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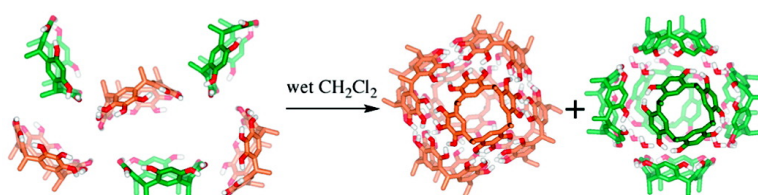# Stability, Dynamics, and Selectivity in the Assembly of Hydrogen-Bonded Hexameric Capsules

Elizabeth S. Barrett, Trevor J. Dale, and Julius Rebek

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Stability, Dynamics, and Selectivity in the Assembly of Hydrogen-Bonded Hexameric Capsules

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Abstract: Fluorescence resonance energy transfer (FRET) was employed to monitor the dynamics of hydrogen-bonded hexameric assemblies formed from resorcin[4]arenes and pyrogallol[4]arenes. Studies were designed to provide further insights into the degree of assembly and stability of these self-assembled capsules at the micro- to nanomolar concentration ranges that are not accessible by NMR studies. The results of this investigation reveal factors that influence the self-assembly of these macrocycles into hexameric capsules. Pyrogallolarenes are very sensitive to the concentration of mixing, with an increase in the equilibration half-life from 36 min at 250 nM to 156 min at 10 μM. The resorcinarenes showed little difference in exchange rates over the same concentration range. The temperature of mixing of the macrocycles was found to be important for both systems with a 12-fold increase in exchange rates over a 20 degree range for the pyrogallolarenes and a 2-fold rate increase for the resorcinarenes over the same temperature range. The stability of the capsules to polar additives such as methanol was probed, with the pyrogallolarenes requiring a higher percentage (1.6% v/v in dichloromethane) of methanol to disassemble the capsules than the resorcinarenes (1.0% v/v in dichloromethane). Pyrogallolarenes assemble in both anhydrous and wet solvents whereas water-saturated solvents are necessary to facilitate the formation of resorcinarene capsules. In addition to these studies, evidence of strict self-sorting in the formation of distinct pyrogallolarene and resorcinarene hexamers was obtained.

Introduction

Resorcin[4]arenes and pyrogallol[4]arenes (Figure 1), spontaneously self-assemble into hydrogen-bonded hexameric capsule hosts when appropriate guests are present. The sizable interior of the hexameric assemblies and applications as nanometric reaction chambers has generated much interest.[1] The ease of synthesis and even commercial availability of the macrocycles offer additional potential in areas such as drug delivery, molecular transport, selective catalysis, and cell mimicry. The most useful synthesis of resorcinarenes was reported by Högberg,[2] while Dalcanale et al.[3] provided the best route to pyrogallolarenes. The macrocycles differ only in the presence of an additional OH on each aromatic ring but assemble similarly in organic media. A seminal paper by MacGillivray and Atwood revealed the structure of a self-assembled hydrogen-bonded capsule in the solid state, comprising six resorcinarene molecules and eight water molecules.[4] Related hexameric assemblies of pyrogallolarenes were crystallized in the absence of water molecules by the groups of Mattay[5] and Atwood.[6] These inspired the studies that have led to the characterization
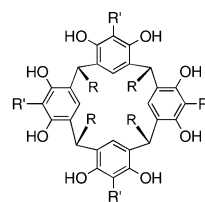
1a R' = H, R = C$_{11}$H$_{23}$ **2a** R' = OH, R = C$_{11}$H$_{23}$

1b R' = H, R = isobutyl   **2b** R' = OH, R = isobutyl

Figure 1. Resorcinarene **1** and pyrogallolarene **2** macrocycles.

of the same hexameric assemblies in common organic solvents by NMR,[7] and even in the gas phase by ESI mass spectrometry.[8] The encapsulation of guest molecules in solution has been extensively investigated[7] and more recently fluorescent guests have been crystallized in the pyrogallolarene hexamer to probe the inner space of the hexameric assemblies,[9,1c] yet little is known about the dynamic behavior of the capsules. While NMR has been the primary tool for the solution-state study of the

