Drug encapsulation within self-assembled microglobules formed by thermoresponsive supramolecules†

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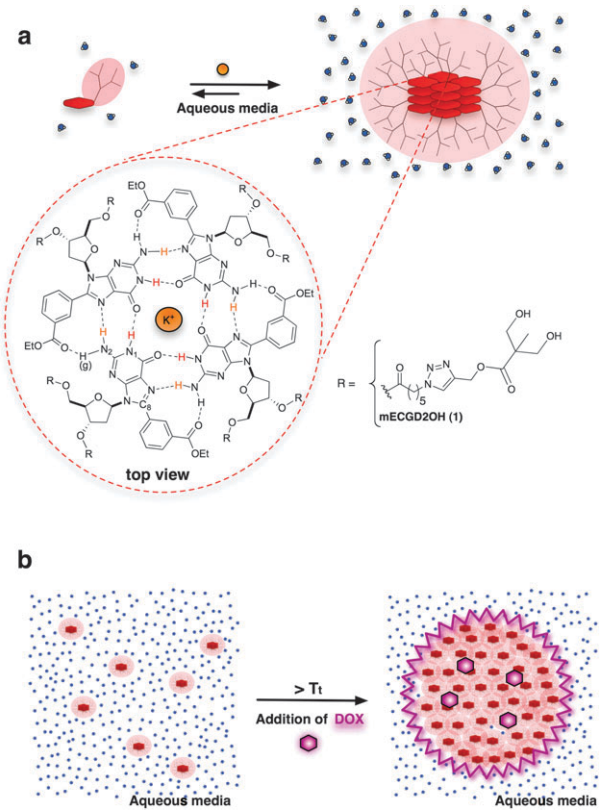
An 8-(phenyl)-2′-deoxyguanosine derivative self-assembles in aqueous media into discrete hexadecamers that further self-assemble above 32 °C into microglobules that encapsulate the drug doxorubicin.

Advances in supramolecular chemistry have led to the design of a variety of biomimetic materials that are suitable for the development of stimuli responsive nanocarrier systems.[1–3] Light, pH, magnetic fields and temperature are among the most frequently used stimuli.[4] Nano- and microscopic globular assemblies made from thermoresponsive polymers are likewise promising systems for responsive drug carriers.[5,6] Besides the elastin-like polypeptides,[7] most of the work in this area has relied on the poly(*N*-isopropylacrylamide)[8] scaffold, and its copolymers, as environmentally responsive materials due to their sharp coil-to-globule transition at biocompatible temperatures of around 32 °C.[9]

Supramolecular self-assembly offers a complementary strategy to the use of polymers for the development of functional nanostructures.[10] To circumvent some of the limitations shown by polymers (*e.g.*, polydispersity, lack of self-correcting synthesis) and to obtain new thermoresponsive scaffolds, recently, our lab developed an 8-(*m*-acetylphenyl)-2′-deoxyguanosine (mAG) derivative that self-assembles in aqueous media into discrete supramolecular hexadecamers that exhibit the Lower Critical Solution Temperature (LCST) phenomenon.[11] Such LCST phenomenon occurs with a transition temperature (T_t) of 58 °C, above which the supramolecular hexadecamers engage in a temperature induced assembly to form solid nanoscopic globules of low polydispersity.[11] We hypothesized that these globules could provide a versatile scaffold for host–guest recognition in aqueous media. Furthermore, if the T_t were reduced to a value closer to and below body temperature, these systems may become suitable to prepare thermoresponsive nano- or microcarriers for bioactive materials such as drugs. Herein, we report our initial attempts towards achieving these goals.

