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Assembly and Exchange of Resorcinarene Capsules Monitored by Fluorescence Resonance Energy Transfer

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

The Skaggs Institute for Chemical Biology and Department of Chemistry, The Scripps Research Institute, MB-26, 10550 North Torrey Pines Road, La Jolla, California 92037

Received January 5, 2007; E-mail: jrebek@scripps.edu

Almost a decade ago MacGillivray and Atwood reported the extraordinary crystal structure of a self-assembled hydrogen-bonded capsule comprising six resorcinarene molecules and eight water molecules.[1] This report inspired numerous solution studies of resorcinarenes that revealed the same assembly in wet organic solvents.[2] The primary tool for their solution-state study has been NMR analysis; however, the encapsulation process itself and the dynamic behavior of the monomers are not easily observed because of the millimolar concentrations required. At lower (nanomolar) concentrations where monomers are likely, fluorescence resonance energy transfer (FRET) is an appropriate tool, and we report its application here. It revealed the real-time exchange of monomer subunits between hexameric capsules and the first direct observation of an encapsulated fluorophore in a resorcinarene hexamer.[3]

Applications of FRET in the study of biological systems have been extensive over the past few decades,[4] but its use in synthetic supramolecular systems has only just emerged.[5] The sensitivity of the technique allows for detection of interacting species at nanomolar concentrations, but labeling synthetic receptors with appropriate fluorophores has proven challenging. Earlier we reported[5a] the attachment of coumarin dyes to calixarenes, and Diederich[5b] has attached two suitable fluorophores to an extended cavitand. For the case at hand, an improved monofunctionalization of resorcinarenes[6] permitted the attachment of appropriate donor (**D**) and acceptor (**A**) fluorophores to the "feet" of resorcinarene monomers (Figure 1). When the **D** and **A** are present in the same hexameric assembly FRET is observed on excitation of the donor dye.

Pyrene and perylene were chosen as the respective donor and acceptor fluorophores (Figure 1). Starting from the *tert*-butyldimethylsilyl (TBS) protected monohydroxyl resorcinarene **1**,[6a] esterification with 1-pyrene carboxylic acid, via the acid chloride **2**, provided the protected pyrene ester **3**. For the acceptor, the known perylene alkyne **6**[7] was reacted with resorcinarene azide **5**, generated from alcohol **1** via the mesylate, in a copper mediated "click" reaction[8] to afford the protected **7**. Following TBAF deprotection of **3** and **7**, the requisite pyrene and perylene resorcinarenes **4** (**D**) and **8** (**A**) were obtained (Scheme 1).

Separately, the labeled resorcinarenes **4** (**D**) and **8** (**A**) in wet chloroform with tetrahexyl ammonium bromide (a known guest for the hexameric assembly) showed clean formation of the respective encapsulation complexes by ^{1}H NMR analysis. Accordingly, the presence of six somewhat bulky substituents on the periphery of the system does not interfere with the assembly or the encapsulation process at millimolar concentrations.

Dilute solutions (250 nM) of **4** (**D**) and **8** (**A**) resorcinarene hexamers in wet chloroform were mixed at room temperature, and the change in the fluorescence emission spectra was followed with time (Figure 2). Although multiple isomeric and combinatorial hexamers can be formed when resorcinarenes **4** (**D**) and **8** (**A**) are mixed, FRET can be expected whenever at least one **D** and one **A** resorcinarene are present in the same hexamer. Initially, only the emission from the donor was observed but over time there was an increase in acceptor emission. This slow development of a FRET signal indicates the exchange of monomers and the formation of the mixed hexamers. Control experiments showed no FRET between the free dyes at these concentrations (see Supporting Information).

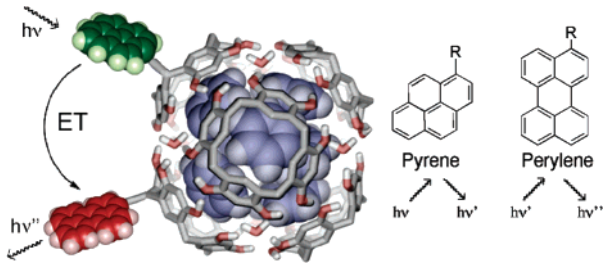
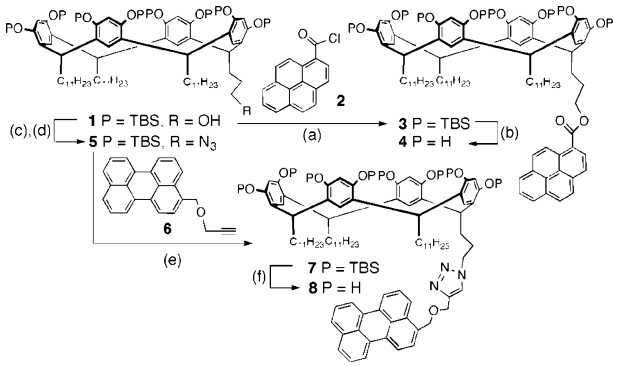


Figure 1. Representation of a **D** and **A** labeled resorcinarene brought within FRET distance in a hexameric assembly. Pyrene and perylene are the donor and acceptor fluorophores, respectively.

