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PAPER

An aquatic host–guest complex between a supramolecular G-quadruplex and the anticancer drug doxorubicin†

José M. Rivera,* Mariana Martín-Hidalgo and Jean C. Rivera-Ríos

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We describe the synthesis of a fluorescent deoxyguanosine derivative that co-assembles (in water) with an unlabeled analogue into a heteromeric supramolecular G-quadruplex, which forms a host–guest complex with doxorubicin as evidenced by FRET experiments.

Biological systems from cells to whole organisms remain an open frontier for supramolecular chemistry.[1] Developing effective strategies for interfacing the naturally occurring supramolecules with their synthetic counterparts poses both great challenges and opportunities to improve human health. Two key elements for moving forward are the invention of biocompatible systems with the concomitant development of suitable characterization strategies. For example, while NMR still represents an indispensable tool for the characterization of most supramolecular assemblies in solution, the current trend towards increasingly complex supramolecules requires adopting techniques more commonly used in biochemistry and molecular biology.

The Förster Resonance Energy Transfer (FRET) technique has been used extensively in biophysical studies with a wide variety of biological complexes (*e.g.*, DNA–protein, protein–protein) and for the development of biosensors.[2] In particular, FRET has been used to study the thermal stability[3] of the folding–unfolding of oligonucleotide based (*e.g.*, DNA/RNA) G-quadruplexes (OGQs)[4] made from G-rich sequences in the presence of suitable cations. It has also been used to study their function as host molecules when binding to proteins,[5] and even small molecule ligands.[6]

Although the use of FRET remains somewhat limited in the field of supramolecular chemistry, it has, nonetheless, gained increasing prominence after the pioneering reports by the Rebek group.[7,8] They validated the versatility of the technique for studying the kinetics and thermodynamics of self-assembled heteromeric supramolecular capsules in the presence of suitable guests. These studies relied on the use of labeled assembling subunits (*e.g.*, calixarenes, resorcinarenes) with appropriate donor–acceptor pairs and/or by using suitable fluorescent guests.

In recent years we have developed 8-aryl-2′-deoxyguanosine (8ArG) derivatives that self-assemble into precise supramolecular G-quadruplexes (SGQs) in both organic and aquatic media.[9] SGQs are coaxially stacked planar G-tetramers whose formation is promoted by cations such as potassium.[10] Although NMR spectroscopy continues to be essential for studying SGQs, the need to study these systems at lower concentrations and/or in complex environments (*e.g.*, cells, organisms) moved us to develop alternative characterization strategies. Here we report the results of our first attempts towards this goal where a fluorescent 8ArG derivative (**1**) and the anticancer drug doxorubicin (**DOX**) act, respectively, as a donor–acceptor pair in a FRET process (Fig. 1).

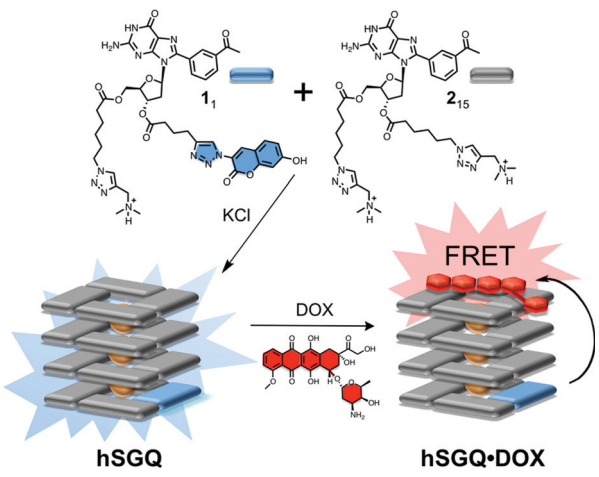

Fig. 1 Top: Kekulé structures of the hydrophilic *d*G derivatives **1** (labeled with a coumarin and represented with a blue rectangle) and **2** (unlabeled and represented with a grey rectangle). Bottom: Cartoon depictions of one of the putative heteromeric hexadecamers (**hSGQ**), obtained after mixing **1** and **2** in the presence of KCl, and its resulting host–guest complex with the anti-cancer drug doxorubicin (**hSGQ·DOX**; the cartoon depicts only one of the possible configurations for the complex).