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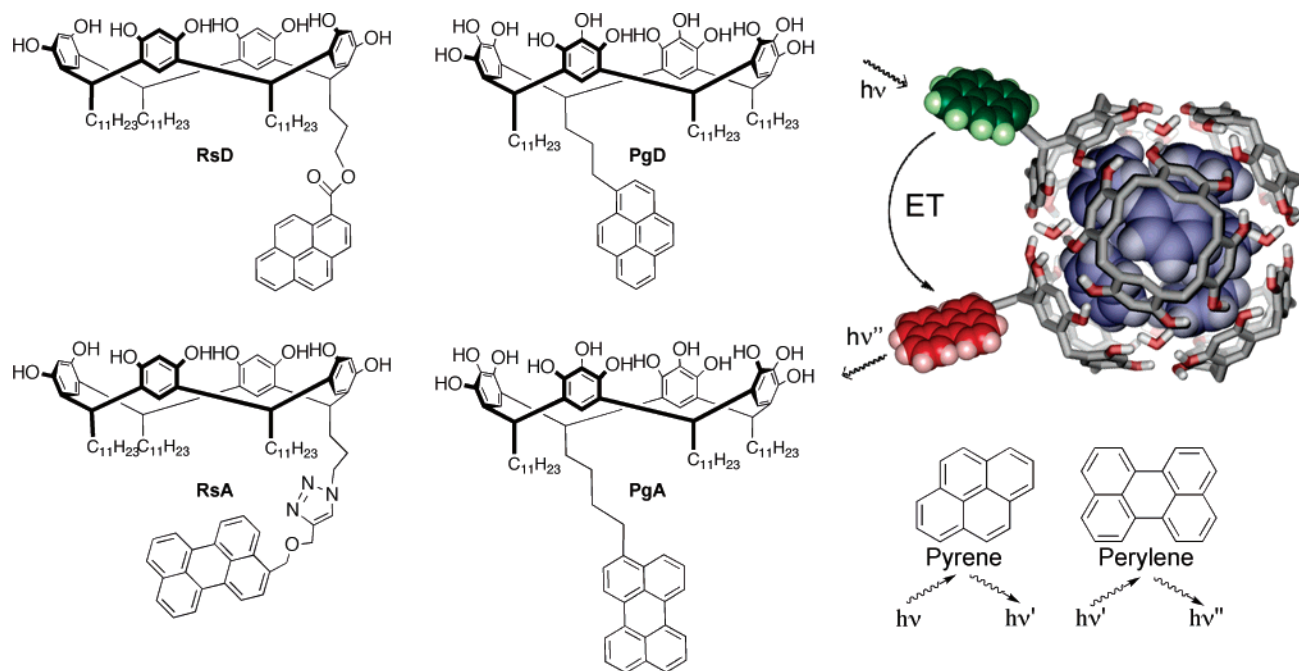
Figure 2. Pyrene donor and perylene acceptor labeled resorcinarenes **RsD** and **RsA** and pyrene donor and perylene acceptor labeled pyrogallolarenes **PgD** and **PgA**.

hexameric assemblies at millimolar concentrations,[7] the assembly dynamics of the hydrogen-bonded capsules are best followed at micro- to nanomolar concentrations. Fluorescence resonance energy transfer (FRET), which has been extensively employed in the study of assembly and dynamic processes in biological systems,[10] has proven to be appropriate for monitoring the dynamics of other self-assembled capsules in this concentration range,[11] and we report here its application to the case of subunits of 1_6 and 2_6. The results of this investigation reveal the factors that influence the dynamic assembly of macrocycles into hexameric capsules. They include the concentration of mixing, the importance of temperature, stability of the capsules to polar additives, and the necessity of water to facilitate the capsule formation.

We recently reported the synthesis of pyrogallolarenes **PgD** and **PgA** and resorcinarenes **RsD** and **RsA** labeled with donor (D) and acceptor (A) fluorophores (Figure 2).[12] There, we presented preliminary results on their use in FRET studies to probe the dynamic behavior of hexameric capsules 1_6 and 2_6. Here we discuss a number of subsequent studies intended to illuminate the degree of assembly and stability of the self-assembled hexameric 1_6 and 2_6 capsules; in addition, we examine the *self-sorting behavior* of resorcinarenes and pyrogallolarenes at the micro- to nanomolar concentration ranges that are not accessible in NMR studies.

Self-assembly implies some level of recognition and selectivity that distinguishes self from nonself. Yet recent studies[13,7g] have shown many multimeric species (including some capsules) strictly self-sort, while others assemble to heteromeric capsules instead.[14] For pyrogallolarenes and resorcinarenes, evidence of strict self-sorting has been shown by NMR at millimolar concentrations,[7f] but heteromeric capsules have been observed in the gas phase by ESI mass spectrometry from dilute solutions.[8] Through the use of the fluorescently labeled monomers and FRET studies, we show strict self-sorting selectivity in the formation of separate pyrogallolarene and resorcinarene hexamers in solution (Figure 3).

Results and Discussion

I. Investigations into the Dynamics of the Pyrogallolarene Hexamer. Effect of Concentration on the Exchange of Pyrogallolarene Subunits. When a donor and an acceptor are

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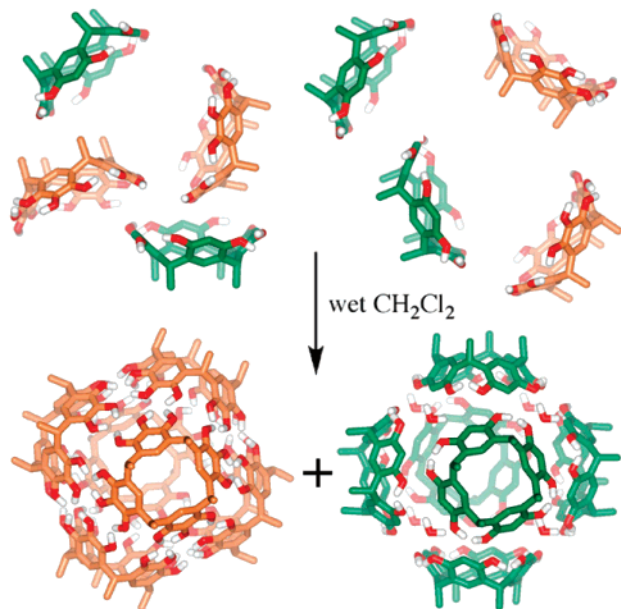
Figure 3. Mixing of pyrogallolarenes **1** (orange) and resorcinarenes **2** (green) results in the formation of two distinct hexamers. Peripheral solubilizing groups (R) and solvent molecules filling the inner space of the hexamers are omitted for clarity.

