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Hierarchical self-assembly of noncovalent amphiphiles†

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Amphiphiles defined by noncovalent inclusion complexes between an alkylated β-cyclodextrin and PEG-conjugated guests assemble into higher-ordered structures whose thermodynamic stability reflects that of the defining intermolecular interactions.

The assembly of amphiphiles into *e.g.* micelles, vesicles, or lamellae, is central to many fundamental areas of science, including biological cell structure and function, detergents, and drug delivery.[1] Increasing numbers of synthetic block copolymer amphiphiles have been found to form stable aggregates,[2] the morphologies of which depend on the composition of the hydrophobic and hydrophilic blocks and solution conditions. Recently, Schubert *et al.* reported a metallo-supramolecular approach to block copolymers in which metal–ligand complexes act as linkers between different blocks.[3] The micelles formed from these metallo-supramolecular amphiphiles offer new forms of responsiveness due to the electro- and photochemical properties of the metal–ligand complexes. Rotello's group has reported supramolecular vesicles (≈ 5 μm) formed through self-assembly of random copolymers with complementary interchain hydrogen bonding.[4]

Here we report new, noncovalent amphiphiles and preliminary studies of the interplay between two levels of supramolecular assembly: the intermolecular junction between blocks and the subsequent organization of the amphiphiles. A hydrophilic poly(ethyleneglycol) tail is attached to a guest (**1**, Fig. 1) whose inclusion into β-cyclodextrin (β-CD) unites it with the hydrophobic alkyl tails on **2**. Inclusion complexes of β-CD are well-suited for these studies for two reasons. First, the nature of the hydrophobic guest on **1** determines the thermodynamics of the association.[5–8] Second, upon complexation the variable guest is hidden within the CD and it therefore has at most a minimal effect on the structure of the amphiphile.

For initial studies, hydrophilic block **1a** was obtained by reacting PEG monomethyl ether of the appropriate molecular weight with 1–adamantyl isocyanate. Dihexadecylthio-β-CD **2** was prepared following methods reported by Davis's group.[9] The association constants for β-CD and adamantane derivatives are on the order of 10^4 M^{-1},[5] and inclusion complexes of the two components give rise to an amphiphilic structure in aqueous solution (Fig. 1).

Quasielastic light scattering (QELS, Wyatt Technology) measurements reveal that the amphiphilic structure leads to larger assemblies. The individual components are soluble in water, and both **1a** and **2** (each an amphiphile on its own) aggregate only into relatively small clusters of hydrodynamic radius 70–100 nm. No evidence for larger aggregates was observed by QELS up to concentrations of 3.0mg mL^{-1}. Aqueous suspensions of equimolar **1a·2** behave quite differently. At concentrations up to 0.55 mg mL^{-1}, the QELS results are indistinguishable from those of the individual components. At and above 0.60 mg mL^{-1}, however, the intensity of scattered light abruptly and reproducibly increases to a level that exceeds the detection limits of the QELS photodiodes. Because even higher concentrations of the individual components do not scatter light with the same intensity, the result suggests the formation of larger aggregates formed by a mixture of the two components. The transition is also observed at the same concentration using turbimetric titrations at 700 nm (see ESI†). The behavior is not due to impurities; when solutions of the putative aggregates are diluted to a concentration below 0.6 mg mL^{-1}, the scattering intensity and R_h distribution returns within minutes (the time necessary to introduce a sample in the QELS detector) to that of the individual components. Competitive inhibition also reverses the aggregation. When 5 equivalents of unalkylated β-CD are added to a 1 mg mL^{-1} solution of **1a·2**, the size of the aggregates is similarly reduced to that of the individual components. Thus, for **1a·2**, we assign a critical micelle concentration (CMC) of 0.60 mg mL^{-1} (0.020 mM),[10] above which the accompanying phase transition is dependent upon molecular recognition events between the two constituents.

To test whether the phase behavior reflects the stability of the inclusion complex, hydrophilic **1b** was substituted for **1a**. Molecule **1b** has the same PEG chain as **1a**, but the association constant of the 2-naphthyl end group with β-CD is significantly lower than that of the adamantyl group in **1a** ($K_{ass} = 4 \times 10^2$ *vs.* 10^4 M^{-1}).[5,7] The QELS and turbidimetric titration data again reveal aggregation in **1b·2**, but the CMC occurs at 1.3 mg mL^{-1} (0.061 mM in the individual components), higher than that observed for **1a·2**. The onset of aggregation is consistent with a critical concentration of the noncovalently linked amphiphiles, rather than the individual components. If the equilibrium constants above are operative in

