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The ins and outs of molecular encapsulation

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Molecular capsules can act as hosts for appropriate guests, and bring them into well-defined nanoenvironments. Various spectroscopic methods have been used to deduce the mechanism of guest exchange in such systems. Generally, the guests get in and out of capsules through the opening and closing of host "flaps" but smaller capsules can exchange simply by dissociation of the host subunits.

Introduction

Encapsulation complexes consist of self-assembled host structures that more or less completely surround molecular guests. The dynamic character of reversible encapsulation is responsible for the facile exchange of constituents and results in the thermodynamic rather than kinetic selectivity of host–guest assemblies. In previous reviews of molecular encapsulation,[1] we have reported in some detail the synthesis and characterization of self-assembled capsules; their assembly with smaller species to give molecule-within-molecule complexes; the asymmetric microenvironments: on the outside, in the lining, and of their cavities; the molecular architecture of curved systems capable of forming closed-shell structures, and the special problems associated with large cavities. Here we emphasize the dynamics of guest encapsulation and the exchange process. We concern ourselves exclusively with reversibly formed capsules in organic media; for contributions to the dynamics of aqueous[2] host–guest systems, such as cucurbiturils,[3] cyclodextrins,[4] and metal-based capsules,[5] as well as organic carcerands and hemicarcerands,[6] we refer the reader to the recent literature.

Timescales

It is useful to recall the timescales involved in molecular recognition phenomena. At one extreme, there are diffusion complexes where two molecules arrive in the same solvent shell (Fig. 1). These typically last less than a billionth of a second ($\leq 10^{-9}$ s) and set the limits for reactivity between two molecules: When they react at every encounter, the kinetics are said to be diffusion controlled. As the encounter lasts longer, that is, when the two molecules have weak intermolecular attractions, then molecular recognition is said to take place. When only the two molecules and the solvent are involved, the lifetime of the complex is proportional to the attractive forces. This results from the diffusion-controlled encounter rates for small molecules, *i.e.*, the constant birthrate divided by the variable deathrate (dissociation). At the other extreme, two molecules may be mechanically linked. That is, even though they are not directly bound to each other by covalent bonds, they are topologically linked as in catenanes, rotaxanes, and carcerands. These systems can diffuse apart only when covalent bonds are broken. For carbon–carbon bonded systems at room temperature, the process would take hundreds of years—roughly 10^{10} seconds. Phenomena we describe in this review are roughly half way between these two extremes, which span 20 decades. Specifically, the reversible encapsulation complexes have lifetimes on the order of one second—give or take a few orders of magnitude; typically

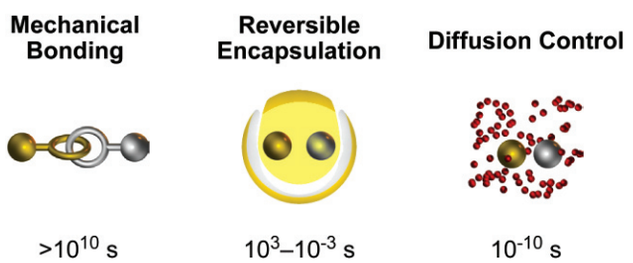

Fig. 1 Different types of intermolecular interactions and their timescales.

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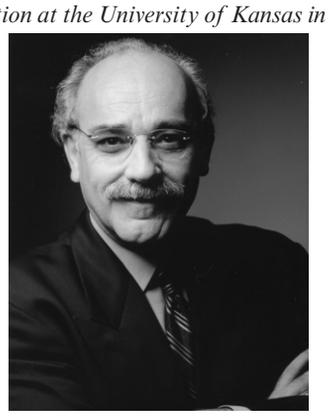
Liam C. Palmer



Julius Rebek, Jr.

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ranging from milliseconds to hours. The timescale does not so much reflect the guest molecule's affinity to the host capsule or two guest molecules' affinities to each other (which may be very small indeed) as it does the affinity of the components of the capsule for each other. That is, the encounters inside are controlled by a higher order of assembly. This is long enough for two molecules detained within such a complex to interact and even react.[7–10] Most conveniently, as we shall see, these encapsulation complexes form and dissipate at a rate that is generally slow on the NMR timescale and separate signals can be seen for free and bound species.