Department of Chemistry, University of Puerto Rico Río Piedras Campus, San Juan, PR 00931, USA. E-mail: jmrivortz@mac.com

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Our strategy relies on the use of the coumarin-labeled 8-(*m*-acetylphenyl)-2′-*d*G derivative **1** as the donor component. We have shown[9b] that the *m*-carbonylphenyl group is an essential element for the high fidelity formation of hexadecameric SGQs. Of the potential fluorophores available, we selected the coumarin moiety because its small size was expected to minimize non-specific interactions and poor aqueous solubility after its conjugation with the 8ArG fragment. Furthermore, in order to maximize the hydrophilicity, we chose to co-assemble **1** with the unlabeled analogue **2** to form heteromeric supramolecular G-quadruplexes (**hSGQ**) (Fig. 1) with a *putative statistical* distribution of both subunits. The solvent-exposed (outer) G-tetrads provide a suitable platform to interact with planar aromatic molecules, a fact that has been exploited in the development of ligands for OGQs.[4,6,11] Specifically, the Neidle group have reported that the anthraquinone-containing drug daunomycin interacts with OGQs *via* end-stacking interactions.[11] We selected the closely related anthraquinone **DOX** because of the latter precedent and also because its absorbance spectrum (acting as an acceptor) complements very well the emission of the coumarin moiety in **1** (donor), which would enable the desired FRET studies (ESI, Fig. S12–S13†). Molecular modeling reveals that the donor and acceptor groups could coexist at a distance below the limit required (~10 nm) to observe FRET even in a configuration (one of the multiple possible) where the coumarin moiety and the **DOX** are at the opposite ends of the **hSGQ** (Fig. 2c and ESI, Fig. S24†).[11b]

The synthesis of **1** begins with the selective protection of the 5′ hydroxyl group of **3** to generate the *tert*-butyldimethyl silyl ether **4**. Esterification of the 3′ hydroxyl group with 5-hexynoic acid afforded the alkynyl-containing **5**, which was converted to **6** after deprotection and subsequent esterification with 6-bromohexanoic acid. Coupling **6** and 3-azido-7-hydroxycoumarin,[12] using the copper-catalyzed Huisgen 1,3-dipolar cycloaddition ("click") reaction, yields compound **7**. Finally, displacement of the bromine with NaN_3 followed by a second click reaction furnished the desired fluorescent derivative **1** (Scheme 1).

Self-assembly studies were performed by mixing dye **1** (1 equiv.) with **2** (15 equiv.)[9b] in a lithium phosphate buffer (LiPB). As expected, the ^{1}H NMR of **1** shows no detectable discrete assembly since lithium only promotes the formation of loosely bound aggregates (**LBA**) or thermodynamically unstable SGQs (Fig. 2a) (500 MHz, 10% D_2O in LiPB, pH 7.4, 298.2 K).[13] Nevertheless, the simple addition of the anti-cancer drug **DOX** (1 equiv.) seemed to be enough to induce the stabilization of some **LBA** and even a small amount of hexadecamers as evidenced by the appearance of signature peaks (*e.g.*, double sets of N1H peaks; Fig. 2b). Furthermore, those signature pairs of peaks increase significantly (to a total of 65%; Fig. 2c) after the addition of KCl (1 M), indicating the formation of robust and discrete hexadecamers, as previously reported for **2** under similar conditions.[9b]

Photophysical characterization of **1** and **DOX** confirmed that the two compounds could serve as a donor–acceptor pair, due to the excellent overlap between the emission of the former (477 nm) and the absorbance of the latter (490 nm) (ESI, Fig. S12–S13†). FRET titration measurements were performed to study the changes in the emission intensity of the donor (**1**) following the addition of the acceptor (**DOX**). Incremental

