More Chemistry in Small Spaces

DARIUSH AJAMI AND JULIUS REBEK JR.*

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

CONSPECTUS

This Account is about coaxing molecules into spaces barely big enough to contain them: encapsulation complexes. In capsules, synthetic modules assemble to fold around their molecular targets, isolate them from the medium for relatively long times, place them in a hydrophobic environment, and present them with functional groups. These arrangements also exist in the interior spaces of biology, and the consequences include the familiar features of enzymes: rapid reactions, stabilization of reactive intermediates, and catalysis. But inside capsules there are phenomena unknown to biology or historical chemistry, including new structures, new stereochemical relationships, and new reaction pathways.

In encapsulation complexes, as in architecture, the space that is created by a structure determines what goes on inside. There are constant interactions between the container and contained molecules: encounters are not left to chance; they are prearranged, prolonged, and intense. Unlike architecture, these reversibly formed containers emerge only when a suitable guest is present. The components exist, but they cannot assemble without anything inside. Modifications of the capsule components give rise to the results of the present Account. The focus will be on how seemingly small changes in the encapsulation complexes, exchanging a $C=S$ for a $C=O$, reducing an angle here and there, or replacing a hydrogen with a methyl, can lead to unexpectedly large differences in behavior.
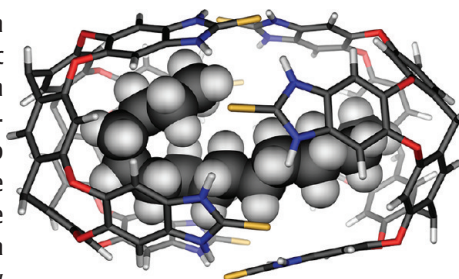
Recognition in Chemistry and Biology

Molecular recognition and the intermolecular forces that drive it are largely invisible to synthetic chemistry, even though a recognition event precedes practically every bimolecular reaction. Physical organic chemists were involved in recognition studies and found it useful and convenient to determine intermolecular forces in nonaqueous solutions: synthetic reactions were most often carried out in organic solvents, anyway. But molecular recognition in water has been cast as one of the outstanding problems of the day and it might appear that physical organic chemistry is not up to or less well-equipped to deal with its challenges. Yet the historical record shows otherwise: the enthalpic hydrophobic effect,[1] the cation/π interactions,[2] and the 55% solution[3] are all products of physical organic chemistry rather than, say, biochemistry. More specifically, these interpretations emerged from synthetic receptors *that more or less completely surrounded their targets* in either water or other solvents, but the phenomena that were identified have since been confirmed in proteins. The controlled, small spaces of the synthetic structures are defined in large part by aromatic panels, so this *Accounts* issue is particularly apt; the perspective is physical organic chemistry, but if the results are relevant to biology, so much the better.

Molecules Inside Molecules

At the outset of our work on hydrogen-bonded capsules nearly 20 years ago, we incurred a disadvantage: each capsule required a de novo synthesis, some of which involved 20 steps to reach the goal of creating assemblies that completely surrounded their targets. These arrangements, molecules inside molecules, had been introduced earlier by Collet in Lyon[4] and Cram in Los Angeles.[5] They used covalent structures that can last many years. We arrived at dynamic systems with reversible uptake and release of target molecules for studies in recognition. Moreover, copies of a single module that could self-assemble around the target offered obvious advantages in synthesis. A single molecule of methane or ethane could be accommodated in the earliest capsule, the "tennis ball",[6] and a
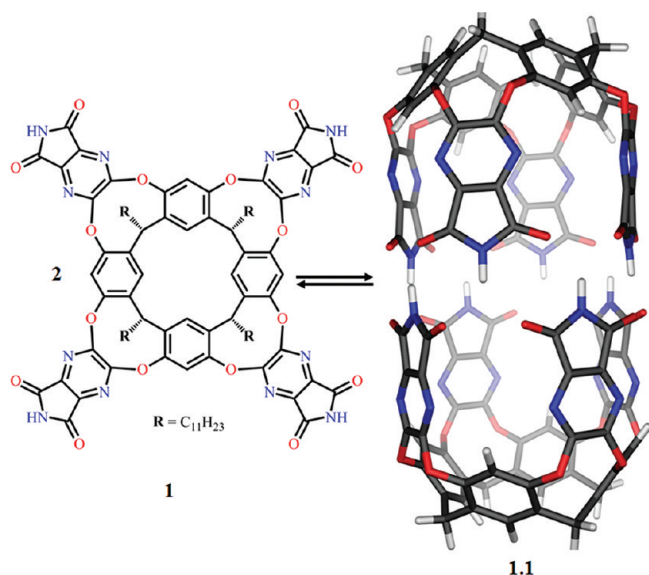
FIGURE 1. Structure of the imide **1**. Its self-assembled capsule is modeled as a DFT-minimized (B3LYP/6-31G*) representation. The peripheral alkyl chains are omitted for clarity.

FIGURE 2. Structure of the thiourea-containing cavitand **2**. Its self-assembled capsule is modeled as a DFT-minimized (B3LYP/6-31G*) representation. The peripheral alkyl chains are omitted for clarity.

number of other roughly spherical shapes of different sizes followed.[7] But it was not until we prepared **1.1**[8] (Figure 1) with a cylindrical shape that new arrangements of molecules held inside could be created.[9]

A Wider Capsule

One of the simplest modifications had to do with the width of the capsule **1.1**. Modeling indicated that the girth could be increased without altering the length through replacing the imides with thioureas as in **2.2** (Figure 2). The corresponding urea capsules had already been prepared,[10] and the target thiourea was easily synthesized.[11] Thioureas have increased acidity[12] and are better hydrogen-bond donors than the corresponding ureas but the lower electronegativity of S versus O weakens its action as an acceptor, a key to the success of thioureas in organocatalysis.[13]

The tetrathiourea dimerized in the presence of suitable guests and a capsule was formed as shown. We studied the encapsulation of a series of normal alkane guests in mesitylene-d_{12} as reported earlier for tetra-imide capsule **1.1**, and the spectra are shown in Figure 3. The longest such alkane that is seen inside **1.1** is *n*-tetradecane, but it must assume a helical conformation that shortens its length and makes it thicker.[14] The coiling introduces energetically pricey *gauche* interactions along the chain, but at the same time permits attractive C−H/π interactions between alkane and capsule. An uneasy, spring-loaded equilibrium results where repulsive and attractive forces are delicately balanced. The *trans*-tetradec-7-ene is also taken up, but all attempts to encapsulate *n*-pentadecane in **1.1** failed.
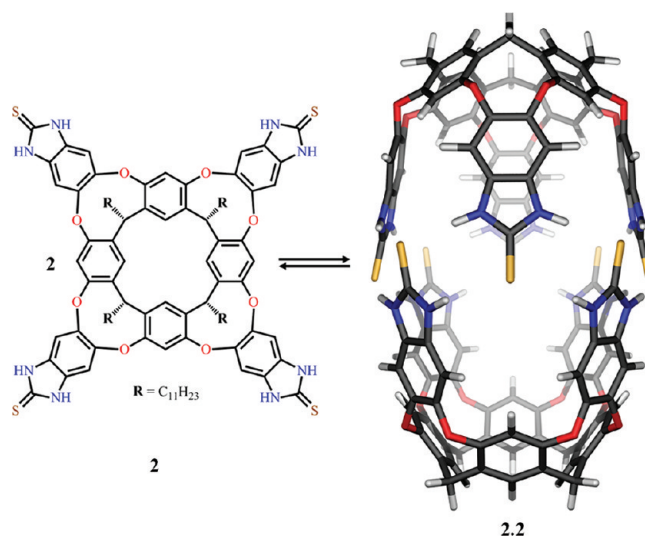
A parallel encapsulation study using **2.2** gave the NMR spectra shown, and the trend of alkane signals is similar to those inside capsule **1.1** for the shorter alkanes *n*-$C_{10}H_{22}$ to *n*-$C_{13}H_{28}$. The longer *n*-$C_{14}H_{30}$ is also accommodated in **2.2**, but the spectrum now indicates a change of guest shape. Unexpectedly, *n*-$C_{15}H_{32}$ is also encapsulated, and the signals are even more revealing. The end methyl groups are in different magnetic environments but exchange rapidly on the NMR time scale. Apparently, the two longer alkanes *n*-$C_{14}H_{30}$ and *n*-$C_{15}H_{32}$ are in a "folded" conformation where one end is deep in the cavitand while the other is near the walls. This shortens the guest enough to fit inside the capsule. Every signal of the alkane is an averaged chemical shift of two magnetic environments. Unfortunately, further details of the conformation cannot be deduced from the spectra, but a schematic proposal is given in Figure 4 in which a "kink" can move rapidly up and down the aliphatic chain.