Ultrafast dynamics ($<10^{-9}$ s) can give insight into various thermal (vibrational) and electronic affects far beyond of the range of normal NMR experiments.[11] The interested reader is directed to a recent review describing the ultrafast dynamics of guests held within cyclodextrins.[12]

Exchange in a cavitand

The earliest indications that the exchange of guests could be followed by conventional NMR came not from a capsule, but an open-ended vessel, called a cavitand. Cram[13] and Dalcanale[14] developed methodologies for synthesizing cavitands based on Högberg's resorcinarene platform.[15] These were characterized as two observable conformations, the vase and kite, and their inter-conversions were monitored through their spectroscopic earmarks. We used intramolecular hydrogen bonding to stabilize the vase form by holding together the "walls" of the cavitand. This was accomplished by condensation of the resorcinarene (**1**) with the activated difluoride **2** as described by Cram, then reduction and acylation of the octaamines with various acid chlorides to give octaamides such as **3** (Fig. 2).

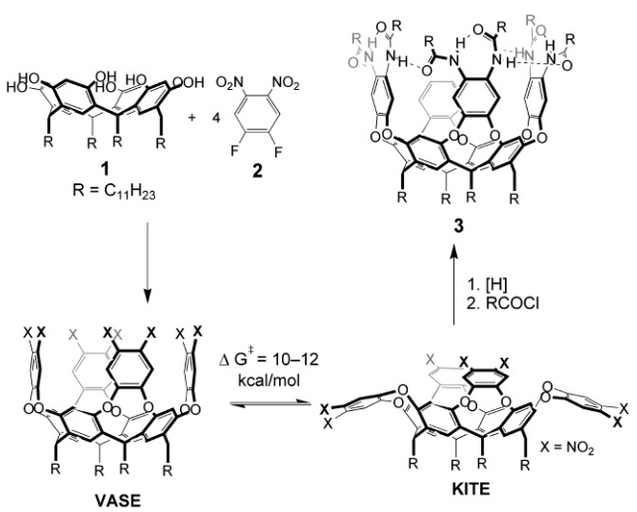

Fig. 2 Synthesis and conformational dynamics of cavitands.

The NMR spectra of these octaamides showed two distinct N–H signals shifted sufficiently downfield to indicate strong hydrogen bonding. These signals coalesce upon heating, and the activation barrier for the dynamic process at the coalescence temperature could be calculated as approximately 17 kcal mol^{-1}.[16] The upper rim of each structure features the head-to-tail arrangement of the hydrogen-bonded amide functions in either a clockwise or counterclockwise sense: these are mirror images. The exchange process involves the racemization of the system. As the amides are arranged in a coherent and probably cooperative way, all of the hydrogen bonds have to be broken in converting one enantiomer to the other.

What made this cavitand special was that its binding of small molecules showed slow exchange on the NMR timescale, so that separate signals could be seen for free and bound guests. This is in stark contrast to the cavitands previously studied in solution by Dalcanale.[14] Even more peculiar was the very low affinities for the host–guest interaction, as low as a few tenths of a kilocalorie. How then to reconcile the slow exchange and its implication of a high kinetic barrier with the weak binding? We believe the answer comes through coupling the racemization process described above with the guest exchange; they are related processes that proceed through a common intermediate (Fig. 3).

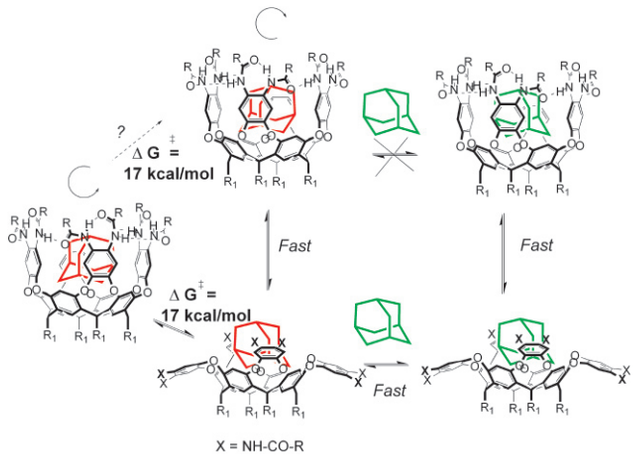

Fig. 3 Racemization and guest exchange in the cavitand proceed through a common "kite" intermediate. The direction of the hydrogen bond seam is indicated by a curved arrow.

