

Influence of Backbone Conformational Rigidity in Temperature-Sensitive Amphiphilic Supramolecular Assemblies

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S Supporting Information

ABSTRACT: Molecular design features that endow amphiphilic supramolecular assemblies with a unique temperature-sensitive transition have been investigated. We find that conformational rigidity in the backbone is an important feature for eliciting this feature. We also find that intramolecular hydrogen-bonding can induce such rigidity in amphiphile backbone. Guest encapsulation stability of these assemblies was found to be significantly altered within a narrow temperature window, which correlates with the temperature-sensitive size transition of the molecular assembly. Molecular design principles demonstrated here could have broad implications in developing future temperature-responsive systems.

Stimuli-responsive systems have garnered significant attention due to their utility in several applications, such as drug delivery, sensing, tissue engineering, and diagnostics.¹ Self-assembling systems such as micelles and liposomes have been quite popular in this context, as the morphology of these assemblies can provide an observable response to a specific environmental change.^{1a,2} Among various stimuli, temperature has been a stimulus of interest, where the thermo-responsive components of the assembly undergo a phase transition at a specific temperature, commonly referred to as cloud point or lower critical solution temperature (LCST).³ Among thermo-sensitive components, oligo(ethylene glycol) (OEG)- and poly(ethylene glycol) (PEG)-based systems have been subjects of exploration in recent years.⁴ The LCST of OEG-containing amphiphilic assemblies is the result of the fact that OEG units are hydrophilic, because they hydrogen-bond with water molecules.⁵ When the temperature of the solution is increased, the OEG units become hydrophobic because of the temperature-induced decrease in hydrogen-bonding. This change in the hydrophilicity of the molecule is the reason for the observed LCST transitions. The structure–property correlations studied with temperature-sensitive polymers are most often studied as a phenomenon that involves a noticeable macroscopic phase change at higher temperatures.³ It has been recognized only recently that there exists an interesting morphological transition in these amphiphilic aggregates at temperatures below the typical LCST, the so-called sub-LCST transition.⁶ In our efforts to understand the underlying structural requirements that endow molecules with sub-LCST characteristics (Figure 1), we have uncovered an interesting effect of the shape of amphiphiles on their aggregation state and

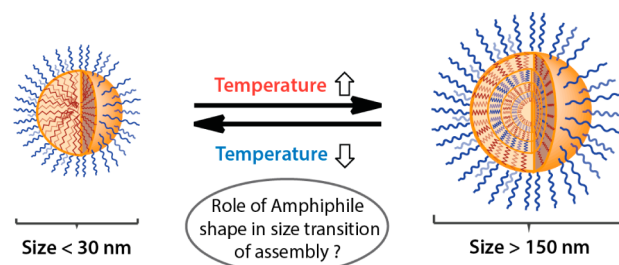
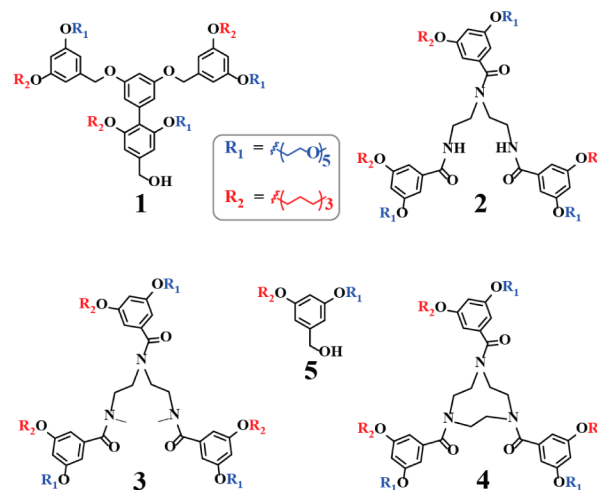


Figure 1. Schematic representation of temperature-dependent size transition of amphiphilic assemblies, and the potential role of amphiphile shape in this phenomenon.

Scheme 1. Structures of the Amphiphiles Used in This Study



their temperature-sensitive behavior. We disclose these preliminary findings in this Communication.

