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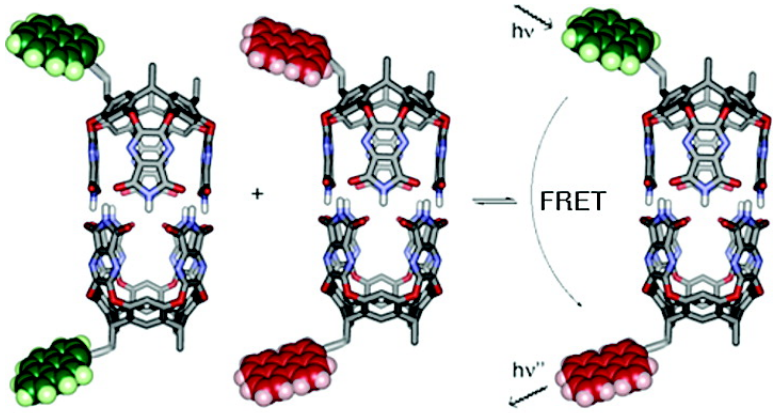
J. Am. Chem. Soc., **2007**, 129 (28), 8818-8824• DOI: 10.1021/ja071774j • Publication Date (Web): 26 June 2007



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Self-Assembly Dynamics of a Cylindrical Capsule Monitored by Fluorescence Resonance Energy Transfer

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Abstract: The constituent cavitands of a cylindrical capsule were labeled with donor and acceptor fluorophores, and fluorescence resonance energy transfer (FRET) was employed as a tool to study the dynamics of self-assembly. When donor and acceptor dyes are present in the same capsular assembly, they are brought within 25 Å of each other, a distance suitable for efficient energy transfer to occur between them. This allowed for the study of interacting species at nanomolar concentrations providing information unattainable from NMR experiments. The kinetic stability of the capsule in the presence of various guest molecules was investigated which revealed a range of more than 4 orders of magnitude in the rates of cylindrical capsule exchange. While the thermodynamic stability of the capsule generally dictates the self-assembly dynamics, it was discovered that longer rigid guests can impart a significant kinetic barrier to monomer exchange.

Introduction

Reversibly formed capsules are assemblies held together by weak intermolecular forces.[1] Hydrogen bonds,[2] hydrophobic forces,[3] salt bridges,[4] and metal/ligand interactions[5,6] have been used to create spaces that act as hosts for a range of guests.[7−9,5a] A great deal is known about their recognition properties and their use as nanometric reaction chambers,[10] as means to stabilize reagents,[11] and as spaces where new forms of stereochemistry can emerge.[12] The dynamic nature of the reversible molecular assemblies is responsible for the facile exchange of constituents, and exchange between the environments inside and outside the capsule becomes a means of regulating reactivity.[13] The capsules are at thermodynamic equilibrium at ambient temperatures and in the liquid phase. Naturally, NMR has been the method most used as it reports the magnetic environment experienced by the guests: the aromatic panels that are universally present in the hosts cause easily recognized and dramatic upfield shifts of the guest's signals.[14] Considerably less is known about the rates and equilibria of the assembly process. The association constants for assembly are invariably high, typically $>10^5\ M^{-1}$, values beyond the reach of millimolar concentrations of NMR titrations. At the relevant nanomolar concentrations, fluorescence resonance energy transfer (FRET) is a technique which has been extensively employed in the study of assembly and dynamic processes in biological systems.[15] Reports on the application of FRET in the study of synthetic supramolecular systems have been rare: we recently reported its use with a hydrogen-bonded hexameric assembly of resor-

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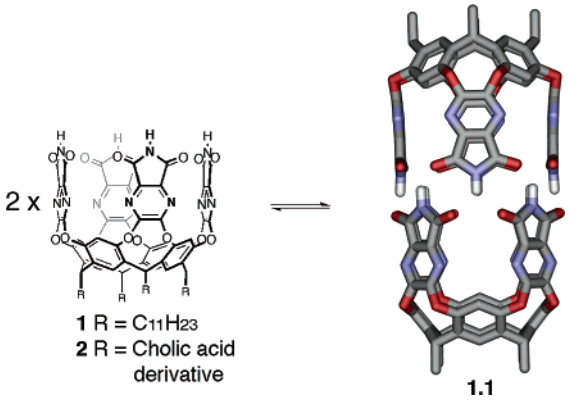

Figure 1. Line drawing of the synthetic receptor **1** and energy minimized dimeric structure of the capsule **1.1** (R groups in the model structure have been removed for clarity).

cinarenes[16] as well as earlier in the study of calixarene capsule assembly in real time,[17] and Diederich applied FRET to follow the conformational switching of resorcinarene cavitands.[18] We report here its application to probe the dynamics of subunit exchange and guest exchange for the self-assembled capsule **1.1** (Figure 1).