The studies of Cram[13] had established a barrier of some 10 to 12 kcal mol^{-1} for the vase-to-kite interconversion. If this conformational change is performed on the guest-containing cavitand, four hydrogen bonds need to be broken: those that hold together adjacent rings. The typical costs of such ruptures in organic solvents are roughly 1 to 2 kcal mol^{-1} per hydrogen bond,[17] so the additional 5 to 7 kcal mol^{-1} is quite reasonable for the overall 17 kcal mol^{-1} activation barrier to racemization. Once the kite conformation is achieved the racemization or exchange of hydrogen-bonding sites on a single ring is very rapid as shown by model compounds. The conversion to the kite also has consequences for the guest held inside. The newly exposed surfaces of the cavitand walls are now available to solvent or other solutes; these are poised to displace the resident guest in an orderly manner, after all, no cavity remains in the kite conformation.

Exchange spectroscopy (2D EXSY)[18] is a relatively simple and direct NMR method for studying kinetics of reversible systems that exchange slowly on the ^{1}H and ^{13}C chemical shift timescale, but fast on the spin-lattice (T_1) relaxation timescale. In practice, the experiment is best applied to rate constants between 0.01 and 10 s^{-1} at a given temperature. Around ambient temperature this translates to $\Delta G^{\ddagger}$ = 16–20 kcal mol^{-1}, although almost any temperature can be used. We used 2D EXSY to study the exchange of free and bound adamantane with the cavitand. The barrier for this guest exchange is approximately 17 kcal mol^{-1}, a value we suspect is no mere coincidence but a consequence of the closely related processes of racemization and guest exchange for this cavitand.

Because we use NMR as the means to determine equilibrium binding constants, we also use the NMR timescale as an arbitrary measure of a complex's kinetic stability, and only those assemblies that form and dissipate slowly on the NMR timescale are considered here. Such cases allow determination of two species (the guest inside and its counterpart in the bulk solution) and permit deductions concerning their whereabouts. The chemical shifts report on the magnetic environment of the guest molecules.

Exchange rates for the tennis ball

The dissociation/reassembly of a capsular host is often the only way to interconvert enantiomeric assemblies, so racemization can be used as a probe of exchange processes. As a mechanism for guest exchange, it sets certain limits; intuitively, no other process

can be as energetically costly (and, therefore, slower) as breaking all the intermolecular bonds that hold the system together. Accordingly, when guest exchange is faster than racemization, we have a reliable indicator for the existence of intermediates in mechanistic studies—a case that we have detected repeatedly. Guest exchange of close-shelled capsules like the tennis ball ($\mathbf{4}_2$; Fig. 4)[19] and the softball ($\mathbf{5}_2$; Fig. 6)[20] were initially examined.

The original tennis ball was based on dimerization of **4** with formation of eight hydrogen bonds surrounding a cavity of about 60 Å^3 (Fig. 4).[19] Chirality was introduced to this structure through synthesis: different substituents were placed on the two ends of the subunits (at the glycoluril bridgeheads).[21] This removed the mirror planes of symmetry in the assembly and left the dimeric capsule with only two-fold axes of symmetry. The assembly exists as a racemate; the enantiomers can interconvert only by complete dissociation then recombination of the two subunits. This process, which breaks the hydrogen bond seam of the tennis ball, should exact an energetic penalty of between 8 and 16 kcal mol^{-1} in the solvent $CDCl_3$. The inversion of the seven-membered ring is also likely to facilitate exchange as it provides a means of staging the breakage of the hydrogen bonds. This inversion cost has been estimated as ~15 kcal mol^{-1}.[22]

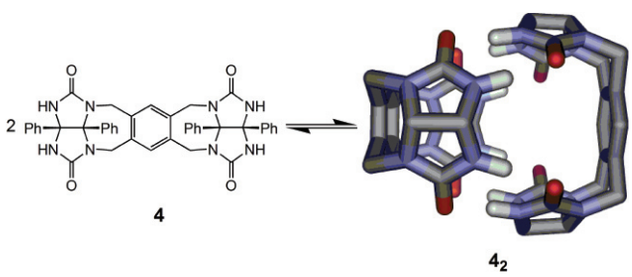

Fig. 4 Tennis ball structure. Bridgehead phenyl groups have been omitted for clarity.

