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Patchy supramolecules as versatile tools to probe hydrophobicity in nanoglobular systems

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Abstract

We describe precise supramolecules that enable evaluating the effective hydrophobicity of amphiphilic or “patchy” nanoglobular systems. These supramolecules exhibit the Lower Critical Solution Temperature (LCST) phenomenon, which provides a quantitative measure of their effective hydrophobicity. Specifically, two isomeric 8-aryl-2'-deoxyguanosine derivatives, with a transposed pair of methylene groups, self-assemble into hexadecameric nanoglobular supramolecular G-quadruplexes (SGQs) showing large differences in their transition temperatures as determined by turbidity and differential scanning calorimetry studies. Molecular modeling studies suggest that the differential clustering of the hydrophobic patches on the surface is responsible for the striking differences between the two isomeric supramolecules.

Keywords

LCST; hydrophobicity; self-assembly; supramolecular

The juxtaposition of hydrophobic and hydrophilic regions (patches) on the surface of proteins play a pivotal role in both health and disease.¹ For example, hydrophobic patches on the surface of proteins mediate protein-protein interactions and have provided a means for the evolution of multimeric systems.² Conversely, the emergence of a hydrophobic patch on a mutant hemoglobin results in a detrimental polymerization leading to sickle cell anemia.³ Yet, despite its long and interesting history,⁴ in addition to recent seminal findings,⁵ there are still critical details to be elucidated regarding the hydrophobic effect; like in the context of the rough amphiphilic (patchy) surfaces on precise nanoglobular systems such as many soluble proteins.⁶

The development of model systems with patchy or amphiphilic surfaces has the potential of clarifying some of those details, in addition to enabling technological applications.⁷ A number of model systems such as polymers or micelles could be envisioned to address this challenge. They have, however, multiple limitations such as their polydispersity and the difficulty of precisely controlling their composition and structures. Here we describe a family of hexadecameric nanoglobular supramolecular G-quadruplexes (SGQs) with precise structures and amphiphilic ‘patchy’ surfaces that offer a complement to polymeric systems (Figure 1).⁸ These systems are thermoresponsive (i.e., showing the lower critical solution temperature or LCST phenomenon), which gives us a quantitative measure of their effective hydrophobicity.⁹ We demonstrate that the distribution of the patches can in fact be used to

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ASSOCIATED CONTENT

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modulate the transition temperature for the onset of the LCST phenomenon, thus, their hydrophobicity.

Compounds **1**, **2**, and **3** were prepared using the methodology described earlier for this family of compounds (Figure S1).^{11–13} In aqueous phosphate-buffered solutions (pH 7.4, 2 M KI) all three compounds (10 mM) showed signature peaks in the ¹H NMR spectra confirming the formation of the corresponding hexadecamers **1**₁₆, **2**₁₆, and **3**₁₆ (Figures S18–20).¹³ Turbidity experiments (transmittance at 500 nm) confirmed the thermoresponsive behavior of these SGQs, and provided the first indication that the different patchy surfaces indeed impact their effective hydrophobicity (Figure 2). The cloud point temperature (T_{cp}) for **1**₁₆ is 10 °C, while for its isomer **3**₁₆ is 32 °C, and for **2**₁₆ is 60 °C.¹⁴ These results suggest that **1**₁₆ is substantially more hydrophobic than **3**₁₆ ($\Delta T_l \sim 22$ °C), while **2**₁₆ appears to be the least hydrophobic of all.¹⁵ The turbidity above T_{cp} results from the formation of a colloidal suspension of microglobules, which dynamic light scattering (DLS) indicated have average hydrodynamic diameters (D_H) in the range between 4.5–21.7 μ m (Figures S25–S26).^{11, 16}

Differential scanning calorimetry (DSC) experiments provided further support to the turbidity measurements while revealing additional data associated with the thermodynamic parameters of these SGQs (Figures S23–S25).¹³ After data deconvolution, the DSC endotherms revealed two different processes: the transition temperature (T_l) corresponding to the LCST and, at higher temperatures, the melting temperature (T_m) of the SGQs within the microglobules (Figures S23–S25).¹³ The endotherms for **1**₁₆ and **3**₁₆ show maxima corresponding (respectively) to T_l values of 6.4 °C and 56.2 °C ($\Delta T_l \sim 50$ °C), while for **2**₁₆ (with two less methylene groups per subunit) shows a T_l of ~ 55.0 °C (Table 1). Furthermore, these measurements reveal these systems have melting temperatures (T_m) in the range between 59–70 °C, with **1**₁₆ being the least thermally stable of the three assemblies. This is likely the result of its larger steric bulk imposed by the butoxy group near the core of the assembly (versus ethoxy in **2** and **3**; Figure 1).