The molecule in which the sub-LCST phenomenon was previously observed is shown as structure 1 in Scheme 1.⁶ We started with a simple hypothesis that the presence of OEG moieties in molecule 1 and its ability to self-assemble to form amphiphilic aggregates are the key criteria for the observed sub-LCST behavior. If this were the case, then other ethylene glycol-based amphiphiles, which do form amphiphilic assemblies in water, should also exhibit similar features. We tested this hypothesis by studying several PEG-containing amphiphiles: Tween-20, Brij-35, Triton-X-100, and Brij-L4. A simple

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size analysis of these assemblies at different temperatures suggests that these molecules do not have the propensity to exhibit a sub-LCST transition (Figure S1), akin to that observed with molecule 1. These results suggest that although the sub-LCST behavior is indeed a temperature-responsive event, the presence of PEG moieties in the amphiphile alone is not a sufficient criterion for this phenomenon.

Next, we tested the possibility that it is the functional group composition inherent to molecule 1 (e.g., pentaethylene glycol and decyl unit as the amphiphile combination) that dictates this behavior. We have already shown that the second generation dendron with the same amphiphilic units does not exhibit this behavior.⁶ We attributed this observation to a frozen aggregate formed by the higher generation amphiphile. To fully test this possibility, however, it is essential that we also test the lower generation amphiphile containing the same amphiphilic units. Accordingly, we synthesized molecule 5, which exhibited an LCST phase transition at ~ 35 °C, but no sub-LCST features were observed in this molecule (Figure S2).

From the observations so far, we next hypothesized that the sub-LCST transition might be associated with the geometry of the functional groups presented, in addition to the presence of thermo-sensitive OEG moieties. Considering this, we next designed and synthesized two linear trimeric amphiphiles 2 and 3, with similar amphiphilic functionalities as 1 (Scheme 1). Our rationale for choosing these two amphiphiles was to test if the assembly properties observed with 1 is due to its trimeric architecture. The difference between 2 and 3 lies simply in the nature of amide backbone. Molecule 2, which is composed of two secondary amides and a tertiary amide, is expected to be slightly more hydrophilic and hence have better water solubility in comparison to molecule 3, which contains three tertiary amides. We envisioned that these two molecules would therefore also help us understand the role of amphiphile's backbone in obtaining the temperature-sensitive assembly characteristics observed with molecule 1. The synthetic scheme and characterization of these amphiphiles are discussed in the Supporting Information (Scheme S1).

We first tested the LCST of amphiphilic assemblies 2 and 3 by monitoring the turbidity with temperature (Figure S3) and found both their LCSTs to be ~ 45 °C. In addition, we also noticed that molecule 2 assembly showed a small change in turbidity at ~ 25 °C, well before reaching its LCST (Figure S3a). This indicated that there is a possible transition in the nanoscale assembly at temperatures below LCST. To understand this further, the size of these assemblies was measured at different temperatures using dynamic light scattering (DLS). Indeed, we found that the assembly from molecule 2 showed temperature-dependent size transition at ~ 25 °C, consistent with the slight change in turbidity observed during the LCST measurements (Figure 2a,b and Figure S4).

It is also noteworthy that the size transition observed with 2 is very sharp (± 2 °C) and reversible (Figure S5), underlining the utility of such property in thermoresponsive applications. Interestingly, on the other hand, assemblies from molecule 3 did not exhibit any sharp size transition (Figure 2c). The size of the assembly changed from about 18 nm at 40 °C to 7 nm at 15 °C. But, these size changes are not sharp at any particular temperature; they are rather small and systematic changes. Since both 2 and 3 are trimeric amphiphiles with very similar structural features, the stark contrast in their assembly properties was indeed surprising. A simple-minded conclusion

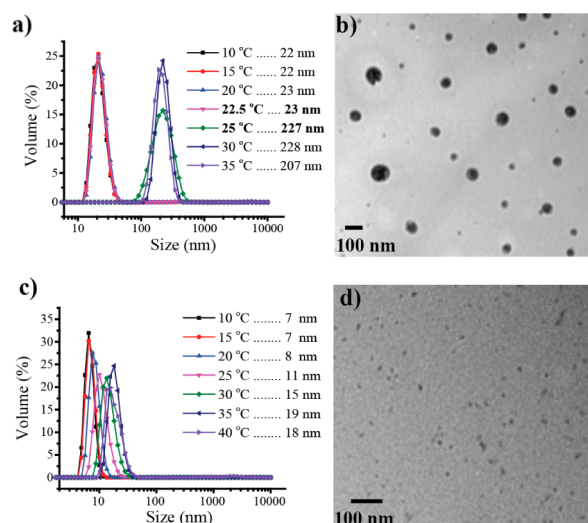


Figure 2. Temperature-dependent size variation as observed with dynamic light scattering (DLS) of (a) molecule 2 and (c) molecule 3. Corresponding TEM images of (b) 2 and (d) 3 assemblies at 25 °C, indicating formation of spherical assemblies.

here is that the sub-LCST behavior is not applicable to all trimeric amphiphiles.