What causes the different behavior in the two capsules **1.1** and **2.2**? Apparently, the thiourea maintains hydrogen-bonding that keeps the capsule intact while the kink in the alkane guest moves along the chain. The thiourea capsule is some 2.2 Å wider at the middle; the increased girth allows the encapsulated species to experience contortions beyond simple *gauche* interactions.[15] These shapes, the helical and folded alkane, are unknown in solution; and even enzyme interiors show only bent alkyl structures.[16] Tight-fitting alkane chains appear in other synthetic assemblies[17] and covalent complexes.[18] Their contortions and tight packing are now becoming advantageous in catalysis.[19,20]
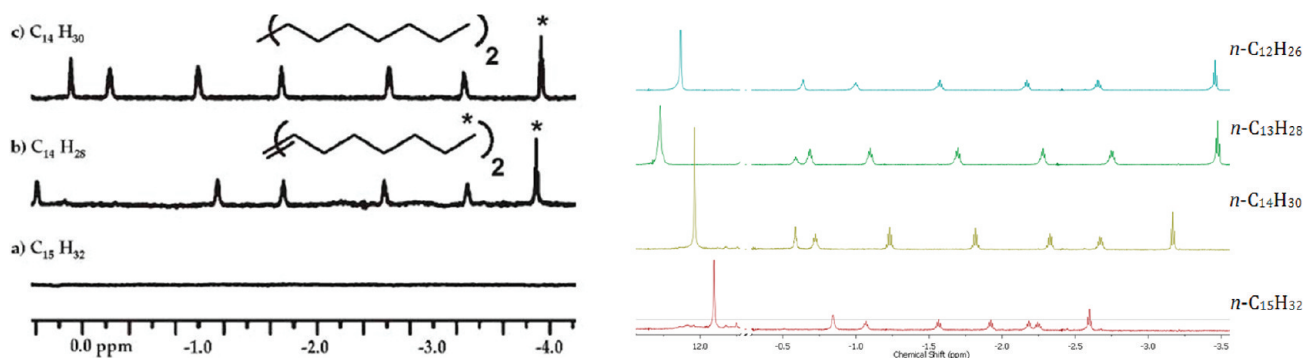
FIGURE 3. Upfield regions of the ^{1}H NMR spectra (600 MHz, mesitylene-d_{12}, 300 K) of encapsulated guests. Left: *n*-tetradecane and *trans*-tetradec-7-ene in **1.1**. Right: alkanes (*n*-C$_{10}$H$_{22}$ to *n*-C$_{15}$H$_{32}$) in **2.2**.
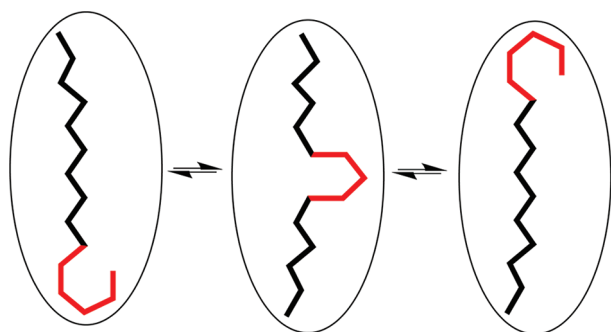

FIGURE 4. (a) Cartoon representation of the folding of *n*-C$_{15}$H$_{32}$ in **2.2**. A mechanism for chemical exchange between the two ends involving a moving "kink" (red) is proposed.

Slightly Different Spacers

The problems posed by de novo capsule synthesis quickly evaporated when it was found that **1.1** could be expanded merely by adding suitable spacer modules in solution (Figure 5a). The cylindrical capsule host **1.1** shows a spontaneous response to glycoluril **3** (Figure 5b) when appropriate guests are present. New assemblies emerge. A "belt" of four glycolurils inserts into the hydrogen bonds in the middle of the capsule in an unexpected twisted arrangement. This results in a chiral assembly **1.3$_4$.1**. The glycolurils act as spacer elements[21] that increase the length by some 7 Å and the capacity of the inner space by some 200 Å^3. The elongation of **1.1** with **3** appears unlimited: longer guests can drive the assembly toward further extension and incorporate two, three, or four belts of glycoluril spacers.[22] The glycolurils were intended to fit into the corners of the cavitand **1** by offering superior hydrogen bond acceptors to the imides' N—H donors. The twisted belt arrangement arises, in part, from angular mismatch between cavitand and glycoluril. The adjacent walls of cavitand **1** are at right angles (Figure 5d), but the ureido functions of **3** (Figure 5b) are presented at a larger angle of approximately 113°.[23]
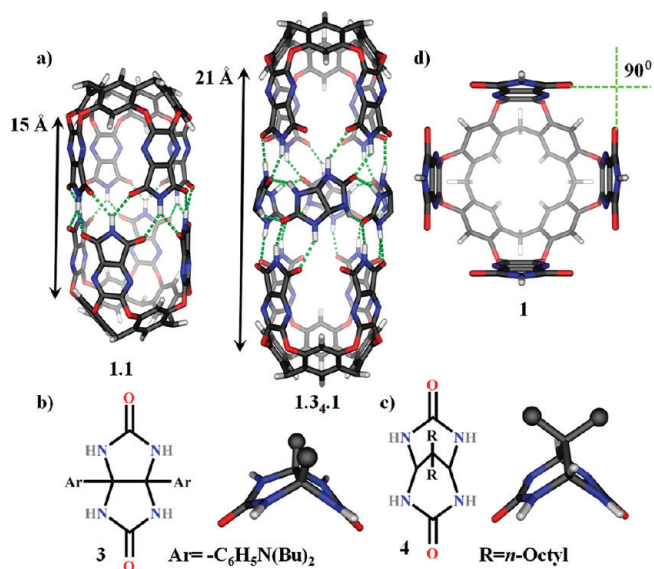

FIGURE 5. (a) Models of the cylindrical capsules **1.1** and the extended **1.3$_4$.1**. (b and c) Structures of the glycoluril **3**, and propanediurea **4**. (d) Top view of cavitand **1**. Peripheral alkyl and aryl groups have been deleted.

