Recent advances in hydrogen-bonded hexameric encapsulation complexes

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Basic research in the chemistry of hexameric resorcin[4]arenes and pyrogallol[4]arenes during the last decade is reviewed. Applications of NMR methods to determine solution structures, host guest properties and exchange dynamics are discussed. The scientific issue is the behavior of molecules in small spaces; the challenge is to translate this information to practical applications in, say, catalysis or transport.

In response to the invitation to write a short review on calixarene capsules in the new millennium, we have limited the topic to resorcinarenes and closely related structures. There are extensive reviews1 that cover the subject up to 1996, just before an epiphany occurred that changed the way resorcin[4]arenes and pyrogallol[4]arenes have since been viewed in the community. The eye-opener was due to Atwood and MacGillivray2 who published the X-ray structure of 1a (Fig. 1). Crystals of this compound obtained from hot nitrobenzene revealed a huge hexameric assembly, essentially an inflated cube, with resorcinarenes as the sides and water molecules at the corners.2 The space inside was nearly 1400 Å3, occupied by an unknown number of disordered solvent molecules (we show 8 benzenes modeled inside). There was vapor pressure osmometry evidence that the assembly persisted in solution as well. Resorcinarenes as modules offering curvature for larger structures – carcerands and cavitands – had already, in the decades since the large-scale synthesis developed by Högberg,3 made a gigantic impact on supramolecular chemistry.4 But it was the self-assembly

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of resorcin[4]arenes *per se* that inspired most of the work of the last decade. The demonstration, by Mattay’s group, that pyrogallol[4]arenes (2) also form gigantic hexameric capsules in the solid state further increased the interest in these systems.5

In 1998, Atwood published the structure of a crystalline dimeric resorcinarene 3 (Fig. 2) held together by isopropyl alcohols that encloses some 230 Å³ of space. This was co-crystallized with the parent fullerene C₆₀ and no guest could be identified inside.6 At nearly the same time, Aoki crystallized a dimeric structure 4 with a Et₄N⁺ ion inside.7 Here the seam of hydrogen bonds holding the capsule together included 8 water molecules. Whether either of these capsules persisted in solution could not be established, but two years later Shivanyuk, Rissanen and Kolehmainen8a crystallized a dimeric capsule 5 bridged by water molecules with Et₃NH⁺ inside (Fig. 3). Its existence in solution depended on the solvent: competitive solvents such as methanol or DMSO disrupted the capsule, but NMR using wet chloroform showed the dimeric encapsulation complex. The guest signals featured sizeable upfield shifts and slow exchange with the free species (on the NMR timescale) and integration indicated a dimeric capsule.8b However, it was not totally clear if the two to one ratio found for this system implies that a dimer is the major species in solution. When the self-assembly of 1c in the presence of tetrathyllum ammonium salts was studied, hexamers encapsulating two guests were found.8d Even earlier, Aoyama’s research group had found that the β-anomer of methyl D-glucopyranoside was selectively extracted into a CCl₄ solution of 1c to give a host-guest complex with a 2 : 1 stoichiometric ratio.9a Accordingly, a dimeric capsule complex 6 was proposed and seemed a reasonable fit, given the volume of the guest (136 Å³), a proposition that later was found to be erroneous (*vide infra*).9b

One of the new structural developments in the last decade was the introduction of pyridine-derived resorcinarenes 7 (Fig. 4) by Mattay and coworkers.10a They showed that dimeric capsule 7₂ exists in solution and in the solid state, as well as in the gas phase.10b In the chloroform solution of 7, two species were observed and were assigned to the monomer and dimer of the pyridine-derived resorcin[4]arene. However, a few years later it was shown, with the aid of diffusion NMR,11 that in chloroform solution the dimeric and hexameric capsules prevail (Fig. 4c).10c Another innovation involved the use of fluorous “feet”,12a halogenated lower rims12b and side chain trapped solvent.12c
These observations all caution that the most abundant structure in solution is not necessarily the one found in the solid state, and that extrapolation from solid state to solution in these systems is risky. The direct observation of guests inside the hexameric host capsule in solution had to await the new millennium. Like many others, the La Jolla group had already adopted and adapted the resorcinarene platform for use in molecular recognition as capsules and deep cavitands. They found that in wet chloroform, which was the widely used standard solvent for these studies, the resorcinarene actually dissolved (a good sign, since in scrupulously dried solvents the resorcinarenes have low solubility and give broad, uninterpretable NMR spectra). Addition of Hex$_4$N$^+$Br$^-$ to the solution resulted in separate sets of signals for the bound and free ammonium salt, with the bound material exhibiting large upfield shifts in the NMR spectrum. The separated signals indicate that large energetic barriers separate the inside and outside of the capsule, since many hydrogen bonds must be broken to allow the exchange of molecules between the two environments (about which, more later). Integration of the spectra clearly showed the 6:1 ratio of resorcinarene to ammonium salt. The use of quaternary ammonium salts was inspired by the nature of the inner surface of the capsule. The 24 aromatic rings provide $p$-bonds that may be regarded as a thin coating of negative charge on the concave inside surface of the capsule. The partial positive charges of the hydrogens on the convex outer surfaces of the ammonium guests are ideal complements for the capsules. Smaller salts also made capsular assemblies, a hint that other occupants were also inside. Typically, capsules have slightly more than half the interior space filled by guests; in these cases, the counterions and some solvent chloroform molecules were coencapsulated to reach this level of occupancy. In 2001 the La Jolla group showed that even neutral molecules would go inside, provided that they filled the space properly.

