon incorporation into a gel network, the presence of surfactant in the hydrogels led to a significant destabilization of the gel network, which was manifested by a decrease in the T_{gs} value upon increasing the surfactant concentration (see Supporting Information). Interestingly, this destabilization was dependent on the morphology of the surfactant aggregate: overall, lipid bilayers destabilized the gel network less than spherical micelles. To understand this result, the molar fraction of hydrogelator molecules in solution (X_m) and in the solid fibers ($X_f = 1 - X_m$) were measured by ^{1}H NMR spectroscopy as a function of surfactant concentration. In the presence of surfactants that form spherical micelles, the fibrous fraction remained constant at $X_f \approx 0.95$ below the CMC (15 mM for $C_{12}TAB$), but decreased progressively above the CMC. This difference indicates a partial solubilization of the gel fibers by the micelles leading to macroscopic destabilization of the gel network. In the presence of CTAT, which first forms spherical micelles that evolve into cylindrical micelles, the X_f value passes through a minimum just above the CMC and then increases again upon formation of cylindrical micelles above

approximately 20 mM. Evidently, the morphological transition from spherical to cylindrical micelles leads to the expulsion of hydrogelator molecules from the surfactant aggregates and favors the formation of separate self-assembled architectures. This behavior most probably results from stronger surfactant–surfactant interactions, which accompany the increase of their packing parameters,[11] as compared to surfactant–hydrogelator interactions. This conclusion is supported by the X_f value measured upon addition of dioctadecyldimethylammonium bromide (DODAB) vesicles, which remained constant at around 95%. This finding indicates no substantial dissolution of hydrogelator molecules by the bilayers, which is in excellent agreement with the unchanged melting temperature (T_m) measured by DSC. These results demonstrate clearly that the mutual interference of the self-assembly of, on the one hand, gelator molecules and, on the other hand, surfactants and lipids, not only depends on their hydrophobicity, but importantly also on the morphology of the surfactant or lipid aggregate. In the case of cylindrical micelles and bilayer aggregates the two self-assembly processes are truly orthogonal, most probably because of stronger interactions between surfactants in the latter type of aggregates compared to spherical micelles. This finding holds particular relevance to natural systems, where lipid bilayers are one of the predominant structures that coexist with other self-assembled architectures.

The formation of new supramolecular architectures through orthogonal self-assembly of hydrogelators and surfactants by the cooling of isotropic solutions was investigated by cryo-transmission electron microscopy (cryo-TEM). Figure 2a shows the formation of well-defined fibers with diameters of about 5 nm (typical for a hydrogel of **1**), which was unaffected by the presence of micelle-forming surfactants such as C_{14}TAB at below or above its CMC, although fiber ends were more frequently observed. At an increased magnification, spherical micelles encapsulated in a fibrillar network were observed when **1** was combined with C_{14}TAB above its CMC (Figure 2a inset). This observation indicates that gel fibers of **1** and micelles of C_{14}TAB can coexist, even if a fraction of the hydrogelator monomers is dissolved by spherical micelles. When this strategy was extended to CTAT surfactants and **1** or **2**, the coexistence of gel fibers and cylindrical micelles were observed by cryo-TEM experiments.

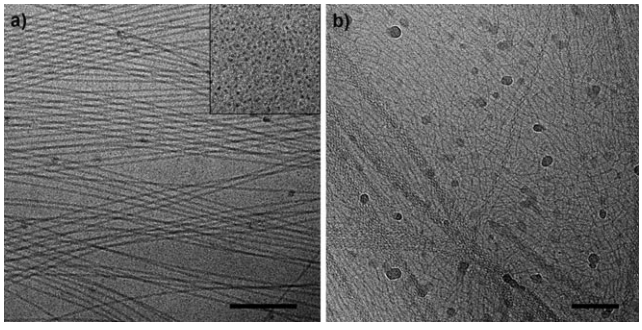

Figure 2. Cryo-TEM images of fibrous networks of a) **1** (4 mM) coexisting with spherical micelles of C_{14}TAB at 20 mM; b) **2** (4 mM) in the presence of CTAT at 100 mM (cylindrical micelles). Scale bar is 100 nm.

Figure 2b shows elongated, stiff fibers with diameters of 7–20 nm formed by **2** (left side). In the background one can distinguish unambiguously the presence of highly entangled cylindrical micelles with diameters of a few nanometers. To the best of our knowledge, this system represents the first example of interpenetrating networks based on orthogonal self-assembly of two different components: both nanostructures evolve from noncovalent interactions between units that self-assemble and disassemble independently. Interestingly, these self-assembled interpenetrating networks display new viscoelastic properties, which are unattainable when using the individual components alone. When we combined these two types of networks, an intermediate and adjustable rheological response was observed: by progressively increasing the [hydrogelator]/[surfactant] ratio, the samples transform from viscoelastic systems to more solidlike samples without any macroscopic phase separation.

