The 55 % Solution: A Formula for Molecular Recognition in the Liquid State

Sandro Mecozzi and Julius Rebek, Jr.*

Abstract: Evidence is presented that molecular recognition through encapsulation processes is largely determined by the volumes of the guest and host. Binding of molecules of suitable dimensions in the internal cavity of a molecular receptor in solution can be expected when the packing coefficient, the ratio of the guest volume to the host volume, is in the range of 0.55 ± 0.09. Larger packing coefficients, up to 0.70, can be reached if the complex is stabilized by strong intermolecular forces such as hydrogen bonds. These considerations also apply to situations in which more than one molecule is encapsulated. Organic liquids are generally characterized by the same packing factors as encapsulation complexes, and it is proposed that the short-range structure of liquids and the complexes resulting from encapsulation are two aspects of the same phenomenon. Similar volume considerations are expected to apply to the binding of substrates in biological receptors.

Keywords: host–guest chemistry · molecular dynamics · molecular recognition · self-assembly · supramolecular chemistry

Introduction

Modern molecular recognition in chemistry began in 1967, when Pedersen[1] described stoichiometric complexes of ammonium salts with crown ethers in solution. Since then the field has expanded and evolved, and a large variety of supramolecular systems (molecular complexes held temporarily together by weak forces) have been synthesized and characterized in solution. We focus here on molecules within molecules,[2] that is, complexes made by hosts that completely surround their guests. These systems present opportunities for studying aspects of recognition phenomena related to volume. In the recent past we have described several self-complementary subunits that assemble to form pseudospherical spaces in dimeric capsules.[3] These self-assembled capsules display selectivity in their choice of guests and we explore here the physical basis of this type of recognition. We propose that the selection is, to a first approximation, governed by volumes, and this notion is generalizable for other types of recognition, for example, drug design.

Finding the ideal guest for our hosts is an exercise that has much in common with rational drug synthesis: the structure of the target is well-defined by crystallography or highly-refined computer modeling; the formulation of a congruent and complementary surface follows, first virtually on the computer screen and then in vitro. In practice, random screening has the higher yield in high-affinity lead compounds in medicinal chemistry, a record that will only be further improved through the sheer numerics of combinatorial chemistry. So it is with recognition. Synthetic accessibility—and sometimes serendipity—dictates which receptor is made, then targets are screened until a good fit is found. When the affinity is optimized, the successful match is announced and the word design is much overused in the publication. This procedure, though effective, requires the availability of some simple and useful rules that can aid in predicting the formation of an ideal host–guest complex. We propose here such a rule.

[*] Prof. J. Rebek, Jr., Dr. S. Mecozzi
The Skaggs Institute for Chemical Biology and
Department of Chemistry
The Scripps Research Institute, MB-26
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 619-784-2876
E-mail: jrebek@scripps.edu

Experimental Section

Background: Consider first some details of the structure and properties of liquids. Short-range intermolecular interactions differentiate liquids from gases, while the lack of long-range order differentiate them from solids. The focus of the solution chemist has been on the behavior and properties of the reacting molecules rather than on the structure of the surrounding environment. However, several useful insights are gained by considering the space that organic liquids occupy. In crystallography the volumes of filled space are known as the packing coefficients (*PC*s), but there is limited literature available with regard to the liquid state.[4, 5] The *PC* of a compound is defined as the ratio between the sum (V_w) of the van der Waals volumes (v_w) of the n molecules in a given volume (V) and the volume (V) [Eq. (1)].

$$PC = \frac{\sum_{i=1}^{n} v_w^i}{V} = \frac{V_w}{V} \quad (1)$$

0947-6539/98/0406-1016 $ 17.50+.50/0

Packing coefficients, often called packing densities, have been the object of several studies related to the physical properties of liquids and solids, and to the protein-folding problem. Earlier estimates of *PC*s for liquids range between 0.44 and 0.56, while *PC*s of organic crystals and the interior of globular proteins are reported to be in the range 0.66–0.77.[5a, 6] Table 1 reports the packing coefficients that we have calculated for some common organic liquids.[7] Usually, slightly more than half of the available space is physically filled by the molecules of the liquid. The remaining empty space is apparently required by the thermal motion of the molecules. There is a compromise between the entropic freedom of the molecules and the enthalpic comforts of contacting their temporary neighbors. As a liquid is cooled, the magnitude of molecular motions decreases until the molecules no longer move past each other, but are confined to specific positions (the lattice sites): a solid is formed. Take, for example, a solid composed of hard spheres. The simplest and most compact close-packed lattices for this system are the hexagonally close-packed (hcp) and the cubic close-packed (A1-ccp) lattices. Both these lattices have a theoretical packing coefficient of 0.74. However, packing coefficients can go up to 0.90 when a close-packed array of infinitely long cylinders is considered.[5a] Theoretically, a hard-sphere close-packed solid at 0 K has a density of about 1.16 times the density of the corresponding liquid at its melting point, that is, the volume of the liquid phase can be expected to be 16% larger than the volume of the solid phase.[5a,b] This corresponds to an increase in the mean intermolecular separation of only 5% and to a packing coefficient of 0.64. This is, however, an ideal rule. The shape of the molecules and the intermolecular forces acting among them can yield very different results, the most egregious case being water.