The clear evidence that the resting state of resorcin[4]arenes (and even pyrogallol[4]arenes) in conventional wet organic solvents is that of hexameric capsules was provided by the Tel Aviv group. Using diffusion NMR they showed that the ammonium guests or other neutral guests are not needed for the capsules to assemble in solution; solvents such as chloroform and benzene alone could be the occupants. Indeed, using diffusion coefficients, which is a means for obtaining the effective size of the molecular species under investigation and hence its weight, it was shown that lipophilic resorcin[4]arenes and pyrogallol[4]arenes self-assemble spontaneously into hexameric capsules in non-polar organic solvents. It was also shown that the

Fig. 3 A third dimeric capsule also uses water molecules to encapsulate Et$_3$NH$^+$ in the solid state. It persists in wet CHCl$_3$ solution as shown by NMR. A cartoon of the dimeric structure proposed for the encapsulated $\beta$-anomer of methyl-$\alpha$-glucopyranoside is shown as 6.

Fig. 4 (a) The structure of 7, (b) The X-ray structure of 7 showing a dimeric capsule$^{10}$ (c) The DOSY of a 20 mM CDCl$_3$ solution of 1e and 7 showing that 7 forms both hexameric (just as 1e) and dimeric capsules in solution. Image reproduced with permission from Ref. 10c

Fig. 5 The $^1$H NMR signal for benzene alone in 1e (a); 8 benzenes are inside. When a small amount of CHCl$_3$ is added new capsule species appear (b). When benzene and CHCl$_3$ are cosolvents, a distribution of capsules are present (c) The signals of encapsulated chloroform (d) and dichloromethane (e) molecules (400 MHz, 298 K) in a 20 mM CHCl$_3$ or CH$_2$Cl$_2$ solution of 2b, respectively.
The closely related pyrogallol[4]arenes, which were shown to form hexameric structures in the solid state, were also probed by diffusion NMR. Here, diffusion NMR provided the proof that in the case of the hexameric pyrogallol[4]arene capsules, water molecules are not part of the supramolecular structure in solution. Based on this observation, the La Jolla group speculated that such pyrogallol[4]arene hexamers should prevail in solution in extremely non-polar organic systems, which cannot accommodate water molecules. Indeed, they found that the hexameric capsule of pyrogallol[4]arene can spontaneously self-assemble when a suitable hydrocarbon is present, and the guest will even contort itself to fill the space. For example, they found that six molecules of octane are encapsulated as modeled in Fig. 7.6

In the hexameric capsule of pyrogallol[4]arene the $^1$H NMR signals of the encapsulated hydrocarbon solvent molecules showed a peculiar pattern, as shown in Fig. 8. The NMR spectra and assignments for encapsulated octane (a) and heptadecane (b) are shown in Fig. 8. The gradual downfield shifts for the signals of octane from the methyl groups ($C_1/C_3$ to $C_4$) reflect their average distances from the pyrogallolarene centers. When a long chain hydrocarbon such as $C_{17}$ is taken up inside the same capsule, it appears to be neatly folded. This folding follows from the NMR spectra of the encapsulated hydrocarbon $C_{17}$ where the largest upfield shifts occurred in the central methylene ($C_9$) of the hydrocarbon and at its ends. That is, both ends and the middle are near the shallow bowls of the pyrogallolarene subunits.