Figure 4. Development of FRET with time upon mixing of **PgD** and **PgA** solutions at 1 μM in CH_2Cl_2 (times from 0 to 6.5 h): $\lambda_{exc} = 346$ nm. The inset shows first-order kinetic treatment of the data.

Figure 5. Variation in the rate of exchange of pyrogallolarene monomers in the hexameric capsules with different concentrations of donor and acceptor labeled macrocycles **PgD** and **PgA**: $\lambda_{exc} = 346$ nm; $\lambda_{em} = 453$ nm. (Uncertainties in the half-lives are $\pm$ 10%.)

Figure 6. Scaled final FRET levels of the pyrogallolarene solutions in CH_2Cl_2 at equilibrium with different concentrations of donor and acceptor labeled macrocycles **PgD** and **PgA**: $\lambda_{exc} = 346$ nm.

present in the same capsular assembly, FRET is observed on photoexcitation of the donor fluorophore. Our preliminary FRET studies monitoring the dynamics of the assembly of the pyrogallolarene hexamer were conducted at a concentration of 1 μM in dichloromethane.[12a] Pyrene pyrogallolarene **PgD** and perylene pyrogallolarene **PgA** were mixed and a half-life of ca. 60 min was obtained for the solution to reach equilibrium. In diffusion NMR studies of Cohen et al., conducted at millimolar concentrations,[7f] pyrogallolarenes **2a** and **2b** were found to exchange at a slower rate than what we observed for the corresponding labeled pyrogallolarenes **PgD** and **PgA** at the nanomolar concentrations of the fluorescence experiments. To probe these differences we undertook a systematic study into the influence of concentration on the exchange rates of pyro-gallolarene macrocycles. Solutions of the pyrene and perylene labeled pyrogallolarenes **PgD** and **PgA** were mixed at concentrations varying from 250 nM to 10 μM in macrocycle.[12,15] Solutions at each concentration were monitored over time by simultaneously following the donor and acceptor fluorescence intensities until equilibrium was reached, that is until maximum FRET was obtained (Figure 4).

The gradual development of FRET—a decrease in the donor emission and an increase in the acceptor fluorescence—indicates the formation of pyrogallolarene hexamers containing both donor and acceptor labeled macrocycles, that is, exchange of the monomers. These results produced a clear trend, the macrocycles exchanged faster as the concentration was lowered (Figure 5). For the pyrogallolarene hexamers, the half-life for exchange increased from 36 min at 250 nM to 156 min at 10 μM.

In addition to the differences in the rate of exchange of the macrocycles at varying concentration, the final level of FRET achieved provides information about the degree of assembly of the hexamers in dilute solutions. When the final fluorescence
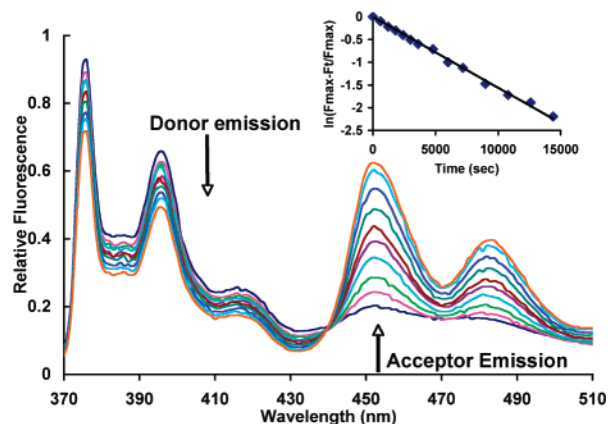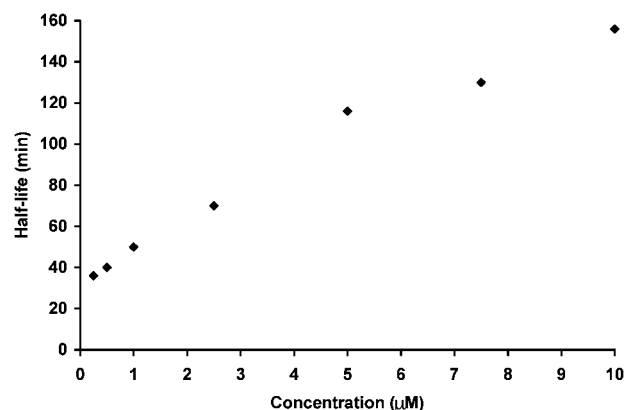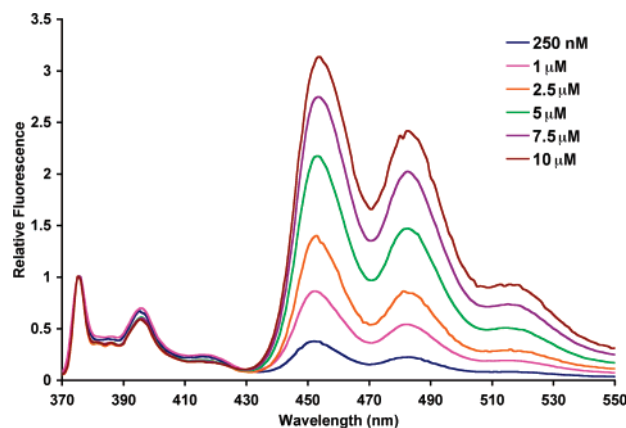
emission spectra for solutions mixed at different concentrations are scaled for comparison, as shown in Figure 6, it is evident that there is a greater amount of energy transfer occurring at higher concentrations.