Table 1. Packing coefficients for some common organic liquids.

Organic liquid	Packing coefficient[a]
benzene	0.54
toluene	0.54
n-hexane	0.51
cyclohexane	0.56
methylene chloride	0.54
chloroform	0.53
carbon tetrachloride	0.53
diethyl ether	0.51
acetone	0.52
acetonitrile	0.53
N,*N*-dimethylformamide	0.61
methanol	0.54
ethanol	0.55
water	0.63

[a] The packing coefficients were calculated according to [Eq. (1)]. The van der Waals molecular volumes v_W were calculated with the program GRASP.[7]

How can the information about the structure of a liquid be used to make predictions in the recognition events between a host and guest? The connection lies in the similarity of the intermolecular forces and freedoms that in one case organize the short-range structure of a liquid and in the other case make formation of a noncovalent complex possible. Just as the volume occupied by the molecules of a generic liquid is little more than half-filled, a favorable recognition event is reached when the guest only occupies about half of the space defined by the cavity of the host. Although this notion may sound surprising—or even naive—we present here evidence that the mere filling of space in our capsules and in the container molecules of others is a dominant factor in the behavior of synthetic host–guest systems.

Volume calculations: Until recently, calculation of cavity sizes has not been a trivial matter. For example, Cram[8] describes a charming method by which plaster forced into a Correy–Pauling–Koltun (CPK) model is used to estimate cavity size and shape, while we have used a computational method with the MacroModel program with overlapping virtual spheres of various sizes packed into the cavity.[9] Neither of these methods provide a satisfactory answer to the question: Where do the holes in the structure end? Nor is there total agreement on the meaning of the volume of a molecule: Where do the atoms end?

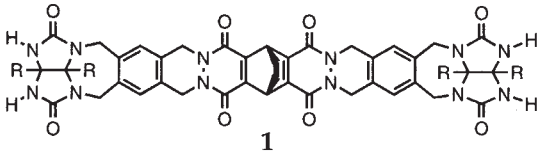
We first minimized hosts **1·1**–**10** by use of the force field Amber*[10] in the MacroModel program.[11] We then used the program GRASP[12] to estimate the volume of the internal cavities of the capsules. The calculation involves rolling a spherical probe along the interior surface. A small probe is ideal, but can frequently fall out of the holes in the structure; a large probe prevents fallout by defining a closed molecular surface, but fails to define the smaller dimples and invaginations of the already concave surface of the interior. Inevitably, there is error and compromise: the larger probe underestimates the interior volume while the smaller overestimates it. The default size of the probe in the software package GRASP has a diameter of 1.4 Å. This probe proved suitable for cavity–volume calculations for capsules **1·1**, **2·2**, **3·3**, **4·4**, **7**, **8**, **9**, and **10**. In fact, a change of ±0.3 Å in the size of the probe translated in an averaged ±5% error in the cavity volumes of these capsules. However, use of the default probe for the smaller capsules **5·5** and **6·6** introduced a large error in the estimation of the volume of the internal cavity. In fact, if the cavity in capsules **5·5** and **6·6** is assumed to be spherical, the diameter of the probe is as big as the radius of the sphere. This implies a large error in the mapping of the cavity surface by the rolling of the probe. Reduction in the probe size to 1.0 Å, for example, is of no advantage and introduces an unwarranted discrepancy with the larger capsules in which smaller probes do not yield a closed cavity surface. In order to overcome this problem, another approach to the calculation of volumes was explored.[9] This method is based on the filling of the cavities with either a lattice of carbon atoms or with branched hydrocarbons. The size and shape of these virtual guests were chosen in such a way that their peripheral atoms overlap with the van der Waals radii of the adjoining capsule atoms. The volume of the overlapping regions was then determined and subtracted from the volume of the guest. The result is a rough estimate of the cavity volume.

Cavity volumes calculated with both methods are reported in Table 2. As expected either method gives consistent results for the larger capsules, but there is a dramatic difference for the small capsules. We attribute this difference to the intrinsic error associated with measuring small cavity volumes by probing the molecular surface of the cavity with a necessarily large rolling sphere. Therefore, the cavity-filling method has to be preferred in cases where the probe size and the size of the cavity are of comparable size. A second source of uncertainty in the volume calculation is due to the set of atomic radii used. As there is no universally accepted set, we used the following values: aliphatic carbon = 1.70 Å, aromatic carbon = 1.75 Å, oxygen = 1.60 Å, nitrogen = 1.65 Å, aliphatic hydrogen = 1.20 Å, aromatic hydrogen = 1.00 Å, and chlorine = 1.75 Å.

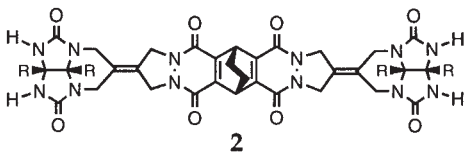
Table 2. Volume of the internal cavities of capsules **1·1**–**10** as calculated according to the Grasp program (**A**) and to the cavity-filling method (**B**).

