

Guest Rotations within a Capsuleplex Probed by NMR and EPR Techniques

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With the help of ¹H NMR and EPR techniques, we have probed the dynamics of guest molecules included within a water-soluble deep cavity cavitand known by the trivial name octa acid. All guest molecules investigated here form 2:1 (host/guest) complexes in water, and two host molecules encapsulate the guest molecule by forming a closed capsule. We have probed the dynamics of the guest molecule within this closed container through ¹H NMR and EPR techniques. The timescales offered by these two techniques are quite different, millisecond and nanosecond, respectively. For EPR studies, paramagnetic nitroxide guest molecules and for ¹H NMR studies, a wide variety of structurally diverse neutral organic guest molecules were employed. The guest molecules freely rotate along their *x* axis (long molecular axis and magnetic axis) on the NMR timescale; however, their rotation is slowed with respect to that in water on the EPR timescale. Rotation along the *x* axis is dependent on the length of the alkyl chain attached to the nitroxide probe. Overall rotation along the *y* or *z* axis was very much dependent on the structure of the guest molecule. The guests investigated could be classified into three groups: (a) those that do not rotate along the *y* or *z* axis both at room and elevated (55 °C) temperatures, (b) those that rotate freely at room temperature, and (c) those that do not rotate at room temperature but do so at higher temperatures. One should note that rotation here refers to the NMR timescale and it is quite possible that all molecules may rotate at much longer timescales than the one probed here. A slight variation in structure alters the rotational mobility of the guest molecules.

Introduction

Understanding the physical and chemical behavior of supramolecular assemblies has been an important goal of chemists during the last two decades.^{1,2} In this context, aspirations are directed at gaining insight into the behavior of guest molecules in confined spaces (often called reaction cavities) and the exploitation of weak intermolecular noncovalent bonds between host and guest to alter the behavior of guest molecules.³ To achieve this goal requires the identification and understanding of the physical characteristics of the interior of a suitable host, knowledge of factors controlling the ratio and stability of host–guest complexes, and the dynamics of a guest within such a complex.^{4,5} During the last 5 years, we have been exploring the use of a deep cavity cavitand, trivially known as octa acid (OA),^{6,7} as a host for carrying out selective photoreactions in water.^{8,9} Our significant success in terms of photoproduct selectivity with this host prompted us to probe the dynamics of OA–guest complexes. We have recently established that, depending on the structure of the guest, the stoichiometry of OA–guest complexes could have a

host/guest ratio of 1:1, 2:1, or 2:2.¹⁰ (Throughout the text, the ratio in such complexes will be expressed in this manner only.) We refer to the complexes with the latter two ratios as capsuleplexes. Through fluorescence and electron paramagnetic resonance (EPR) techniques, we inferred the interior of OA–guest capsuleplexes to be nonpolar and benzene-like despite being surrounded by water molecules.¹¹ In this report, we focus on the dynamics of a guest molecule within an OA capsuleplex, the 2:1 complex in particular, by employing ¹H NMR and EPR techniques. Our interest in organic capsules relates to their use as reaction cavities for manipulating photochemical processes. In this context, we are interested in understanding the dynamics of the host, the guest, and the host–guest complex on the excited-state timescale. To our knowledge, no such studies have been carried out with OA capsule. It is also important to note that the dynamics of host–guest assemblies are time-dependent. Because the timescales of NMR, EPR, and excited singlet and triplet states are different, the system may be static on one timescale but dynamic on a different timescale. With this understanding, we probe the mobility of guests within the OA capsule by NMR and EPR techniques. The structure and cavity dimensions of OA are provided in Scheme 1. Structures of the guests (diamagnetic **1–6** and paramagnetic **7** and its diamagnetic equivalent **8**) used in this study are provided in Scheme 2.

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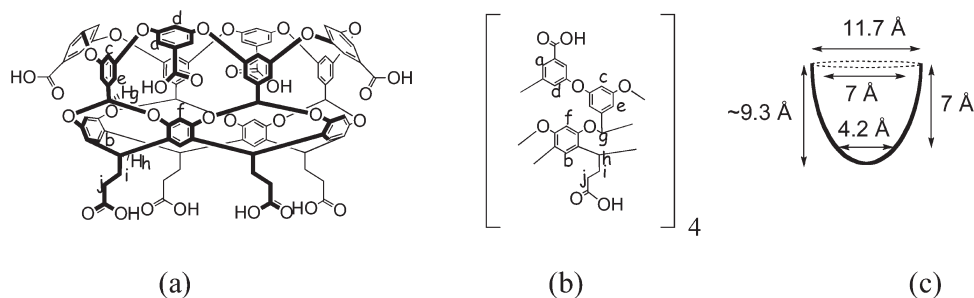
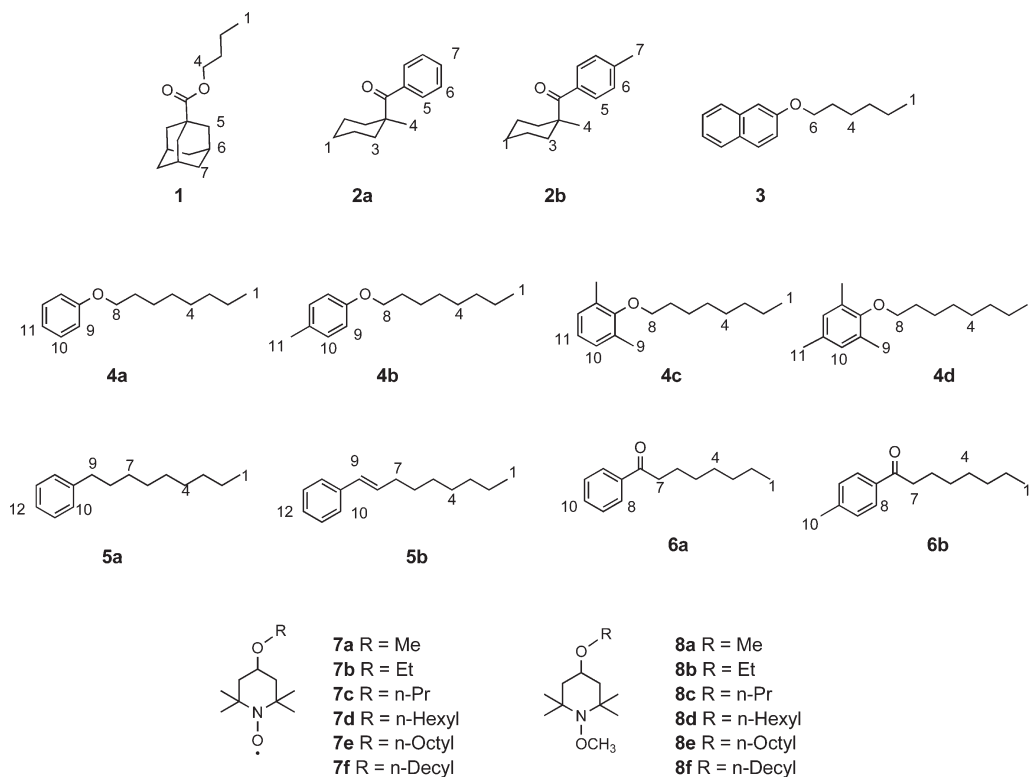
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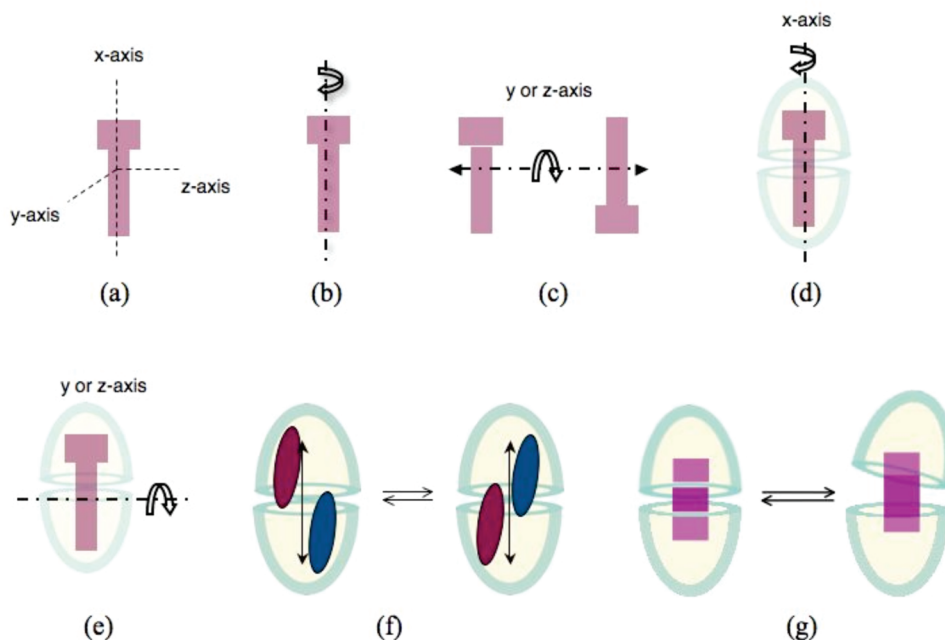
Scheme 1. Chemical Structure (a), Structure of a Single Panel (b), and (c) Cartoon Representation with Dimensions of the Host (Octa Acid) Used in This Study**Scheme 2. Chemical Structures of the Guests Used in This Study**

