Size control and compartmentalization in self-assembled nano-structures of a multisegment amphiphile†

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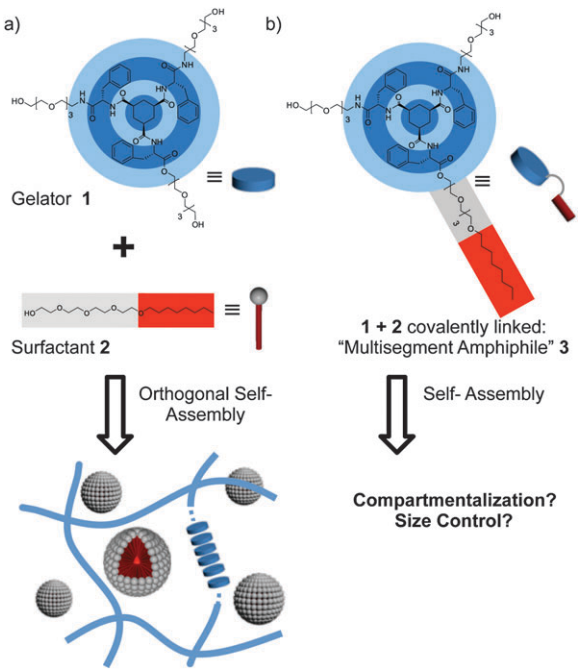
A "multisegment amphiphile" has been synthesized by covalently connecting two well known building blocks, a gelator and a micelle forming surfactant. Self-assembly results in the formation of compartmentalized nano-object displaying properties inherited from both parents.

Amphiphiles continue to attract widespread attention in science and technology, not only because of their fascinating self-assembly properties, but also because their numerous, diverse applications in *e.g.* detergent formulations, catalysis, and drug delivery. Despite their large chemical diversity, amphiphiles share a common structure consisting of a hydrophilic and a hydrophobic segment, giving rise to a limited range of morphologies like micelles, vesicles, bilayers, and the corresponding inverted phases.[1] More recently, other building blocks such as small molecule gelators and peptides have been used to construct novel self-assembled architectures like ribbons, helices and tubes[2] with regular, well-defined structural features at molecular length scales.[3] Nevertheless, small molecule based self-assembled structures cannot yet compete with the sophisticated architectures found in natural systems, especially with regard to the levels of compartmentalization, size control, and hierarchical structure formation.[4] Currently, such multicompartment structures of defined size and 3–50 nm structural features can most easily be fabricated by controlled phase separation of multiple covalently connected segments in block copolymers, giving rise to a variety of multicompartment assemblies[5] and hierarchically structured materials.[6] Only few examples have been reported of self-assembled structures from small molecules with a block structure.[7] The challenge remains to develop self-assembling systems based on small molecules that allow size control, hierarchical structure formation and compartmentalization at (sub)-molecular length scales. Previously we and others showed that the orthogonal self-assembly of certain hydrogelators and surfactants is a valid strategy towards new nanoarchitectures, including self-assembled interpenetrating networks and various organizations of vesicles or micelles with fibrous networks (Scheme 1a).[8,9] We anticipated that the covalent connection of two orthogonally self-assembling molecular building blocks would give rise to microphase separation phenomena at the molecular length scale.



Scheme 1 (a) Schematic representation of hydrogelator **1** and micelle forming surfactant **2**, which undergo orthogonal self-assembly into their individual architectures. (b) Multisegment amphiphile **3** is expected to give assemblies with structural features and compartments inherited from the parent compounds **1** and **2**. Light areas represent hydrophilic domains whereas dark areas represent hydrophobic domains.

Here we present a first example of such a "multisegment amphiphile", as the small molecule counterpart of block copolymers (Scheme 1b). We found that this multisegment amphiphile self-assembles into well-defined aggregates with compartments and structural features at molecular length scales inherited from its parent compounds. Our results suggest that this behavior results from strong spatial constraints on a phase separating system at the molecular scale.

A first requirement for a multisegment amphiphile is that the self-assembly of each segment is driven by incompatible interactions. In this study we have combined a low-molecular-weight hydrogelator and a surfactant, separated by a hydrophilic linker. The parent hydrogelator **1** is a member of the well-studied class of 1,3,5-cyclohexyltrisamide hydrogelators,

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which self-assemble by hydrogen bonding and hydrophobic interactions.[10] In this study hydrogelator **1** has been functionalized with three hydrophobic phenyl alanine amino acids and three tetra-ethylene glycol tails. In water **1** forms a turbid viscous solution at concentrations below 7 mM, whereas above this concentration turbid gels are formed. The surfactant segment used in this study is based on tetra-ethylene glycol mono-octyl ether **2** which self-assembles into micelles above a critical micelle concentration (cmc) of 8.4 mM due to hydrophobic interactions.[11] Previous work showed that self-assembly of non-ionic surfactants and cyclohexanetrisamide based hydrogelators like **1** are orthogonal processes.[8a]