We sought a more appropriate complement to the right angles of the cavitand and arrived at propanediurea **4**. The corresponding angle of **4** (Figure 5c) is ∼99°,[24] and we expected its smooth integration into **1.1**. Typical self-assembly processes that incorporate multiple subunits create cavity shapes of high symmetry such as spheres, polyhedra, and cylinders. So we were unprepared for the new capsules featuring "S"- and "banana"-shapes that resulted from the miscegenation of **1** and **4**.

We used *n*-alkanes as guest probes since we had extensive experience with their behavior in the glycoluril-extended capsules: the characteristic NMR signals that reveal their positions and dispositions and their flexibility in filling the spaces. Figure 6 shows the ^{1}H NMR spectra recorded at 280 K, since all the assemblies exhibited sharp signals at that
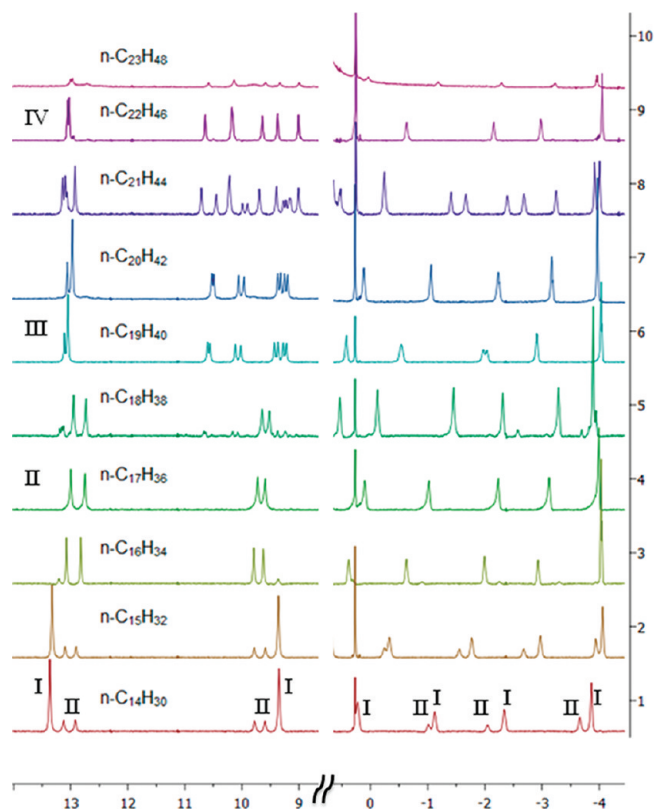
FIGURE 6. ^{1}H NMR spectra of extended capsules formed with guests from *n*-tetradecane to *n*-tricosane. The concentrations are 4.8 mM in cavitand **1**, 10.4 mM in propanediurea **4** (20.8 mM in **4** for guests *n*-$C_{19}H_{40}$ to *n*-$C_{23}H_{48}$), and 24 mM in guest (148 mM for guests *n*-$C_{21}H_{44}$ to *n*-$C_{23}H_{48}$) with mesitylene-d_{12} as solvent. All spectra were recorded at 280 K.
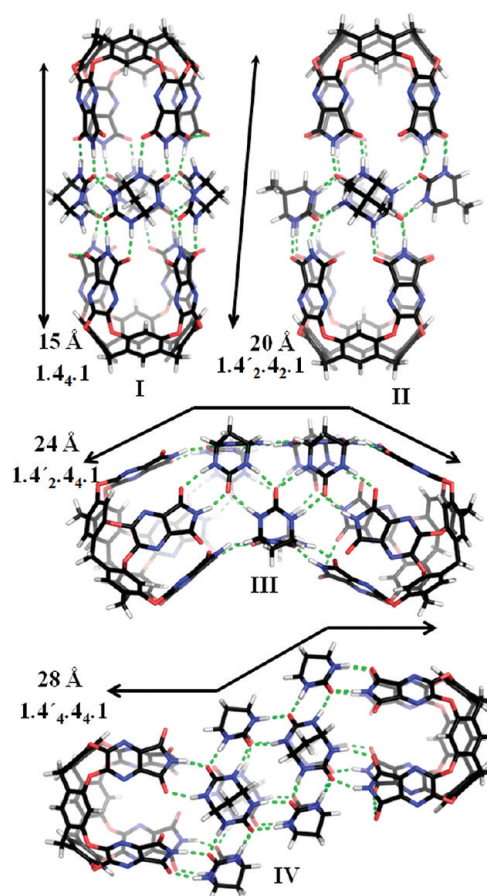

FIGURE 7. Models of the capsule assemblies **I**–**IV** and their approximate cavity lengths. (Peripheral alkyl and aryl groups have been deleted.)

temperature. The shortest alkane *n*-tetradecane (*n*-$C_{14}H_{30}$), gave *two* new assemblies (labeled **I** and **II**; Figure 6, line 1). Integration of the spectra showed both arose from insertion of four molecules of **4** into capsule **1.1**. The magnetic environment at the two ends of either capsule is the same and signals are identical for C_1/C_{14}, C_2/C_{13}, C_3/C_{12}, and C_4/C_{11}. The upfield shifts of the guest signals, and especially their spacing, indicate an extended conformation with little or no compression (coiling). At ambient temperature or higher, both assemblies appear achiral, but as the sample is cooled, diastereotopic geminal guest protons are observed for the guest in the major assembly (**I**), indicating a chiral environment on the NMR time scale. A D_4-symmetric structure **1.4$_4$.1** (Figure 7) is indicated for this assembly, which is the propanediurea version of the chiral assembly **1.3$_4$.1**. The glycoluril-derived capsule, however, racemized at higher temperatures with longer and compressed *n*-alkane guests (C_{17}–C_{19}) which exerted pressure from the inside. The faster racemization of **1.4$_4$.1** suggests a weaker H-bonded network compared to **1.3$_4$.1**.

The minor assembly **II** is observed at lower temperatures ($\leq$280 K), and the *n*-$C_{14}H_{30}$ guest inside behaves as if it experiences a longer space. Moreover, the guest's methylene protons in **II** are not diastereotopic (even at low temperatures) indicating an achiral structure for this extended capsule. The methylene signals are shifted slightly downfield (ca. 0.1−0.3 ppm, Figure 6) when compared to the corresponding signals in assembly **I**. As the sample is heated, the two assemblies interconvert and show coalescence of the signals at ca. 300 K. Similar behavior was observed on encapsulation of *n*-pentadecane, during which a ROESY experiment showed exchange between the guest signals in the two different assemblies. What is the structure of **II**? Molecular modeling suggests a C_{2h}-symmetric assembly **1.4′$_2$.4$_2$.1** (the prime ['] denotes the horizontal orientation of the propanediurea carbonyls as shown in Figure 7). The C_{2h}-symmetry is responsible for the appearance of two imide N−H signals, four different ureido N−H-resonances, three PD-bridgehead C−H signals, two different methine C−H's, and six aromatic C−H signals. Many of the signals

are also enantiotopic due to the plane of symmetry in the assembly. The structure may have a glycoluril counterpart: in the glycoluril-derived $1.3_4.1$, interconversion of the enantiomers is believed to proceed through an intermediate with two planes of symmetry; however, the structure proposed for **II**, *mutatis mutandis*, would also explain the racemization.