Scheme 1. Synthesis of Donor and Acceptor Labeled Resorcinarenes[a]



[a] Conditions: (a) **2**, NEt_3, CH_2Cl_2, 18 h, 49%; (b) 1 M TBAF in THF (12 equiv), AcOH (12 equiv), THF, 0 °C, 30 min, 80%; (c) MsCl, NEt_3, CH_2Cl_2, 0 °C → room temp, 18 h; (d) NaN_3, DMF/THF, 18 h, 60 °C, 70% over two steps; (e) **6**, CuI (0.10 equiv), TBTA ligand (0.01 equiv), THF, 60 °C, 1 h, 87%; (f) 1 M TBAF in THF (12 equiv), AcOH (12 equiv), THF, 0 °C, 30 min, 63%.

Disproportionation of the mixed hexamers at these concentrations was confirmed when the solution was titrated with methanol, a solvent known to disrupt the hydrogen bonds of the capsules. At 1.5% v/v methanol the hexamer was disassembled and, as the resorcinarenes were monomeric in solution, FRET was no longer observed.

The kinetics of the dynamic behavior of the resorcinarene monomers in the hexameric assemblies could be extracted from these studies. Treatment of the data as first-order dissociation kinetics gave the rate for the exchange of monomers. The assemblies

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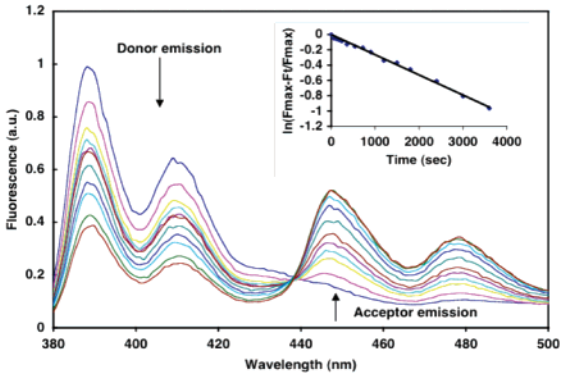

Figure 2. Development of FRET with time upon mixing **4** (**D**) and **8** (**A**) hexamer solutions at 250 nM, (times shown from 0 to 4 h). $\lambda_{exc} = 350$ nm. The inset shows the first-order kinetic treatment of the data.

Table 1. Relative Rates of Exchange of Resorcinarenes in the Hexamer

solvent[a]	added guest	half-life[b] (min)	k_{rel}[b]
$CHCl_3$		46	21
C_6H_6		3	292
CH_2Cl_2		10	100
CH_2Cl_2	$Hex_4N^+Br^-$	14	70
CH_2Cl_2	$Bu_4N^+Br^-$	14	70
CH_2Cl_2	Bu_4SbBr	16	59

[a] In all cases water-saturated solvents were used. [b] Half-life for the system to reach equilibrium; uncertainties in k_{rel} and half-lives are ±10%.

were monitored in different wet solvents and in the presence of known guest molecules (Table 1). The capsules were slowest to exchange resorcinarene monomers in chloroform compared to dichloromethane or benzene; apparently, a more stable hexameric capsule is formed in chloroform. Known guests for the hexamer showed a longer half-life for monomer exchange: as expected a guest has a stabilizing effect on the assembly and a more robust capsule exists when a guest other than solvent molecules is inside.

The exchange of resorcinarene monomers was relatively fast at these low concentrations, in contrast to a diffusion NMR study of Cohen et al. where more than 24 h was required for a mixture of two hexamers to reach heteromeric equilibrium.[2e] We attribute this disparity to the vastly different concentration of the experiments. At the nanomolar concentrations of the fluorescence studies there are more monomeric resorcinarenes in solution relative to the hexamers and the rate of exchange of the assembly is increased. A similarly fast exchange of monomers was observed in a recent mass spectrometric study.[9]

To further probe the assembly, a fluorescent guest *inside the capsule* made it possible to observe FRET across the mechanical boundary of the hexamer. A number of pyrene derivatives were examined as potential guests by ^{1}H NMR analysis and the pyrene salt **9**[10] was encapsulated. A solution of 5:1 unlabeled resorcinarene to perylene resorcinarene **8** (**A**) was prepared and pyrene salt **9** was added. Comparison of the emission spectra of the individual components to that of the mixture of **8** (**A**) and **9** revealed FRET from the guest inside the capsule to the perylene resorcinarene appended to the capsule (Figure 3). Upon addition of methanol, the FRET disappeared as the capsule was reduced to its constituent monomers and the guest was released.

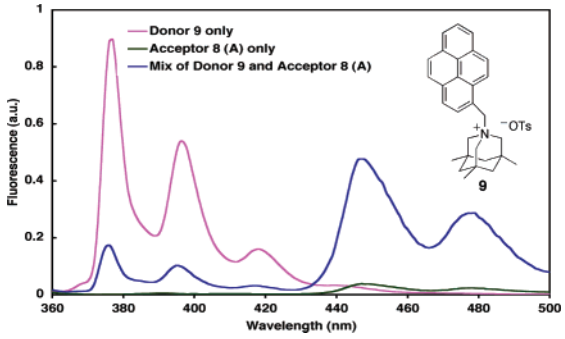

Figure 3. FRET from pyrene salt **9** inside the hexamer to the perylene resorcinarene **8** (**A**) in the hexamer. $\lambda_{exc} = 350$ nm.

In summary, resorcinarenes labeled with donor and acceptor fluorophores probe the dynamic behavior of hexameric capsules in solution through FRET observation. Additionally, FRET occurs from a fluorescent guest to a labeled resorcinarene host. While several examples exist of FRET in mechanically linked rotaxanes,[11] to our knowledge, this represents the first observation of FRET across a capsular boundary.

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Supporting Information Available: Key experimental and characterization of new compounds, absorption and emission spectra, details of kinetic experiments and methanol titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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