Multisegment amphiphile **3** was constructed by the covalent connection of **1** and **2** (ESI†). Contrary to surfactant **2**, multisegment amphiphile **3** did not dissolve in water at room temperature. Heating of the samples resulted in the formation of a transparent solution, which is common for gelators like **1**.[10] Cooling to room temperature led to the formation of a viscous transparent solution at [**3**] <0.25 mM and a turbid gel for [**3**] >5 mM. In between these concentrations a turbid viscous solution was formed (ESI†). The critical gelation concentration (cgc) is comparable to **1** (7 mM), suggesting that **3** aggregates in water in a similar way to **1**. FT-IR showed that N–H and C=O vibrations of a freeze-dried xerogel of **3** appeared at wavenumbers characteristic for hydrogen bonded amides.[12] Similar behavior was observed for **1**, indicating both **1** and **3** are involved in hydrogen bonding (ESI†).

To see to which extent the C8-tails contribute to the stability of the fibers, the thermostability of gels of **3** was investigated by dropping ball measurements (Fig. 1a). Especially for concentrations below 15 mM the gel-to-sol transition temperature (T_m) was higher for **3** than for **1**. These observations suggest that gels of **3** display higher thermal stability as compared to **1**, which is most likely due to hydrophobic interactions between the C8-tails.

The presence of hydrophobic interactions between the C8 tails of **3** was further investigated by fluorescence spectroscopy using the solvatochromic probe Nile Red (NR).[13] In the presence of **3** the emission intensity of NR showed a significant increase together with a blue shift of the maximum emission wavelength (λ_{max}) from 660 nm in pure water to 622 nm in aqueous solutions of **3** ([**3**] = 1.0 mM) (Fig. 1b and ESI†), indicative of the formation of hydrophobic domains. For **1**, neither increase of the emission intensity nor blue shift of λ_{max} of NR was observed. These results clearly show that self-assembly of **3** is also driven by hydrophobic interactions between the C8 tails, leading to the formation of hydrophobic microdomains which are not present in assemblies of gelator **1**. In a control experiment with **2** the λ_{max} of NR only shifted by 25 nm to 635 nm, which is significantly less than the 39 nm blue shift observed with **3**, indicating that the hydrophobic domains in aggregates of **3** are more hydrophobic than in surfactant **2**. The onset of the blue shift of λ_{max} of NR with **2** occurred at 8.0 mM, which is in nice agreement with the reported value for the cmc of **2** (8.4 mM).[11] Interestingly, the hydrophobic microdomains in aqueous solutions of **3** are already formed at a concentration around 0.01 mM, which is more than two orders of magnitude lower than with surfactant **2**, clearly showing that the gelator segment contributes significantly to the stability of the hydrophobic domains.

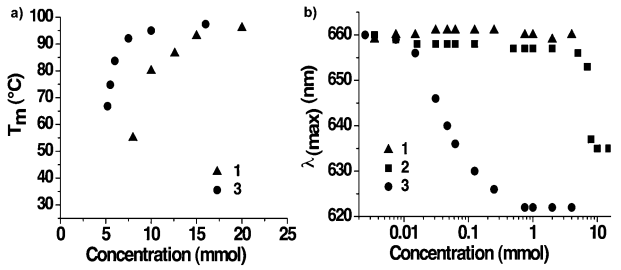

Fig. 1 (a) Gel-to-sol phase transition temperature (T_m) of aqueous gels of **1** and **3** as a function of concentration, as determined by dropping ball measurements. (b) Emission maximum (λ_{max}) of NR (0.1 μM) as a function of concentrations **1**, **2** and **3**. Nile Red was excited at 550 nm.

The morphology of aggregates of **1** and **3** was investigated in more detail with Cryo Transmission Electron Microscopy (Cryo-TEM). Cryo-TEM of gelator **1** in water at 0.25 mM and 1.0 mM revealed the presence of fibers and extended sheets, which is in good agreement with the observed viscosity and turbidity of the samples (ESI†). Both the fibers and sheets formed by **1** are highly disperse in width, with sizes ranging from 50–500 nm, comparable to similar gelators.[10b]

In the case of multisegment gelator **3** Cryo-TEM revealed that transparent and viscous samples of **3** at a concentration of 0.25 mM consisted primarily of monodisperse, elongated fibers with a diameter of 9 nm. The diameter of these fibers was very uniform pointing to a cylindrical shape. Furthermore, these 9 nm fibers did not fuse or intertwine, but appeared to be rigid with an estimated persistence length of approximately 0.5 μm (Fig. 2a). In some instances a splitting of the 9 nm fibers into smaller and thinner fibrils with a diameter of