The enantiotopic signals of capsule **II** ($1.4'_2.4_2.1$) were revealed by encapsulation of a chiral guest. Waldvogel et al. had earlier[25] shown that 2-tetradecanol in capsule 1.1 induced local stereoselective helical folding, and we applied this approach to $1.4'_2.4_2.1$ with a suitable guest, 2-heptadecanol. Indeed, doubling of the enantiotopic host signals of **II** occurred with this guest and the imide N—H signals of the host became diastereotopic.[26]

Hydrocarbons are moving targets that can compress and lengthen depending on the available space. Likewise, the capsules can incorporate or shed spacers to accommodate the alkane guest: these systems display an extensive and mutual induced fit behavior. Each methylene added to an extended *n*-alkane chain increases the length by 1.25 Å and each carbon atom added to the coiled chain increases the length by 0.93 Å.[27] It was no surprise, then, that in the presence of **4** and the longer *n*-octadecane (n-$C_{18}H_{38}$) the emergence of yet a new encapsulation assembly **III** (Figure 6, line 5) in addition to **II** could be detected. The guest is in a more relaxed conformation, and separated imide NH signals are seen. The formation of **III** is exclusive with *n*-nonadecane and a chiral structure is required since a diastereotopic CH_2-group of this guest can be observed at 240−300 K. Unaccountably, *six* spacer units of **4** are taken up, an unprecedented number, since all other extensions involve multiples of four units. We arrived at the unusual "banana"-shaped structure $1.4'_2.4_4.1$ (Figure 8) of C_2-symmetry, apparently formed by the insertion of two units of **4** into assembly **II**. The NMR spectrum shows four imide N—H's, twelve different urea NH-resonances, six bridgehead C—H signals for **4**, four different methine C—H signals, and eight aromatic C—H signals.

The structure was supported by the NOE-signals observed, the simulated NMR-spectrum at the DFT-level of theory (B3LYP/6-31G*) and molecular modeling. Additional evidence for the unusual shape of **III** came from its shape-selective encapsulation of rigid (nondeformable) guests. For example, the rectilinear *p*-pentaphenyl (**5**) was taken up by the previously described doubly extended capsule $1.3_8.1$, (Figure 8), but it is inconveniently shaped for the bent **III** and was not encapsulated. However, the bent dialkynylketone **6**
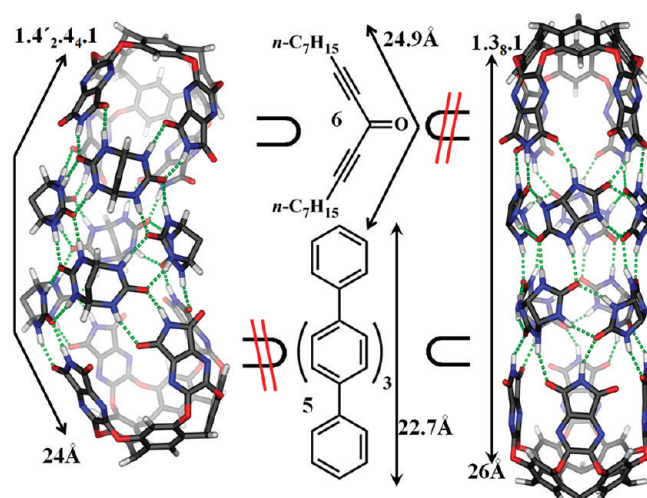

FIGURE 8. Selective encapsulation of complementary shaped guests in assembly **III** and the previously reported, doubly extended cylindrical capsule $1.3_8.1$.

was encapsulated in the congruent **III** but not in the incompatibly linear capsule $1.3_8.1$.

With *n*-eicosane (n-$C_{20}H_{42}$) as guest, the same assembly **III** ($1.4'_2.4_4.1$) was observed, and, as expected, the guest signals are shifted upfield (Figure 6 line 7; up to 0.5 ppm for protons at C_4/C_{17}), indicating a more compressed guest conformation. Yet another new species was induced in response to *n*-heneicosane (n-$C_{21}H_{44}$) as guest. The new assembly was seen competing with **III** but with the longer *n*-docosane as guest a single complex (**IV**, Figure 6 line 9) emerged. Integration of the spectrum revealed *eight* units of **4** were present in the new, elongated assembly. An achiral structure for this doubly extended capsule was indicated, since guest methylene signals did not become diastereotopic even at low temperatures. We favor the D_2-symmetric structure for **IV** ($1.4'_4.4_4.1$) shown in Figure 7; this assignment is, admittedly, an extrapolation but is supported by ^{1}H NMR data and results of NOE experiments. The assembly features two imide N—H signals, eight different urea NH-resonances, six bridgehead C—H signals of **4**, two different methines C—H, and six aromatic C—H signals. With the longer *n*-tricosane (n-$C_{23}H_{48}$), assembly **IV** is still formed, but the signal-to-noise ratio is poor even in the presence of 60 equivalents of guest (Figure 6 line 10).

Harnessing π/π Attractions

Although individual π/π interactions are weak, they are typically numerous in protein interiors and contribute in sum to protein folding and thermal stability.[28] We also found that a number of these weak interactions in the new capsules[29] can impart selectivity to the self-assembly
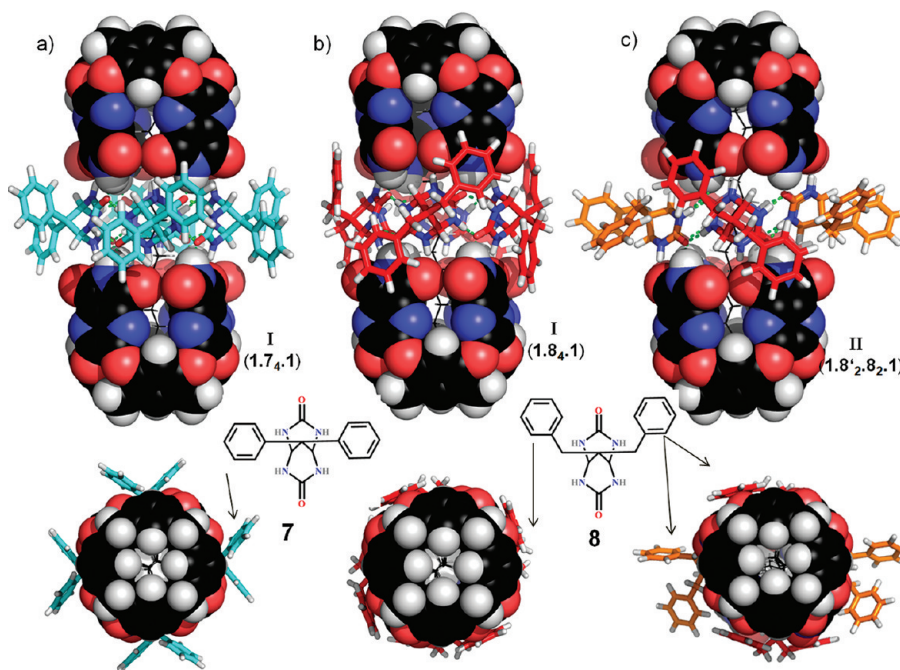
FIGURE 9. Renderings of assemblies **I** and **II** with different spacers **7** and **8**. In (a) the phenyls of the spacer **7** are fixed away from the capsule's surface. In (b) assembly **I** with the spacer **8** is bound in a "twisted" fashion (marked red). The phenyls are folded back onto the capsule's surface. In (c) assembly **II** with the spacer **8** (marked red when bound in a "twisted" fashion and orange if bound "horizontally" to the cavitand) has fewer stabilizing $\pi-\pi$ interactions with the assembly's hydrogen-bonding array. (Peripheral alkyl groups have been deleted for easier viewing.)