Scheme 3 presents a cartoon depiction of the three types of motion of the 2:1 capsuleplex in which we are interested. The three axes of the guest are defined in Scheme 3a. To be consistent with the EPR literature, we have labeled both the magnetic and the long molecular axis as x although we are aware that one would prefer to label the long molecular axis as z . A free guest could undergo overall rotation along the x , y , and z axes (Scheme 3b,c). We visualize two types of guest rotational motion in a capsuleplex: (1) guest rotation along its x axis (Scheme 3d), a motion that would not lead to major changes in the structure of the host–guest complex and (2) guest motion along the y or z axis (Scheme 3e), a motion that would, especially with an unsymmetrical guest, displace the two ends (arbitrarily named its head and tail) from one part of the capsule to a different one (arbitrarily named the top and bottom of the capsule). With respect to the positional change, both types of motions lead to the same structural change. The rotation of the guest along the y or z axis within the capsule as illustrated would be prohibited by the walls of the host. Therefore, rotation along the y or z axis as illustrated in the Scheme would not occur when the guest molecule is contained in a confined space. We visualize that a motion

equivalent to that of y or z axis rotation could be achieved by a coordinated “wiggly” motion of the various parts of the guest molecule within the confined space of the capsule. In the text, we use such a rotation only to represent the final outcome of the guest structure with respect to the two halves of the host. It must be noted that the possible rotations of the capsuleplex as a whole along the x , y , or z axis would alter neither the relative orientation of the guest with respect to the host nor the scope of this article.

The presence of more than one unit of the same or two different guest molecules within a capsule (2:2 capsuleplex) raises the possible sliding motion of these molecules (shown in Scheme 3f).¹⁰ With ¹H NMR data, we have recently established that two guest molecules such as naphthalene and 1,4-diethylbenzene exhibit a sliding motion of the type shown in Scheme 3f within an OA capsule at room temperature. In addition to these guest motions, the capsule made up of two host molecules can open and close (Scheme 3g). We are currently investigating this process through photophysical techniques, but the results of these studies are not included here. In this report, we are concerned only with the guest motion corresponding to the x , y , or z axis (Scheme 3a,b) within the 2:1 OA capsuleplex. We have employed EPR and ¹H NMR

Scheme 3. Cartoon Representations of Various Motions of Host–Guest Complexes Being Investigated (b–f), Host Opening and Closing (g), and the Three Axes of the Guest (a)



techniques to gain information about guest rotational motions within the complex on nanosecond and millisecond timescales, respectively. Experimental results presented below lead us to conclude that all guest molecules investigated (Scheme 2) rotate along their *x* axes on the NMR timescale (ms). Most importantly, rotation about the *y* or *z* axis is structure-dependent. An analysis of EPR spectra of OA-bound nitroxide probes (**7a–d**) suggested that rotation along the *x* axis occurs on the nanosecond timescale.

Results and Discussion

Techniques Used. The current study consists of ^1H NMR, COSY, NOESY, and ROESY experiments.¹² All COSY and ^1H NMR titration spectra are included as Supporting Information (SI) and are not discussed in the text. The ^1H NMR signals assigned to various hydrogens of the host and guest through COSY are marked in displayed Figures. ^1H NMR titration data were used to identify host–guest stoichiometry. It is important to note that for all investigated host–guest systems (Scheme 2, structures **1–6** and **8**) the ^1H NMR signals for free guests were seen only when the amount of the guest was in excess of the 2:1 ratio, suggesting that they form 2:1 capsuleplexes. During titration experiments, ^1H chemical shifts of the OA-bound guests remained constant, independent of the amount of host in the solution (the host concentration was changed from 0 to 2 equiv of the guest), suggestive of the lack of equilibrium between the free and complexed guest, and thereby precluding the determination of the binding constants of host–guest complexes. NOESY spectra recorded at a standard mixing time of 300 ms allowed us to gain information on guest orientation within the OA capsule. In specific cases, NOESY spectra were also recorded at additional mixing times of 150 and 50 ms. To ensure that the NOESY correlations are not an artifact, in select cases ROESY experiments were also carried out. NOESY and conventional ^1H NMR spectra recorded at various temperatures provided information on the dynamics of the guest along the *y* and *z* axes within the OA capsule.

Choice of Probes. For ^1H NMR studies, probes **1–6** (Scheme 2) were chosen. All 12 probes listed in Scheme 2 formed 2:1 capsuleplexes with OA (^1H NMR titration spectra in SI). Probes with different structures and different substituents were chosen to explore the structural influence on the rotational mobility of guests within a capsule. For EPR studies, nitroxide probes **7a–f** (4-alkoxy-2,2,6,6-tetramethyl-piperidine-1-oxyl; NET-*n*, series where *n* is the chain length) series) were chosen.^{13,14} On the basis of the NMR spectral behavior of corresponding diamagnetic derivatives **8a** and **8b** (where R is OCH_3 and OC_2H_5), we believe that 4-methoxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (**7a**) and 4-ethoxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (**7b**) formed 2:2 capsuleplexes and therefore were not investigated further by EPR experiments. All other paramagnetic probes (**7c–f**) used in this study were presumed to form 2:1 complexes. Because of the broad NMR signals, no ^1H NMR characterization of the complex could be carried out. Expecting that the length of the alkyl chain would affect the rotational mobility of the probe, various lengths of alkoxy groups were attached at the 4 position of the above nitroxide probe (**7c–f**).

Methodology. Most information regarding the guest motion within the OA capsule was extracted from ^1H NMR spectra of host–guest complexes. The OA host has 4-fold symmetry and is made up of four identical panels (Scheme 1a,b) containing 10 sets of chemically nonequivalent hydrogens (the total number of hydrogens is 14) giving a maximum of 10 NMR signals in the δ region of 2–8 with 6 of these appearing in the δ region of 6–8 (shown in Figure 1a). With a symmetrical guest such as 4,4'-dimethylstilbene, a capsule is formed from one guest molecule and two molecules of OA. Such a capsuleplex is symmetrical (the top and bottom halves of the capsule are identical) and displays (despite the presence of eight identical panels, four on top and four on bottom) only six signals in the δ region of 6–8 in the ^1H NMR spectrum (Figure 1b). When OA complexes to an

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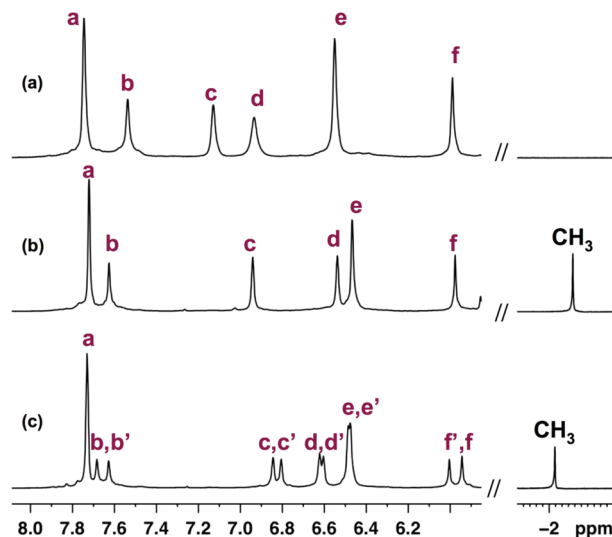


Figure 1. Partial ^1H NMR spectra (500 MHz, D_2O) of (a) OA, (b) 4,4'-dimethylstilbene@OA₂, and (c) 4-methylstilbene@OA₂. ([OA] = 1 mM in 10 mM sodium tetraborate buffer and [guest] = 0.5 mM in all cases. Aromatic resonances of the host are labeled from a to f, and encapsulated guest methyl groups are marked as CH_3 ; for assignments of host hydrogens, see Scheme 1a,b.)