	Volume of the internal cavity [Å^3]	
	A	**B**
1·1	313	322
2·2	225	240
3·3	240	248
4·4	190	197
5·5	61	68
6·6	37	52
7	159	159
8	95	97
9	117	119
10	119	117

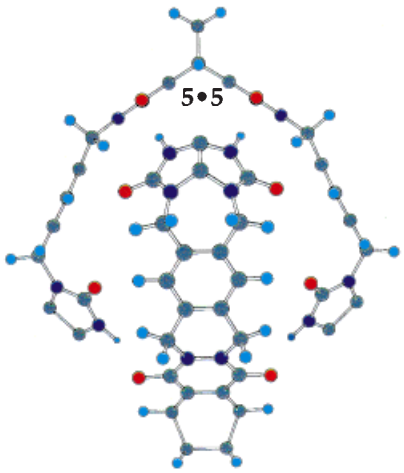
In order to explore the stability of the complexes, a molecular dynamics simulation of each capsule was run. The simulation temperature was set at 300 K, the total simulation times were 200 ps and the time step was 1 fs. GB/SA chloroform solvation was used. Structures were sampled every 10 ps, and the volume of the internal cavity was recalculated. In all of the capsules examined, the volume of the cavity was not constant, but oscillated in a 10% range from the starting minimized volume. Accordingly, the resulting value is crude: it depends greatly on the parameters used for modeling hydrogen bonds and it neglects the breathing dynamics—the



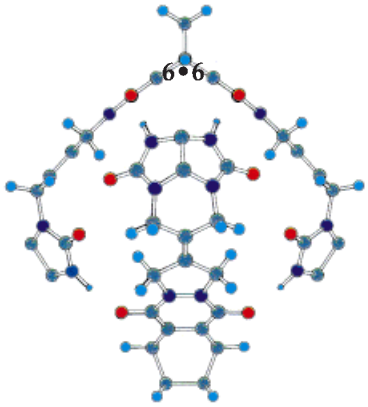
1



2



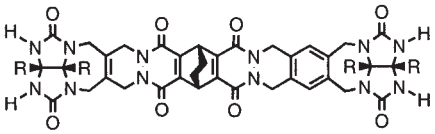
5•5



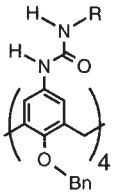
6•6

1•1

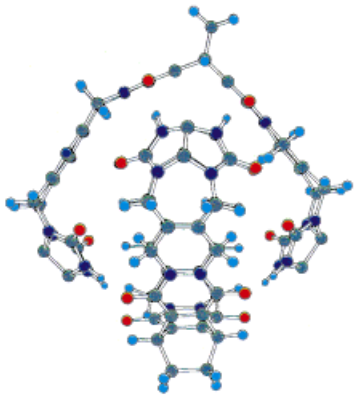
2•2



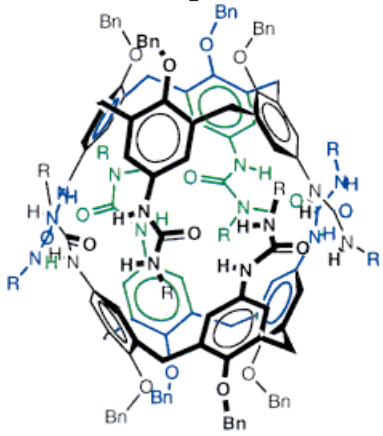
3



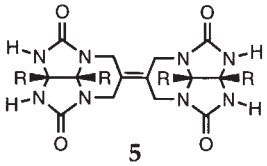
4



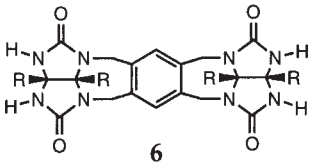
3•3



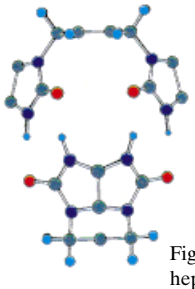
4•4



5



6



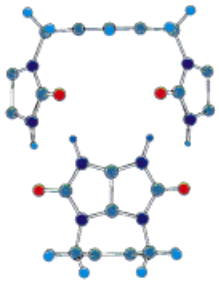


Figure 1. Molecules **1**–**6** and their dimers **1**·**1**–**6**·**6**. **1**: R = CO_2-*n*-heptyl, **2**, **3**: R = *p*-(*n*-heptyl)-phenyl, **4**: R = *p*-fluorophenyl, **5**, **6**: R = CO_2ethyl. Residues R have been omitted from the 3D representations of dimers **1**·**1**–**6**·**6** for clarity.