Cavitand **1** self-assembles into a cylindrical dimer **1.1** held together by eight bifurcated hydrogen bonds in most apolar organic solvents, with the solvent generally filling in the inner space (Figure 1).[19] When mesitylene is used as solvent, it is too large to be adequately accommodated in the capsule and does not compete with intended guests.[20] Since the capsule cannot be empty, suitable guest molecules are required to form a well-defined encapsulation complex. The lifetimes of the complexes vary from milliseconds to days, a range that makes for slow exchange on the ^{1}H NMR time scale so separate signals are observed for free and bound guests. The 16 aromatic rings of **1.1** provide strong magnetic shielding for encapsulated guest molecules, and the guest proton resonances can be shifted upfield to -4 ppm.[21]

Background

Although binding of molecules in the cylindrical capsule is well-studied, little is known about the exchange of the capsule monomer units and the dependence of this exchange on the encapsulated guest.[14] It was clear from the earliest studies that the uptake, release, and exchange of small guests in the capsule was rapid on the human time scale.[22] A ^{1}H NMR magnetization transfer spectroscopy study, conducted with a capsule containing one molecule of benzene and one molecule of *p*-xylene, revealed that guest exchange occurs within seconds and without capsule dissociation.[23] In the case of large guests, replacement of the ill-fitting mesitylene occurred rapidly; however, exchange rates between large guests were slow and took days to reach equilibrium.[22] A ^{1}H NMR study into the replacement of 4,4′-dimethylbiphenyl by 4,4′-dimethylstilbene suggested that guest exchange was possible without the dissociation of the capsule.[23] When the exchange of both the capsule halves and the guest were examined in mesitylene with 12% methanol, similar rates were found for the two processes, and it was concluded that complete dissociation of the capsule was a requirement for guest exchange in protic solvents.[24]

Additionally, a study into the capsule subunit exchange was conducted with a chiral version of the cylindrical capsule.[25] A solution of the chiral capsule **2.2** containing 4,4′-dimethylbiphenyl was mixed with a solution of the achiral capsule **1.1** with the same guest, and exchange of the capsule halves was monitored by ^{1}H NMR spectroscopy. An equilibrium mixture was obtained with a half-life of approximately 10 min. The equilibration of the chiral and achiral capsules was found to be independent of the incoming guest concentration.

While the chiral version of the capsule allowed for a preliminary investigation into the subunit exchange of the capsules, the study was limited by the requirement that different sets of guest peaks occur for the mixed capsule in the ^{1}H NMR spectra. From a selection of over 30 known guests for the cylindrical capsule, only two were found to satisfy these criteria; hence, a thorough examination of subunit exchange could not be conducted by ^{1}H NMR spectroscopy. We report here the successful labeling of the cylindrical capsule monomers with suitable donor and acceptor fluorophores for FRET analysis. When a donor and an acceptor fluorophore are present in the same capsular assembly, FRET is observed upon excitation of the donor. This has enabled the study of capsule subunit exchange by FRET with the detection of interacting species at nanomolar concentration.

Results and Discussion

Synthesis of Labeled Capsules. Recently we reported the synthesis of resorcinarenes **4** and **7** labeled with pyrene and perylene as the respective donor and acceptor fluorophores.[16] This synthesis used the newly developed efficient monofunctionalization of resorcinarenes.[26] Employing the same mild conditions that form **1**, we reacted resorcinarenes **4** and **7** with 5,6-dichloropyrazine-2,3-dicarboxylic acid imide, to afford the respective donor and acceptor cavitands **5** (**D**) and **8** (**A**) (Scheme 1).[21] The cavitands were accessed in good yields and upon dissolution in d_{12}-mesitylene and addition of a suitable guest such as 4,4′-dimethylstilbene, clean formation of each of the complexes **5.5** (**D**) and **8.8** (**A**) was observed. This confirmed that the presence of the substituents on the base of the cavitand did not hinder capsule formation. Two isomers are present, and the assembly is, strictly speaking, a heterodimer,[27] but the NMR spectra of encapsulated guests showed only a single set of resonances.

Control Fluorescence Experiments. The fluorescence and absorption spectra of the pyrene and perylene-labeled cavitands **5** (**D**) and **8** (**A**) confirm that the absorption of the acceptor overlaps significantly with the emission of the donor, allowing

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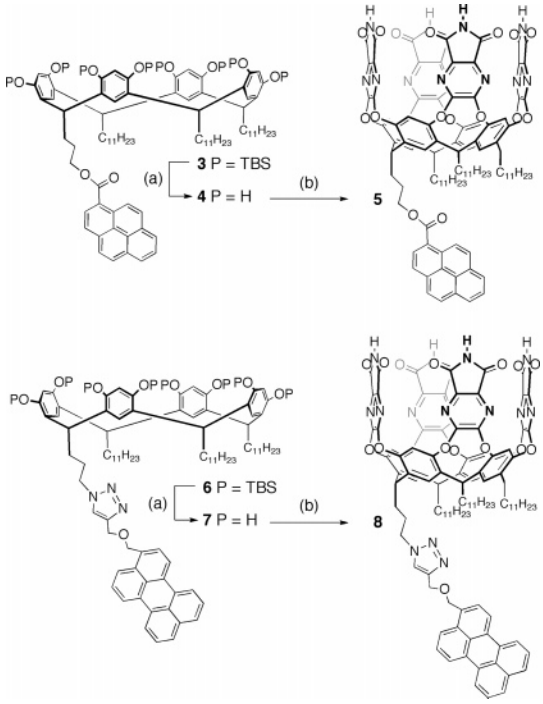
Scheme 1. Synthesis of Pyrene and Perylene-Labeled Cavitands **5** (**D**) and **8** (**A**)[a]



[a] Conditions: (a) 1 M TBAF in THF (12 equiv), AcOH (12 equiv), THF, 0 °C, 30 min, **4**, 80%; **7**, 63%; (b) 5,6-dichloropyrazine-2,3-dicarboxylic acid imide (4.4 equiv), NEt_3, DMF, RT, 50 min, **5**, 82%, **8**, 85%.

for nonradiative energy transfer between them when the donor is excited. To ensure that any FRET we observe is from the desired capsular assembly, the possibility of intermolecular FRET had to be ruled out. Protected resorcinarenes **3 (D)** and **6** (**A**) served as control compounds for dilution experiments.[16] As they lack the hydrogen-bonding sites required for capsule formation, any FRET between the two compounds can only arise from intermolecular FRET between the dyes in solution. Dilution studies were performed with equimolar mixtures of **3** (**D**) and **6** (**A**) in toluene. At 25 μM a small amount of intermolecular FRET was observed, but on 10-fold dilution to 2.5 μM, no FRET occurred. All subsequent experiments were conducted at concentrations where no intermolecular FRET was observed.