unsymmetrical guest such as 4-methylstilbene, the capsule as a whole is unsymmetrical along the y - z plane. In this case, the top and bottom halves of the capsule are not identical. The inclusion of an asymmetrical guest destroys the symmetry of the capsule and its peaks (equivalent protons in the top and bottom halves) are split, the extent being dependent on the electronic differences between the top and bottom parts of the guest molecule. As seen in Figure 1c, many host peaks are split (H_b , H_c , H_d , and H_f). One might note that the symmetry of the capsuleplex would be restored by the free rotation of the guest along the y or z axis. We believe from our recordings of split peaks that such rotation does not occur on the NMR timescale. It is quite likely that the guest tumbles on a longer timescale, but if it does, it would be invisible to 1D NMR. However, if one can monitor interactions between the host and guest signals on a longer timescale, then one might be able to detect the guest tumbling within the capsule. With this in mind, we recorded NOESY and ROESY correlations between the host and guest signals. We are aware that NOESY correlations are subject to artifacts when the molecular weight of the system falls in the range of 1–4 kDa. The OA capsule with the guest and associated Na^+ ions is slightly above this range. In spite of this, to be sure that the NOESY correlations are trustworthy we also recorded ROESY spectra for select examples. This allowed us to make conclusions based on the NOESY correlations presented here. An examination of the correlations in NOESY spectra provided information relating to the rotational mobility of the guest. The head and tail portions of a stationary guest molecule within the capsuleplex would be placed in different halves of the capsule and thus complexed to two molecules of OA. NOESY interactions that should reflect such binding, especially when the host signals for each OA are distinguished as in the case of guest 4-methylstilbene, are illustrated in Figure 2. Here there are two signals due to H_f of the host, one due to the top half and the other due to the bottom half of the capsule, and the methyl group of 4-methylstilbene interacts with only one of the two. A similar correlation was observed with H_b . If the guest is tumbling on the NOE timescale, then one would expect the correlation of the methyl group of 4-methylstilbene with protons present at both ends of the capsule. To be sure that our observation is correct, we

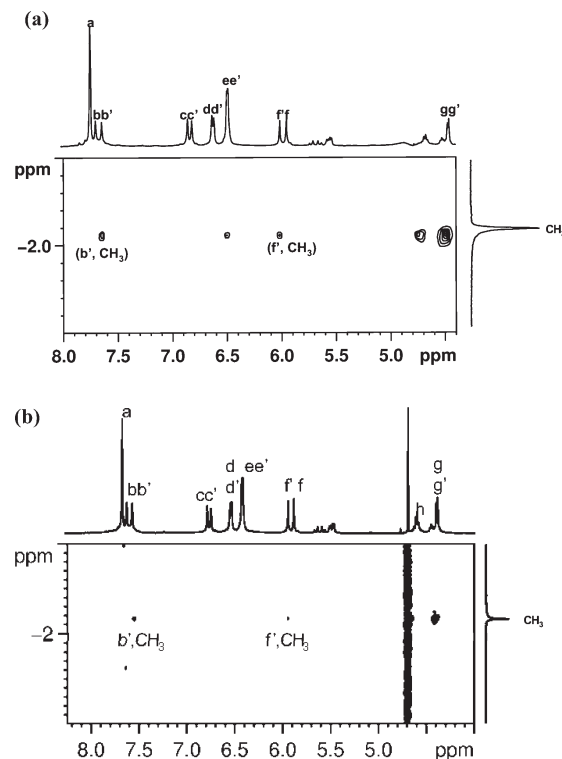


Figure 2. (a) 2D NOESY partial spectrum of 4-methylstilbene@OA₂. ([OA] = 5 mM in 50 mM sodium tetraborate buffer and [guest] = 2.5 mM. Aromatic resonances of the host are labeled from a to f, and encapsulated guest methyl groups are marked as CH_3 ; for assignments of host hydrogens, see Scheme 1a,b.) (b) Partial 2D ROESY spectra of 4-methylstilbene@OA₂. ([OA] = 5 mM in 50 mM sodium tetraborate buffer, [guest] = 2.5 mM, and the ROESY spin lock is 300 ms.)

also carried out ROESY experiments, and the results are presented in Figure 2. We interpret the absence of correlation between the methyl hydrogens and both H_f (and both H_b) signals of the host in both NOESY and ROESY to mean that the guest does not undergo motion equivalent to rotation along the y or z axis for 300 ms, which is the mixing time used for the accumulation of NOESY data. This would also mean that on the photochemical scale, the timescale that is of interest to us, the guest is stationary within the capsule. We discuss the results from utilizing these spectral features (signal split in ^1H NMR and NOESY correlations) to provide information on the rotational motion of guests 1–6 along their y or z axis within OA at various temperatures and mixing times (NOESY) in the following sections.

In the hypothetical case of a nonrotating guest along the x axis, the 4-fold symmetry of OA would be lost and chemically equivalent hydrogens present on the four panels could become magnetically nonequivalent and, in principle, multiple ^1H NMR signals for the host protons could be expected. None of the capsuleplexes that we have investigated distinguished the four panels of each OA molecule. This suggests that the guest rotation along the x axis occurs on a much shorter timescale than that of NMR. To further probe the rotational process along the x axis, we recorded EPR spectra of nitroxide probes (7c–f) included within the OA capsule. We hypothesized that the guest rotation along the x axis could be probed by EPR (on the nanosecond timescale). Rotational correlation times were measured by EPR for various nitroxide probes appended with alkyl chains of different lengths.

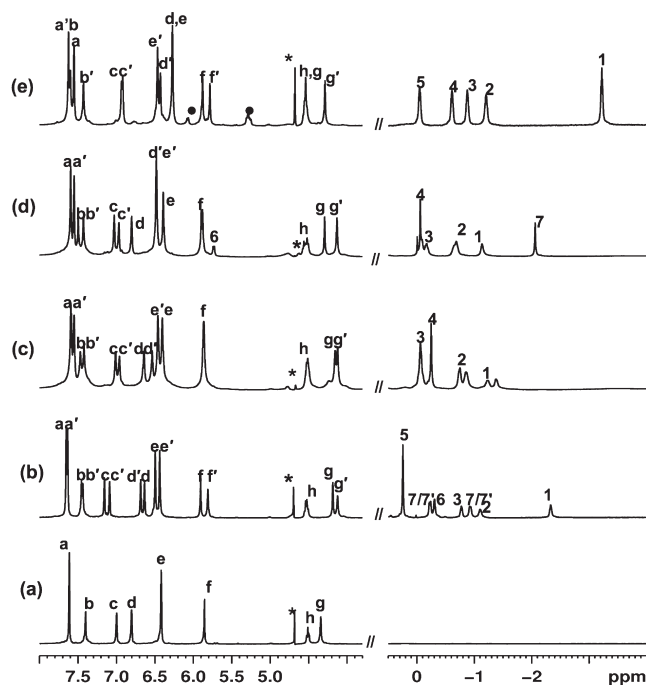


Figure 3. ^1H NMR (500 MHz, D_2O) spectra of (a) OA (1 mM in 10 mM borate buffer), (b) $1@OA_2$, (c) $2a@OA_2$, (d) $2b@OA_2$, and (e) $3@OA_2$. Aromatic guest resonances of the host OA are represented by labels a–g, and bound guest resonances are labeled 1–7. For assignments of host hydrogens, see Scheme 1a,b; for assignments of the guest, see Scheme 2. Labels * and • represents the residual water and bound aromatic guest resonances, respectively.

The rotational correlation times of the probes in solution were on the sub-nanosecond timescale and almost independent of the length of the alkyl chain, while the correlation times of the probes within the OA capsule were on the nanosecond timescale and were dependent on the alkyl chain length. The details are discussed in the following sections.