Higher T_l values relate to the energy required to expel the associated water molecules, while the corresponding enthalpy indicates the number of water molecules associated within the hydration shells. For example, the fact that there are less hydration water molecules associated with **1**₁₆ is reflected in the lower magnitude of the enthalpy for the process (0.75 Kcal/mol) when compared to the corresponding values for **2**₁₆ and **3**₁₆ (~ 12 Kcal/mol) (Figure 2 and Table 1). The apparent large discrepancy between the onset of turbidity (T_{cp}) and the maximum of the DSC peak (T_l) for **3**₁₆ results from an athermal association process between the nanoglobular assemblies, which is likely mediated by interactions between the peripheral hydroxyl groups and water molecules. This phenomenon is similar to the one reported by Winnik *et al.*¹⁷ using telechelic PNIPAM derivatives that form micellar assemblies. According to Winnik, this water mediated association (of their micelles, in their case) leads to globules that are large enough to scatter light (thus the onset of turbidity), but since it does not involve the partition of the hydration water molecules to the bulk, it seems to be below the detection limit of the calorimeter. The fact that this occurs exclusively with **3**₁₆ (since for the other two assemblies the onset of turbidity occurs slightly above the T_l) will be the subject of future molecular dynamics studies aimed at illuminating this particular phenomenon.

Molecular modeling studies of these SGQs provide vital clues to help explain why **1**₁₆ shows a significantly higher hydrophobicity than its isomer **3**₁₆ (Figure 1). The former shows clusters of patches concentrated around the core's surface, while the latter presents a more scattered distribution of such patches. These smaller and more distributed patches on the surface of **3**₁₆ can be accommodated in the tetrahedral water network with minimal

disruption of the hydrogen bonds. Interestingly, this remodeling of the surface of **3**₁₆ patches has an effect very similar to the actual removal of the hydrophobic patches (**2**₁₆). While the distribution of patches enables the formation of clusters of **3**₁₆ at lower temperatures (i.e., lower T_{cp}) relative to **2**₁₆, the former shows a T_i as well as an enthalpy of the transition very similar to that of the latter (although the sharper endotherm transition for **3**₁₆ (Figure 2) hints to a more cooperative dehydration process). These results underscore the importance of having systems that are modular and molecularly precise, which could enable future molecular dynamics studies to further illuminate these phenomena.

The hydrophobic effect never ceases to provide surprising twists in its manifestations. Although much progress has been made since its first descriptions in the early twentieth century,⁴ it remains a fertile ground for new discoveries and applications. We have demonstrated an additional use for SGQs in the elaboration of useful tools for studying the hydrophobic effect in a precise nanoglobular system. We have shown how the three-dimensional distribution of hydrophobic elements leads to a profound impact on the hydrophobicity of the assembly. We expect these results to enable further studies on the dynamics of water molecules in contact with patchy surfaces of different sizes and shapes, which should complement studies performed with polymers. For example, related systems should enable the evaluation of how the specific nature of the patches (e.g., saturated versus unsaturated patches) in conjunction with their relative sizes and distributions affect the hydrophobicity. We feel that the modularity of the system will aid both experimental and theoretical approaches aimed at answering these and related questions.

Supplementary Material

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Acknowledgments

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12. The monomers **1** and **2** (Figure 1) were synthesized as previously reported for **3** (Ref. 11; Figure S1). All the data for **3**, including its self-assembly and LCST properties, have previously been reported in reference 11b.
13. See Supporting Information.
14. The values of T_f for these SGQs show no significant changes as a function of concentration in the range between 5–10 mM.
15. Cooling all of the SGQs below their respective T_l (**1**₁₆ to 0 °C; **2**₁₆ and **3**₁₆ to 25 °C) reestablishes the initial state of solubility with fairly transparent solutions. Furthermore, the process can be repeated at least seven times for each sample without signs of fatigue as seen in Figures S21–S22 in the Supporting Information.
16. Below the T_l the D_H values are 3.6–4.0 nm (**1**₁₆), 4.0–4.6 nm (**2**₁₆) and 5 nm (**3**₁₆) 5 nm.
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Figure 1.

Kekulé structures for **1**, **2** and **3**. Addition of two methylene groups (red) to **2** (control), produce the structural isomers: **1** with two methylenes in the aryl group and **3** possessing one methylene in each side chain attached to the ribose. Addition of KI in aqueous solution generates self-assembled nanoglobules (**1**₁₆, **2**₁₆, and **3**₁₆) with patchy surfaces. The models on the right represent the solvent-accessible surface area of the SGQs with the methylenes highlighted in red. They were generated using Maestro after minimizations in MacroModel with the AMBER 94 force field.¹⁰

Figure 2. (Top) Turbidity curves (measured at 500 nm) and (Bottom) DSC endotherms for **1**₁₆ (blue), **2**₁₆ (green) and **3**₁₆ (red).¹² The measurements were performed in aqueous phosphate-buffered solution (all at 10 mM in each derivative **1–3**; pH 7.4, 2 M KI).

Table 1Thermodynamic parameters gathered from with DSC measurements for supramolecules **1**₁₆, **2**₁₆, **3**₁₆.

	T_{cp}	T_t	T_m	$\Delta H_{(T_t)}(^{\circ}C) (Kcal/mol)$
1 ₁₆	10	6.35 ± 0.03	59.50 ± 0.03	0.75 ± 0.04
2 ₁₆	60	55.1 ± 0.4	68.4 ± 0.1	12.23 ± 0.5
3 ₁₆	32	56.19 ± 0.06	69.98 ± 0.05	12.4 ± 0.1

 T_{cp} = transition temperature as determined by cloud-point measurements; T_t = transition temperature as determined by DSC