Fluorescence Studies in Toluene. When capsules **5.5** (**D**) and **8.8** (**A**) are mixed in an equimolar ratio, a statistical distribution of products would result in 25% of each of the homomeric capsules **5.5** (**D**) and **8.8** (**A**) and 50% of the heteromeric capsule **5.8** (**DA**) (Figure 2). Excitation of the donor fluorophore would produce a FRET signal only when capsule **5.8** (**DA**) is present. Again two isomers are possible but either places the dyes well within their Förster radii to give efficient energy transfer, i.e., the system behaves as though there are only two states.[28] It is also noted that even at maximum FRET, a significant amount of donor emission can be expected from homodimer **5.5** (**D**).

Initially toluene was employed as the solvent for the fluorescence studies as the capsular assembly forms cleanly around two toluene molecules.[19] Dilute solutions (500 nM) of **5.5** (**D**) and **8.8** (**A**) in toluene were mixed at room temperature, and the change in the fluorescence emission spectra was followed with time (Figure 3). At first, only emission from the donor was observed, but over time there was an increase in acceptor emission accompanied by a decrease in donor emission. This gradual development of a FRET signal indicates exchange of capsule subunits and the formation of the mixed capsule **5.8** (**DA**). As discussed above, FRET between "free" dyes does not occur at this concentration.

The data obtained was treated as first-order dissociation kinetics as the rate of disassembly of the formed capsule is the rate-limiting step in the exchange of the capsular subunits. Capsule association in suitable solvents or in the presence of appropriate guest molecules is known to occur very rapidly.[22] For the capsules in toluene a rate of 1.9×10^{-3} s^{-1} was obtained for the exchange of the capsule subunits, which corresponds to a half-life of 6 min. Titration with methanol, a solvent that competes effectively for the hydrogen bonds, resulted in the expected loss of the FRET signal, as the capsules dissociate to their constituent cavitands. The disassembly of the capsules occurred rapidly and required only 2% v/v methanol (see Supporting Information for details).

Effect of Concentration of Capsules on Mixing. An investigation was then conducted into the effect of varying the concentration at which the capsules are mixed. Solutions of the pyrene and perylene capsules **5.5** (**D**) and **8.8** (**A**) in toluene were mixed at 5 μM, 1 μM, 500 nM, and 50 nM (Figure 4). Over these concentration ranges, some variation in the rate of capsule exchange was observed with equilibrium being established faster at higher concentrations. We had initially expected a faster rate of exchange at lower concentrations if there was a higher relative concentration of monomers;[25] however, calculations revealed that even at 50 nM the percentage of monomers is negligible (under 3%) because of the high association constants of these capsules. The concentration dependence of the rate of exchange of the capsule subunits suggests an intermolecular interaction between the capsules in solution. The nature of this interaction and the mechanism by which capsule exchange is accelerated at higher host concentration will be the subject of further investigations.

Fluorescence Studies in Mesitylene. Toluene has its limitations as a solvent for the study of the cylindrical capsule; it is too good a guest itself for other guests to be encapsulated at the nanomolar concentrations of our experiments. Consequently, mesitylene was employed as the solvent for the investigations into the effect of guests on the exchange rate of capsule subunits. Although mesitylene is an ill-fitting guest for the capsule, the capsule is notorious for its ability to encapsulate trace impurities from any solvent.[14] Even with distilled mesitylene with a purity of 99.9%, impurities are present in millimolar amounts. These impurities are in large excess relative to the nanomolar concentration of the capsule and have the potential to template capsule formation. On mixing of mesitylene solutions of **5** (**D**) and **8** (**A**) in the absence of additional guest molecules a very slow development of a FRET signal was observed over a period of days. First-order dissociation kinetic treatment of the data yielded a rate of approximately 6.0×10^{-6} s^{-1}, which corresponds to a half-life of 32 h for the exchange of the capsule in mesitylene (some variation occurred with different batches of distilled mesitylene; benzene

(28) In a study with the pyrene and perylene dye pair a Förster distance of 22.3 Å was obtained providing a distance determination range of 11−32 Å: Masuko, M.; Ohuchi, S.; Sode, K.; Ohtani, H.; Shimadzu, A. *Nucleic Acids Res.* **2000**, *28*, e34, ii−viii.