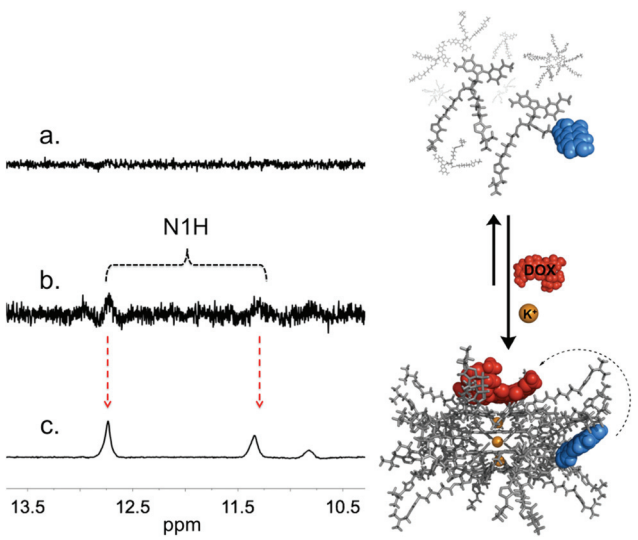
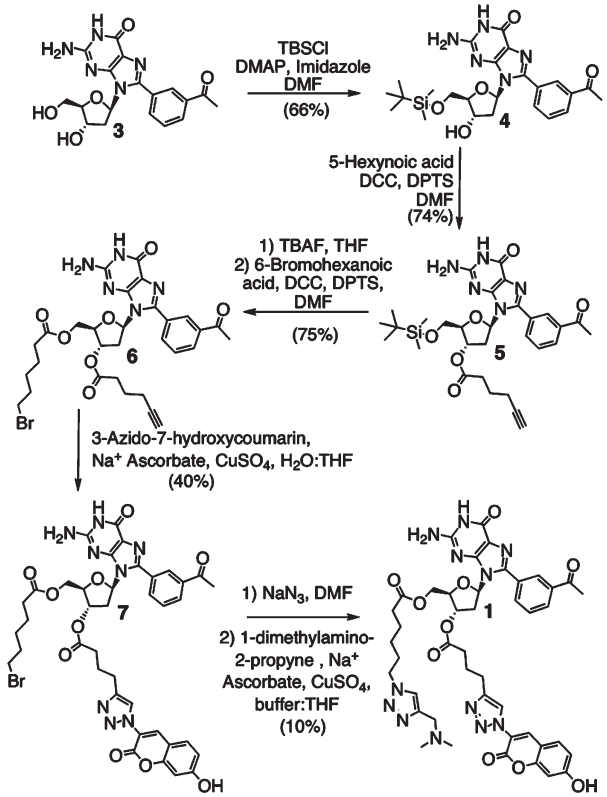


Fig. 2 Partial ^{1}H NMR spectra (500 MHz, 10% D_2O in LiPB, pH 7.4, 298.2 K) of a 5 mM sample with: (a) a mixture of 0.06 equiv. of **1** and 0.94 equiv. of **2**; (b) same as (a) but with 0.06 equiv. of **DOX**, where the N1H peaks (corresponding to the formation of a hexadecamer) begin to show; (c) same as (b) but with 1 M KCl showing a clearly defined double set of N1H signals. The right panel illustrates the equilibrium between (top) monomeric subunits of **1** and **2** and loosely bound aggregates (**LBA**); and (bottom) one of the potential complexes between **DOX** and the various possible heteromeric supramolecular G-quadruplexes (**hSGQ**·**DOX**).



Scheme 1 Synthesis of coumarin labeled *d*G derivative **1**.

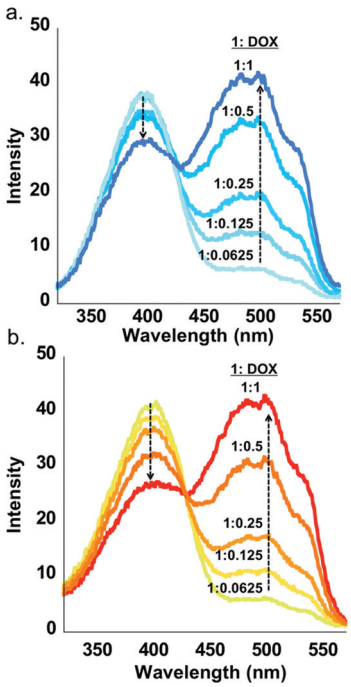


Fig. 3 Overlaid excitation spectra (590 nm) of $\mathbf{1}_1\mathbf{2}_{15}$ (5 mM mixture in a LiPB pH 7.4) with increasing amounts of the acceptor **DOX** showing the quenching of the former (donor) with the concomitant increase in intensity for the latter (acceptor) as indicated by the trend arrows; (a) sample without and (b) with 1 M KCl.

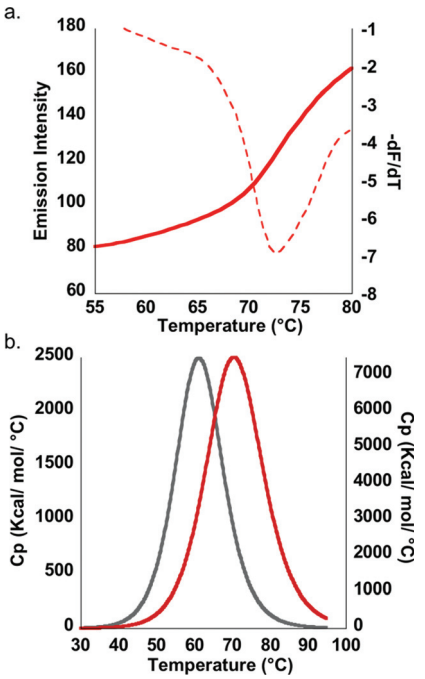


Fig. 4 Melting profiles for **hSGQ·DOX** (LiPB pH 7.4) as determined by (a) variable temperature FRET experiments, monitoring the donor emission at increasing temperatures. The reported T_m is the inflection point in the melting profile as determined by the minimum in the first derivative plot (dashed red line). (b) Overlay of the DSC endotherm for **hSGQ** (grey curve) and for **hSGQ·DOX** (red curve), showing an enhancement in the thermal stability of the hexadecamer when interacting with the drug.