Realizing that the only difference in molecular designs of 2 and 3 is the amide backbone, we were interested in investigating its role. Our initial hypothesis of the difference in hydrophilicity of the amide backbones could not explain this sub-LCST observation as molecule 3 (less hydrophilic backbone) surprisingly had much better solubility than molecule 2 at room temperature. Since the presence of secondary amides in 2 is not contributing to its solubility in water, we hypothesized a scenario where the molecule 2 is stabilized by intramolecular hydrogen-bonding between the two secondary amides at the termini, as shown in Figure 3. The

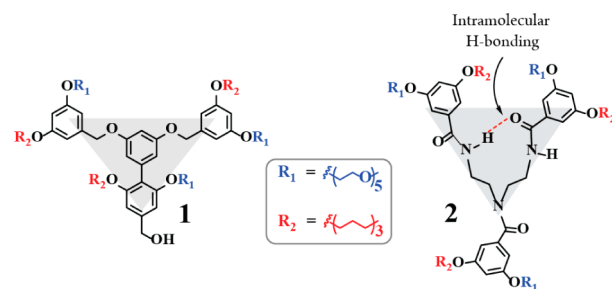


Figure 3. Similarity in the shape of dendron amphiphile 1, and hypothetical representation of hydrogen-bond-stabilized trimer 2.

reason for this hypothesis stems from the possibility that such a conformation would have molecule 2 assume a shape similar to that of molecule 1 (Figure 3), which could be the reason for the similarity in their temperature-sensitive assembly features. On the other hand, such a conformational stabilization is not possible in molecule 3 due to the lack of hydrogen-bonding protons in its backbone, which could explain the deviation of its assembly properties.

Since all these studies are carried out in water, it is reasonable to question the existence of such intramolecular hydrogen-bonding as water (bulk solvent) can effectively compete for hydrogen-bonding. However, there are two reasons for our

assertion on the existence of such a possibility: (i) Note that our initial assembly size is >200 nm. Considering that the observed aggregates are solid spheres and not water-filled (as observed from TEM in Figure 2 and hydrophobic guest incorporation in Figure S6), these complex micelle-like aggregates have a high percentage of the amphiphiles within their solvent-excluded interior. This water-excluded environment is conducive for intramolecular hydrogen-bond formation in natural and artificial systems.⁷ (ii) Amide–amide hydrogen-bond strengths have been found to be comparable or greater than amide–water hydrogen-bond strengths.⁸ These factors prompted us to first investigate the possibility of intramolecular hydrogen-bonding in **2**. However, this proved to be hard to decipher, because molecule **2** exists as nanoscopic assembly in water and NMR peaks were not easily discernible. It is understandable that the buried amides in a large aggregate are particularly difficult to visualize, because of low segmental mobility.

We were, however, interested in evaluating whether molecule **2** is capable of intramolecular hydrogen-bonding. We utilized a nonpolar solvent for this purpose, as the internal environment of the assembly is nonpolar. Thus, we studied the hydrogen-bonding possibilities in **2** in CD_2Cl_2 using NMR. The ^1H NMR spectra of molecule **2** showed a downfield shift of amide protons clearly indicating the presence of hydrogen-bonding. To decipher if this is due to intramolecular hydrogen-bonding rather than the possible intermolecular hydrogen-bonding, we monitored the chemical shifts of amide protons at several concentrations. We found that the hydrogen-bonding in molecule **2** is indeed intramolecular, as the chemical shifts of the amide protons were independent of concentration change (Figure S7).