Guest Molecules That Do Not Rotate along Their y or z Axis within an OA Capsule on the NMR Timescale at Room Temperature. First we discuss the behavior of four guest molecules (**1**, **2a**, **2b**, and **3**) within the OA capsule. We group these molecules that all form 2:1 capsuleplexes with OA in water (see SI for ^1H NMR titration spectra) and are stationary within the OA capsule in the NMR timescale. ^1H NMR spectra of OA complexes of **1**, **2a**, **2b**, and **3** are displayed in Figure 3. Guest and host signals are assigned on the basis of the COSY data that are provided as SI. An upfield shift (with respect to that in CDCl_3) of the guest proton signals is a clear indication that these molecules are included within the OA capsule.^{15,16} For example, the terminal methyl group present in the alkyl chain of **1** and **3** and the para methyl in **2b** are shifted to below $\delta -2.0$ because of paramagnetic shielding by the four phenyl rings present at the base of the OA capsule. Furthermore, adamantyl protons in **1** and cyclohexyl ring protons in **2a** and **2b** have chemical shifts below $\delta 0$. The important point to note is that in each of these examples many of the host signals are split into two, which suggests that in the presence of these guest molecules the top and bottom halves of the capsule are not identical, at least not on the NMR timescale. This feature was further confirmed by recording NOESY spectra

at 300 ms of mixing time. The region of relevance to the current discussion, correlations between the guest signals and the two host signals (H_g and $H_{g'}$), are displayed in Figure 4. The interesting observation is that the two sets of H_g hydrogens, arbitrarily marked as H_g and $H_{g'}$, correlate with different parts of the guest molecule, which is suggestive of the stationary nature of the guest within the OA capsule. For example, in **1**, we believe that the correlation of the adamantyl hydrogens with $H_{g'}$ and the alkyl chain hydrogens with H_g suggests the residence of these guest molecule groups in two different regions of the capsule without any positional exchange within 300 ms. A similar trend is also seen in **2a**, **2b**, and **3**. In **2a**, the cyclohexyl-ring hydrogens correlate with H_g and the methyl group on the phenyl ring correlates with $H_{g'}$. For the sake of clarity, only the cyclohexyl hydrogens in **2a** and the alkyl chain hydrogens in **3** are shown. All of these examples suggest that the two parts of the guest molecule reside in two different regions of the capsule. We conclude this section by reiterating the lack of rotation of the above four guest molecules corresponding to the y or z axis on the 300 ms timescale at room temperature. To make sure that the conclusions drawn above are not based on experimental artifacts, we carried out ROESY studies on $3@OA_2$ and $1@OA_2$ (ROESY spectra provided in SI). In the case of $3@OA_2$, even in ROESY the methyl group of the alkyl chain correlated with only one b, e, f, or g proton of the host, suggesting that the molecule is stationary on the NOESY/ROESY timescales. Similar relationships between NOESY and ROESY data were seen in the case of $1@OA_2$. To further confirm the absence of rotation of **3** within the OA capsule, we recorded ^1H NMR of $3@OA_2$ at various temperatures. There was only a minor variation in the NMR between 26 and 60 °C (Figure 5). Similar variable-temperature NMR studies with $1@OA_2$ (Figure S28 in SI) suggested to us that even at 60 °C guest molecules **1** and **3** undergo very little motion along the y and z axes within the capsule on the NMR timescale.

Guest Molecules That Rotate along Their y or z Axis within OA at Room Temperature on the NMR Timescale. In this section, we discuss the dynamics of **4a**, **5a**, and **6a** within the OA capsule. These three guest molecules such as the four molecules discussed above (**1**, **2a**, **2b**, and **3**) are unsymmetrical with respect to their y – z plane. In spite of such a feature, the NMR spectra of these molecules are distinctly different from those of **1**, **2a**, **2b**, and **3**. They do not show splitting of the host hydrogens (Figures 6a and 7a,c). The identical signals obtained for the two halves of the capsule suggest that on the NMR timescale these guest molecules undergo motion that could be translated into rotation along the y and z axes to lead to magnetically equivalent halves of the capsule. We recognize that simple C_2 rotation along either the y or z axis is not possible within the capsule. We visualize that the guest molecules, through the coordinated movement of various parts of the molecule, undergo a motion that is similar to rotation along the y or z axis.

Guest Molecules That Do Not Rotate along Their y or z Axis within OA at Room Temperature on the NMR Timescale but Do Rotate on Longer Timescales. By hypothesizing that the rotational flexibility of **4a** could be manipulated by methyl substitution that would provide $C-H\cdots\pi$ ¹⁷ and/or steric interaction between the guest and the host, we examined the behavior of **4b**, **4c**, and **4d**. Expectedly, the hydrogens on the alkyl chain were significantly upfield shifted, suggesting that this part is located in one-half of the capsule (Figure 6b–d). More importantly, signals of the para methyl group in **4b** and **4d** were also

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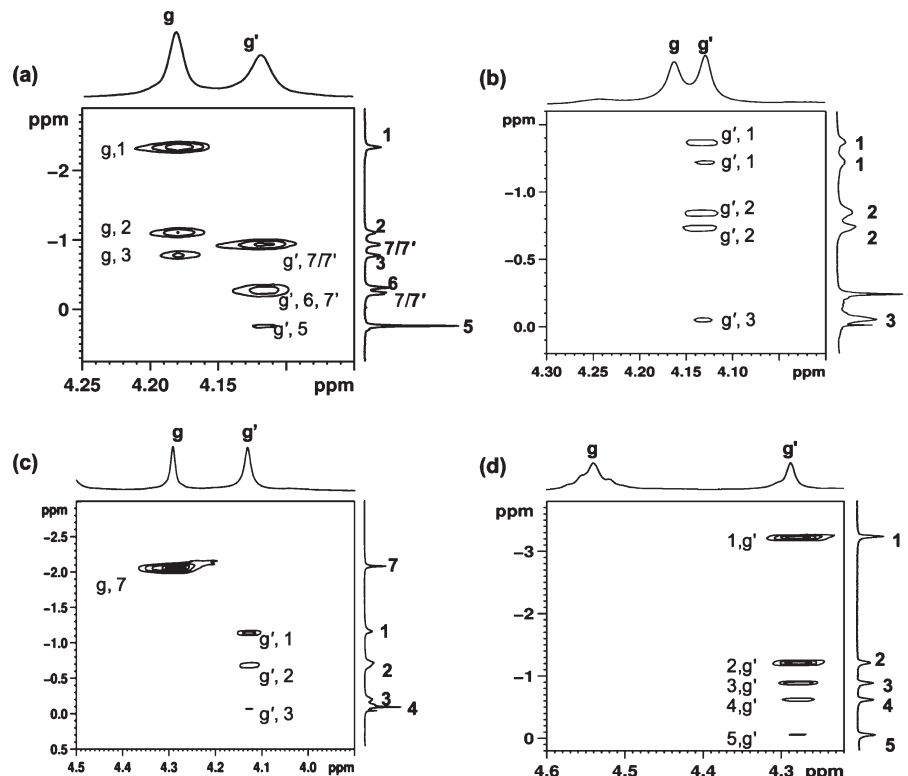


Figure 4. 2D NOESY partial spectrum of (a) **1**@OA₂, (b) **2a**@OA₂, (c) **2b**@OA₂, and (d) **3**@OA₂ (500 MHz, 5 mM) in buffered D₂O (50 mM), [OA] = 5 mM, and [guest] = 2.5 mM. (OA peaks are marked as g and g', and guest peaks are marked as 1–7). The mixing time was 300 ms.

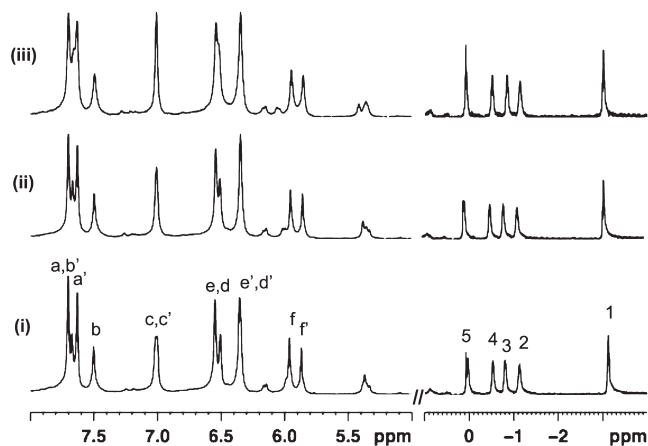


Figure 5. Partial ¹H NMR spectra of **3**@OA₂ (400 MHz, [OA] = 1 mM) in 10 mM borate buffer (D₂O) at (i) 26 °C, (ii) 40 °C, and (iii) 60 °C. (OA peaks are marked as a–f, and guest peaks are marked as 1–5.)