addition of **DOX** (0.0625–2 equiv.) to samples with KCl (**hSGQ·DOX** = $[\mathbf{1}_1\mathbf{2}_{15}{\cdot}3K^+]{\cdot}$**DOX**) and without potassium (**LBA·DOX**) (5 mM, LiPB pH 7.4) showed a linear quenching of the donor emission upon excitation of both samples at 390 nm (ESI, Fig. S15–S16†). The excitation spectra (emission at 590 nm) demonstrate a stronger quenching for **hSGQ·DOX** when compared to **LBA·DOX** (Fig. 3). A plot of the excitation intensity of **1** (390 nm) as a function of the concentration of **DOX** showed a more linear decrease (R^2 = 0.98) and a steeper slope for the sample with KCl than for the sample without it (R^2 = 0.90) (ESI, Graph S4†). A more linear quenching suggests that the addition of **DOX** is the main cause for the quenching of **1** while a less linear quenching is suggestive of other factors such as non-specific aggregation. The steeper slope in the presence of KCl hints at a more efficient energy transfer as would be expected for a well-defined supramolecular complex like **hSGQ·DOX**.

Variable temperature (VT) FRET experiments with **hSGQ·DOX** resulted in a sigmoidal melting profile with a calculated melting temperature (T_m) of 72.5 °C (Fig. 4a).[5] In contrast, similar measurements in the absence of KCl lead to more gradual emission increments of the donor with no significant inflection points (ESI, Fig. S18†). This suggests that the molecules of **DOX** promote the formation of random aggregates in addition to the small amounts of the hexadecamer detected in the NMR experiments (Fig. 2b), even in the absence of KCl. Addition of the potassium salt, as also seen with NMR measurements, displaces the equilibrium further towards the high fidelity formation of hexadecamers (Fig. 2, right panel).

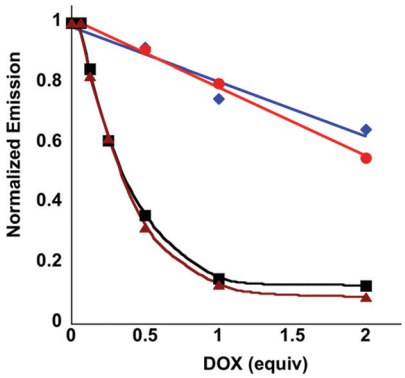


Fig. 5 Normalized fluorescence emission intensity (471 nm) of **8** (0.312 mM) in either LiPB (red circles) or LiPB with additional 1 M KCl (purple diamonds) as a function of the equivalents of added **DOX** (excited at 390 nm). The corresponding solutions of **8** mixed with **1** and **2** (5 mM) show significantly lower emissions in both LiPB (black squares) and LiPB with additional 1 M KCl (brown triangles).

Control experiments were performed in order to evaluate the quenching due to concentration effects. The coumarin derivative **8** (ESI, Fig. S19†)[14] has UV/Vis and fluorescence spectra similar to **1** but does not self-assemble because it *lacks the guanine moiety*. Mixing it with **DOX** under identical conditions to those

used for the aforementioned FRET experiments shows a slight quenching of **8** (Fig. 5) with a slight further quenching after the addition of KCl (ESI, Fig. S20†). In addition, variable temperature experiments for this mixture do not show significant changes in donor emission over the relevant temperature range (ESI, Fig. S21†). These observations reveal that a simple mixture of the donor and acceptor groups is not enough for efficient energy transfer, thus highlighting the essential role of the guanine moiety.