We propose that the increased exchange rates at low concentration could be attributed to the higher relative concentration of monomeric macrocycles to hexamers in these dilute solutions. A higher proportion of free monomers in solution should lead to a more facile exchange of the subunits in the

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(15) Control dilution experiments have previously been conducted with protected versions of **RsD**, **RsA**, **PgA,** and **PgD** to establish that no intermolecular FRET occurs over this concentration range.
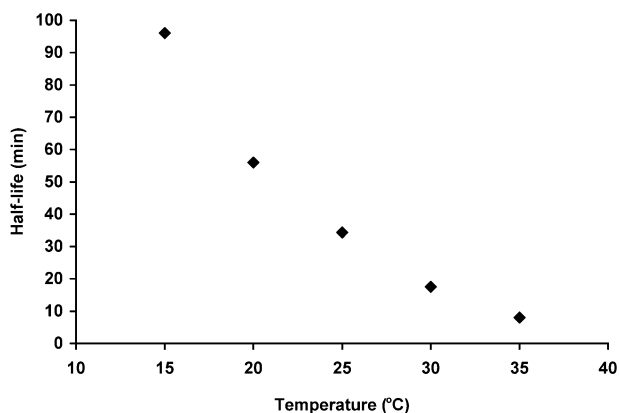
Figure 7. Change in equilibration half-life for 1 μM pyrogallolarene solutions in CH_2Cl_2 containing donor and acceptor labeled macrocycles **PgD** and **PgA** to equilibrate at different temperatures. (Uncertainties in the half-lives are ± 10%.)

hexamers. The lower level of FRET obtained at lower concentrations is also consistent with the hexamers being less assembled, with a higher relative number of free monomeric macrocycles that do not participate in energy transfer.

Importance of Temperature on Rates of Exchange. The influence of temperature on the dynamics of pyrogallolarene hexamer assembly is also relevant to future applications and we varied the temperature of mixing to probe the stability of the hexameric assembly. For the pyrogallolarene system, solutions of **PgD** and **PgA** were mixed at temperatures ranging from 15 to 35 °C and the development of FRET was monitored over time. The first point to note is that dramatic differences in the rates were observed for the solution to reach equilibrium, with a half-life of ca. 96 min at 15 °C compared to ca. 8 min at 35 °C (Figure 7). The faster exchange of the monomeric units is consistent with previous observations by Cohen et al. that heating a mixture of two different pyrogallolarenes accelerated the equilibration of the solution at NMR concentrations.[7f] In addition to the difference in exchange rates, a decreased level of FRET is observed at high temperature, suggesting that the pyrogallolarene hexamers are less assembled (see Supporting Information for details).

These results clearly demonstrate that the pyrogallolarenes exhibit a high level of sensitivity to temperature in relation to both the rate of exchange of the monomeric units and the degree of hexameric assembly.

Stability of the Pyrogallolarene Hexamers to Polar Additives. To further probe the stability of the hexamers under different conditions, titrations with methanol into solutions of the hexamer were performed. The addition of methanol to an equilibrated mixture of pyrene and perylene labeled pyrogallolarenes **PgD** and **PgA** disrupts the hydrogen-bonding network and results in dissociation of the capsule. With these labeled monomers, this is evidenced by a loss of FRET, that is, loss of perylene fluorescence and a concomitant increase in the pyrene fluorescence (Figure 8). By gradually titrating methanol into the equilibrated mixed pyrogallolarene hexamer solutions we can determine the amount of methanol required for complete dissociation to the constituent monomers.

Solutions of pyrogallolarene hexamers containing **PgD** and **PgA** equilibrated at concentrations ranging from 250 nM to 10 μM in donor and acceptor macrocycle were titrated with methanol. As shown in Figure 9 the hexamers tolerated the first
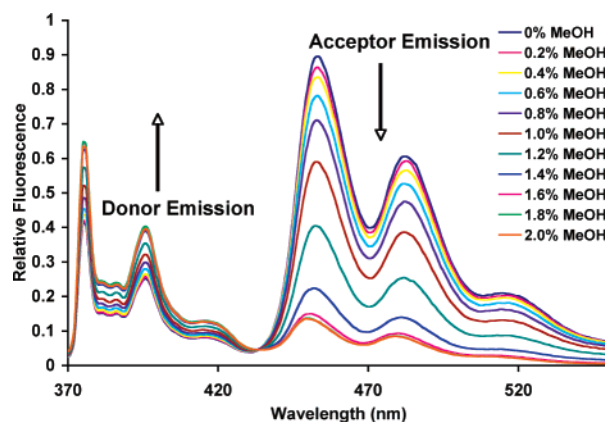