0947-6539/98/0406-1018 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 6

changes in shape (and therefore dimensions) of the capsules that occur with the deformation of hydrogen bonds—a process generally agreed to involve small energy changes. The overall error on a cavity volume calculation is determined by several factors. Changes in volume as a result of molecular motions account for most of the error. These changes are taken into account by the 10% variability in cavity volume associated with the molecular dynamics simulations. The importance of the error associated with the generation of the molecular surface is minimized by use of the same probe size for all the complexes. Finally, it is reasonable to expect that refinements in van der Waals radii and in the computational methods used to calculate cavity volumes will generate a range of packing coefficients different from the ones the we report. However, we want to emphasize that the important concept is not the absolute numerical value of this range, but rather the existence of a narrow range of packing coefficients for which encapsulation of a guest is optimal. We estimate this to be in the range 0.55 ± 0.09. We next examined several possible guests by calculating the volume enclosed by their van der Waals molecular surface. The volumes calculated in this way are in agreement with the few known molecular volumes.[13]

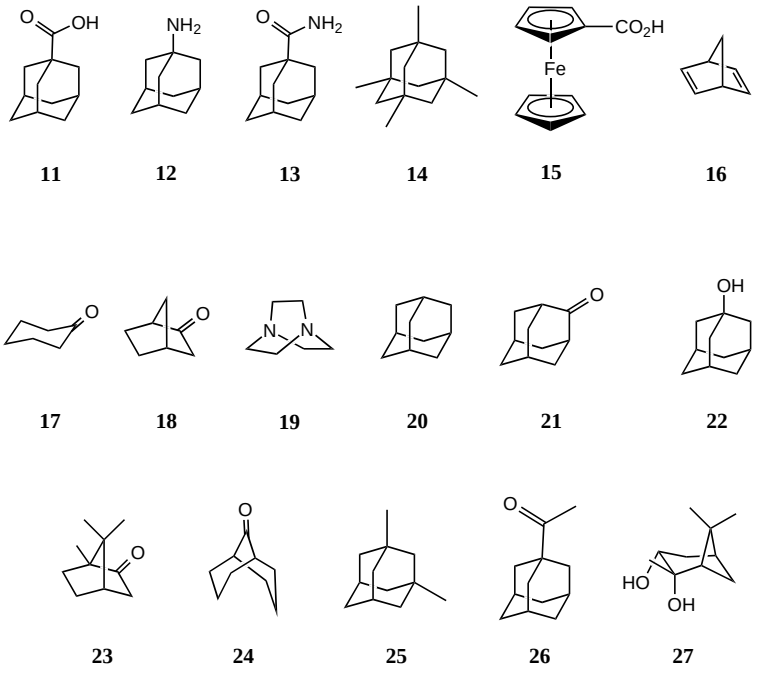


Figure 2. Structures of the guests used in the encapsulation studies.

Results and Discussion

Structures **1**–**6** (Figure 1) represent molecular capsules. These systems involve two identical subunits that are held together through series of hydrogen bonds. The two halves are self-complementary with respect to their hydrogen-bonding donors and acceptors. These dimers resemble carcerands[14] and cryptophanes,[15] in that they form molecule within molecule complexes, but they are formed reversibly, and this dynamic quality makes them suitable for measuring equilibria at ambient temperatures and in organic media. The capsules form and dissipate on a time scale that varies from milliseconds to hours.[16] These time intervals are long enough to allow many types of intermolecular interactions, including covalent-bond formation, to be established.[17]

Dimer **1·1**—the softball—is the largest capsule synthesized to date with an internal cavity of approximately 313 Å^3.[4c, 18] The sheer size of the cavity reduces the error on the volume calculation and allows equilibrium–binding studies on a greater range of guest sizes (Figure 2).

The relative affinity or ability of several guests to induce the capsular form **1·1** at room temperature is recorded in Table 3. The best guests are polar adamantane derivatives such as 1-adamantane carboxylic acid (**11**) and 1-adamantane amine (**12**), but even the nonpolar tetramethyl adamantane (**14**), when present in large excess, was encapsulated. The derivatives with hydrogen-bond donors are welcome because almost all of the atoms that line the interior of the cavity are sp^2 hybridized with their π surfaces lining the cavity. Hydrogen-bond donors can easily find their complements on these surfaces and at the heteroatoms. Hydrogen-bond acceptors can interact with the seam that holds the system together by way of bifurcated hydrogen bonds. One cannot predict how the enthalpy of those hydrogen bonds are changed when, for example, the adamantane is inside. Nor can one predict whether the van der Waals contacts between encapsulated solvents and the concave surface of the capsule are more or less favorable than those interactions of the hydrocarbon portion of the adamantane guest. In short, there is no reliable way of predicting what the enthalpy of the process should be. Even so, we have found that when the dimensions of the guest are suitable for the internal cavity of these capsules, optimal binding is reached if about 55% of the volume is filled.

Table 3. Binding constants, volume, and packing coefficients for some selected guests in capsule **1·1**.

	Binding constant	Volume [Å^3]	Packing coefficient
11	780	175	0.56
12	190	157	0.50
13	310	177	0.56
14	6.7	211	0.67[a]
15	280	169	0.56

[a] This guest showed signs of encapsulation only when a large excess (ten equivalents) was added to the softball in solution.