If the intramolecular hydrogen-bonding were indeed present in **2**, the molecule is likely to be conformationally more rigid and thus be similar to **1** in the relative placements of the amphiphilic moieties within the trimer (Figure 3). To test this hypothesis, we designed and synthesized molecule **4**, which should have similar amphiphile shape as the conformationally rigid version of **2**, but is structurally similar to **3** in that it does not have any secondary amides (Figure 4). In fact, the only

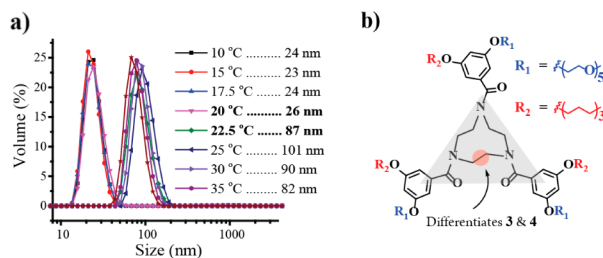


Figure 4. (a) Temperature-dependent size variation of molecule **4** assembly, from DLS. (b) Cyclic trimer **4** depicted with shape similar to **1** and **2**.

difference between these molecules is that the terminal methyl groups of **3** have been tied together to form **4**. Interestingly, size characterization of molecule **4** at different temperatures by DLS has revealed that this molecule also exhibits a rather drastic and sharp size transition feature at ~ 23 °C (Figure 4) akin to molecule **2**. Considering that **4** is structurally similar to **3**, but conformationally similar to the proposed intramolecularly hydrogen-bonded **2**, these results signify the importance of conformational rigidity for the observed sub-LCST transition.

Finally, we were interested in understanding the implications of the sub-LCST behavior exhibited by **2** and **4** on the encapsulation stabilities of these assemblies. We have done this by analyzing the exchange dynamics of encapsulated guest molecules at different temperatures, using a recently reported fluorescence resonance energy transfer (FRET)-based method.⁹ Briefly, poor encapsulation stability should result in a dynamic exchange of encapsulated guest molecules, and therefore an exponential FRET evolution; in contrast, there should be minimal or no evolution of FRET with time for assemblies with good encapsulation stability. We observed that both **2** and **4** assemblies show stable encapsulation at higher temperatures (Figure 5). However, significant increase in guest exchange was

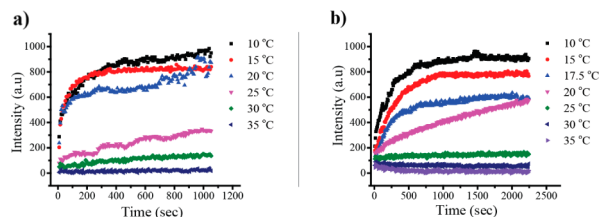


Figure 5. Guest exchange dynamics as a function of temperature in (a) trimer **2** and (b) cyclic trimer **4** assemblies.

observed when the temperature approached their corresponding size transition points, 25 and 23 °C for **2** and **4** respectively (Figure 5). At first sight, this seems counterintuitive that the guest exchange dynamics is faster at lower temperature. Note however that these results simply suggest that the smaller supramolecular assembly below the sub-LCST transition is a poorer host for guest encapsulation compared to the larger supramolecular assembly observed at higher temperatures. Since these are two different assemblies, the observations do not violate any laws of thermodynamics.

We interrogated molecule **3** for encapsulation stability as well. Note that there were small size changes with temperature in molecule **3** as well, which we dismissed as relatively insignificant. However, if there were any significant guest encapsulation differences with this molecule at different temperatures, these small changes can be significant. We found that assemblies from **3** exhibited spontaneous guest exchange irrespective of temperature as shown in Figure S8 (i.e., poor encapsulation stability at all temperatures). These results support our earlier hypotheses.

In summary, we have investigated the structural requirements for oligomeric amphiphiles to exhibit a unique temperature-sensitive transition below its LCST (sub-LCST). We find that the mere presence of a temperature-responsive oligoethylene (or polyethylene) glycol unit does not endow molecules with sub-LCST behavior. Rigidity in the amphiphile backbone was found to be an essential criterion, in addition to the presence of OEG moieties. We also found that intramolecular hydrogen-bonding within the amphiphile can induce such rigidity in the amphiphile backbone. The guest encapsulation property of these assemblies was found to be significantly altered as a result of temperature-responsive size change. We show that excellent guest encapsulation stability can be achieved at higher temperatures in assemblies from more rigidified amphiphiles. The reversibility and sharpness of the size change illustrate the applicability of this phenomenon in many temperature-responsive applications. The supramolecular design parameters studied here can be translated into more robust and

biodegradable systems, which is one of the current foci in our laboratories.

■ ASSOCIATED CONTENT

📄 Supporting Information

Synthesis, characterization, and experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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