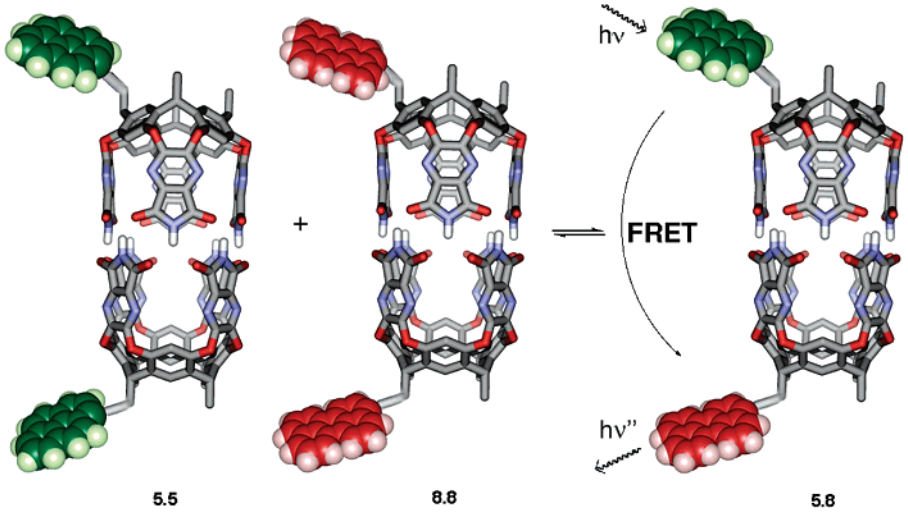


Figure 2. Equilibrium between the donor- and acceptor-labeled capsules **5.5** (**D**) and **8.8** (**A**) and the heteromeric capsule **5.8** (**DA**) (The structures drawn are schematic representations of the actual molecules). When the donor and acceptor cavitands are present in the same capsule, FRET is observed on excitation of the donor dye.

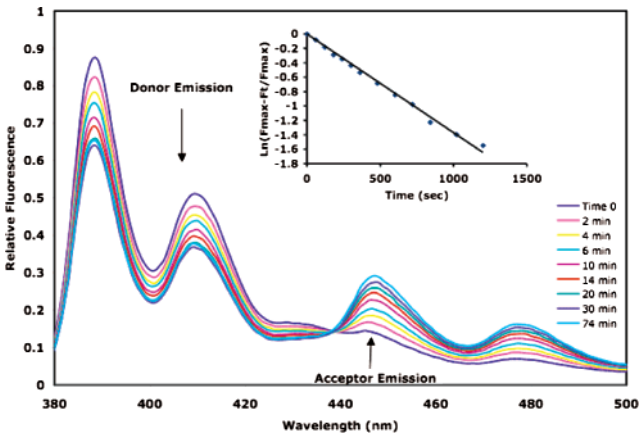


Figure 3. Development of FRET with time upon mixing **5.5** (**D**) and **8.8** (**A**) solutions in toluene at room temperature. $\lambda_{exc} = 350$ nm. Inset: First-order kinetic treatment of the data.

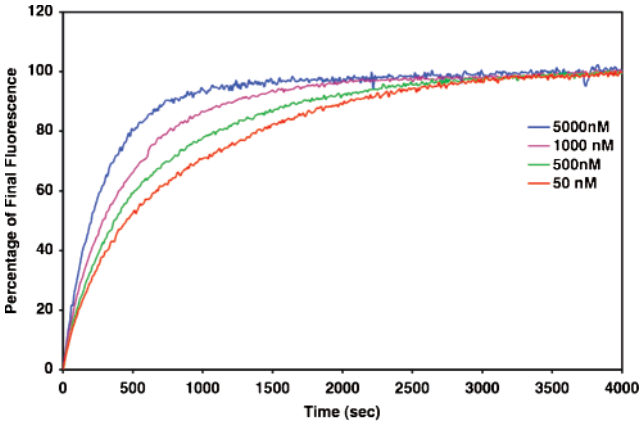


Figure 4. Variation in the rate of exchange of capsules with different concentrations of donor- and acceptor-labeled capsules **5.5** (**D**) and **8.8** (**A**) in toluene. $\lambda_{exc} = 350$ nm. $\lambda_{em} = 447$ nm.

and *p*-xylene were earlier identified as impurities in the commercial solvent, and this combination makes for a well-filled capsule).[19] The significantly faster exchange in toluene (6 min) can be attributed to the ability of toluene to solvate the cavitand, filling the necessary space as the capsule dissociates and recombines.

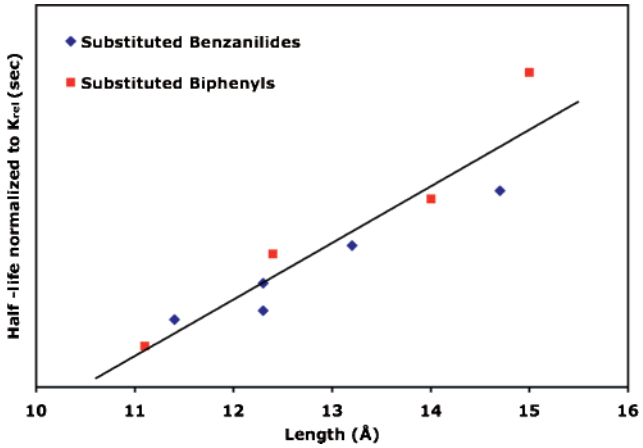
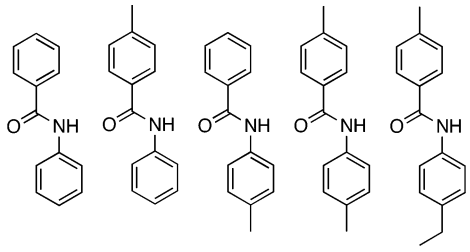


Figure 5. Half-lives for capsule exchange normalized relative to K_{rel} for the substituted benzanilide and substituted biphenyl guest series as a function of guest length. A trend line has been added for clarity. The half-lives are presented on a logarithmic scale.