significantly upfield shifted ($\Delta\delta \sim 4.0$ with respect to CDCl₃). We believe the aryl and alkyl parts of **4b** and **4d** would be present at two different ends of the capsule because the narrow end of the capsule is too small to accommodate them both. Most interestingly similar to **1**–**3**, with **4b**, **4c**, and **4d** the hydrogens due to the host molecules are split, suggesting that the two halves of the capsule experience different magnetic environments (Figure 6) as a result of the absence of rotation along their y or z axis within the OA capsule on the NMR timescale. However, OA complexes of the latter group of probes showed NOESY correlations at 300 ms of mixing time (Figures 7 and 9) with both H_g and H_{g'} suggestive of the overall motion corresponding to rotation along their y or z axis, thus suggesting that time plays a critical role in guest rotation

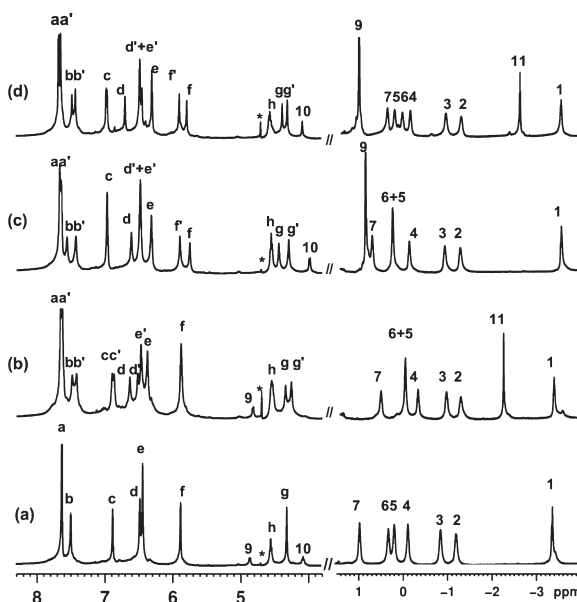


Figure 6. ¹H NMR (500 MHz, D₂O) spectra of (a) **4a**@OA₂, (b) **4b**@OA₂, (c) **4c**@OA₂, and (d) **4d**@OA₂. [OA] = 1 mM in 10 mM borate buffer. Aromatic guest resonances of the host OA are represented as a–g, and bound guest resonances are represented as 1–11. * represents the residual water resonance.

within the capsule. On the basis of conventional ¹H NMR and NOESY spectra, we believe that **4b**, **4c**, and **4d** do not rotate on the order of milliseconds but do so at > 300 ms.

One possible interpretation is that the NOESY correlations seen at longer mixing time (300 ms) are due to spin diffusion. If this is true, then we should have observed this phenomenon with guests **1**–**3** discussed in the previous section. It is not obvious to us

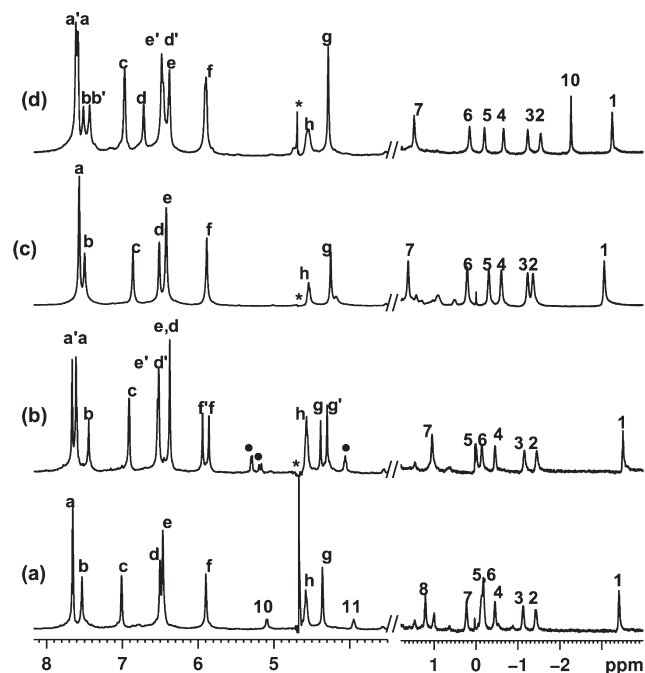


Figure 7. ^1H NMR (500 MHz, D_2O) spectra of (a) $5\text{a}@OA_2$, (b) $5\text{b}@OA_2$, (c) $6\text{a}@OA_2$, and (d) $6\text{b}@OA_2$. $[OA] = 1\text{ mM}$ in 10 mM borate buffer and $[\text{guest}] = 0.5\text{ mM}$. Aromatic guest resonances of the host OA are represented as a–g, and bound guest resonances are represented as 1–11. * and • represent the residual water and bound aromatic guest resonances, respectively.

why spin diffusion would occur only with **4b**, **4c**, and **4d** but not with **1–3**. On the basis of the results observed with guests **1–3**, believing that reliable conclusions could be drawn from NOESY data, we carried out NOESY experiments at different mixing times to specify the needed time range for rotation. Surprisingly, the spectra recorded at 50 ms of mixing time for **4b**, as shown in Figure 9 where the terminal methyl hydrogens of the alkyl chain correlate with only H_g , suggest that this molecule that rotated freely at 300 ms did not do so at 50 ms. The NOESY results suggest that the tolyl ring of **4b** alternately occupies the two narrow corners of the capsule in the 300 ms time period but is limited to only one corner for a shorter (50 ms) time period. The results presented above suggest that guest motion along the y or z axis within a capsule can be controlled by simple methyl substitution. This also became evident when the spectra of **6a** and **6b** were compared (Figure 7c and d). The difference in the NMR spectra of **6a** and **6b** is identical to that for **4a** and **4b**. This observation further confirmed that the C–H $\cdots\pi$ interaction could restrict the rotational mobility of a guest molecule within an OA capsule. To further probe the structural effect on the rotational mobility of guests, we compared the NMR spectra of **5b** and **5a** (Figure 6a and b). Once again, the molecule with a C=C bond was unable to undergo coordinated motion within the capsule on the NMR timescale. The conclusion that molecules **5b** and **6b** are stationary within the capsule is also supported by the NOESY data presented in Figure 8c and d. In conclusion, the analysis of the NMR data of **4a–d**, **5a,b**, and **6a,b** leads us to conclude that guest molecules within a capsule are capable of undergoing motion along the y and z axes but minor structural changes can influence the extent of their mobility.

Guest Molecules That Do Not Rotate along Their y or z Axis within OA at Room Temperature on the NMR Timescale but Do Rotate at Higher Temperatures. The effects of temperature on guest motion within the OA capsules with **4b** and

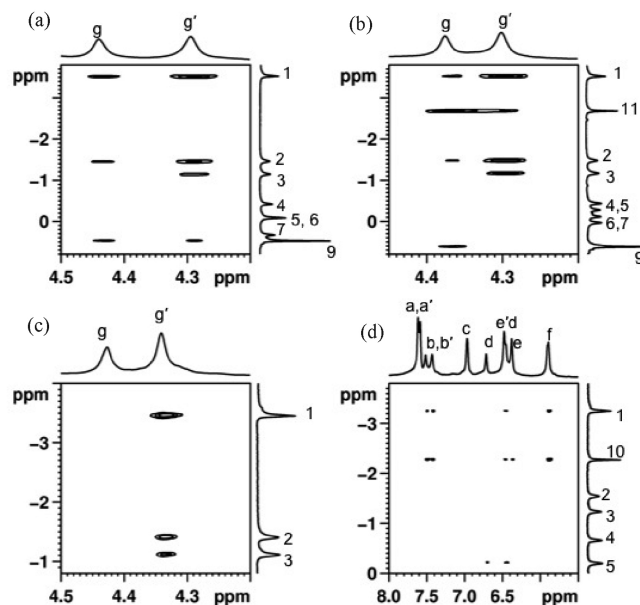


Figure 8. 2D NOESY partial spectra (500 MHz, D_2O) of (a) $4\text{c}@OA_2$, (b) $4\text{d}@OA_2$, (c) $5\text{b}@OA_2$, and (d) $6\text{b}@OA_2$. $[OA] = 5\text{ mM}$ in 50 mM borate buffer, and $[\text{guest}] = 2.5\text{ mM}$ (OA peaks are marked as a–g, and guest peaks are marked as 1–11). The mixing time is 300 ms.