Figure 8. Decrease in FRET of a 5 μM solution of pyrogallolarene hexamer containing **PgD** and **PgA** in CH_2Cl_2 upon titration with methanol.
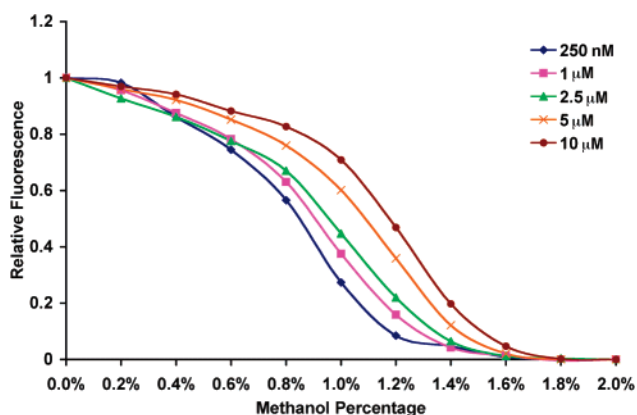

Figure 9. Change in the fluorescence at the perylene maxima upon titration of methanol into the equilibrated solutions of pyrogallolarene hexamers in CH_2Cl_2 with varying concentrations of donor and acceptor labeled macrocycles **PgD** and **PgA**: λ_{exc} = 346 nm; λ_{em} = 453 nm.

ca. 0.4% v/v methanol addition with very little loss of FRET; however, between 1.0% and 1.6% v/v methanol a sharp decrease in the perylene fluorescence was observed as the hexameric capsules dissociated to the monomeric macrocycles. Further addition of methanol has a negligible effect on the fluorescence levels as the capsule is not associated at high concentrations of methanol.[7f] Another fact that arose from these results is that the more dilute solutions required less methanol to dissociate the hexamers to their constituent monomers.

The Role of Water on the Assembly of Pyrogallolarene Hexamers. In contrast to the resorcinarenes it has been shown, both from examination of crystal structures[5] and from diffusion NMR studies,[7d,f] that pyrogallolarenes do not require water to facilitate the assembly of hydrogen-bonded hexamers. Consequently exchange experiments with pyrogallolarenes discussed thus far were conducted in dry dichloromethane. When we investigated the role of water and employed water saturated solvents in place of the dry solvent, a significant increase in the exchange rate for the pyrogallolarene hexamers to reach equilibrium was observed. Furthermore, the level of FRET was noticeably lower in the water saturated solvent, suggesting a smaller degree of hexameric assembly in the presence of water. These results are consistent with a weaker capsule in the presence of water, with water destabilizing the intermolecular hydrogen bonds necessary for the hexamer to assemble. This was further supported by titrating the pyrogallolarene hexamer solutions equilibrated in water saturated solvent with metha-
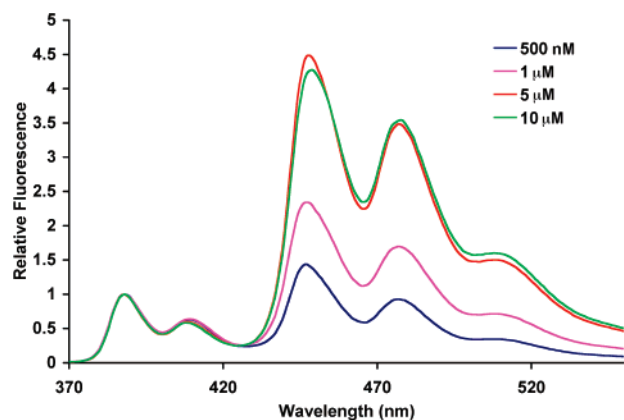
Figure 10. Scaled final FRET level of the resorcinarene solutions in water-saturated CH₂Cl₂ at equilibrium with different concentrations of donor and acceptor labeled macrocycles **RsD** and **RsA**: λ_{exc} = 350 nm.

Figure 11. Change in the fluorescence level at the perylene maxima upon titration of equilibrated solutions of resorcinarene hexamers in water-saturated CH₂Cl₂ with varying concentrations of donor and acceptor labeled macrocycles **RsD** and **RsA** with methanol: λ_{exc} = 350 nm; λ_{em} = 446 nm.

nol: less methanol was required to fully dissociate the capsule compared to the corresponding hexamers formed in dry solvent.

II. Investigations into the Dynamics of the Resorcinarene Hexamer. Effect of Concentration on the Exchange of Resorcinarene Subunits. We have previously reported our preliminary results on the dynamics of the resorcinarene hexameric assemblies in water saturated solvents.[12b] The experiments were conducted at 250 nM of **RsD** and **RsA**, and in water saturated dichloromethane the half-life for the solutions to reach equilibrium was ca. 10 min. This is significantly faster than the time for **1a** and **1b** to equilibrate at the millimolar concentrations of the diffusion NMR studies of Cohen et al.[7d] As in the study of the pyrogallolarene system discussed above, we conducted an investigation into the influence of concentration on the exchange of resorcinarene subunits in the hexamers at concentrations from 500 nM to 10 μM.[12,15] Much to our surprise, very little variation in the rate of exchange was observed over this concentration range, with half-lives between 10 and 14 min being obtained.