Differential Scanning Calorimetry (DSC) experiments for **hSGQs** reveal an endothermic peak corresponding to a T_m of 61 °C, while similar measurements for the complex **hSGQ·DOX** indicate a significant increase in the melting temperature (ΔT_m = +9 °C; Fig. 4b).[15] DSC measurements lead to a value for the T_m having an excellent correlation with the one determined by the VT FRET experiments. These results underscore that the drug is interacting with and stabilizing the **hSGQ** in a manner reminiscent to that reported for OGQs with small molecule ligands.[4,6,11]

We have recently reported the discovery of an amphiphilic hexadecameric SGQ that showed the phenomenon of thermally induced self-assembly known as Lower Critical Solution Temperature (LCST).[16a] At temperatures above the LCST this SGQ formed microglobules that encapsulated **DOX**, which we demonstrated by sedimentation experiments and fluorescence microscopy.[16b] Up until now, we had little evidence to demonstrate the direct interactions between **DOX** and the SGQs within the microglobules. The results presented here fill that gap, and also open the door to further studies with other biomedically important small molecules or macromolecular guests (*e.g.*, DNA/RNA, proteins), in a manner similar to nucleic acid based supramolecular receptors.[5] The results of such studies will be reported in due course.

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Notes and references

1 J. W. Steed and J. L. Atwood, *Supramolecular Chemistry*, John Wiley & Sons, LTD, Chichester, 2000.
2 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.
3 P. A. Rachwal and K. R. Fox, *Methods*, 2007, **43**, 291–301.
4 S. Neidle and S. Balasubramanian, *Quadruplex Nucleic Acids*, The Royal Society of Chemistry, Cambridge, 2006.
5 D. Margulies and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2009, **48**, 1771–1774.
6 A. Benz, V. Singh, T. U. Mayer and J. S. Hartig, *ChemBioChem*, 2011, **12**, 1422–1426.
7 R. K. Castellano, S. L. Craig, C. Nuckolls and J. Rebek Jr., *J. Am. Chem. Soc.*, 2000, **122**, 7876–7882.
8 E. S. Barrett, T. J. Dale and J. Rebek Jr., *J. Am. Chem. Soc.*, 2007, **129**, 3818–3819.
9 SGQs refer exclusively to homomeric assemblies as the ones described in: (*a*) V. Gubala, J. E. Betancourt and J. M. Rivera, *Org. Lett.*, 2004, **6**, 4735–4738; (*b*) M. García-Arriaga, G. Hobley and J. M. Rivera, *J. Am. Chem. Soc.*, 2008, **130**, 10492–10493; (*c*) J. E. Betancourt and J. M. Rivera, *Org. Lett.*, 2008, **10**, 2287–2290; (*d*) M. d. C. Rivera-Sánchez, I. Andujar-de-Sanctis, M. Garcia-Arriaga, V. Gubala, G. Hobley and J. M. Rivera, *J. Am. Chem. Soc.*, 2009, **131**, 10403–10405; (*e*) L. R. Rivera, J. E. Betancourt and J. M. Rivera, *Langmuir*, 2011, **24**, 1409–1414.
10 (*a*) J. T. Davis and G. P. Spada, *Chem. Soc. Rev.*, 2007, **36**, 296–313; (*b*) J. T. Davis, *Angew. Chem., Int. Ed.*, 2004, **43**, 668–698.
11 (*a*) S. Neidle, *Therapeutic Applications of Quadruplex Nucleic Acids*, Elsevier, 2012, pp. 67–91; (*b*) G. R. Clark, P. D. Pytel, C. J. Squire and S. Neidle, *J. Am. Chem. Soc.*, 2003, **125**, 4066–4067. The crystal structure of the complex daunomycin-d(TGGGGT) was used as the reference to model **DOX** interacting with [$\mathbf{1}_1\mathbf{2}_{15}$·3K$^+$]; see ESI, Fig. S24† for further details.
12 K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill and Q. Wang, *Org. Lett.*, 2004, **24**, 4603–4606.
13 Lithium phosphate buffer was prepared instead of the standard sodium based PBS, because it is known that small cations such as lithium preclude the formation of stable GQs. N. V. Hud and J. Plavec, in *Quadruplex Nucleic Acids*, ed. S. Neidle and S. Balasubramanian, RSCPublishing, Cambridge, 2006, pp. 100–130.
14 V. Hong, S. I. Presolski, C. Ma and M. G. Finn, *Angew. Chem., Int. Ed.*, 2009, **48**, 9879–9883.
15 $\Delta T_m = T_m$ ([$\mathbf{1}_1\mathbf{2}_{15}$·3K$^+$]·**DOX**) − T_m ($\mathbf{1}_1\mathbf{2}_{15}$·3K$^+$).
16 (*a*) J. E. Betancourt and J. M. Rivera, *J. Am. Chem. Soc.*, 2009, **131**, 16666–16668; (*b*) J. E. Betancourt, C. Subramani, J. L. Serrano-Velez, E. Rosa-Molinar, V. M. Rotello and J. M. Rivera, *Chem. Commun.*, 2010, **46**, 8537–8539.