Subunit Exchange with Added Guest Molecules. A number of known guest molecules for the cylindrical capsules were chosen which rapidly replace mesitylene impurities and template capsule formation. The ^{1}H NMR spectra for each of these guests in the unlabeled capsule **1.1** revealed the clean formation of kinetically stable complexes. Pairwise competition experiments were conducted by ^{1}H NMR spectroscopy to establish the relative binding affinity (K_{rel}) of the guests for the capsule. To do this the capsule was exposed to an excess of two different guests, the NMR spectra were then recorded over time until thermodynamic equilibrium was reached, and the guest resonances were integrated to provide K_{rel}. For fluorescence studies, solutions of the pyrene and perylene capsules **5.5** (**D**) and **8.8** (**A**) in mesitylene were prepared with these guests. In all cases an excess of the guest was employed (1000 equiv), and the solutions were allowed to stand until equilibrium was reached. Aliquots of these solutions were diluted into mesitylene and mixed at room-temperature such that the starting concentration of each capsule was 250 nM. The change in the fluorescence emission spectra was monitored with time until equilibrium was reached, a period that ranged from minutes to weeks depending on the guest used to fill the capsule. The dynamic behavior of

Table 1. Measured Rates (k) and Half-Lives for the Exchange of Capsule Subunits with Substituted Benzanilide Guests **9**−**13** in Mesitylene, Relative Binding Affinities (K_{rel}) of the Capsule for the Guests in d_{12}-Mesitylene, and Guest Lengths

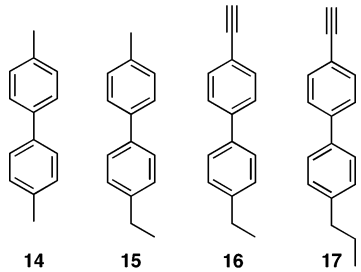


9 **10** **11** **12** **13**

guest	k (s^{-1})[a]	half-life[a]	K_{rel}	length (Å)[b]
9	2.0×10^{-2}	34 s	<0.1	11.4
10	6.1×10^{-4}	18 min	2.7	12.3
11	2.0×10^{-4}	58 min	5.3	12.3
12	5.4×10^{-6}	36 h	100	13.2
13	1.1×10^{-4}	108 min	1.8	14.7

[a] Uncertainties in k and the half-life are ±10%. [b] The structures were energy-minimized by using Spartan with MM2. The length was measured between the two most remote hydrogen atoms. Centers of atoms were used for all measurements.

Table 2. Measured Rates and Half-Lives for the Exchange of Capsule Subunits with Substituted Biphenyl Guests in Mesitylene, Relative Binding Affinities of the Capsule for the Guests in d_{12}-Mesitylene, and Guest Lengths



14 **15** **16** **17**

guest	k (s^{-1})[a]	half-life[a]	K_{rel}	length (Å)[b]
14	1.6×10^{-3}	7 min	2.0	11.1
15	6.3×10^{-6}	31 h	100	12.4
16	2.7×10^{-6}	71 h	85	14.0
17	1.4×10^{-5}	14 h	1.7	15.0

[a] Uncertainties in k and the half-life are ±10%. [b] The structures were energy-minimized by using Spartan with MM2. The length was measured between the two most remote hydrogen atoms. Centers of atoms were used for all measurements.

the capsules could then be extracted from these studies using first-order dissociation kinetic treatment which provided the rates, k, and half-lives for these exchange processes. The results from these studies are presented in Tables 1−3.

Shown in Table 1 are the first series of guests to be examined, substituted benzanilides **9** to **13**. A number of points can be made in relation to these results. First, 4,4′-dimethylbenzanilide **12** is an excellent guest for the capsule and forms the most thermodynamically favorable capsule as revealed by K_{rel}. A correlation between the stability of the capsule and the rate at which the capsule subunits exchange is apparent, as this complex has by far the slowest exchange rate with a half-life of 36 h for the process. At the other end of the scale is benzanilide **9**, which exhibits an extremely fast capsule exchange rate, the fastest of any guest measured with a half-life of only 34 s. Although benzanilide **9** is cleanly encapsulated and forms kinetically stable complexes by ^{1}H NMR spectroscopy, it is readily displaced by any of the other guests in this series. A similar relationship between guest affinity and capsule exchange rates is observed for monomethyl benzanilides **10** and **11**, that is, slower exchange in the presence of the better guest. The slight anomaly in this series is 4-methyl-4′-ethylbenzanilide **13**. Although the ^{1}H NMR competition experiments reveal that guest **13** forms a less thermodynamically stable complex than guests **10** or **11**, the subunit exchange is slower in the presence of this longer guest.

Results from the next series of guests, the substituted biphenyls, are shown in Table 2. In the case of 4,4′-dimethylbiphenyl, guest **14**, capsule exchange is considerably faster than with any of the other substituted biphenyls, with a half-life of only 7 min. As was discussed previously, 4,4′-dimethylbiphenyl **14** has been employed in ^{1}H NMR experiments to examine both guest exchange (with its deuterated analogue) and capsule subunit exchange (employing a chiral cavitand). The two rates measured for these exchange processes were similar and yielded half-lives of 5 to 10 min, remarkably consistent to the rate obtained from the fluorescence experiments. Some other intriguing results were obtained with this series of guests. With 4-ethyl-4′-methylbiphenyl **15**, possessing one additional methylene unit compared to **14**, a dramatic difference in both binding affinity and capsule exchange rates is observed with an increase from 7 min to 30 h for the half-life for the exchange process. Although guests **16** and **17** have similar binding affinities for the capsule to guests **15** and **14**, respectively, the longer guests **16** and **17** gave significantly slower exchange rates than their shorter counterparts.