Controlling the T_t *via* intrinsic parameters (*i.e.*, structural information in the building blocks of supramolecules) enables the reliable construction of nanostructures of well-defined size and composition. In recent years we have developed a family of 8-aryl-2′-deoxyguanosine (8ArG) derivatives as versatile recognition motifs for the construction of supramolecular nanostructures in both organic[12,13] and aqueous media[14] (Scheme 1). Our studies suggest that properly placed functional groups can increase the stability and specificity of the resulting G-quadruplex supramolecules by enhancing non-covalent interactions such as hydrogen bonds and π-π.[15] To this end, we synthesized the 8-(*m*-ethoxycarbonylphenyl)-2′-deoxyguanosine (**mECGD2OH**, **1**) derivative, which shows a lower T_t than our previous mAG-based system. This new 8-(*m*-carbonylphenyl)-2′-deoxyguanosine derivative (**1**) was synthesized using a methodology similar to that previously reported by us.[11] The pure derivative **1** was obtained after column chromatography as confirmed by NMR, IR, and mass spectrometry (for details see ESI†, Fig. S3–S4). Monomer **1** is



Scheme 1 (a) Representation for the (a) formation of a hexadecamer by **mECGD2OH** (**1**) in aqueous media; and (b) stimuli-responsive behavior of $\mathbf{1}_{16}$ that enables the encapsulation of doxorubicin·HCl (**DOX**) within the resulting microglobule.

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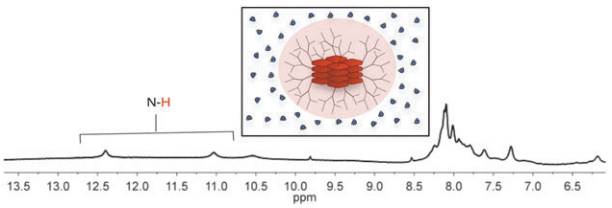

Fig. 1 Partial ^{1}H NMR spectrum showing the region of the N1–H and aromatic signals of $\mathbf{1}_{16}$ (500 MHz, 10% D_2O in phosphate-buffered solution, pH 7.4, 2 M KI). The red cartoon represents $\mathbf{1}_{16}$.

poorly soluble at room temperature (*ca.*, 23 °C) in an aqueous phosphate-buffered solution (pH 7.4), but goes in solution upon addition of KI (2 M). ^{1}H NMR experiments of homogeneous solutions of **1** (10 mM, pH 7.4, 2 M KI) in H_2O–D_2O (9 : 1, potassium buffer) reveal the signatures for its self-assembly. The ^{1}H NMR spectrum shows the characteristic double set of signals corresponding to two pairs of tetrads in different chemical environments (Fig. 1). The 2D NOESY spectrum further supports the formation of $\mathbf{1}_{16}$, by showing the signature cross peaks characteristic of a hexadecamer in water (ESI†, Fig. S6).[14]

The thermoresponsiveness of $\mathbf{1}_{16}$ was determined by transmittance experiments. Aqueous phosphate-buffered solutions (pH 7.4, 2 M KI) of $\mathbf{1}_{16}$ were completely homogeneous and highly soluble at room temperature. The turbidity for solutions of $\mathbf{1}_{16}$ increased upon heating, becoming cloudy at ~32 °C. Transmittance measurements at 500 nm confirm these observations (Fig. 2a). Upon cooling it back to 25 °C, the solution again becomes transparent. This process is reversible and can be repeated at least ten times with no signs of fatigue (*e.g.*, decomposition, irreversible aggregation) (Fig. 2b). Dynamic light scattering (DLS) studies provide essential information regarding the sizes of the various supramolecules as a function of temperature (25–76 °C). Below the T_t, the average hydrodynamic diameter (D_H) for $\mathbf{1}_{16}$ is 5.0 nm (Fig. 2c). Upon reaching T_t, the D_H of the newly formed species in solution increased by four orders of magnitude