The folding of alkyl groups is a general feature of encapsulation in these systems and was studied with a series of alkyl ammonium salts with resorcin[4]arene. As shown in the spectra of Fig. 9, the methyl group of the tetrahexyl salt enjoys the furthest upfield shift, and presumably, can penetrate the cavity of a resorcinarene panel. What folding there is – and the diastereotropic signals of $C_2$ are consistent with this behavior – must take place near the N atom. The longer heptyl and octyl groups “buckle” and $C_4$ is nearest the cavitation.

A peculiar observation is that despite the ability of the resorcin[4]arenes capsules to accommodate tertiary amines and tetraalkyl ammonium salts, the corresponding hexameric capsules of pyrogallol[4]arene were found to encapsulate only tertiary amines in chloroform solution. Moreover, it was found that protonation of encapsulated tertiary amines results in the ejection of the ammonium salts formed. Clearly, this is not a steric effect, as diffusion NMR showed that the protonated amine was ejected from the capsule cavity while the hexameric capsule was still intact. The power of diffusion NMR in characterizing such capsular host–guest systems was demonstrated again when the complexation of...
the lipophilic resorcin[4]arene 1c with glutaric acid and the β-anomer of methyl-d-glucopyranoside (see 6) was revisited.96 There we could show that the 1:1 and 2:1 ratio between 1c and glutaric acid or glucoside, respectively, represent hexamers of 1c encapsulating six or three molecules of the guests, respectively.96  

Fig. 10 shows the signal decay as a function of the gradient strength of a representative peak of 1c, encapsulated glucoside and biscalix[5]-arene (11). The molecular weight of 11 is 2398 g mol⁻¹, which is slightly higher than that of the dimer of 1c. This figure also shows a graphical presentation of the normalized signal decay of 1c, encapsulated glucoside, and 11, as compared with that of the encapsulated tetrahexyl salt. According to Fig. 10, the same signal decays are obtained for the encapsulated glucoside, encapsulated tetrahexyl salt and 1c, which are all significantly smaller than the signal decay of the biscalix[5]-arene (11).

How things get in and out of these hexamers is puzzling and we have made some attempts to answer this in the case of large tetralkylammonium guests.27 There is a size dependence of the exchange (exit) rate, with the larger guests leaving more slowly. For R₄N⁺ the activation free energies for exit as a function of R are: R = C₃ 13.1; C₄ = 14.8; C₅ = 16.7 and C₆ = 17.1 kcal M⁻¹. At first glance, this could merely reflect tighter binding of the larger guests, but equilibrium studies and competition experiments show the opposite trend. For R₄N⁺ the apparent association constants for encapsulation as a function of R are: R = C₃ 5 > 10⁴; C₅ = 1200; C₇ = 450 and C₈ = 150 M⁻¹. Accordingly, it is necessary to propose an opening of limited size!27

A mechanism is shown in cartoon form (Fig. 11) where the guest exits from a capsule through an opening created by removal of one resorcinarene panel.

Alcohols can, in principle, replace the water molecules in the structure of the hexameric capsules but can also be encapsulated like other guests.23,24 The different sites that alcohols can occupy in such systems are shown in Fig. 12. Using diffusion NMR it was possible to show that some alcohols are only encapsulated, while others are part of the hexameric capsules (just like water molecules) and other alcohols occupy both sites.24 Interestingly, this conclusion was reached by monitoring the effect of exchange on the diffusion measurements performed using the longitudinal eddy current (LED) diffusion NMR sequence.29 This sequence is generally used in diffusion ordered spectroscopy (DOSY),30 and is more sensitive to the effects of exchange.31 It was also demonstrated with the aid of diffusion that tertiary alkylamines and quaternary alkylammonium interact with the hexameric capsules both from the inside and the outside.32

Self-sorting has become one of the earmarks of self-assembling systems because the corrections that occur during the assembly process involve constant distinctions between “self” and “non-self”. Resorcinarenes and pyrogallol-arenes share so much in terms of size, shape and chemical surfaces that determine their recognition properties. But do they self-sort? The Tel Aviv group20 first approached this problem using diffusion NMR techniques and showed that no evidence of scrambling of the components of the two respective hexameric capsules occurs, while scrambling does occur when two resorcin[4]arenes or two pyrogallol[4]-arenes are used. When 1b and 2b were mixed no change in the starting diffusion coefficient was observed, as shown in Fig. 13a. However, when 1b and 1c were mixed, equilibration in the starting diffusion coefficients was observed with time, as shown in Fig. 13b.