2a are discussed below. Temperature-dependent ^1H NMR spectra obtained with **2b**, **4c**, and **4d** are presented in the SI section. Although to save space these spectra are not discussed, the behavior of these three molecules are similar to that of **4b** and **2a** discussed in this section. The ^1H NMR spectra of $4\text{b}@OA_2$ recorded at 5, 25, and 55 $^\circ\text{C}$ and displayed in Figure 10 show that the split host signals appearing at 5 and 25 $^\circ\text{C}$ merge into a single set of peaks at 55 $^\circ\text{C}$. We believe that the change observed at 55 $^\circ\text{C}$ is due to the two halves of the capsule becoming magnetically equivalent from the fast tumbling of **4b** within the capsule. By carefully analyzing the temperature dependence of one of the host peaks (Figure 11) with the help of the WINDNMR-Pro simulation program,¹⁸ we obtained the kinetic parameters for the tumbling motion of **4b** within the capsule. The ΔH^\ddagger , ΔS^\ddagger , and ΔG^\ddagger parameters are indicated in Figure 11. The estimated large negative entropy of activation (–18.5 eu) is consistent with the restricted space available for the guest molecule as it reorients itself between the two halves of the capsule. Recording the ^1H NMR spectrum of **2a** at various temperatures (Figure 11) to ascertain if such a large negative entropy of activation is unique to **4b** proved it to be otherwise (with **2a** it was –22.8 eu). On the basis of these and additional examples (**2b**, **4c**, and **4d**) presented in the SI section (Figures S29–S38), we believe that the loss of entropy plays an important role in the rotation of guest molecules within the OA capsule. We visualize that the coordinated motion initiated by the coiling and bending of the alkyl chain displaces the aromatic group from its initial location. Thus, rotation along the y or z axis is not a simple linear rotation but is achieved through the coordinated zigzag motion of different parts of the guest molecule.

We wish to highlight one more observation related to temperature-dependent ^1H NMR spectra. An examination of Figure 10 suggests that the host signals in the case of the $4\text{b}@OA_2$

(18) (a) Reich, H. J. *J. Chem. Educ.* **1995**, *72*, 1086. (b) The DNMR simulations were performed with an updated version of WINDNMR (<http://www.chem.wisc.edu/areas/reich/plt/windnmr.htm>): Reich, H. J. *WinDNMR Dynamic NMR Spectra for Windows. J. Chem. Educ.: Software, Ser. D* **1996**, *3D*, No. 2.

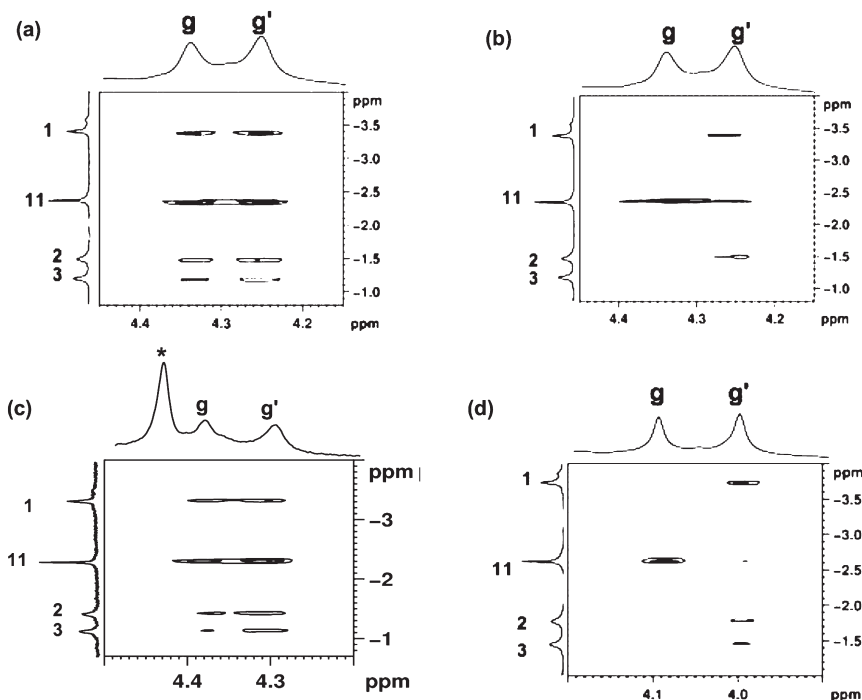


Figure 9. 2D NOESY partial spectra of **4b**@OA₂. (a) 300 ms mixing time, 25 °C, (b) 50 ms mixing time, 25 °C, and (c) 300 ms mixing time, 25 °C, OA/guest (4:1), free host peak marked as *, and (d) 300 ms mixing time, 5 °C 500 MHz, and [OA] = 5 mM in buffered D₂O (50 mM). (OA peaks are marked as g and g', and guest peaks are marked as 1–11.)

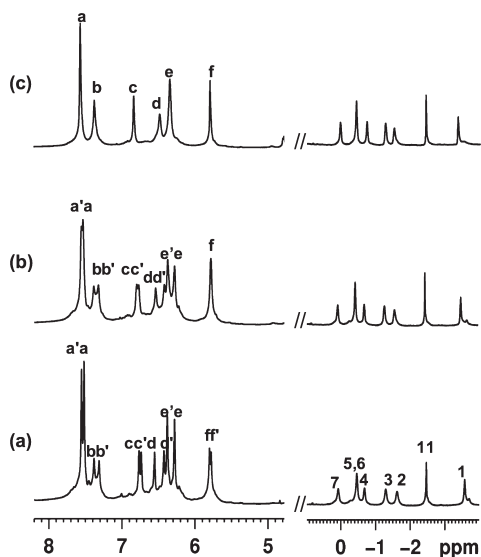


Figure 10. Partial ¹H NMR spectra of **2a**@OA₂ (500 MHz, [OA] = 1 mM) in 10 mM borate buffer (D₂O) at (a) 5 °C, (b) 25 °C, and (c) 55 °C. (OA peaks are marked as a–f, and guest peaks are marked as 1–11.)

capsuleplex are well split at 5 °C but less so at 25 °C. Such line broadening is an indication that the guest molecule rotates more freely at the latter temperature. Upon examining the NOESY data presented in Figure 9 in this context, it is interesting that at 25 °C (data collected at a mixing time of 300 ms) the guest alkyl methyl and the aromatic methyl signals correlated with both H_g and H_{g'}, suggesting residence on the NMR timescale of the two ends of the molecule for both halves of the capsule. However, at 5 °C, the correlation of the alkyl methyl and the aromatic methyl signals of the guest molecule with H_{g'} and H_g, respectively, suggests that the two ends of the guest molecule are localized at

the two halves of the capsule. This observation also rules out the experimental artifact of spin diffusion at 300 ms being responsible for methyl signals correlating with both H_g and H_{g'}. If this is the case, then one should have seen the same type of correlation even at 5 °C. We believe the observed effects could be readily understood on the basis of rotational dynamics of the guests within the capsule.

Evidence for dynamics within the capsule of **2a** at higher temperature (55 °C, Figure 10) that was absent at 25 °C, at least on the 300 ms timescale, prompted the examination of the behavior of **1**, **2b**, and **3** at 60 °C. There were no changes in the ¹H NMR spectra of these capsuleplexes in the temperature range of 25–60 °C (see SI for temperature-dependent ¹H NMR spectra; Figures S28–S31). It could be that these latter molecules are more strongly held within the capsule or are too rigid for the needed coordinated bending and twisting motions for relocation from one end of the capsule to occur.

Role of Capsule's Opening and Closing on Its Guest Rotation. Rotations described above could be visualized to occur in two ways. In the one shown in Scheme 4a, guest motion occurs within a single capsule (i.e., it is an intrasupramolecular process), with the capsule remaining closed during the entire process. Second, an apparent rotation of the guest could be visualized where the host molecules exchange their positions via capsuleplex dismantling (Scheme 4b). Both processes, if occurring on the NMR timescale, would make the two host molecules of the capsule magnetically equivalent (to result in unsplit host NMR signals). We believed these two mechanisms should be distinguishable by NOESY experiments in the presence of excess OA. Our reasoning is illustrated in a cartoon fashion in Scheme 5 with the top and bottom OA molecules that form the capsule and the free uncomplexed OA color-coded.

We hypothesized that if there is excess OA present in solution (blue in Scheme 5), when the capsule opens and closes it is quite possible that reassembly could result in three types of capsules.