Using a series of NMR experiments, we measured the rates of in–out guest exchange and the host subunit exchange simultaneously for the molecular tennis ball ($\mathbf{4}_2$, Fig. 4). Rate constants for the racemization process, as deduced from a 2D EXSY experiment, correspond to an activation energy of 17.5 kcal mol^{-1} at 295 K.[21] This exchange rate was also measured in the presence of methane or ethane as a guest. As expected, the presence of good guests helped stabilize the capsule towards dissociation and either of these guests raised the activation energy for racemization by about 1 kcal mol^{-1}. While the stabilization was anticipated, the results of guest exchange rates were not. A priori, two plausible exchange mechanisms can be imagined: (1) a dissociative mechanism in which the two capsule subunits completely separate, or (2) a gating mechanism[22] whereby one "flap" of the tennis ball opens by ring inversion of the seven-membered ring (as shown in Fig. 5). The latter mechanism opens a sizeable hole in the tennis ball at the cost of four hydrogen bonds. The partly exposed guest can then be replaced in a substitution reaction by a solute molecule. The remaining four hydrogen bonds can maintain this partial assembly long enough for many exchanges of the resident guest with the incoming guests to take place. Earlier experience in molecular recognition studies[23] showed that as few as five hydrogen bonds can hold a complex together for milliseconds under these conditions, long enough for substitution to occur. For the case at hand, methane exchange was 8 times faster than subunit dissociation while ethane exchange was some 5 times faster. In short, there exists a mechanism for guest exchange that does not require complete dissociation of the capsule, and supports the "gating" mechanism as the dominant one for guest exchange.

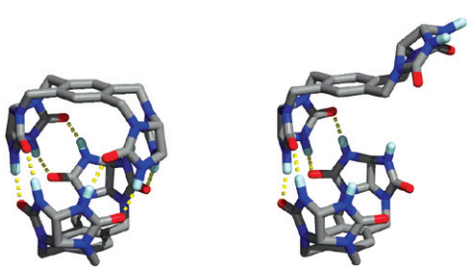

Fig. 5 The intact tennis ball, left, and the conformation with an open flap, right. The structure on the right represents intermediate of a possible "gating" mechanism.

The plausibility of the two models was further appraised by computation.[22] According to molecular mechanics studies with the AMBER* force field, a complete dissociation of the capsule in chloroform should cost 29 kcal mol^{-1} at room temperature. Capsule dissociation should be accompanied by 30–40 eu of favorable entropy, worth 9–12 kcal mol^{-1} of free energy at room temperature. The computed dissociation energy (17–20 kcal mol^{-1}) accords well with the experimental NMR data above. The alternative gating mechanism requires a ring inversion with an estimated 15 kcal mol^{-1} penalty and little entropic compensation. When this cost is added to the breaking of four hydrogen bonds (estimated at an additional 15 kcal mol^{-1}), the resulting energy barrier for the gating mechanism (30 kcal mol^{-1}) is significantly higher than for the dissociative mechanism. These calculations indicate guest exchange in the tennis ball more likely occurs by capsule dissociation as the rate-limiting step.

Slow exchange in the "softball"

Another early system that proved convenient for kinetic studies of exchange involved the softball $\mathbf{5}_2$ (Fig. 6).[20] It was observed that free [2.2]paracyclophane gradually replaced encapsulated adamantane from the softball in xylene-d_{10} solution (Fig. 7).[24] Because the rate of substitution was directly proportional to the concentration of entering cyclophane, we view the process as a supramolecular counterpart of the S_N2 reaction. Slow exchange was usually the case when substitutions involved larger incoming guests (paracyclophane and *o*-carborane) but the use of smaller incoming guests (ferrocene) showed rapid replacement of resident guests (adamantane) (Fig. 7). As expected, high concentrations of incoming guest show S_N1-like rates (saturation kinetics), since guest exit becomes rate limiting.