While little difference in the exchange rates was observed, the amount of FRET did change with significantly higher FRET at 10 μM compared to 500 nM. Shown in Figure 10 are the scaled final FRET levels of the equilibrated resorcinarene solutions at different concentrations.[16] These differences in the amount of FRET are consistent with the resorcinarenes being less assembled into hexamers at these lower concentrations, with more free monomers in solution.

Importance of Temperature on Rates of Exchange. To investigate the influence of temperature on the exchange of the resorcinarene hexamers, 1 μM solutions of **RsD** and **RsA** in water-saturated dichloromethane were mixed at temperatures ranging from 15 °C to 35 °C. While a trend was observed, with slower exchange at the lower temperatures, the differences in half-lives are only small, ranging from ca. 18 min at 15 °C to 8 min at 35 °C. A decreased level of FRET was again observed at high-temperature (see Supporting Information for details).

Stability of the Resorcinarene Hexamers to Polar Additives. As with the pyrogallolarene hexamers, titration of equilibrated solutions of resorcinarene hexamers with methanol results in disassembly of the capsule. Solutions in water saturated
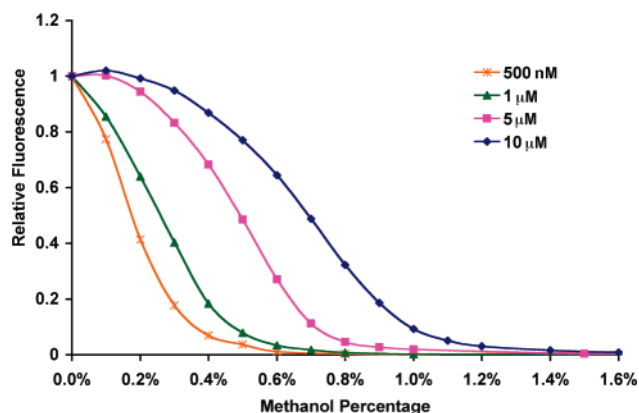
(16) Perylene is known to undergo spectral shape changes upon π-stacking and the more concentrated 10 μM spectral trace reveals that there is some π-stacking: Clark, A. E.; Changyong, Q.; Li, A. D. Q. *J. Am. Chem. Soc.* **2007**, *129*, 7586−7595.

dichloromethane of **RsD** and **RsA** at 500 nM, 1 μM, 5 μM, and 10 μM were equilibrated until the maximum FRET was obtained. At this point the solutions were titrated with methanol in increments of 0.1% v/v and the results are presented in Figure 11. A definite trend was observed with the more dilute solutions requiring less methanol to disassemble the capsule than the more concentrated solutions. The solutions at 500 nM or 1 μM are very sensitive to even 0.1% v/v methanol, as evidenced by the sharp decrease in the perylene fluorescence, and the hexamers are essentially completely dissociated at 0.6% v/v methanol. In contrast, the solutions at 5 and 10 μM are tolerant of the first 0.2% v/v methanol and the 10 μM solution requires the addition of as much as 1.2% v/v methanol for complete dissociation. These results again point to the incomplete assembly of the resorcinarenes into hexamers at low concentrations, with a higher relative concentration of resorcinarene monomers.

Alternative experiments to probe the effect of polar additives on the exchange rates of the resorcinarene hexamers involved addition of a small percentage of methanol to the wet dichloromethane prior to the addition of pyrene and perylene labeled resorcinarenes at 1 μM. As before, the gradual development of FRET was observed over time under these different conditions indicating hexamer formation. While 0.1% v/v methanol was tolerated with a minimal change in the time for the solution to equilibrate, when the methanol percentage was increased to 0.2 and 0.3% v/v, the capsules exchanged far more rapidly with half-lives for the system to reach equilibrium decreasing from 10 min to 2.6 min and 45 s, respectively. In addition to the different rates for the exchange process, the final level of FRET was significantly lower with a higher methanol percentage. These results point to a weaker capsule in methanol as the polar, protic solvent clearly disrupts the hydrogen bonds necessary for assembly leaving only a small number of the resorcinarenes as hexamers.

The Role of Water on the Assembly of Resorcinarene Hexamers. It has previously been demonstrated that water is necessary for the assembly of resorcinarene hexamers, both in solution and in the solid state, with eight water molecules participating in the hydrogen-bonding assembly.[4,17] Consequently all exchange experiments for the resorcinarene hexamers were conducted in water-saturated solvents. Some variation in

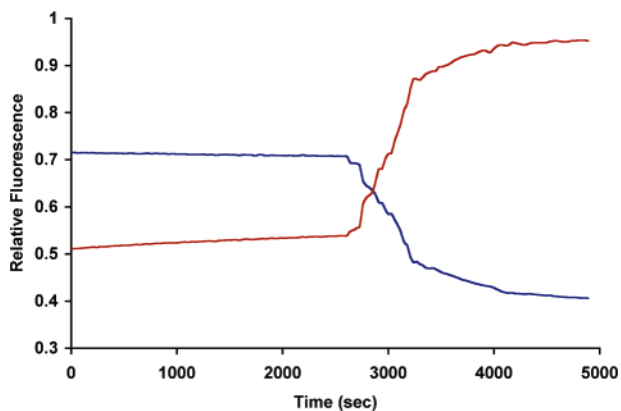(17) Avram, L.; Cohen, Y. *Org. Lett.* **2002**, *4*, 4365−4368.