process itself. The existence of mixtures of complexes **I** and **II** with guests $C_{14}-C_{16}$ indicates that they are comparable in free energy. This thermodynamic balance suggested that contributions from even small aromatic $\pi-\pi$ attractive interactions forces could tip the balance for formation of one assembly over the other. The system might serve to measure $\pi-\pi$ interactions, in the same way as Wilcox et al. measured edge-to-face interactions with a torsion balance.[30]

Specifically, we installed aromatic panels on the propanediurea units in two different ways. In the first, direct attachment of phenyl groups to the framework (as in spacer **7**) resulted in aromatic units that are directed *away* from assembly **I** (**1.7₄.1**, Figure 9a). In the second, connection was through a CH₂ unit (as in the benzyl groups of spacer **8**) inserted between the frame and phenyls. This allowed enough flexibility for the aromatic panels to fold back on each other or onto the outer surface of capsule **I** (**1.8₄.1**). The latter arrangement offers stabilizing $\pi-\pi$ interactions to the assembly's hydrogen-bonding array (Figure 9b). Molecular modeling estimated the distance between the aromatic π surface to the hydrogen-bonding array at ∼3.5 Å, a value in agreement with literature precedents.[31−33]

In the capsule arrangement **1.8₄.1**, eight benzene rings (two on each of the four spacers) can make these contacts, but assembly **II** (**1.8′₂.8₂.1**), loses half of these interactions, since the

spacers (highlighted in orange in Figure 9c) cannot stack their benzylic functions back onto the hydrogen bonding surface.

With the benzyl spacers, the shorter guests (n-$C_{14}H_{30}$ to n-$C_{16}H_{36}$) induced only assembly **I** and even the longer $C_{17}H_{36}$ was bound *exclusively* **I**. Control experiments with benzyl spacers having bulky peripheral groups showed that stacking was the driving force for the assembly.

But returning to the cases at hand, what governs the structural changes in the two assemblies? Longer guests apply pressure on the two ends of the capsule. The pressure of the compressed guests favors spacer orientations that give the capsule longer dimensions. For example, the accessible cavity length in **II** is approximately 1 Å longer than that in **I**. An additional factor is the filling of space: complexes which stay very close to the ideal packing coefficient[3] of slightly more than 50% are favored over a wide range of guest lengths. The induced fit of the host and the adaptability of the guest appear inextricably linked. But even the $\pi-\pi$ interactions *on the outside of the assembly* can provide stabilizing forces that outweigh the size issues. What do the properties of spacer **5** teach us about self-assembly? Folding onto the outer surface minimizes vacuums: surfaces that are not solvated. Folding, here or in biology, inevitably liberates solvent and increases overall intermolecular interactions that stabilize assemblies.
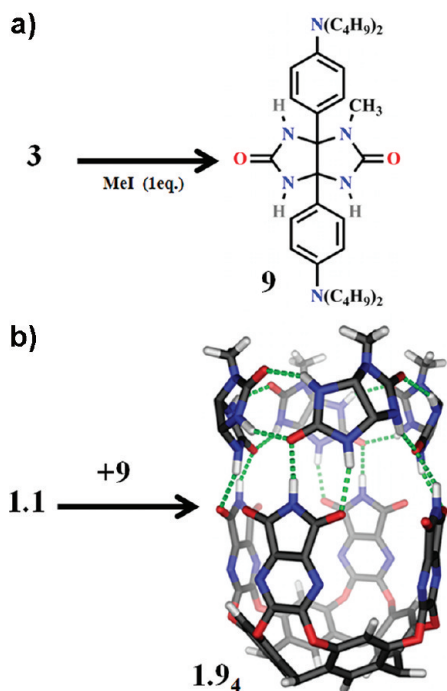
a)

b)

FIGURE 10. (a) Line drawing of N-methylated glycoluril **9**. (b) Modeled structures of the new cavitand **1·9₄**.

FIGURE 11. (a) ^{1}H NMR spectra (600 MHz, 280 K, mesitylene-d_{12}) of **1·9₄** complex with normal alkanes (n-$C_{10}H_{22}$ to n-$C_{14}H_{30}$) (2 mM). A small amount of **1·1** persists with C_{11}.

Capsules to Cavitands: N–H to N–CH₃

Early attempts to further deepen the cavitands[34] that form the capsules were unsuccessful: incorporating larger aromatic panels by covalent synthesis led to conformational changes that can be broadly ascribed to solvophobic collapse,[35] and the resulting aggregates were structures that had no internal spaces.[36] So a depth of ~1 nm was easily achieved for cavitands, and the application of glycolurils or propanediureas expanded the capsules through noncovalent synthesis. The use of a N-monosubstituted glycoluril such as **9** would prevent hydrogen bonding, at least along that edge of the module, and prevent closed capsule formation. Instead, we expected that **1·1** would be separated into deeper cavitands as shown in Figure 10. The superior hydrogen bonds should stabilize[37] the new cavitand and, in the arrangement shown with a single enantiomer of the spacer, provide an asymmetric nanoenvironment[38] near its rim.

In the event, addition of N-methylated glycoluril **9** to a solution of encapsulated normal alkanes in **1·1** gave new cavitand host **1·9₄** (Figure 10) with alkanes partially inside.[39] The alkanes ranged from n-$C_{10}H_{22}$ to $C_{14}H_{30}$, and only with n-undecane which is an ideal guest[14] for **1·1**, did a small amount of that original complex persist. The longer alkanes gave only the new, open-ended assemblies. There is an obvious monotony to the spectra (Figure 11), unlike the case
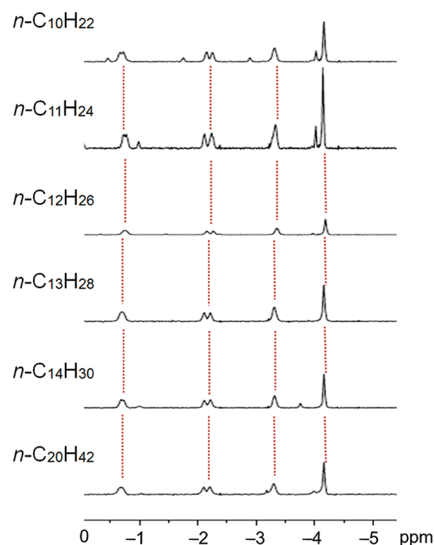
with capsules, where there is a gradual upfield shift with alkane length as coiling sets in. Here, all alkanes have the same pattern, at least for the first four carbons of the chain; these atoms experience the same aromatic envelope of the cavitand and get in and out the same way.[40] Judging from the chemical shifts, none of these guests are coiled but exist[41] in an extended conformation with their proton signals shifted upfield by as much as $\Delta\delta = -5$ ppm. The diastereotopic signals of the CH₂ groups indicate a chiral magnetic environment.