The behavior of the softball in various solvents provides another example of this rule. The NMR spectra in $CDCl_3$, [D_{10}]*p*-xylene, and [D_6]benzene are shown in Figure 3. In $CDCl_3$, two solvent molecules leave too much unoccupied space, while three solvent molecules leave too little. The broadened spectrum observed with suggests a dynamic system in which well-defined capsules are minority components. For the larger solvent xylene, the spectrum is likewise broad: the dimeric capsule is destabilized. Calculations reported elsewhere[4c] indicate that a constellation of two xylenes can only be accommodated inside when they are forced to a face-to-face distance of < 3.3 Å, this distance being less than optimal for stacking. Only in benzene is the sharp spectrum of the capsule seen, and only in benzene do two solvent molecules nicely fill the appropriate volume. In other words, the benzene molecules experience little change in free volume on entering

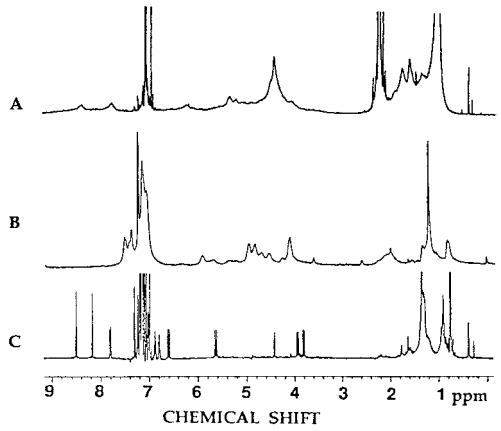


Figure 3. NMR spectra of **4** in [D_{10}]*p*-xylene (**A**), $CDCl_3$ (**B**), and [D_6]benzene (**C**).

the capsule. The concentration of benzene inside the capsule is similar to that in bulk solution (approximately 10 M). Evidence that two molecules of solvent benzene are indeed present inside the softball is given elsewhere;[19] their release by a single large guest provides much of the driving force for the encapsulation process and leads to unusual thermodynamic parameters observed.[20]

Dimer **2·2** has a spherical cavity characterized by a volume of 225 Å^3.[21] This intermediate size (when envisioned with the softball or tennis ball) allows the binding of a variety of guests (Table 4). While most of these guests show a binding behavior close to that seen with the softball, molecules **11** and **23** seem to violate the proposed rule by filling much more than just about half the volume of the cavity. These anomalies point to an important aspect in the encapsulation process: the nature and magnitude of the intermolecular forces at work in the complex cannot be neglected. Guest design based on volume and shape considerations only gives good results with the weakest forces: dipole–dipole, dipole–induced dipole, and London dispersion forces. These same forces are also responsible for keeping molecules together in apolar aprotic solvents and are at work in the encapsulation of the most apolar guests in **1·1** and **2·2**. However, different and stronger intermolecular interactions are also available for binding. Guests such as **11**, **13**, and **23**, have hydrogen-bonding acceptor and/or donor functionalities, and it is reasonable to assume these groups are engaged in a hydrogen-bonding network with the host donor/acceptor groups following encapsulation. The extra stabilization enthalpy coming from these interactions can then be enough to compensate for the entropy lost and therefore to allow the encapsulation of guests with packing coefficients up to 70%. That this is the case is evident from Table 4. Guests **25** and **26** have a volume and shape similar to **11**, but they are not encapsulated. Amber* minimization of the complex between **2·2** and **11** shows the participation of the acidic hydroxyl group in a hydrogen bond with the carbonyls on the glycolurils. In this respect, molecule **3**,[22] which forms dimer **3·3** featuring a chiral cavity, is particularly revealing. A molecular dynamics simulation of the complex between this dimer and **27** ($V = 173$ Å^3, $PC = 0.71$, $K = 190\ \text{M}^{-1}$) shows the formation of a hydrogen-bond network between the two hydroxyl groups of the guest and the carbonyls on the host. The hydrogen bonds between the host and the encapsulated guest are formed in a reversible way: due to the dynamics of the complex, they constantly slide along the interior surface by use of different donor/acceptor groups on the host. The encapsulation of a specific guest is therefore dependent on the nature of the intermolecular forces engaged in the binding process. However, the encapsulation process cannot produce systems that are denser than organic crystals and super-dense solids, which are characterized by packing coefficients around 75%.

Table 4. Binding constants, volume, and packing coefficients for selected guests in capsule **2·2**.

	Binding constant	Volume [Å^3]	Packing coefficient
16	12[a]	97	0.43
17	1700[b]	103	0.46
18	1800[b]	110	0.49
19	500[b]	102	0.45
20	3800[b]	125	0.56
21	5.2×10^5[b]	132	0.59
22	5.2×10^5[b]	135	0.60
23	910[a]	160	0.71
11	130[a]	154	0.68
24	510[b]	142	0.63
25	0	154	0.68
26	0	181	0.80

[a] Measured by direct binding. [b] Measured by competitive binding.

Dimer **4·4** is the capsule made by two calix[4]arenes.[9] Here, the two monomers are kept together by a head-to-tail hydrogen-bonding pattern of the urea functional groups fixed on the upper rim of the calixarene. The cavity presents two domains of different polarity; the hydrophobic poles that have aromatic π systems and a hydrophilic equator that offers hydrogen-bonding possibilities to the urea functions. The volume calculated for the cavity is 190 Å^3, a value supported by a recent crystal structure.[23] An excellent guest for this cavity was cubane ($V = 103$ Å^3, $PC = 0.54$). The calixarene capsule also binds smaller guests such as 1,4-difluorobenzene ($PC = 0.44$) and pyrazine ($PC = 0.38$). The reason for the binding of these smaller guests is again to be found in the specific interactions available to these guests with the lining of the capsule, and the positions of the guests within it. The partially positive C–H bonds of both pyrazine and 1,4-difluorobenzene are directed toward the π surfaces at the poles of the capsule and the partially negative heteroatoms are directed toward the seam of urea hydrogens at the equator. This positioning of pyrazine inside another type of capsule was also deduced by Sherman,[24] about more of which later. These specific attractive contacts with the host ultimately determine whether the guest will be encapsulated and how far on either side of the 55% solution it can be accommodated.