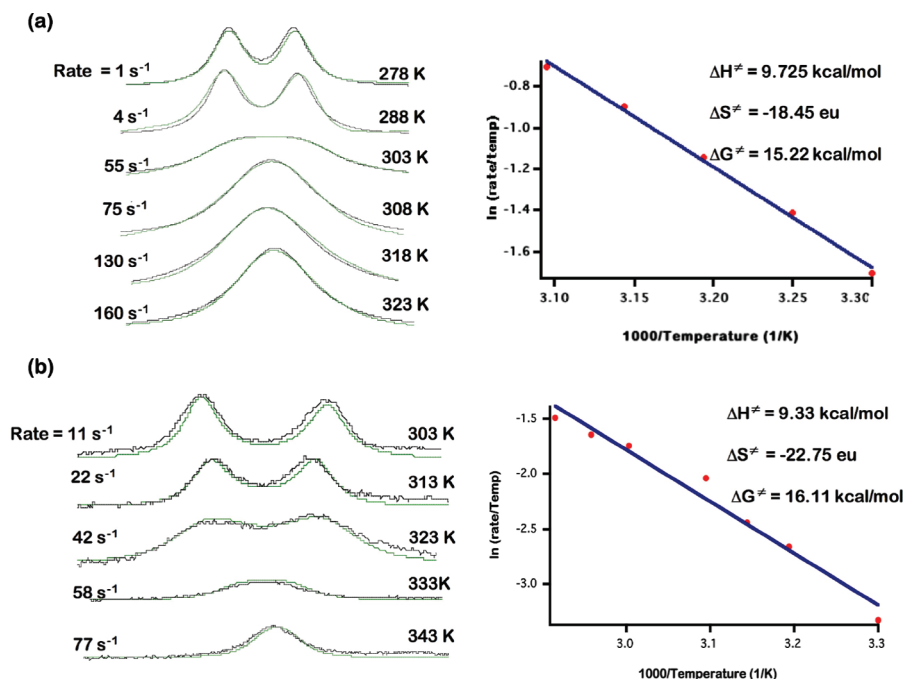
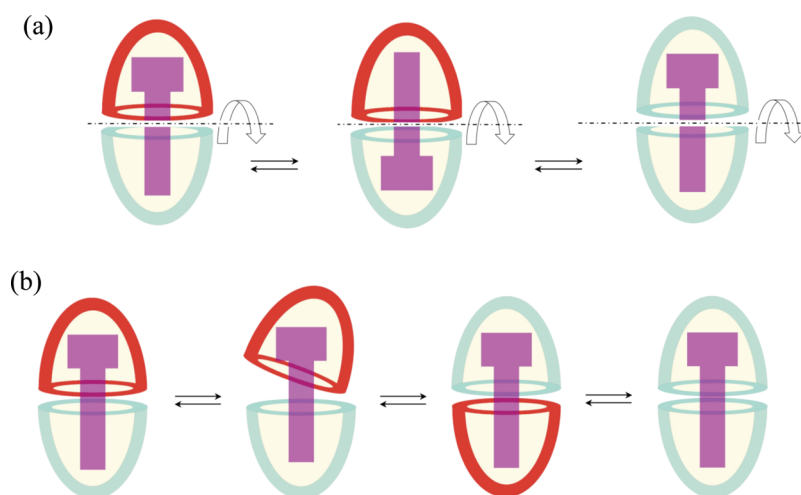


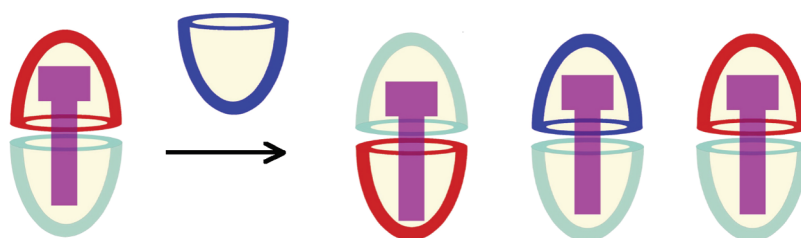
Figure 11. (a) NMR simulations (WINDNMR-Pro) for the H_c resonance in $4b@OA_2$ and the Eyring plot. (b) NMR simulations (WINDNMR-Pro) for the H_c resonance in $2a@OA_2$ and the Eyring plot.

Scheme 4. Two Possible Mechanisms for Making the Two Halves of the Capsule Equivalent^a



^a (a) The guest rotates freely inside and (b) the two halves of the capsule exchange their positions.

Scheme 5. Capsule Components' (Two Halves of the Capsule) Exchange with Free Cavitand Resulting in Three Possible Arrangements of the Capsule with Respect to the Host Inside^a



^a The blue cavitand is the free one in water.

If this occurs, then the guest molecule should show NOESY correlations not only with two halves of the capsule but also with the free OA present in solution. With this idea in mind, we examined the NOESY spectrum of the capsuleplex of $4b$ and OA

in the presence of excess OA. The 1H NMR spectrum of the capsuleplex in the presence of excess OA showed three distinct signals for the H_g proton of OA (Figure 8c). Two of these (H_g and H_g') are due to top and bottom halves of the capsule, and the third

one (marked as *) is due to free OA. Obviously, on the NMR timescale, as discussed above, the guest is stationary within the capsule. However, when the NOESY spectra were recorded at a mixing time of 300 ms, the para methyl of **4b** correlated with both H_g and H_g' of the capsule but not with excess OA (Figure 8c). This suggested that excess OA present in solution plays no part in guest rotation within a capsule. On the basis of this, we believe that the motion of the guest occurred via an intrasupramolecular pathway. It is quite likely that the rotational process was facilitated by the partial opening of the capsule, but according to the NOESY data presented above, during this process the capsule does not disassemble and reassemble.

We conclude this section by pointing out that guest rotation within the OA capsule would be prohibited by the walls of the container. However, molecules could accomplish structural changes equivalent to rotation along y and z axes through coordinated wiggly motions of various parts of the molecule. In principle, every guest molecule could undergo such motions, but depending on the weak forces that hold the guest within a host, the timescale would vary. The technique used to probe in this study provides an understanding of molecular motion on the millisecond timescale. Even with this technique, by varying the mixing time and temperature, we have been able to gain an understanding of guest motions within a closed nanocontainer.

Guest Rotation along the x Axis as Monitored by EPR Spectroscopy. We mentioned above that guest molecules probably rotate within the capsule faster (along the molecular x axis) than the NMR timescale. Therefore, we probed this motion through the EPR technique where the timescale is much shorter (ns). To monitor the guest rotational motion, the EPR spectra of paramagnetic probes **7c–f** (Scheme 2) were recorded at room temperature. The three-line EPR spectra (three hyperfine lines: $2I_N + 1 = 3$) were simulated by the well-established procedure of Freed and co-workers¹⁹ and matched the experimental spectra. The main input parameters were the g_{ii} components, which were assumed to be $g_{xx} = 2.009$, $g_{yy} = 2.006$, and $g_{zz} = 2.0025$, and the A_{ii} principal values of the A_N tensor for the coupling between electron and nuclear spins, which were taken to be $\langle A_N \rangle = (A_{xx} + A_{yy} + A_{zz})/3$. An increase in the environmental polarity enhances the A_N tensor components owing to the increased electron spin density on the nitrogen nucleus of the NO group of the nitroxide probe.^{20,21} Monitoring the A_N tensor helped us to ascertain the location of guests in the presence of OA. The correlation time (τ) for the rotational diffusion motion of the spin probe provided information about the guest rotation within the capsule. Brownian motion was assumed in the calculation of the main component of the correlation time, that is, the perpendicular one (τ_{perp}).