Four N-methylated glycourils are present but the singlet at ~13.5 ppm indicates that all the imide N–H's are equivalent. Accordingly, *each assembly involves a single enantiomer of the glycoluril*. This is exquisite self-sorting and was confirmed by resolving the glycoluril: both racemic and optically active compounds gave the same spectra. The optically active assembly with n-$C_{13}H_{28}$ and n-$C_{17}H_{36}$ guests showed characteristic CD spectra. Ordinarily, n-alkanes cannot compete with an organic solvent for a cavitand.[42] Apparently, the belt of glycolurils provides attraction to normal alkanes, since these flexible molecules are generally not good guests for open-ended cavitands. The proposed model of the chiral cavitand **1·9₄** shown is supported by DOSY and 2D NOESY experiments.

And Cavitands Back to Capsules

The use of higher alkanes (from n-$C_{15}H_{32}$ to n-$C_{19}H_{40}$) gave unexpected results: a new *extended capsule* emerges. The hydrocarbon chains compress to fit into the new capsule in

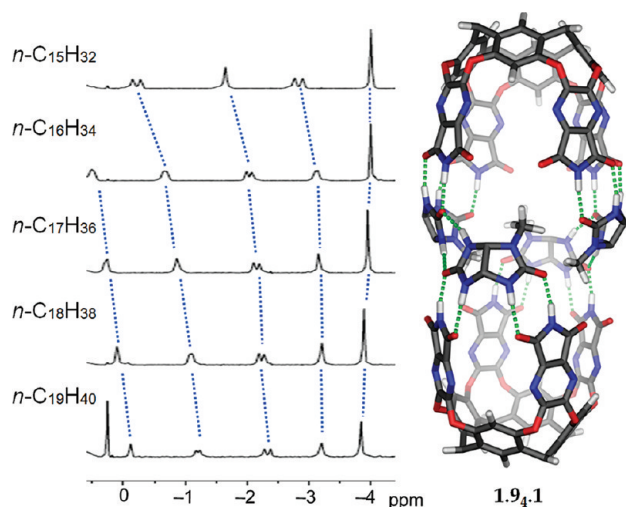**FIGURE 12.** ^{1}H NMR spectra (600 MHz, 300 K, mesitylene-d_{12}) of complexes with normal alkanes (n-C$_{15}$H$_{32}$ to n-C$_{19}$H$_{30}$), and proposed model of the capsule **1•9$_4$•1** (the peripheral groups are removed for clarity).
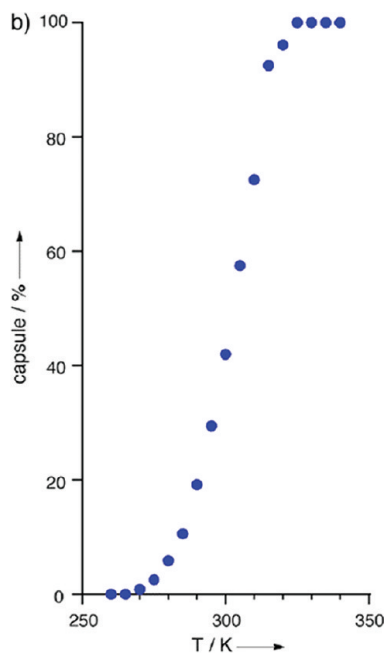

FIGURE 13. Equilibrium between capsule **1•9$_4$•1** and cavitand **1•9$_4$**; n-C$_{18}$H$_{38}$ is the guest. As the temperature is raised, the concentration of **1•9$_4$•1** increases and glycoluril **9** is released.

a manner similar to other completely surrounded alkanes, and the signals characteristic of coiling appear in the NMR spectra (Figure 12). The assembly involves four N-methyl glycourils, two cavitands, and one guest. Two different imide N–H signals (at 13–14 ppm) indicate a reduced symmetry for this capsule, and the NOESY spectrum of this assembly shows unusual crosspeaks between the N-methyl signal and the imide N–H signals. The spectra indicate a capsule of composition **1•9$_4$•1** as shown in Figure 10, formed by bringing together two cavitands **1•9$_4$** with the loss of four glycolurils. Only four hydrogen bonds (and the adhesive forces to the guest) hold the two halves of the assembly together in the proposed structure. A DOSY experiment with both **1•1** and **1•9$_4$** in solution showed a smaller diffusion coefficient for **1•1**, while a DOSY spectrum of a mixture of **1•9$_4$•1** and **1•1** showed a smaller diffusion coefficient for the former.

Unlike extended capsule **1•3$_4$•1**, which responds to increasing guest length by recruiting two, three, or four belts of (unsubstituted) glycoluril spacers, the N-methyl groups of **1•9$_4$•1** prevent incorporation of more spacers. Accordingly, n-C$_{20}$H$_{42}$ and longer alkanes are no longer guests for **1•9$_4$•1**. But ironically, n-C$_{20}$H$_{42}$ appears to be a good guest for extended cavitand **1•9$_4$**; the ^{1}H NMR spectrum of the complex is much like those of the shorter alkanes of Figure 9. It is even possible that a dumbbell-shaped assembly is formed with an extended cavitand on either end of the alkane chain. The progression of species in this system with increasing alkane length is **1•9$_4$** (C$_{10}$ to C$_{14}$), **1•9$_4$•1** (C$_{15}$ to C$_{19}$), and **1•9$_4$** again (C$_{20}$ and above).

A unique feature of this system is the coexistence of the cavitand **1•9$_4$** and capsule **1•9$_4$•1** in the presence certain alkane guests. When n-C$_{18}$H$_{40}$ was used as a guest, the distribution of the assemblies varied with temperature (Figure 13). The steep curve indicates a cooperative phenomenon as higher temperatures force the system from two to five particles.[15] The trend is consistent with the equilibrium:

$$2\,\mathbf{1•9_4}(\text{guest}) \leftrightarrow \mathbf{1•9_4•1}(\text{guest}) + 4\,\mathbf{9} + \text{guest}(\text{free})$$
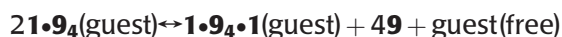
While this is a simplistic view of a complex system, it is the best we can do, for now.

Conclusions

Why study molecular behavior in small spaces? For more than a century, the accepted setting for much of chemistry was dilute solution where reagents are surrounded by a dynamic sea of solvent. What has changed now is that biochemistry and chemical biology influence the chemical researcher. Water is the only acceptable medium, and organic solvents are deemed as irrelevant (and even unwholesome). Model compounds or reactions are also out; site-directed mutagenesis can manipulate proteins or nucleic acids with exotic substitutes for the naturally occurring components inserted at will. Systems chemistry rather than linear processes is in vogue with systems biology as the paradigm. But the present work was done in organic

solutions with only a few components. We have given evidence here that incremental changes in capsule and component structure give disproportionate differences in behavior of the guest molecules that occupy the space. The difficulty in predicting which small geometrical changes in one of the building blocks affects the outcome of multicomponent assemblies has been encountered by others.[43,44] Nor is this news for the biochemist: single changes in amino acid side chains of an enzyme in the confined space of the active site can drastically alter function and selectivity. We have also encountered how minimal changes in guest structure provoke strange adaptations in the capsular assembly, again hardly news to the practitioners of in vitro evolution of nucleic acids. The intimate, mutual adaptations of the container and the contained provide a dynamic that shifts an assembly pathway to an unanticipated outcome. Finally, we note that, unlike the covalent carcerands or cryptophanes, these spectacularly unlikely containers have no independent existence; they appear only when a suitable guest is present. Filling their small spaces properly is the common, final instruction for the assembly to emerge.