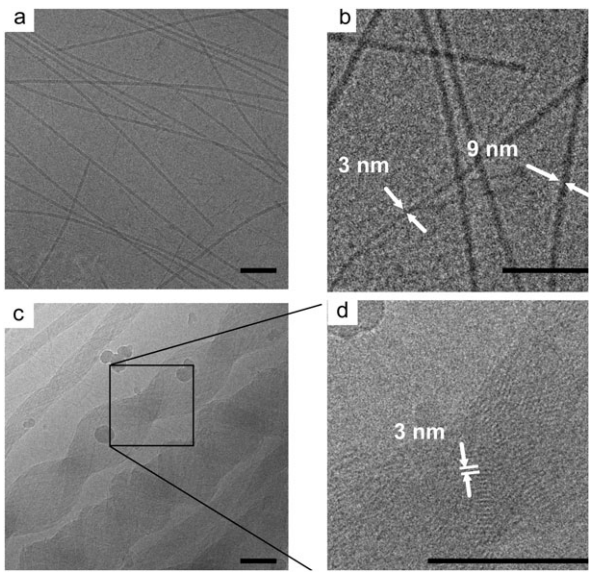

Fig. 2 Cryo-TEM images of (a) fibrous network of **3** at 0.25 mM displaying fibers with a diameter of 9 nm; (b) magnification of **3** at 0.25 mM displaying both 9 nm fibers and 3 nm fibrils; (c) fibrous network of **3** at a concentration above 0.25 mM. Polydisperse twisted tapes of 50 to 200 nm in diameter are formed; (d) magnification of Fig. 3c showing substructures consisting of fibrils of 3 nm in diameter. Scale bar: 100 nm.

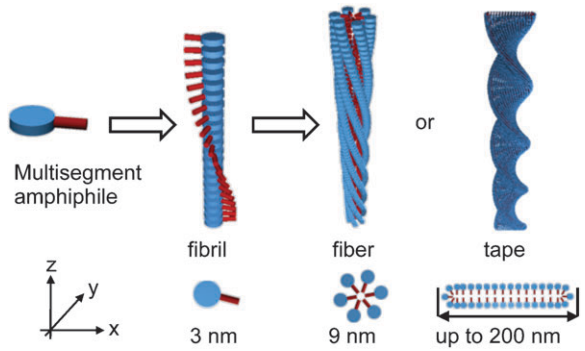

Fig. 3 Schematic representation of the self-assembly of **3** into fibril, hydrogen bonded in the *z*-direction. Additionally, due to hydrophobic interactions between the surfactant segments, 5 to 7 fibrils assemble into 9 nm fibers or up to 200 nm tapes depending on the concentration.

approximately 3 nm was observed (Fig. 2b). Increasing the concentration of **3** to above 0.25 mM resulted in the gradual appearance of polydisperse, twisted tapes with diameter ranging from 50–200 nm (Fig. 2c). At higher magnifications the tapes showed a periodic fine structure of stripes parallel to the long fiber axis, with a spacing of ~3 nm (Fig. 2d), similar to the diameter of the thinner fibrils observed at concentrations below 0.25 mM. Most likely, both the 9 nm fibers and the wider, twisted tapes are superstructures of the 3 nm fibrils.

Based on these observations a tentative model is proposed for the self-assembly process and resulting aggregate structures formed by multisegment amphiphile **3** (Fig. 3). The smallest observed aggregates are the 3 nm fibrils, which nicely correspond to the diameter of a single discotic molecule of **3**. The high aspect ratio of these fibrils is typical for the morphology of other cyclohexane–trisamide gelators which are known to form extended stacks of hydrogen-bonded moieties on top of each other.[10b] Therefore, the 3 nm fibrils are most likely single stacks of molecules of **3** hydrogen bonded in the *z*-direction.

In an additional step these thinner fibrils associate into parallel bundles corresponding to the 9 nm fibers. A simple geometric model revealed that a 9 nm bundle consists of 5–7 fibrils with a diameter of 3 nm, which most likely are held together by hydrophobic interactions between the aliphatic chains pointing to the center of these cylindrical fibers (ESI†). The overall fibrous morphology is dominated by the highly anisotropic interactions between the gelator segments, but the regular diameter of the 9 nm fibers is reminiscent of the well-defined size of micelles and results from competing interactions including hydrophobic attraction between the alkyl moieties and steric constraints from the gelator segments. At higher concentrations twisted tapes are formed, which are, most likely, not a superstructure of the 9 nm fibers, but also of the 3 nm fibrils. It is not yet clear whether these twisted tapes are formed by a morphological transition from the 9 nm fibrils, or directly from the 3 nm fibrils. The periodic striped pattern observed at higher magnifications is a strong indication that the twisted tapes consisted of a layered structure of 3 nm fibrils, which are also held together by hydrophobic interactions in the plane of the layer. Alternative models for the structure of the twisted tapes remain possible.

In conclusion, starting from a molecular gelator and a surfactant we have been able to develop a so-called multisegment amphiphile, which self-assembles in a cooperative fashion into compartmentalized fibers, showing well-defined structural features at molecular length scales. These fascinating self-assembly properties and the resulting structures originate from orthogonal self-assembly of the parent compounds, spatially constrained by their covalent connection within the multisegment amphiphile. The multisegment approach offers new opportunities for the rational development of novel, molecular based, multicompartment architectures thereby expanding the scope of classic surfactant morphologies. Moreover, by separately addressing the self-assembly processes of the individual segments it will be possible to gain control over morphological transitions and hierarchical structure formation.

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