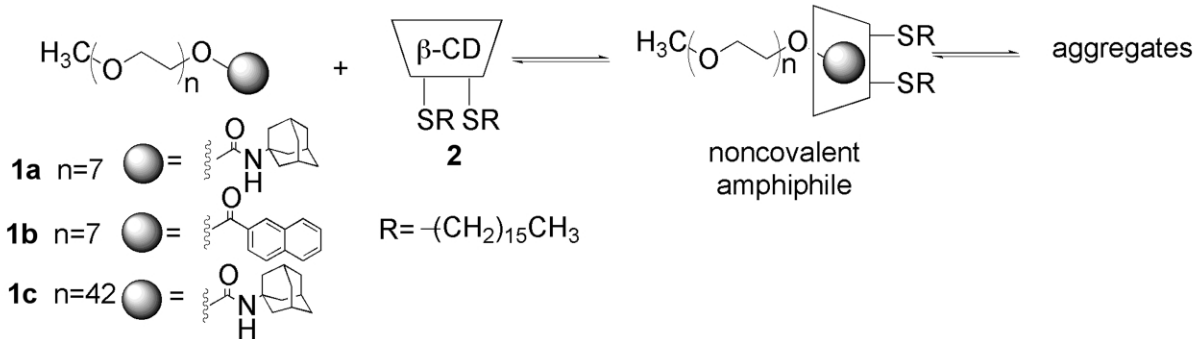

Fig. 1 Schematic representation of noncovalent amphiphiles defined by host–guest inclusion, and the molecular structures of their components.

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† Electronic supplementary information (ESI) available: Synthesis, representative ^1H and ^{13}C NMR, turbidimetric titrations and QELS data. See http://www.rsc.org/suppdata/cc/b4/b405982d/.

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these systems, the expected concentration of dimer is ≈ 0.4 mM for **1a·2** and **1b·2** at their respective CMCs.

The high scattering intensities above the CMC precluded our original plan to use static and dynamic light scattering to characterize the size and shape of the amphiphilic aggregates in solution. The morphologies of the aggregates deposited onto solid supports were explored *via* TEM by using uranyl acetate as a negative stain agent. Fig. 2a shows an image obtained from a 1.0 mg mL^{-1} solution of **1a·2** wick-dried onto 200 mesh fomvar/carbon-coated copper grids. The aggregates are long and pseudo-rectangular, with sharp features and widths of 20–50 nm and lengths of 155–480 nm. A magnified image (Fig. 2b) reveals that the finer structure comprises parallel features of very high aspect ratio that are approximately 6 nm in width. While speculative, it is tempting to ascribe this morphology to cylindrical micelles that are either folded or aggregated on the TEM grid. The extent to which the structure of the surface-deposited aggregates reflects that in solution is not known, but the TEM images are sensitive to the solution concentration of the components. For example, an identical solution at double the concentration (2 mg mL^{-1}) gives larger rectangular aggregates, as shown in Fig. 2c. These well-structured aggregates are not observed from solutions below the CMC nor from the individual components, and thus the TEM images reflect a history—albeit revisionist—of the solution structure.

The molecular structure also impacts the aggregation structure, as revealed by samples prepared from 1.0 mg mL^{-1} **1c·2**. TEM images of these constructs show a fine, fibrous network (Fig. 2d) that we attribute to the longer hydrophilic tail of **1c·2** *vs.* **1a·2**. A possible mechanism for the morphological change is that there exists greater steric repulsion between the larger PEG tails on the surface of a cylindrical aggregate, leading to greater curvature and a smaller cylinder diameter. Importantly, we note that the CMC for **1c·2** occurs at the same molar concentration as **1a·2** (0.020 mM).

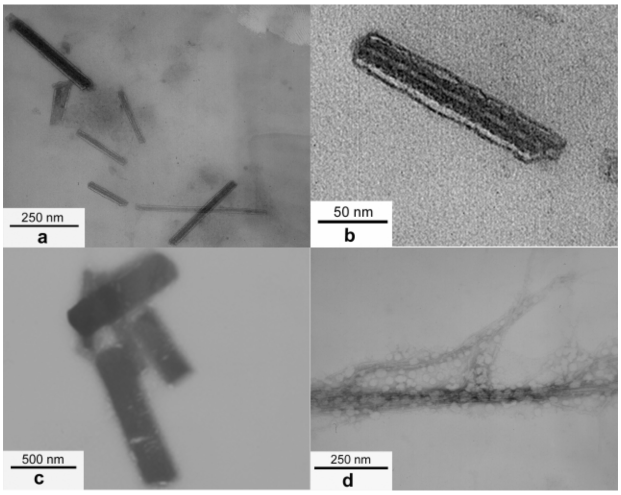

Fig. 2 TEM images of surface-deposited aggregates from noncovalent amphiphiles above their solution CMC. (a) **1a·2**, 1.0 mg mL^{-1}; (b) **1a·2**, 1.0 mg mL^{-1} magnified image; (c) **1a·2**, 2.0 mg mL^{-1}; (d) **1c·2**, 1.0 mg mL^{-1}.

In conclusion, we synthesized hydrophobic and hydrophilic molecules containing host and guest functional groups. Inclusion complexes provide a relatively weak, reversible linkage that defines a supramolecular amphiphile. Above a critical concentration, those amphiphiles self-assemble further into large aggregates that are responsive to their environment through the defining molecular recognition event. Evidence of this responsiveness is seen through competition experiments and the dependence of the critical concentration on the thermodynamic stability of the inclusion complex. Hierarchical structures of this type might be especially sensitive to environment, insofar as cooperativity exists between the two stages of assembly. The system described provides a mechanism through which to further investigate how molecular thermodynamics and structure influence the phase behavior of the aggregates.

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10 We use the term micelle generally, to imply an aggregated phase rather than a specific structure.