Figure 12. Change in the fluorescence intensity with time of the donor emission maximum (409 nm, red) and the acceptor emission maximum (446 nm, blue) upon combination of **RsD** and **RsA** in anhydrous CH$_2$Cl$_2$. After 2500 s, 1 μL of H$_2$O was added and FRET began to occur: λ_{exc} = 350 nm.

Figure 13. Mixing of pyrene resorcinarene **RsD** and perylene pyrogallolarene **PgA** shows no formation of heterohexamers after 4 days: λ_{exc} = 350 nm. the inset shows the change in fluorescence intensity with time of the donor emission maximum (409 nm) and acceptor emission maximum (453 nm) before and after the addition of perylene resorcinarene **RsA**.

exchange rates were observed when different batches of water-saturated solvent were used and this prompted us to further investigate the role of water on the dynamics of the exchange of the resorcinarene monomers and the assembly of hexamers. When strictly anhydrous dichloromethane was employed, no FRET was observed, suggesting no exchange of the resorcinarenes and that the resorcinarenes were monomeric in solution. After ca. 40 min, addition of a small amount of water (1 μL) to the solution resulted in FRET beginning immediately, as evidenced by an increase in the pyrene fluorescence and decrease in the perylene fluorescence (Figure 12).[18]

Solutions were then prepared with varying proportions of water saturated and anhydrous dichloromethane and an increase in the exchange rate of the resorcinarenes was observed with increasing water content. These results clearly show the importance of water to facilitate the hexameric assembly of resorcinarenes.

III. Comparisons Between the Pyrogallolarene and Resorcinarene Hexameric Assemblies. From these studies of two seemingly related hexameric assemblies, a number of differences can be identified in relation to their dynamic behavior. As far as the concentration of mixing is concerned, the pyrogallolarenes exhibited a higher degree of sensitivity to concentration with markedly slower equilibration times at high (10 μM) concentrations than at low (250 nM) concentrations. In contrast the resorcinarenes showed very little variation in equilibration times over a similar concentration range (see Supporting Information for details). The reasons for this are still unclear.

Both systems have a significantly higher amount of FRET at 10 μM than at nanomolar concentrations, consistent with more assembled hexamers and fewer free monomers at these concentrations. While comparison of Figures 6 and 10, particularly the relative level of FRET at 5 and 10 μM in each system, may suggest that there is a greater level of assembly of the resorcinarenes into hexamers than the pyrogallolarenes at the same concentration, caution must be taken in comparison of FRET levels between the two hexameric assemblies. The pyrene and perylene fluorophores are attached to the base of the resorcinarenes by a longer tether than the corresponding pyrogallolarenes, and FRET is very sensitive to both the distance
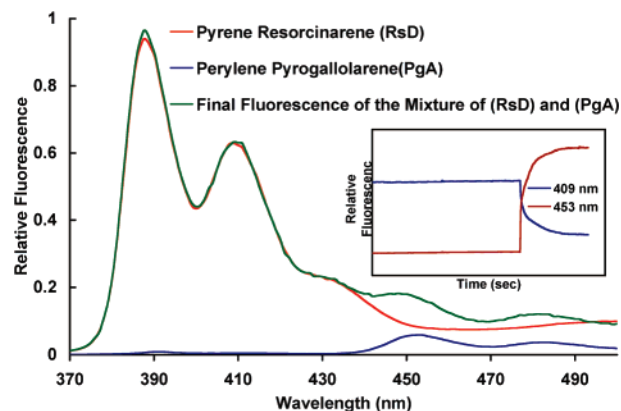
(18) The more erratic data after the addition of water is the result of beads of water on the inside of the cuvette.

between the donor and the acceptor and the mutual orientation of the fluorophores.[10]

In relation to the influence of temperature on the dynamic behavior of the assemblies, the pyrogallolarene capsules were once again far more sensitive to changes, with a variation in the half-life from 8 to 96 min over a range of only 20°. Although the resorcinarenes exhibited a similar trend, i.e., slower exchange at lower temperatures, the differences in half-lives were far less dramatic, varying from 8 to 18 min over the same temperature range (see Supporting Information for details).

Compared to the resorcinarene capsules, the pyrogallolarene hexamers were more stable to methanol and required the addition of a higher percentage to dissociate the capsule. This is consistent with previous observations regarding the stability of pyrogallolarene hexamers to polar solvents.[6,7d] For both systems, the amount of methanol required to disassemble the hexamers was dependent on the concentration of the capsules, with less methanol needed for the more dilute solutions of hexamers. This is again consistent with the hexamers being less assembled at lower concentrations.