Dimer **5·5**, the tennis ball, sports a fused benzene ring as spacer between two glycoluril recognition elements and has an internal volume of 69 Å^3.[25] The binding behavior of this molecule towards apolar guests strictly conforms to the proposed rule of cavity filling. This capsule shows some selectivity between methane ($V = 28$ Å^3, $PC = 0.41$, $K_a = 33\ \text{M}^{-1}$) and ethane ($V = 45$ Å^3, $PC = 0.65$, $K_a = 51\ \text{M}^{-1}$), the

0947-6539/98/0406-1020 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 6

latter being preferably bound. Encapsulation of xenon (van der Waals radius = 2.18 Å, $V = 43$ Å^3, $PC = 0.62$) and argon (van der Waals radius = 1.89 Å, $V = 28$ Å^3, $PC = 0.41$), with xenon being preferably bound, was also shown experimentally. Helium (van der Waals radius = 1.6 Å, $V = 17$ Å^3, $PC = 0.25$) was not bound.

The dimer formed by molecule **6** is the smallest of the capsules with only an ethylene spacer between two glycoluril recognition elements.[26] The volume of the internal cavity of dimer **6·6** is only 52 Å^3 (cavity-filling method see Table 2). This dimer is able to bind methane ($V = 28$ Å^3, $PC = 0.54$), but not ethane ($V = 45$ Å^3, $PC = 0.86$).

The behavior of these systems can be summarized by saying that in cases where encapsulation of the guest occurs, the packing coefficient is statistically distributed in the range 0.55 ± 0.09. Guests with packing coefficients less than 45% are not well-encapsulated because the intermolecular interactions between the host and the guest are not better than those that the guest experiences in the bulk solvent. Moreover, a price is paid for undesirable empty space generated in the capsule: the interior surface becomes desolvated. On the other hand, guests with packing coefficients higher than approximately 65% are not easily encapsulated because this corresponds to the artificial freezing of the molecule in the capsule. The guest becomes restricted in its movements compared with the freedom it enjoys in the solvent. Encapsulation of bigger guests can, however, be attained by extra stabilization from additional intermolecular interactions such as hydrogen bonds. An unusual (and yet untested) corollary is that different guests may be preferred at different temperatures, but such selectivity is likely to be small.

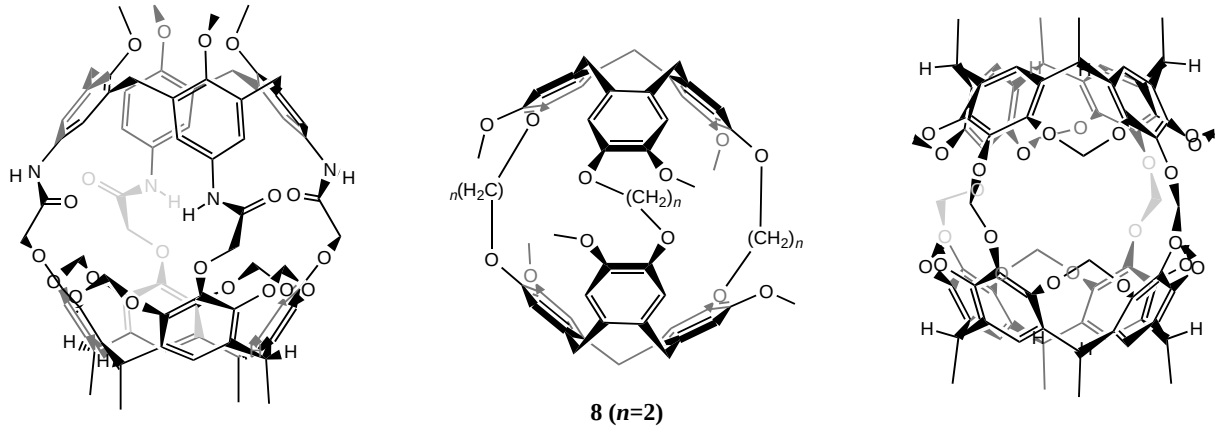
There are a limited number of examples of molecule within molecule systems that have been examined in other laboratories, and they show similar volume dependencies. Calix[4]-arene-carceplex **7** (Figure 4) has an internal cavity of 159 Å^3 and has been studied in detail.[27] Several solvent guests have been used as templating agents in the final step of the synthesis. While the packing coefficients of all these guests range between 0.45 and 0.65, there are striking differences in their efficacies. For example, the reaction in *N*-methyl-2-pyrrolidinone ($PC = 0.58$) gives a yield of 50%, but when 1,5-dimethyl-2-pyrrolidinone ($PC = 0.74$) is used, the yield is reduced to < 5%. The optimal template reaction is reached when the packing coefficient is in the range of the 55% solution, but specific contacts such as hydrogen bonds are also involved and obscure the interpretation.