The La Jolla group studied the problem using Foerster resonance energy transfer (FRET) techniques.33 The hexamers of resorcinarenes bearing either perylene or pyrene readily exchanged their sub-units when mixed (Fig. 14),33 as did hexamers of pyrogallolarenes34 bearing either perylene or pyrene, but no evidence of exchange of the modules from a resorcinarene hexamer to a pyrogallolarene hexamer was found. Moreover, even the assembly process was strictly self-sorting.34c

The gas phase would have things differently.34 Schalley and coworkers showed that in the mass spectrometer
not only hetero-hexamers, but all manner of mixed oligomers could be observed and characterized.34

How does this happen? It may be that in the near vacuum the loss of water molecules is compensated by the additional hydrogen bonding components of the pyrogallol and hetero-oligomerization occurs. Even simpler, there may just be differences of behavior in the two different phases of solution and gas.

It is possible to hybridize in a different sense. Specifically, a cylindrical capsule (Fig. 15) and the hexameric resorcinarene form a heterodimeric species, in the presence of a guest such as chloroform.35 It was possible to monitor the hybridization in real time using FRET techniques. Self-sorting does not occur in this specific system, but the system can be reset to the starting state (the self-sorted state) by offering a good guest for the cylindrical capsule such as benzanilide.

These results emphasize that the issue of self-sorting in the self-assembly of even the simplest hexameric capsules of resorcin[4]arenes (1) and pyrogallol[4]-arenes (2) in the different phases is difficult to reconcile and explain.

A second issue concerns the nature of resorcinarenes in dry solvents. What happens when there are not enough water molecules to complete the seam of hydrogen bonds needed, say, for the hexamer or possibly the dimer? This was approached recently by Mattay and coworkers,36 who used DOSY techniques to detect a 1 : 1 complex of resorcinarene with a 2,2,2-trifluoro-1-phenylethanol, a 2 : 1 complex with 2-butanol and a hexamer (rather than a dimeric capsule) with 2-ethyl-octanol. No encapsulated guests were apparent from the spectra, so it was concluded that these systems are under rapid in/out exchange. However, these conditions are quite different from Aoyama and coworkers’ conditions9 in which two-phase extractions from water gave the encapsulated species in slow exchange.

A third mystery has to do with the peculiar selectivity of the hexamers. Alkyl ammonium salts are excellent guests for the hexameric capsules of resorcinarenes but not for the pyrogallolarenes.20,28 However, trialkylamines are excellent guests for the latter. Cation/π interactions would favor the opposite result, so what causes this selectivity? This is even more peculiar since Philip and Kaifer were able to encapsulate the charged cobaltocenium both in 1c and 2b.37 They were also able to encapsulate a series of nitroxides in 1c.38 The Atwood group was able to load several fluorophores in pyrogallol[4]arenes and study their spectroscopic characteristics but.
here the loading was relatively low.\(^{39}\) So currently there are more examples of guest encapsulation in resorcin[4]arene hexamers than in pyrogallol[4]arene hexamers, but that could soon change.\(^{40}\)

Even fewer guests have been found to be encapsulated in the hexameric capsule of calixpyridine[4]arene (7).\(^{41-43}\) It should be noted, however, that these observations are not only connected to the affinity of the different guests towards the cavities of 1 and 2 but also reflect the affinity of the solvent used (generally CDCl\(_3\)) towards the cavity of the hexamers of 1 and 2.

Conclusions

It is probable that self-assembly of these systems is more than a century old,\(^{42}\) as any synthesis that produces 1 or 2 in the presence of wet organic solvents can give encapsulation complexes, but the capsule can form even without solvents.\(^{43}\) The self-assembly of up to 22 molecules (at least in the case of resorcin[4]arenes with benzene) bodes well for future investigations of encapsulation phenomena. Since some resorcinarenes are commercially available or are easy to prepare, the barrier to entry of experimental work in this field is low. Already, the use of the resorcin[4]arene capsule as a reaction chamber\(^ {34}\) indicates progress and further applications can be expected. Another avenue that should be further explored is the metallo-supramolecular capsules, which were omitted from this review since the subject was thoroughly treated elsewhere quite recently.\(^ {45}\) The behavior of molecules in small spaces is quite different from that in dilute solutions. These capsules offer an essentially hydrophobic environment, isolated from solvent, where single molecule guests enjoy high (~ 1 molar) concentrations. Apart from their use in physical organic chemistry, their resemblance to enzyme active sites makes them appealing models for some biological phenomena.

Notes and references

26 (a) L. C. Palmer and J. Rebek, Jr., Org. Lett., 2005, 7, 787; (b) L. C. Palmer and


44 A. Scarso, personal communication.