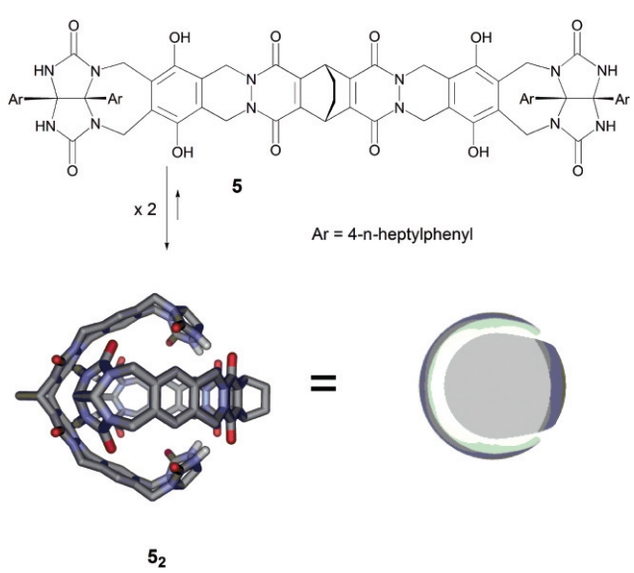

Fig. 6 Softball structure. Aryl groups have been omitted for clarity.

A more detailed study of the exchange process was performed in the smaller "wiffle" ball analog,[25] a system much easier to come by. The results are consistent with the size considerations above: guests which occupy a smaller fraction of the interior volume get in and out quickly, while large guests take their time. An NMR study of the correlation times of the softball and a large guest, paracyclophane provides a clue to the slow entry. The cyclophane does not tumble freely inside the softball;

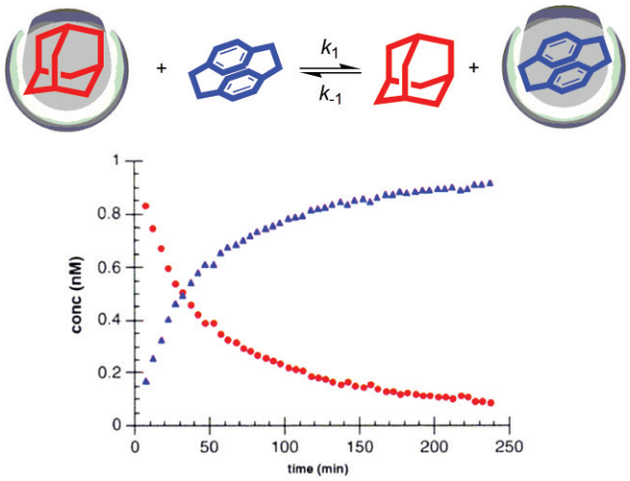

Fig. 7 Paracyclophane gradually replaces adamantane in the softball.[24]

rather its motion is coupled strongly to those of the softball's subunits.[26]

In contrast to the computational results with the tennis ball, calculations by Wang and Houk showed that the softball exchanges by a gating mechanism.[22] Complete capsular dissociation requires breaking 16 hydrogen bonds at an energetic cost of up to 70 kcal mol^{-1}! The alternative gating mechanism requires opening of two separate flaps. The opening of the first flap requires breaking six hydrogen bonds at a cost of about 22 kcal mol^{-1}—a much lower penalty that can be offset by the entropy of guest escape. Fig. 8 shows the two options for the second step either opening of adjacent flaps ("side door" mechanism, path a) or opposite flaps ("back door" mechanism, path b). The side door mechanism appears more likely since it requires breaking fewer hydrogen bonds than the latter mechanism. In either case, the incoming guest probably interacts with the first open flap and then pushes the encapsulated guest out through one of the remaining flaps. A separate, putative solvolysis step (not shown) would follow similar trajectories and may help mediate the guest exchange.

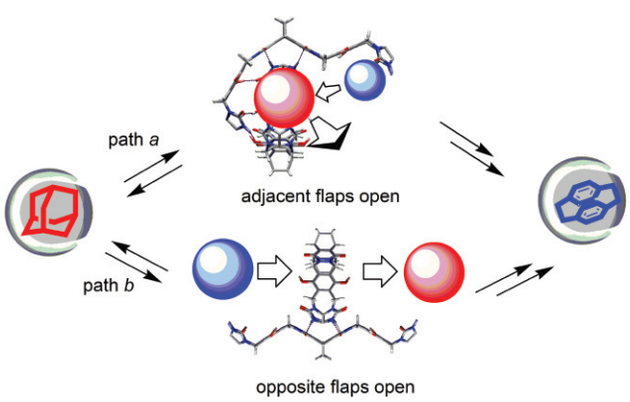

Fig. 8 Guest exchange in the softball requires two flaps to open. Side door (path a) *versus* back door (path b) mechanism of guest exchange. Outgoing adamantane is shown in red, incoming [2.2]paracyclophane in blue.