BIOGRAPHICAL INFORMATION

Julius Rebek, Jr. was born in Hungary in 1944 and lived in Austria from 1945 to 1949. He and his family then settled in the United States in Kansas. He received his undergraduate education at the University of Kansas in 1966 and obtained a Ph.D. from the Massachusetts Institute of Technology (1970) for studies in peptide chemistry with Professor D. S. Kemp. As an Assistant Professor at the University of California at Los Angeles (1970−1976), he developed the three-phase test for reactive intermediates. In 1976, he moved to the University of Pittsburgh where he rose to the rank of Professor of Chemistry and developed models for allosteric effects and cleft-like structures for studies in molecular recognition. In 1989, he returned to MIT, where he was the Camille Dreyfus Professor of Chemistry and devised synthetic self-replicating molecules. In July of 1996, he moved his research group to The Scripps Research Institute to become the Director of The Skaggs Institute for Chemical Biology, where he continues to work in molecular recognition and encapsulation phenomena.

Dariush Ajami received is M.Sc. from Chemistry and Chemical Engineering Research Center of Iran in 1999. He then moved to the University of Braunschweig in Germany to obtain his Ph.D. in 2003 with Professor Rainer Herges. In 2004, he joined the group of

Professor Rebek at The Scripps Research Institute in California as a postdoctoral researcher. He is currently an Assistant Professor of Molecular Assembly at the Skaggs Institute of Chemical Biology.

FOOTNOTES

REFERENCES

1 Ferguson, S. B.; Seward, E. M.; Diederich, F.; Sanford, E. M.; Chou, A.; Inocencio-Szweda, P.; Knobler, C. B. Strong Enthalpically Driven Complexation of Neutral Benzene Guests in Aqueous Solution. *J. Org. Chem.* **1988**, *53*, 5593–5595.

2 Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. Molecular recognition in aqueous media: donor-acceptor and ion-dipole interactions produce tight binding for highly soluble guests. *J. Am. Chem. Soc.* **1988**, *110*, 1983–1985.

3 Mecozzi, S.; Rebek, J. The 55% Solution: A Formula for Molecular Recognition in the Liquid State. *Chem.—Eur. J.* **1998**, *4*, 1016–1022.

4 Canceill, J.; Cesario, M.; Collet, A.; Guilhem, J.; Pascard, C. A New Bis-Cyclotribenzyl Cavitand Capable of Selective Inclusion of Neutral Molecules in Solution - Crystal-Structure of Its Ch2cl2 Cavitate. *J. Chem. Soc., Chem. Commun.* **1985**, *6*, 361–363.

5 Cram, D. J.; Karbach, S.; Kim, Y. H.; Baczynskyj, L.; Kalleymeyn, G. W. Shell Closure of 2 Cavitands Forms Carcerand Complexes with Components of the Medium as Permanent Guests. *J. Am. Chem. Soc.* **1985**, *107*, 2575–2576.

6 Wyler, R.; Demendoza, J.; Rebek, J. A Synthetic Cavity Assembles through Self-Complementary Hydrogen-Bonds. *Angew. Chem., Int. Ed.* **1993**, *32* (12), 1699–1701.

7 Rebek, J. Reversible encapsulation and its consequences in solution. *Acc. Chem. Res.* **1999**, *32* (4), 278–286.

8 Heinz, T.; Rudkevich, D. M.; Rebek, J. Pairwise selection of guests in a cylindrical molecular capsule of nanometre dimensions. *Nature* **1998**, *394* (6695), 764–766.

9 Rebek, J. Simultaneous encapsulation: Molecules held at close range. *Angew. Chem., Int. Ed.* **2005**, *44* (14), 2068–2078.

10 (a) Ebbing, M. H.; Villa, M. J.; Valpuesta, J. M.; Prados, P.; de Mendoza, J. Resorcinarenes with 2-benzimidazolone bridges: self-aggregation, self-assembled dimeric capsules, and guest encapsulation. *Proc. Natl. Acad. Sci.U.S.A.* **2002**, *99* (8), 4962–4966. (b) Choi, H. J.; Park, Y. S.; Cho, C. S.; Koh, K.; Kim, S. H.; Paek, K. Unusually stable molecular capsule formation of a tetraphenyleneurea cavitand. *Org. Lett.* **2004**, *6* (24), 4431–4433. (c) Jeong, K. S.; Tjivikua, T.; Rebek, J., Jr. Relative Hydrogen-Bonding Affinities of Imides and Lactams. *J. Am. Chem. Soc.* **1990**, *112*, 3215–3217.

11 Asadi, A.; Ajami, D.; Rebek, J. Bent alkanes in a new thiourea-containing capsule. *J. Am. Chem. Soc.* **2011**, *133* (28), 10682–10684.

12 Bordwell, F. G.; Algrim, D. J.; Harrelson, J. A. The Relative Ease of Removing a Proton, a Hydrogen-Atom, or an Electron from Carboxamides Versus Thiocarboxamides. *J. Am. Chem. Soc.* **1988**, *110* (17), 5903–5904.

13 (a) Zhang, Z.; Schreiner, P. R. (Thio)urea organocatalysis—what can be learnt from anion recognition? *Chem. Soc. Rev.* **2009**, *38* (4), 1187–1198. (b) Pihko, P. M. *Hydrogen Bonding in Organic Synthesis*; Wiley-VCH Verlag GmbH & Co.: Weinheim, Germany, 2009.

14 Scarso, A.; Trembleau, L.; Rebek, J., Jr. Helical folding of alkanes in a self-assembled, cylindrical capsule. *J. Am. Chem. Soc.* **2004**, *126* (41), 13512–13518.

15 (a) Yamanaka, M.; Shivanyuk, A.; Rebek, J., Jr. Kinetics and thermodynamics of hexameric capsule formation. *J. Am. Chem. Soc.* **2004**, *126*, 2939–2943. (b) Palmer, L. C.; Rebek, J., Jr. The Ins and Outs of Molecular Encapsulation. *Org. Biomol. Chem.* **2004**, *2*, 3051–3059.

16 (a) Zanotti, G.; Scapin, G.; Spadon, P.; Veerkamp, J. H.; Sacchettini, J. C. Three-dimensional structure of recombinant human muscle fatty acid-binding protein. *J. Biol. Chem.* **1992**, *267* (26), 18541–18550. (b) Han, G. W.; Lee, J. Y.; Song, H. K.; Chang, C.; Min, K.; Moon, J.; Shin, D. H.; Kopka, M. L.; Sawaya, M. R.; Yuan, H. S.; Kim, T. D.; Choe, J.; Lim, D.; Moon, H. J.; Suh, S. W. Structural basis of non-specific lipid binding in maize lipid-transfer protein complexes revealed by high-resolution X-ray crystallography. *J. Mol. Biol.* **2001**, *308* (2), 263–278.

17 Petina, O.; Rehder, D.; E. Haupt, T. K.; Grego, A.; Weinstock, I. A.; Merca, A.; Boegge, H.; Szakacs, J.; Mueller, A. Guests on Different Internal Capsule Sites Exchange with Each Other and with the Outside. *Angew. Chem., Int. Ed.* **2011**, *50*, 410–414.