Collet et al. have described the complexation properties and the packing coefficients of various cryptophanes, classifying the corresponding host–guest complexes in terms of pseudoliquids, pseudocrystals, and supercritical fluids. As an example of the complexation properties of these molecules, we refer to cryptophanes **8** and **9** (Figure 4).[4a,b] The former prefers CH_2Cl_2 as a guest to $CHCl_3$, while **9** prefers $CHCl_3$. By applying our methodology to the calculation of cavity volumes of these complexes, we were able to estimate the volume of the internal cavities of **8** and **9** as 95 Å^3 and 117 Å^3, respectively.[28] Accordingly, the packing coefficients of CH_2Cl_2 ($V = 57$ Å^3) and $CHCl_3$ ($V = 71$ Å^3) are 0.60 and 0.75 in cryptophane **8**, and 0.49 and 0.61 in cryptophane **9**, respectively. The binding behavior of these two cryptophanes fits within the range of the proposed rule.

As a final example, we turn to the carceplex **10** (Figure 4, cavity size 119 Å^3), which has been extensively studied by Sherman et al.[29] These authors showed that a large variety of molecules can be used as templates in the final step of the synthesis of **10**. The best yields are reached when pyrazine ($PC = 0.62$) and 1,4-dioxane ($PC = 0.65$) are used. The largest guest to be used for this template step is *N*-methyl pyrrolidinone that has a $PC = 0.77$; a guest that gave the lowest yield. While the process of the template step doubtless involves considerations beyond those involved in equilibrium binding, the results are again in reasonable agreement with the premise developed here.

Conclusions

The complexing properties of a molecular capsule can be estimated based on the packing coefficient of the guest in the internal cavity of the host: the best binding is reached when



7 **8 (*n*=2)** **9 (*n*=3)** **10**

Figure 4. Calix[4]arene-carceplex **7**, cryptophanes **8** and **9**, and carceplex **10**.

the packing coefficient is in the range 0.55 ± 0.09. This ratio corresponds to the packing coefficient of most organic liquids. We propose that this volume-optimized binding behavior is also a feature of natural biological systems, the binding of substrates and inhibitors in the active site of enzymes conforming to the same rule. A suitable guest is one that has, of course, the shape appropriate to fit into the capsule and has the right packing coefficient. We fully expect that building in specific polar interactions will tolerate greater (or smaller) packing coefficients: the enthalpy gained in the interactions will pay for the entropy lost or the vacuums created. Within this steric limitation, the designer of the best guest could do worse than begin with simple volume considerations if a cavity is well-defined. Unhappily, either large holes in the structures or an opening to the exterior do not often allow a precise definition of an internal molecular cavity. In these cases, it may be difficult to judge binding capabilities based on volume considerations alone. The fact that most recognition events in biological system involve just such cases is probably why the 55% solution has taken so long to formulate.

Acknowledgments: We thank the Skaggs Research Foundation and the National Institutes of Health for financial support. S.M. ackwnowledges a NASA-NSCORT Postdoctoral Fellowship. We also thank Michel Sanner and Art Olson for helpful discussions. Finally, we are grateful to Richard Lerner, William Jorgensen, and Charles Brooks, III, for suggestions and advice.

Received: November 27, 1997 [F911]