For the most part there is a direct correlation between the rate of capsule exchange and the binding affinity of the capsule for a particular guest with the thermodynamically favored guests yielding slower capsule exchange rates. This correlation begins to fail as longer guests are used. Shown in Figure 5 is the relationship between the relative half-lives for capsule subunit exchange normalized according to K_{rel} and the guest length for both the substituted benzanilide and the substituted biphenyl guests. Dividing each half-life value by the corresponding K_{rel} for that particular guest normalized the half-lives. The trend can clearly be seen with the increased length of these rigid guests leading to slower exchange of the capsules. This helps to explain the slower capsule exchange observed for 4-ethynyl-4′-propylbiphenyl **17**, compared to the shorter 4,4′-dimethylbiphenyl **14**, despite their similar affinities for the capsule. Likewise the slower than expected exchange rate for the capsule with 4-methyl-4′-ethylbenzanilide **13** can be rationalized when the length of the guest is considered. For these long rigid guests, entry and exit mechanisms may be different from those of the flexible guests discussed below.

The final set of guests to be examined were the alkanes, whose encapsulation in the cylindrical capsule has been well studied.[29] These previous studies revealed very long equilibration times for solutions containing a mixture of two alkanes, indicative of exceedingly slow in/out rates for guest exchange. With the fluorescently labeled capsules **5.5** (**D**) and **8.8** (**A**) in hand, we could quantify the capsule exchange rates, and the results are presented in Table 3.

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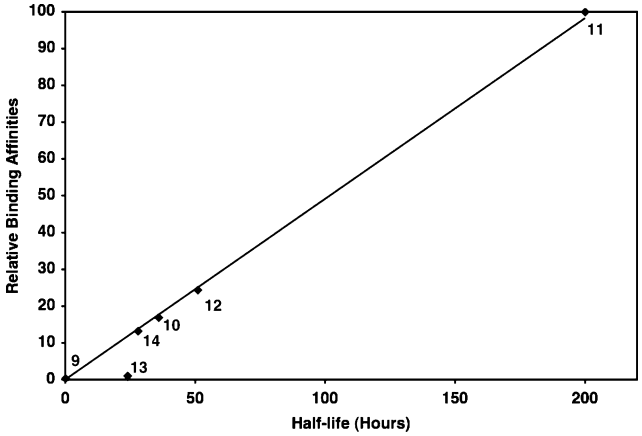

Figure 6. Relative binding affinities as a function of half-life of exchange for the alkane guests. The data point labels refer to the number of carbon atoms in the saturated alkane guests. For undecane the estimated half-life of 200 h from the ^{1}H NMR equilibration experiment is used.

Table 3. Rates and Half-Lives for the Exchange of Capsule Subunits with Alkane Guests in Mesitylene and Relative Binding Affinities of the Capsule for the Guests in d_{12}-Mesitylene

guest	k (s^{-1})[a]	half-life[a]	K_{rel}
C_9H_{20}	1.6×10^{-3}	7 min	0.3
$C_{10}H_{22}$	5.4×10^{-6}	36 h	16.9
$C_{11}H_{24}$	$\gg 2 \times 10^{-6}$	$\gg$100 h	100
$C_{12}H_{26}$	3.8×10^{-6}	51 h	24.4
$C_{13}H_{28}$	8.1×10^{-6}	24 h	1.0
$C_{14}H_{30}$	6.8×10^{-6}	28 h	13.2

[a] Uncertainties in k and the half-life are $\pm$10%.

Undecane is clearly the best alkane guest for the capsule; however, the exchange of the capsule around this guest was unfortunately too slow to be accurately determined. Even after three weeks, there was only a very small increase in FRET, and the errors in the small increases prohibited the complete study of this guest. This result is consistent with previous ^{1}H NMR competition experiments between decane and undecane, where 12 weeks was required for the system to reach equilibrium.[29b] An equilibration period of this length of time corresponds to a half-life of ca. 200 h. Once again, a very rapid exchange rate for the capsules is observed in the presence of the shortest guest, nonane, with a half-life of 7 min for the process. In general, for the flexible alkanes, a direct correlation between the relative binding affinities and the half-life for exchange of the capsule subunits is observed (Figure 6).[30] This is in contrast to the more rigid guests discussed above. Alkane guest length does not appear to affect the rate of exchange for the capsule monomers. We expect this is related to the increased flexibility of these guests.

Summary of Capsule Subunit Exchange in the Presence of Added Guests. The cylindrical capsule is held together through a cyclic seam of eight bifurcated hydrogen bonds and intermolecular forces between host and guest. It is expected that varying the guest will mostly influence the intermolecular forces between the host and the guest (however capsule deformation upon guest complexation can also change the hydrogen bond strength).[29b] In an attempt to explain the remarkably fast rates of capsule exchange in the case of benzanilide **9**, 4,4′-dimethylbiphenyl **14**, and nonane, we propose that although these guests template capsule formation as shown by ^{1}H NMR spectroscopy, they are too small to significantly interact with both halves of the capsule interior. In other words, the intermolecular forces between the host and guest are minimal, and the capsule is held together primarily by the seam of hydrogen bonds. As the guest size increases, the intermolecular forces between the guest and both halves of the host are enhanced, resulting in more stable capsules and slower rates of capsule exchange. Of course, once a guest becomes too long, this again destabilizes the capsule, as observed by the decrease in K_{rel}. However, slow exchange rates are still observed for the longer rigid guests, as they create a kinetic barrier to monomer exchange.