We anticipated that the length of the alkyl chain would influence the rotational mobility of the probe. Figure 11a shows representative EPR spectra for **7d** at different OA/**7d** molar ratios. (Spectra for the others are provided in SI.) It is clear that the EPR spectra of samples containing OA/**7d** < 2 consist of two components. In these spectra, the high-field hyperfine line is split into two. One of these corresponds to the uncomplexed guest, and the other corresponds to the complexed guest. At OA/**7d** = 4:1, the component due to complexed guest was predominant. In Figure 11b, experimental (recorded in the absence and in the presence of 4 equiv of OA) and simulated spectra of **7d** are

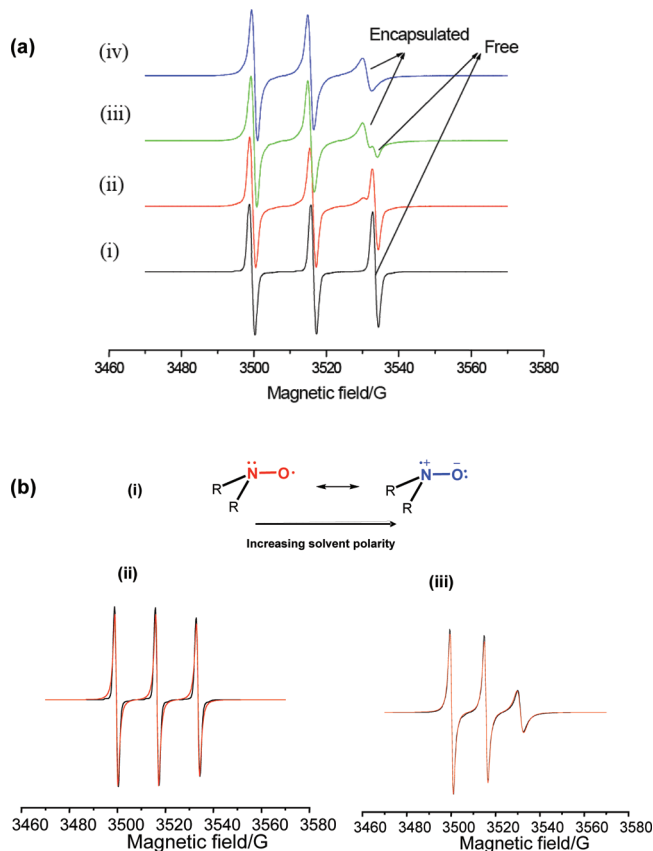


Figure 12. (a) EPR experimental spectra of **7d** at various OA concentrations (in 20 mM sodium tetraborate buffer): (i) OA/**7d** (0:1), (ii) OA/**7d** (1:1), (iii) OA/**7d** (2:1), and (iv) OA/**7d** (4:1). [**7d**] = Amount corresponding to 1 mM in water, and it was partially solubilized; the addition of OA increases the solubility of **7d** in water. The high-field hyperfine lines of the encapsulated and free components are indicated by arrows. (b) (i) Resonance structures of nitroxide derivatives depending upon the polarity of the medium; the distribution depends on the polarity of the medium. (ii) EPR spectra of **7d** in water corresponding to the free component (simulated spectrum shown in red) with $g_{ii} = 2.011$, 2.006, and 2.0028; $A_{ii} = 6$, 6, and 39.05 G; $\langle A \rangle = 17.02$ G; and $\tau_{\text{perp}} = 0.031$ ns. (iii) EPR spectra of OA/**7d** (4:1) corresponding to the encapsulated component (simulated spectrum shown in red) with $g_{ii} = 2.011$, 2.006, and 2.0028; $A_{ii} = 6$, 6, and 34.57 G; $\langle A \rangle = 15.52$ G; and $\tau_{\text{perp}} = 0.66$ ns.

provided. (The main parameters used for computation are provided in the caption.) It is important to note that both the $\langle A_N \rangle$ (polarity) and τ_{perp} (rotational mobility) parameters for **7d** in the absence and in the presence of 4 equiv of OA are significantly different. A sharp reduction in polarity, that is, a decrease in $\langle A_N \rangle$, and in mobility, that is, an increase in τ_{perp} , for OA/**7d** = 4 with respect to OA/**7d** = 0 suggest that the probe at OA/**7d** = 4 is fully included within OA. The environmental polarity measured by A_N for **7d**@OA₂ is close to that of benzene.^{11,20,21} This is consistent with the micropolarity measured earlier for the OA capsule with various fluorescence probes.¹¹ At a host to guest ratio of 4:1, more than 85% of **7c–7f** existed as complexes with OA. The A_N parameters measured for **7c**, **7d**, **7e**, and **7f** at a host to guest ratio of 4:1 were 15.83, 15.52, 15.93, and 16.0 G, respectively. These numbers are smaller than the value measured in water in the absence of OA (17.02 G). Interestingly, **7d** shows a minimum polarity, probably indicating that this molecule snugly occupies the capsule.

Because the host–guest complexes of the above paramagnetic probes gave extremely broad ¹H NMR spectra, we could not

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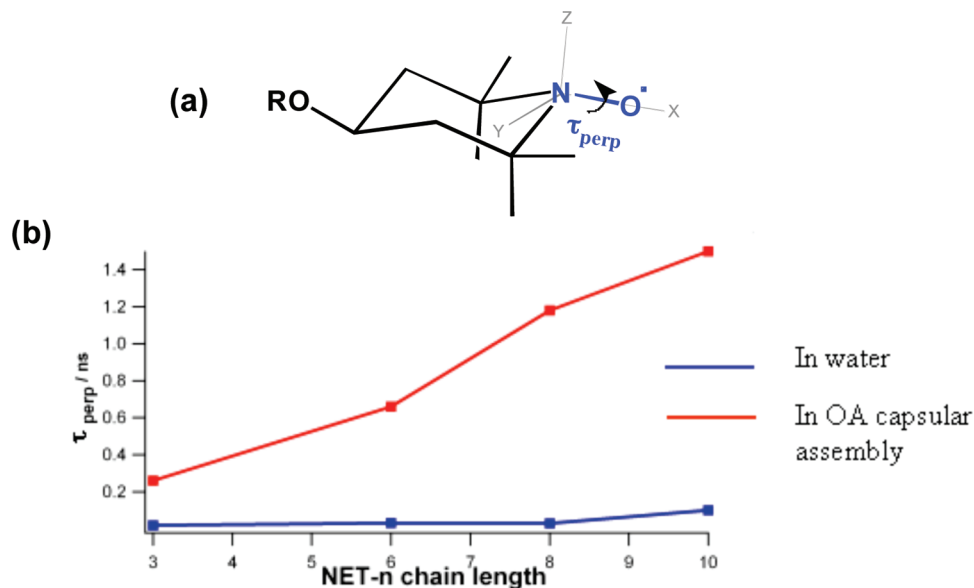


Figure 13. (a) Reference frame that describes the rotational correlation time (τ_{perp}). (b) Rotational correlation times of **7c** (NET-3), **7d** (NET-6), **7e** (NET-8), and **7f** (NET-10) included within OA. **7a** and **7b** form a 2:2 (H/G) capsular assembly; **7c–f** forms a 2:1 (H/G) capsular assembly with OA.

ascertain the complex formation by NMR as we have done with probes **1–6**. Therefore, we indirectly inferred the complexation of the NET series with OA by using the corresponding methylated derivatives (N–O replaced with N–OMe; see **8** in Scheme 2). ^1H NMR titration spectra for **8c** are provided as SI (Figures S25–S27). The titration of OA with **8d–f** gave similar results. All of these data suggested that **8c–f** formed 2:1 capsule-plexes. Extending these results to the paramagnetic probes, we believe that **7c–f** also formed a 2:1 complex with OA.

A plot of τ_{perp} measured from the simulated spectra at a host to guest ratio of 4:1 versus the chain length is provided in Figure 12. The Figure also includes the definition of τ_{perp} , which is the rotational diffusion mobility of the probes along the magnetic x axis. We emphasize that τ_{perp} values for all probes (except **7f**) in water were close to 0.03 ns. For **7f**, it was 0.1 ns. This value together with line broadening indicates a partial self-aggregation of this probe in water. However, for all probes within the OA capsule, τ_{perp} values were an order of magnitude longer than in pure water, suggesting that rotation is restricted within the OA capsule. Given that under the conditions that τ_{perp} was measured >85% of the probe molecules were complexed to OA, we believe that the estimated τ_{perp} value largely reflects the rotational mobility of probes within the capsule. Interestingly, τ_{perp} was dependent on the alkyl chain length. For example, for O-propyl, O-hexyl, O-octyl and O-decyl the numbers were 0.26, 0.66, 1.18, and 1.5 ns, respectively. On the basis of the EPR data, we conclude that paramagnetic guest molecules undergo rotation along the molecular x axis within the capsule and that their rotational motion is dependent on the length of the alkyl

chain. Importantly, the rotation of the probes is restricted within the capsule compared to that in water.

Summary

In this study, we have shown that a number of organic molecules of fairly moderate size complex to a deep-cavity cavitand known by the trivial name octa acid to form a closed container. The guest molecule that is imprisoned within a molecular container is not stationary. The extent of freedom is dependent on weak interactions that hold the guest within the container. We have employed ^1H NMR and EPR to probe the mobility of guests. We find that all guest molecules undergo fairly rapid rotation (nanosecond range) along the x axis. However, the mobility along the y and z axes varies. Even a small structural change such as the addition of a methyl group makes the guest molecule stationary within the container on the NMR timescale. We are in the process of understanding the factors that control the dynamics of the guest within a molecular container.

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Supporting Information Available: Experimental details, synthesis procedures, and additional NMR and EPR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.