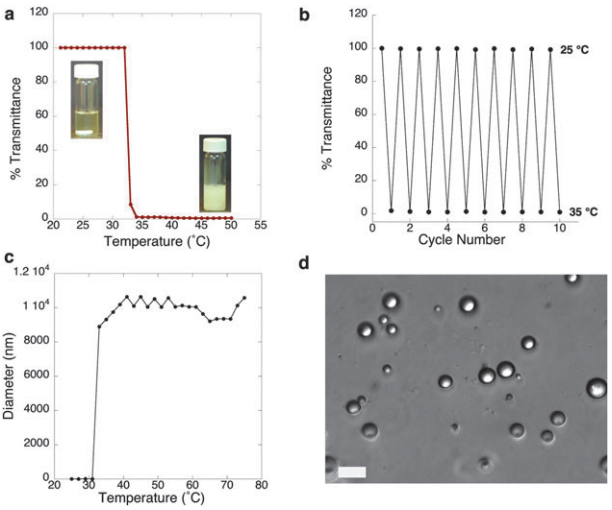

Fig. 2 (a) Turbidity curve (measured at 500 nm) for $\mathbf{1}_{16}$ (red circles). (b) Change in transmittance at 500 nm when alternating the temperature between 25 °C and 35 °C. (c) Average hydrodynamic diameters (D_H) of $\mathbf{1}_{16}$ as a function of temperature measured by DLS. (d) OM micrograph of $\mathbf{1}_{16}$ at 37 °C. All measurements were performed using an aqueous phosphate-buffered solution (pH 7.4, 2 M KI) of **1** (10 mM). The white scale bar represents 20 μm.