Variation in Guest Concentration. In all of the above studies, the concentration of the guest was maintained at 1000 times that of the capsule. It was of interest to examine the importance of guest concentration on the exchange of the capsule subunits. For this study, three different guests were chosen, benzanilide **9**, 4-methylbenzanilide **10**, and 4,4′-dimethylbiphenyl **14**. The number of equivalents of guest was varied from 10 to 1000 (less than a 10 fold excess did not provide reproducible results). For each of the benzanilides, a small dependence on guest concentration was observed with increased exchange rates obtained when the guest concentration was increased. For 4-methylbenzanilide **10** the half-life was 175 min with 10 equiv and 131 min with 1000 equiv of guest, and for benzanilide **9** the change was from a half-life of 55 s (10 equiv) to 35 s (1000 equiv). Addition of these anilide guests increases both the polarity and H-bonding ability of the bulk solution, which should aid in the dissociation of the hydrogen-bonded capsule. It is known that an increase in the concentration of hydrogen-bonding groups can have a dramatic effect on the capsular subunit exchange process.[24] 4,4′-Dimethylbiphenyl **14** does not contain a polar amide group, and variation in the concentration of this guest did not greatly influence the rate of capsule exchange. In general the results suggest that the guest concentration does not have a large impact on the exchange rate of the capsule.

Conclusions

Cavitands labeled with donor and acceptor fluorophores have been successfully synthesized and utilized to probe the dynamic behavior and kinetic stability of the cylindrical capsule by FRET. This enabled the study of interacting species at nanomolar concentrations, providing information unattainable from NMR experiments. The thermodynamic stability of the capsule generally dictates the kinetic stability of the capsule, that is, the capsule subunits exchange slower when a more favorable guest is encapsulated. However, long rigid guests do not follow this trend, as the longer guests impart a kinetic barrier to monomer exchange. Through these FRET studies we could quantify the kinetic stability imparted on the hydrogen-bonded capsule by the host−guest interactions. Since the assemblies all contain the same eight bifurcated hydrogen bonds, the measured differences in monomer exchange rates are mostly due to changes in the host−guest interactions. For the series of guests studied, a remarkable range of more than 4 orders of magnitude was observed in the rates of cylindrical capsule exchange. As for guest exchange, the opening of "flaps" in the assembly seems

(30) The anomaly in the series is tridecane, however guests with a chain length of 13 have previously been found to be bound less favorably in both the *n*-alkane and oligioethylene glycol guest series than the guests of length 12 or 14. The reasons for this are unknown. Purse, B. W.; Rebek, J., Jr. *Chem. Commun.* **2005**, 722−724.

a likely first step, but just how many and which flaps remains a question for the future. Recent observations by Diederich[31] on the dynamics of related systems point to a richer landscape of conformations for these resorcinarene derivatives than is summarized by "vase" and "kite".[32]

Experimental Section

1. General Considerations. All reagents were purchased from commercial suppliers and were used without further purification unless otherwise stated. All reactions were performed under an anhydrous nitrogen atmosphere. Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} coated plates (Merck).

Deuterated solvents were used as purchased from Cambridge Isotope Laboratories. ^{1}H and ^{13}C NMR spectral data were recorded on a Bruker 600-DRX spectrophotometer. Data is expressed in parts per million (ppm) downfield shift from tetramethylsilane with residual protio solvent as an internal reference and is reported as position (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (J in Hz) and integration (number of protons). High-resolution mass spectra (HRMS) were recorded on an Agilent ESI-TOF mass spectrometer by Scripps Center for Mass Spectrometry. UV absorption spectra were recorded on a Varian Cary 50 UV-Visible spectrophotometer. Fluorescence measurements were obtained using a Fluorolog-3 Model FL3-21 spectrofluorometer.

2. Synthesis. All the guests employed are known compounds and were prepared according to literature procedures.[21,33] Capsule **1.1** was prepared and purified according to previously described procedures.[21]