Evidence of a "memory effect" gives further evidence for separate mechanisms of exchange and dissociation.[27] A chiral, enantiopure guest was added to a softball derivative in which the cavity was chiral but racemic. The resulting diastereomeric complex initially showed a 1 : 1 kinetic distribution that slowly changed to a thermodynamic distribution favoring one diastereomer. This equilibration occurred on the same timescale as the capsule dissociation ($t_{1/2}$ = 19 h). Adding excess of the guest's enantiomer rapidly (within minutes) gave a distribution that favored the unstable diastereomeric complex, followed by slow conversion to the thermodynamic product (Fig. 9). This

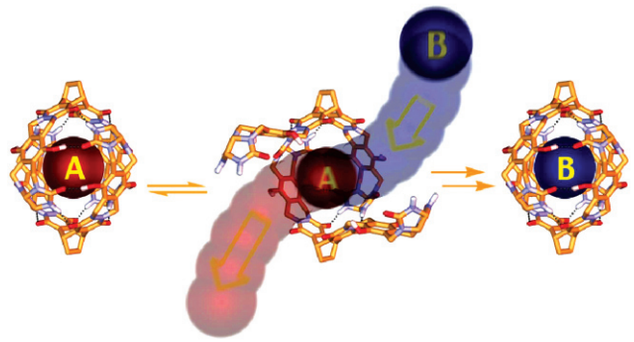

Fig. 9 A memory effect of guest in the softball supports a gating mechanism.

memory effect can only be explained as fast guest entry into a preassembled capsule to give a relatively unstable diastereomer, followed by equilibration to the more stable form through (slower) capsule dissociation. While we cannot directly observe it, the exchange process may be additionally mediated by solvolysis.

Fast exchange in the tetrameric football

The compound **6** was prepared to test whether an assembly composed of four subunits could also form a capsule in analogy to a notional football (American style) (Fig. 10). Here, as elsewhere, the instructions for assembly are written into the hydrogen-bonding sites and the shape of the subunits through chemical synthesis. The hydrogen-bonding preference of sulfamides and ureas to heterodimerize leads to a head-to-tail arrangement of the subunits and when suitable guests are present, they nucleate capsule assembly.[28] Indeed, they are integral to the process. The dynamics of the assembly, exchange of guests, and even the exchange of subunits were something of a surprise to us.

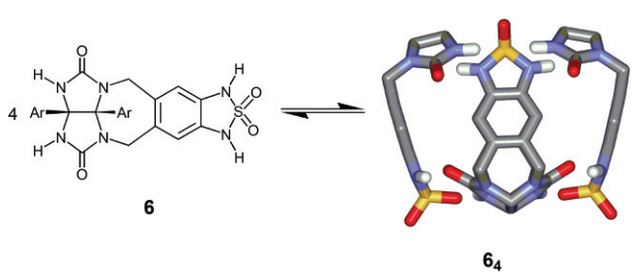

Fig. 10 Structure of the monomeric subunit, left, and the tetrameric (American) football, right. Aryl groups have been omitted for clarity.

Quinuclidinium salts are the best guests for the nucleation of the football into an assembly and are at least a 100-fold more effective than adamantane for the process. It is clear that cation–π interactions are powerful forces for organizational processes. Many of the atoms of these capsules are sp^2 hybridized with the p orbitals directed toward the center of the cavity. Quaternary ammonium ions, coated with a thin layer of positive charge on their hydrogen atoms, are in many ways ideal complements for the cavities. The interior lining may be thought of as coated with a thin layer of negative charge. The quinuclidinium-containing assemblies can be directly analyzed by soft ionization techniques such as ESI mass spectrometry since they are positively charged.[29] Quinuclidinium complexes of two different footballs were prepared in separate solutions. These footballs differed by the peripheral groups on the glycoluril portions that modify their solubilities. But these groups are also useful tags for distinguishing the assemblies in the gas phase. When the two solutions were mixed, complete equilibration of the football components had occurred before the first mass spectrum could be taken, that is, in addition to the complexes of the homotetramers A_4 and B_4, the statistically expected mixture of A_3B, A_2B_2, and AB_3 was present.