[1] C. J. Pedersen, *J. Am. Chem. Soc.* **1967**, *89*, 7017 – 7036.
[2] This phrase first appears as the title of a lecture by D. J. Cram at the C. David Gutsche Symposium, Washington University, St. Louis, Missouri, May 5, 1990; for examples, see D. J. Cram, *Nature* **1992**, *356*, 29 – 36.
[3] J.Rebek, Jr., *Chem Soc. Rev.* **1997**, 255 – 264 and references therein.
[4] a) L. Garel, J. Dutasta, A. Collet, *Angew. Chem.* **1993**, *105*, 1249 – 1251; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1169 – 1171; b) A. Collet,in *Comprehensive Supramolecular Chemistry Vol. 2* (Eds: J. L. Atwood, J. E. Davies, D. D. MacNicol, F. Vögtle), Elsevier, **1996**, pp325 – 365; c) R. Meissner, X. Garcias, S. Mecozzi, J.Rebek, Jr., *J. Am. Chem. Soc.* **1997**, *119*, 77 – 85.
[5] a) A. Bondi, *Physical Properties of Molecular Crystals, Liquids, and Glasses*, Wiley, **1968**; b) J. Israelachvili, *Intermolecular & Surface Forces*, 2nd ed., Academic Press, **1992**; c) A. I. Kitaigorodsky, *Molecular Crystals and Molecules*, Academic Press, **1973**, pp. 18 – 19.
[6] a) M. H. Klapper, *Biochim. Biophys. Acta* **1971**, *229*, 557 – 566; b) *Prog. Bioorg. Chem.* **1973**, *2*, 55 – 133; c) P. L. Privalov, S. L. Gill, *Adv. Protein Chem.* **1988**, *39*, 191 – 234; d) F. M. Richards, *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 151 – 176; e) F. M. Richards, *J. Mol. Biol.* **1974**, *82*, 1 – 14.
[7] These packing coefficients were calculated according to Equation (1), with the van der Waals radii described in the Volume calculations section.
[8] C. N. Eid, Jr., D. J. Cram, *J. Chem. Ed.* **1993**, *70,* 349 – 351.
[9] B. C. Hamann, K. D. Shimizu, J. Rebek, Jr., *Angew. Chem.* **1996**, *108*, 1425 – 1427; *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 1326 – 1329.
[10] Although appropriate force field parameters for all the functional groups in molecules **1 – 6** do not exist, the use of the force field Amber* of MacroModel 5.5 allows a reasonable approximation for the computation and the comparison of volumes.
[11] F. Mohamadi, N. G. J. Richards, W. C. Guidia, R. Liskamp, C. Caulfield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440 – 467.
[12] A. Nicholls, K. A. Sharp, B. Honig, *Proteins* **1991**, *11*, 281 – 296.
[13] J. C. McGowan, A. Mellors, *Molecular Volumes in Chemistry and Biology: Applications Including Partitioning and Toxicity*, Wiley, **1986**.
[14] J. C. Sherman, D. J. Cram, *J. Am. Chem. Soc.* **1991**, *113*, 2194 – 2204.
[15] A. Collet, *Tetrahedron* **1987**, *43*, 5725 – 5759.
[16] a) R. Grotzfeld, N. Branda, J. Rebek, Jr., *Science* **1996**, *271*, 487 – 489; b) K. D. Shimizu, J. Rebek, Jr., *Proc. Nat. Acad. Sci.* **1995**, *92,* 12403 – 12407.
[17] J. Kang, J. Rebek, Jr., *Nature* **1997**, *385*, 50 – 52.
[18] R. Meissner, J. de Mendoza, J. Rebek, Jr., *Science* **1995**, *270*, 1485 – 1488.
[19] J. Kang, J. Rebek, Jr., *Nature* **1996**, *382*, 239 – 241.
[20] For a discussion of thermodynamics of inclusion processes see a) D. J. Cram, H-J. Choi, J. A. Bryant, C. B. Knobler, *J. Am. Chem. Soc.* **1992**, *114*, 7748 – 7765; b) J. D. Dunitz, *Chem. Biol.* **1995**, *2*, 709 – 712; c) B. R. Peterson, P. Wallimann, D. R. Carcanague, F. Diederich, *Tetrahedron* **1995**, *51*, 401 – 421; d) M. S. Searle, M. S. Westwell, D. H. Williams, *J. Chem. Soc. Perkin Trans. 2* **1995**, 141 – 151.
[21] J. M. Rivera, T. Martin, J. Rebek, Jr., *J. Am. Chem. Soc.* **1998**, *120*, 66 – 99.
[22] J. M. Rivera, T. Martin, J. Rebek, Jr., *Science* **1998**, *279*, 1021 – 1023.
[23] O. Mogck, E. F. Paulus, V. Böhmer, I. Thondorf, W. Vogt, *Chem. Commun.* **1996**, 2533 – 2534.
[24] R. G. Chapman, J. C. Sherman, *J. Am. Chem. Soc.* **1995**, *117*, 9081 – 9082.
[25] a) R. Wyler, J. de Mendoza, J. Rebek, Jr., *Angew. Chem.* **1993**, *105*, 1820 – 1821; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1699 – 1701; b) C. Valdès, U. P. Spitz, L. Toledo, S. Kubik, J. Rebek, Jr., *J. Am. Chem. Soc.* **1995**, *117*, 12733 – 12745; c) N.Branda, R. Wyler, J. Rebek, Jr., *Science* **1994**, *263*, 1267 – 1268; d) X. Garcias, J. Rebek, Jr., *Angew. Chem.* **1996**, *108*, 1328 – 1330; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1225 – 1227.
[26] C. Valdés, L. Toledo, U. P. Spitz, J. Rebek, Jr., *Chem. Eur. J.* **1996**, *2*, 989 – 991.
[27] a) P. Timmermann, W. Verboom, F. C. J. M. van Veggel, J. P. M. Duynhoven, D. N. Reinhoudt, *Angew. Chem.* **1994**, *106*, 2437 – 2440; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2345 – 2348; b) A. M. A. van Wageningen, P. Timmerman, J. P. M. van Duynhoven, W. Verboom, F. C. J. M. van Veggel, D. N. Reinhoudt, *Chem. Eur. J.* **1997**, *3*, 639 – 654.
[28] Calculation of the internal cavity volume for cryptophanes **8** and **9** is made difficult by the presence of large holes in the molecular surfaces of the molecules. A closed cavity could only be built by use of a probe of 1.55 Å in diameter, a probe that inevitably underestimates the cavity volumes of **8** and **9**. For the same reason, not all the structures sampled during the molecular dynamics simulation could be used for volume calculations. In some cases the holes were just too large to be closed with a probe of the appropriate size.
[29] R. G. Chapman, N. Chopra, E. D. Cochien, J. C. Sherman, *J. Am. Chem. Soc.* **1994**, *116*, 369 – 370.

  0947-6539/98/0406-1022 $ 17.50+.50/0