Pyrene-Labeled Cavitand 5. To a solution of pyrene resorcinarene ester **4** (68 mg, 55 μmol) and 5,6-dichloropyrazine-2,3-dicarboxylic acid imide (55 mg, 242 μmol) in DMF (3 mL) was added dropwise NEt_3 (63 μL, 451 μmol). The solution was stirred at RT for 50 min, the solvent was removed *in vacuo*, and the residue was sonicated with MeOH and H_2O for 1 h. The product was filtered and washed further with MeOH, H_2O, and more MeOH to afford the pyrene-labeled cavitand **5** (65 mg, 79%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.86 (t, J = 7.3 Hz, 6H), 0.89 (t, J = 6.8 Hz, 3H), 1.10−1.47 (m, 54H), 2.02−2.08 (m, 2H), 2.27−2.35 (m, 6H), 2.57−2.61 (m, 2H), 4.72 (t, J = 6.0 Hz, 2H), 5.68 (t, J = 7.7 Hz, 3H), 5.86 (t, J = 8.0 Hz, 1H), 7.34 (s, 2H), 7.39 (s, 2H), 8.07−8.12 (m, 2H), 8.10 (s, 2H), 8.13 (s, 2H), 8.21 (t, J = 8.2 Hz, 2H), 8.25−8.30 (m, 3H), 8.67 (d, J = 8.1 Hz, 1H), 9.30 (d, J = 9.3 Hz, 1H), 9.77 (bs, 1H), 9.80 (bs, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.2, 28.0 (2 co-incident resonances), 29.5 (2 co-incident resonances), 29.6, 29.7 (2 co-incident resonances), 29.8 (4 co-incident resonances), 32.0, 32.1, 32.3, 32.4, 34.0, 34.2, 34.3, 64.8, 118.8, 123.6, 124.1, 124.2 (2 co-incident resonances), 124.3, 124.8, 124.9, 126.5, 126.6 (2 co-incident resonances), 127.3, 128.4, 129.8, 129.9, 130.4, 131.1, 131.3, 134.6, 136.3, 136.7, 136.9, 137.2, 142.1, 142.3, 152.3, 152.4 (2 co-incident resonances), 152.5, 157.9, 158.2 (2 co-incident resonances), 162.6, 162.8, 168.2; HRMS (ESI-TOF; M + Na^+) calcd for $C_{105}H_{100}N_{12}O_{18}Na$ 1839.7170, found 1839.7235.

Perylene-Labeled Cavitand 8. To a solution of perylene resorcinarene triazole **7** (69 mg, 50 μmol) and 5,6-dichloropyrazine-2,3-dicarboxylic acid imide (48 mg, 220 μmol) in DMF (3 mL) was added dropwise NEt_3 (57 μL, 410 μmol). The solution was stirred at RT for 50 min, the solvent was removed *in vacuo*, and the residue was sonicated with MeOH and H_2O for 1 h. The product was filtered and washed further with MeOH, H_2O, and more MeOH to afford the perylene-labeled cavitand **8** (82 mg, 85%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 6H), 0.89 (t, J = 6.2 Hz, 3H), 1.15−1.47 (m, 54H), 2.03−2.06 (m, 2H), 2.13−2.18 (m, 2H), 2.27−3.37 (m, 6H), 4.51 (bs, 2H), 4.82 (s, 2H), 5.00 (s, 2H), 5.60−5.68 (m, 4H), 7.26 (bs, 2H), 7.29 (bs, 2H), 7.46−7.55 (m, 5H), 7.65−7.67 (m, 2H), 7.91 (d, J = 7.5 Hz, 1H), 8.06 (bs, 2H), 8.08 (bs, 2H), 8.16−8.25 (m, 5H), 9.77 (s, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.2, 22.8, 28.0, 28.1, 29.5, 29.6, 29.8, 29.9, 32.0, 34.2 (2 co-incident resonances), 34.3, 49.6, 64.0, 71.4, 104.7, 118.8, 119.7, 120.5, 120.6 (2 co-incident resonances), 122.6, 123.8, 123.9, 124.1, 124.4, 124.5, 126.7, 126.8, 127.0 (2 co-incident resonances), 127.7, 128.1, 128.2, 128.4, 129.1, 131.0, 131.2, 131.7, 131.8 (2 co-incident resonances), 133.1, 134.6, 135.9, 136.4, 136.9, 137.5, 142.3, 142.4, 145.9, 152.2, 152.4, 152.5, 158.0 (2 co-incident resonances), 158.2 (2 co-incident resonances), 162.7, 162.8; HRMS (ESI-TOF; M + H^+) calcd for $C_{112}H_{108}N_{15}O_{17}$ 1934.8042, found 1934.7984.

3. Preparation of NMR Samples. Solutions for competition NMR experiments were prepared with 2 mM of cavitand **1.1** and an excess (20 mM) of each guest in mesitylene-d_{12}. NMR spectra were recorded directly after mixing and at regular time intervals until thermodynamic equilibrium was reached. The relative binding affinities of the guests to the capsule were determined by direct integration of the corresponding peaks of the encapsulated guests and/or integration of the NH signals.

4. Fluorescence Measurements. Spectrophotometric grade toluene was purchased from Aldrich and used as received. Mesitylene was distilled prior to use to remove fluorescent impurities. All measurements were conducted at room temperature. Solutions of each capsule **5.5** (**D**) and **8.8** (**A**) were prepared at 7.7×10^{-6} M with 1000 equiv of guest unless otherwise stated and allowed to equilibrate for at least 48 h. Equimolar amounts of each solution were then mixed, and an aliquot of the mixture was diluted into 3 mL of mesitylene so that the concentration of each capsule was 250 nM. The fluorescence spectrum was then recorded with an excitation wavelength of 350 nm.

The apparent rate constant, k, for the exchange of the capsule subunits was determined by fitting the data to the first-order equation:

$$\ln((F_{\max} - F_t)/F_{\max}) = -kt$$

where F_t and $F_{\max}$ are the fluorescence intensities at 447 nm at times t and the maximum value obtained after the capsules have reached equilibrium. The negative slope of the line provides the apparent rate constant k.

Acknowledgment. We thank the Skaggs Institute for Chemical Biology and the NIH (GM50174) for financial support. E.S.B. and T.J.D. are Skaggs postdoctoral and predoctoral fellows, respectively. We are also grateful to Prof. Pablo Ballester (Institute of Chemical Research of Catalonia (ICIQ), Spain) for helpful discussions.

Supporting Information Available: Additional fluorescence and absorption spectra showing spectral overlap, details of methanol titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

JA071774J

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