For the study of guest exchange, a single football carrying the salt in CH_2Cl_2 was treated with the deuterated quinuclidinium salt. Equilibration of the two guests within the capsule had occurred before the first NMR spectrum could be taken (within a few minutes). Fig. 11 shows our interpretation, or an artistic rendition of the fast guest exchange in the football system. First one flap can open *via* ring inversion of the seven-membered ring (Fig. 11a). While the subsequent steps are speculative, it is clear that two flaps must open, with or without the assistance of incoming guest (Fig. 11b–d). This breaks eight hydrogen bonds (some good, some weak) at the top of football, and permits the remaining flaps to tilt away from one another. However, the eight hydrogen bonds at lower end of the football remain intact and still surround something in the middle.

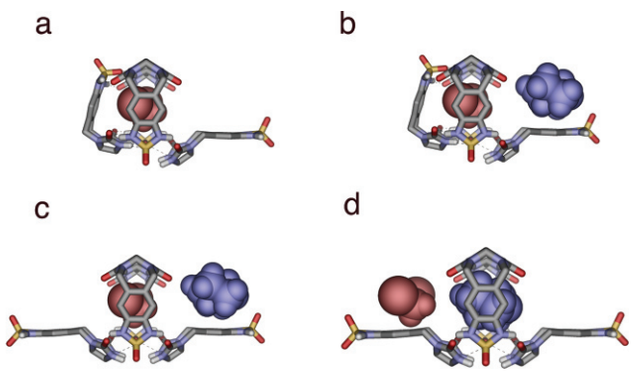

Fig. 11 Proposed mechanism for supramolecular substitution of guests in the football: (a) one flap opens, (b) then a second, (c) then incoming guest approaches, and (d) pushes out the previous occupant of the cavity.

For the exchange of the football subunits, one possibility involves peeling away an entire quarter of the football, again, at a cost of eight hydrogen bonds, but still with eight hydrogen bonds to hold the system together with the nucleating guests (Fig. 12b). Peeling off yet another (adjacent) quarter of the football would cost only four more hydrogen bonds (Fig. 12c) and may give a kinetically competent concentration of the monomer as a true intermediate in the process. Recombination of these monomers with the remnants of other footballs then completes the mechanism of exchange (returning to the assembly Fig. 12a).

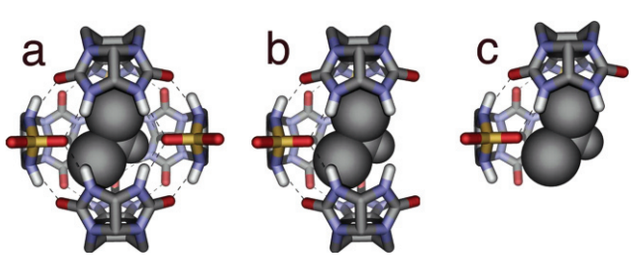

Fig. 12 Possible mechanism for the exchange of football subunits based on sequential monomer removal.

A kinetically stable, elongated tetramer (**7**) was also synthesized and studied (Fig. 13).[30] In this capsule, the lower limit for the barrier to host exchange (subunit dissociation) was estimated at 20 kcal mol^{-1} by 2D EXSY studies. Under these conditions, exchange of tetramethyladamantane guest showed rates of 10 s^{-1} in CD_2Cl_2 and was too slow to measure in CCl_4. This rate difference probably results from a combination of solvent polarity and the role of solvent molecules mediating the exchange process.

Exchange in calixarene dimers

Calixarenes functionalized with ureas on their wider rim (*e.g.*, **8**) dimerize to give capsules with appropriate guests like benzene (Fig. 14). Most of what is known about guest

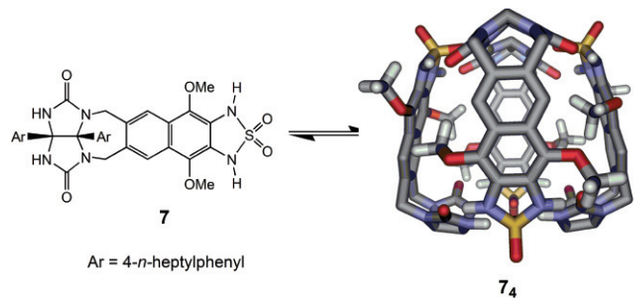

Fig. 13 Extended naphthalene tetramer. Aryl groups have been omitted for clarity.