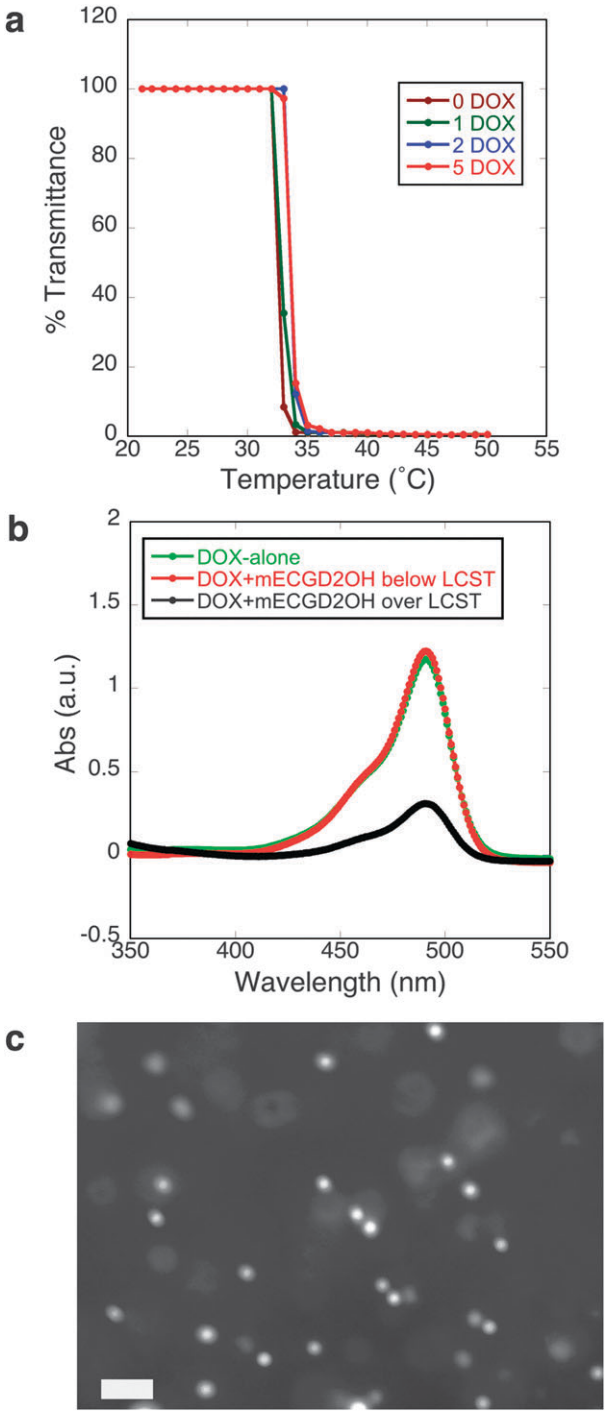

Fig. 3 (a) Effect of increasing the amount of **DOX** in the T_t of $\mathbf{1}_{16}$. (b) Absorption spectra for **DOX** alone (3.13 mM) and $\mathbf{1}_{16}$ (10 mM) with 5 equiv. of **DOX** below (25 °C) and above (40 °C) T_t. The solutions of $\mathbf{1}_{16}$ with **DOX** were incubated (45 min) below or above the T_t and then centrifuged. The supernatant was diluted (800×) with the same buffer (phosphate buffer, pH 7.4, 2 M KI) and the absorption spectra of the diluted solutions were measured. (c) Fluorescence microscopy images of **DOX** encapsulated in the globules at 37 °C. The white scale bar represents 20 μm.

to >10 μm. Continued heating of the solution from 40 to 60 °C leads to a gradual decrease of the D_H until the values stabilized at <9 μm. This behavior is consistent with a steady dehydration of the globules. Conversely, the sudden increase in size above 70 °C and the concomitant increase in polydispersity are likely due to the formation of amorphous aggregates upon the melting of $\mathbf{1}_{16}$ (Fig. S9, ESI†). Optical microscopy (OM), with temperature control (37 °C), showed that these aggregates are discrete microglobules with a relatively uniform distribution of sizes (Fig. 2d).

We next assessed the influence of binding a guest molecule on the thermoresponsive properties from $\mathbf{1}_{16}$. Doxorubicin hydrochloride (**DOX**) was chosen as the guest molecule because of its current use as anticancer agent and its inherent fluorescence properties.[16] Previously, **DOX** has been conjugated to different carrier molecules such as peptides[17] and other macromolecules.[18] Its fluorescence properties has enabled monitoring its distribution, or that of its polymer conjugates, in micelles and even in cells.[19] As shown in Fig. 3a, in the absence of **DOX**, $\mathbf{1}_{16}$ exhibited a T_t of around 32 °C (pH 7.4). However, in the presence of the drug (2 equiv. of **DOX** per hexadecamer) the T_t increases modestly by up to 2 °C. **DOX**, which possesses an anthraquinone moiety, could potentially interact with the core of $\mathbf{1}_{16}$ in a manner reminiscent to that of daunomycin, a similar anthraquinone drug that interacts with G-quadruplex DNA structures.[20] This behavior agrees with our previous report using a hexadecamer related to $\mathbf{1}_{16}$, in which we showed the tuning of the T_t to higher values by its co-assembly with a more hydrophilic derivative.[11] Additional **DOX**, however, does not seem to further increase the T_t, underscoring the potential versatility of this system to encapsulate other drugs with little effect in the thermoresponsive properties.

Sedimentation experiments provide further evidence for the encapsulation of **DOX** within the globules. Host $\mathbf{1}_{16}$ (0.625 mM) was mixed with **DOX** (5 equiv.) in a phosphate solution (pH 7.4, 2 M KI) and incubated (45 min) either below or above the T_t. Afterwards, the solution was centrifuged and the absorbance of the supernatant was measured. The spectra of the solutions of **DOX** alone and with $\mathbf{1}_{16}$, after incubation below the T_t followed by centrifugation, were practically identical (Fig. 3b). In contrast, after incubation above the T_t (40 °C) followed by centrifugation, the supernatant registered a decrease in the absorption from **DOX**. This indicates that the sedimented globules retain the molecules of **DOX**. About 75% of **DOX** was encapsulated within the globules as inferred by the diminished absorbance at 491 nm. Fluorescence microscopy studies are also consistent with the encapsulation of **DOX** within the microglobules (Fig. 3c).

In summary, we have demonstrated that discrete thermosensitive supramolecules assembled from 8ArG derivatives are an attractive and complementary strategy to polymer based systems for drug encapsulation at biocompatible temperatures. The scope and limitations of related systems for the encapsulation of other bioactive molecules are currently underway.

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