18 Ko, Y. H.; Kim, Y.; Kim, H.; Kim, K. U-Shaped Conformation of Alkyl Chains Bound to a Synthetic Receptor Cucurbit[8]uril. *Chem.—Asian J.* **2011**, *6*, 652–657.

19 Cavarzan, A.; Scarso, A.; Sgarbossa, P.; Strukul, G.; Reek, J. N. H. Supramolecular Control on Chemo- and Regioselectivity via Encapsulation of (NHC)-Au Catalyst within a Hexameric Self-Assembled Host. *J. Am. Chem. Soc.* **2011**, *133*, 2848–2851.

20 Dydio, P.; Rubay, C.; Gadzikwa, T.; Lutz, M.; Reek, J. N. H. Cofactor-Controlled Enantioselective Catalysis. *J. Am. Chem. Soc.* **2011**, *133*, 17176–17179.

21 Ajami, D.; Rebek, J. Expanded capsules with reversibly added spacers. *J. Am. Chem. Soc.* **2006**, *128* (16), 5314–5315.

22 Ajami, D.; Rebek, J. Longer guests drive the reversible assembly of hyperextended capsules. *Angew. Chem., Int. Ed.* **2007**, *46* (48), 9283–9286.

23 Moon, K.; Chen, W. Z.; Ren, T.; Kaifer, A. E. A unique hydrogen bonding network in the crystal structure of 3a,6a-diphenylglycoluril. *CrystEngComm* **2003**, *5*, 451–453.

24 Piacenza, G.; Beguet, C.; Wimmer, E.; Gallo, R.; Giorgi, M. 2,4,6,8-tetraazabicyclo-[3.3.1]nonane-3,7-dione and 2,4,6,8-tetraacetyl-2,4,6,8-tetraazabicyclo[3.3.1]nonane-3,7-dione. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1997**, *53*, 1459–1462.

25 Siering, C.; Torang, J.; Kruse, H.; Grimme, S.; Waldvogel, S. R. Enantioselective helical folding inside a self-assembled, cylindrical capsule. *Chem. Commun.* **2010**, *46* (10), 1625–1627.

26 Shivanyuk, A.; Rebek, J. Social Isomers in Encapsulation Complexes. *J. Am. Chem. Soc.* **2002**, *124*, 12074–12075.

27 Ajami, D.; Rebek, J. Compressed alkanes in reversible encapsulation complexes. *Nat. Chem.* **2009**, *1* (1), 87–90.

28 (a) Burley, S. K.; Petsko, G. A. Aromatic-aromatic interaction - a mechanism of protein-structure stabilization. *Science* **1985**, *229*, 23–28. (b) Blundell, T. L.; Singh, J.; Thornton, J.; Burley, S. K.; Petsko, G. A. Aromatic Interactions. *Science* **1986**, *234*, 1005. (c) Galán, A.; de Mendoza, J.; Toiron, C.; Bruix, M.; Deslongchamps, G.; Rebek, J., Jr. A Synthetic Receptor for Dinucleotides. *J. Am. Chem. Soc.* **1991**, *113*, 9424–9425. (d) Jeong, K. S.; Muehldorf, A. V.; Rebek, J., Jr. Molecular Recognition. Asymmetric Complexation of Diketopiperazines. *J. Am. Chem. Soc.* **1990**, *112*, 6144–6145.

29 Kaanumalle, L. S.; Gibb, C. L. D.; Gibb, B. C.; Ramamurthy, V. *J. Am. Chem. Soc.* **2005**, *127*, 3674.

30 (a) Paliwal, S.; Geib, S.; Wilcox, C. S. Molecular Torsion Balance for Weak Molecular Recognition Forces. Effects of ″Tilted-T″ Edge-to-Face Aromatic Interactions on Conformational Selection and Solid-State Structure. *J. Am. Chem. Soc.* **1994**, *116*, 4497–4498. (b) Hof, F.; Scofield, D. M.; Schweizer, W. B.; Diederich, F. A Weak Attractive Interaction between Organic Fluorine and an Amide Group. *Angew. Chem., Int. Ed.* **2004**, *43*, 5056–5059. For a recent review, see: (c) Mati, I. K.; Cockroft, S. L. Molecular balances for quantifying non-covalent interactions. *Chem. Soc. Rev.* **2010**, *39*, 4195–4205.

31 Castellano, R. K.; Meyer, E. A.; Diederich, F. Interactions with aromatic rings in chemical and biological recognition. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210–1250.

32 Salonen, L. M.; Ellermann, M.; Diederich, F. Aromatic Rings in Chemical and Biological Recognition: Energetics and Structures. *Angew. Chem., Int. Ed.* **2011**, *50*, 4808–4842.

33 Bissantz, C.; Kuhn, B.; Stahl, M. A medicinal chemist's guide to molecular interactions. *J. Med. Chem.* **2010**, *53*, 5061–5084.

34 Tucci, F. C.; Rudkevich, D. M.; Rebek, J. Deeper Cavitands. *J. Org. Chem.* **1999**, *64*, 4555–4559.

35 Cram, D. J.; Choi, H. J.; Bryant, J. A.; Knobler, C. B. Host-guest complexation. 62. Solvophobic and entropic driving forces for forming velcraplexes, which are 4-fold, lock-key dimers in organic media. *J. Am. Chem. Soc.* **1992**, *114* (20), 7748–7765.

36 Tucci, F. C.; Rudkevich, D. M.; Rebek, J. Velcrands with snaps and their conformational control. *Chem.—Eur. J.* **2000**, *6* (6), 1007–1016.

37 Park, T. K.; Schroeder, J.; Rebek, J. New Molecular Complements to Imides. Complexation of Thymine Derivatives. *J. Am. Chem. Soc.* **1991**, *113*, 5125–5127.

38 Jeong, K. S.; Muehldorf, A. V.; Rebek, J. Molecular Recognition. Asymmetric Complexation of Diketopiperazines. *J. Am. Chem. Soc.* **1990**, *112*, 6144–6145.

39 Yamauchi, Y.; Ajami, D.; Lee, J.-Y.; Rebek, J. Deconstruction of Capsules using Chiral Spacers. *Angew. Chem., Int. Ed.* **2011**, *50*, 9150–9153.

40 Rieth, S.; Hermann, K.; Wang, B.-Y.; Badjic, J. D. Controlling the dynamics of molecular encapsulation and gating. *Chem. Soc. Rev.* **2011**, *40*, 1609–1622.

41 Trembleau, L.; Rebek, J. Helical Conformation of Alkanes in Hydrophobic Environments. *Science* **2003**, *301*, 1219–1220.

42 Scarso, A.; Trembleau, L.; Rebek, J., Jr. Encapsulation induces helical folding of alkanes. *Angew. Chem., Int. Ed.* **2003**, *42* (44), 5499–5502.

43 Sun, Q.-F.; Iwasa, J.; Ogawa, D.; Ishido, Y.; Sato, S.; Ozeki, T.; Sei, Y.; Yamaguchi, K.; Fujita, M. Self-Assembled M24L48 Polyhedra and Their Sharp Structural Switch Upon Subtle Ligand Variation. *Science* **2010**, *328*, 1144–1147.

44 Lin, Z.; Emge, T. J.; Warmuth, R. Multicomponent Assembly of Cavitand-Based Polyacylhydrazone Nanocapsules. *Chem.—Eur. J.* **2011**, *17*, 9395–9405.