The effect of water on the dynamic assembly of the two capsules was very different. In the case of the resorcinarenes, no FRET is observed when the donor and acceptor labeled macrocycles **RsD** and **RsA** are mixed in anhydrous dichloromethane. It is only upon the addition of water that the resorcinarenes assemble into hexameric capsules. In contrast, the pyrogallolarenes do not require water for the capsular assembly and the monomeric units exchange freely in anhydrous solvent. When water-saturated solvents are employed, the rate of exchange of the pyrogallolarene units is however increased.

IV. Self-Sorting of Pyrogallolarenes and Resorcinarenes. After investigations into the dynamic behavior of both the pyrogallolarenes and the resorcinarenes, we attempted to form heterohexamers of pyrogallolarenes and resorcinarenes. On mixing of 1 μM solutions of pyrene donor resorcinarene **RsD** and perylene acceptor pyrogallolarene **PgA** in water-saturated dichloromethane, no FRET was observed over 6 h—the time required for either the resorcinarene or the pyrogallolarene hexamers to equilibrate under these conditions (Figure 13). After this time the corresponding perylene acceptor resorcinarene **RsA**, partner to the pyrene donor resorcinarene **RsD** in solution, was added. The monomers began exchanging immediately under

these experimental conditions, and FRET began accordingly. The solution of pyrene donor resorcinarene **RsD** and perylene acceptor pyrogallolarene **PgA** could even be left for a period of 4 days and still no evidence of FRET appeared. Alternatively, we started with the pyrene donor pyrogallolarene **PgD** and the perylene acceptor resorcinarene **RsA**, and again no evidence of heterohexamers appeared. FRET was only observed once there were either a pair of donor and acceptor labeled resorcinarenes or a pair of donor and acceptor labeled pyrogallolarenes in solution. These results clearly demonstrate that the pyrogallolarene and resorcinarene macrocycles self-assemble and self-sort exclusively into homomeric hexamers in solution, even at the low concentrations of the fluorescence experiments.

Conclusions

The dynamic behavior of pyrogallol[4]arenes and resorcin-[4]arenes, each labeled with donor and acceptor fluorophores, have been investigated under several different conditions by varying the concentration of mixing, or the temperature of the solution. The importance of water on the assemblies and the stability of the capsules to polar additives were also probed. Pyrogallolarenes and resorcinarenes have potential applications in a number of areas because they are assembled to a certain extent even at nanomolar concentrations. Information regarding the degree of assembly and stability of the hexameric capsules under a variety of conditions has been gleaned, and that makes them promising candidates for these applications. Furthermore, evidence of strict self-sorting in the formation of pyrogallolarene and resorcinarene hexamers was obtained.

Experimental Section

UV absorption spectra were recorded on a Varian Cary 50 UV-Visible spectrophotometer. Fluorescence measurements were obtained using a Fluorolog-3 Model FL3−21 spectrofluometer. Spectrophotometric grade dichloromethane was purchased from Aldrich and used as received or stirred with distilled water and filtered to obtain water saturated dichloromethane. Anhydrous dichloromethane was purchased from Acros and used as received. All measurements were recorded at 25.0 ± 0.1 °C unless otherwise stated.

Solutions of the pyrene and perylene pyrogallolarenes **PgD** and **PgA** and the pyrene and perylene resorcinarenes **PgD** and **PgA** were prepared

at 5×10^{-5} M. Aliquots of the pyrene and perylene solutions were then diluted and mixed such that the concentration of each of the macrocycles was 1 μM, unless otherwise stated. The donor was excited at 346 nm for the pyrogallolarenes and at 350 nm for the resorcinarenes, and the fluorescence spectra were recorded at regular time intervals. For time-based experiments the fluorescence values at the donor and acceptor emission maxima were simultaneously recorded at regular time intervals. A decrease in donor fluorescence as well as the increase in acceptor fluorescence was observed.

The apparent rate constant, k, for the exchange of the resorcinarene or pyrogallolarene monomers in the hexamer was determined by fitting the data to the first-order equation

$$\mathrm{Ln}((F_{\mathrm{fin}} - F_{\mathrm{t}})/(F_{\mathrm{fin}} - F_{\mathrm{o}})) = -kt$$

where F_{t} and F_{fin} and F_{o} are the fluorescence intensities at the perylene maxima at times t and the final and initial value obtained after the hexamers have reached equilibrium. The negative slope of the line provides the apparent rate constant k. From this, the half-life ($t_{1/2}$) for the capsule to reach equilibrium is determined from

$$t_{1/2} = \ln 2/k$$

To investigate whether heterohexamers form between pyrogallolarenes and resorcinarenes a number of experiments were conducted in both water-saturated and dry dichloromethane. In each case 1 μM solutions of a pyrogallolarene and a resorcinarene were mixed: either **RsD** and **PgA** or **RsA** and **PgD**. Time-based fluorescence measurements were run, monitoring both the donor and acceptor emission maxima. After a period of 6 h, another addition was made so that there were now two labeled resorcinarenes (**RsD** and **RsA**) or two labeled pyrogallolarenes (**PgD** and **PgA**) in solution. At this point, FRET began to occur.

Supporting Information Available: Additional fluorescence spectra showing the final FRET level at different temperatures and the compiled results of the change in equilibration half-lives with temperature and concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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