Inspired by the remarkable functionality of cytoskeletons and extracellular matrixes in nature, another new and interesting application for our system could be as a fibrous network coexisting with bilayer vesicles. Cryo-TEM studies revealed that the typical fibrous networks of **1**–**4** remain intact in the presence of bilayer-forming surfactants and lipids such as DODAB and DOPC. Reciprocally, vesicles of DODAB and DOPC are stable in the presence of gel fibers (Figures 3a and b). These observations are in excellent agreement with the DSC and NMR spectroscopy studies described above, and together they show unambiguously that the molecular components do not interact significantly. However, their self-assembled structures influence each other at a supramolecular level: the confined space provided by the pores of

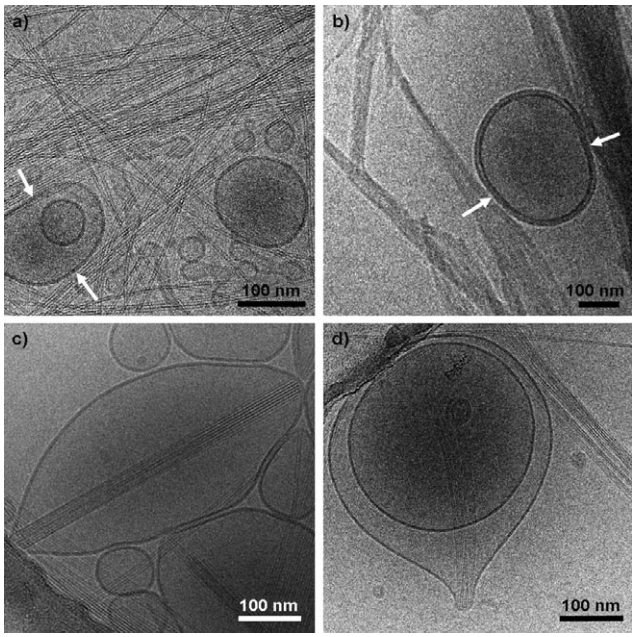

Figure 3. Cryo-TEM images of unilamellar a) DOPC vesicles coexisting with a network of well-defined fibers of **1** with a high aspect ratio; b) DODAB vesicles with thicker fibers of **3**; c) and d) DOPC vesicles deformed by the growth of fibers of **1** directly contained in their aqueous compartment.

the gel network forces the vesicles to accommodate their shape because of the stress exerted on their walls (see arrows in Figures 3a and b).

Encouraged by the above results, we then examined to what extend our strategy could be exploited to gelate the aqueous compartment of vesicles. To this end, DOPC vesicles were prepared in the presence of **1**, which can be reversibly switched from the gel state (basic conditions) to the liquid state (acidic conditions) by adjusting the pH value. Encapsulation of free monomers of **1** in unilamellar liposomes was achieved at pH 2. Fiber formation by **1** was induced by increasing the pH value to neutral. Cryo-TEM images confirm the formation of unilamellar vesicles, with well-defined fibers of **1** having a diameter of 5 nm in the middle of their aqueous compartment (Figures 3c and d), so called "gellosomes".[21] Interestingly, all the cryo-TEM micrographs showed that the growth of the fibers is restricted to a few hundreds of a nanometer by the membrane wall, as indicated by the arrows in Figures 3c and d, thus demonstrating that the fibers are inside the vesicles. The confinement of these fibers also leads to spontaneous alignment, which might be either an entropy or nucleation phenomenon.[22] In turn, the stiffness of the elongated fibers, which originates from highly anisotropic and strong supramolecular interactions, results in such a pressure on the membrane that it leads to a deformation of the vesicles from their otherwise spherical shape, but without rupture of the membrane. (Figures 3c and d). These observations indicate that there is a significant mutual interaction between both of the self-assembled structures at the supramolecular level. The finding that the vesicle shape can be modified by internally self-assembled fibrous aggregates is of great interest because the vesicle shape is an important factor in transport and exchange properties.

In conclusion, the orthogonal self-assembly of hydrogelators and surfactants has proven to be a very attractive method to create a variety of new nanostructures including self-assembling interpenetrating networks and "gellosomes", which are unattainable by the self-assembly of a single component. The architectures described display novel properties, which arise from interactions between self-assembled objects rather than specific intermolecular interactions. These architectures are already much more complex than traditional gelator and surfactant structures, and mark the first step towards self-assembled systems that can mimic the complexity and functionality of natural systems. In principle, the orthogonal self-assembly approach can be extended to other molecular building blocks (provided that they are sufficiently incompatible to avoid coassemble) which should give access to a broad and unexplored range of nanostructures exclusively based on "weak" interactions.

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