exchange in these systems is due to Böhmer and collaborators. They used reduced-symmetry, C_2-symmetric calix[4]arenes in benzene to determine a rate constant for capsule racemization of approximately 0.26 s^{-1} ($\Delta G^{\ddagger}$ = 18 kcal mol^{-1}) by 2D EXSY at 25 °C.[31] Benzene in–out exchange, which requires capsule dissociation, showed a rate constant of about 0.47 s^{-1} ($\Delta G^{\ddagger}$ = 18 kcal mol^{-1}). Racemization can occur by capsule dissociation or by rotation of the ureas on the wider-rim. The similar barriers to exchange and racemization for the two processes suggests that they are coupled; namely, that racemization occurs by capsule dissociation/recombination. In a subsequent report, they showed that steric crowding around the hydrogen-bonding groups significantly increases kinetic stability of the capsule from seconds to hours or even days.[32] Similarly, judicious choice of guest can also increase kinetic stability: capsule lifetimes increase from 2.9 h with chloroform to 78 h with cyclohexane.[33]

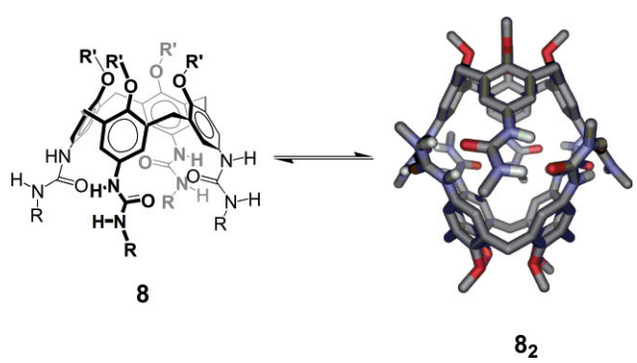

Fig. 14 Calixarene dimer held together with 16 hydrogen bonds. Alkyl groups (R) have been truncated for clarity.

With a cationic guest like tetraethylammonium, a different mode of exchange emerges.[34] Variable-temperature NMR shows a significantly lower activation barrier to racemization ($\Delta G^{\ddagger}$ = 12 kcal mol^{-1}) and a higher barrier to internal guest rotation ($\Delta G^{\ddagger}$ = 13 kcal mol^{-1}). The contributions to the energetic changes were investigated by molecular dynamics simulations. The capsule must expand to accommodate this guest relative to benzene. This weakens some hydrogen bonds, but creates favorable cation–π interactions, stabilizing the capsule. Racemization now seems to occur by the rotation of the wider-rim functionality, rather than complete capsule dissociation. Apparently, the cationic guest can facilitate rotation of the polar (negative) carbonyls just inside the capsule's "equator". Thus, switching guests has the potential to affect both the rates and the pathways of host dynamics and guest exchange.

Fluorescence studies from our lab proved to be an interesting complement to the NMR results.[35] A fluorescently tagged calixarene dimer showed a dissociation rate constant of 6×10^{-4} s^{-1}. The rate difference—nearly three orders of magnitude—was rationalized as a combination of: steric differences at the wider rim, hydrogen-bonding differences at the wider rim, and very different concentrations of the experiments.

Exchange in the cylindrical capsule

The cylindrical capsule ($\mathbf{9_2}$) is prepared by the guest-induced dimerization of the modified, self-complementary cavitand (Fig. 15).[36] This capsule is notorious for its uncanny ability to encapsulate trace impurities from common NMR solvents. We use mesitylene-d_{12} as the solvent because it is the largest deuterated solvent commercially available and with carefully purified material the NMR spectrum of the cylindrical capsule in this medium showed only broad incomprehensible signals. With the commercial material used directly, one of the major sets of observed signals corresponds to a capsule that was unsymmetrically filled. Specifically, one molecule of deuterated *p*-xylene and one molecule of deuterated benzene were held inside. The deuterated mesitylene is roughly 7 M as a pure liquid and impurities at 0.1% are 7 mM—on the order of typical NMR concentrations—so it is not surprising that the capsule would form with these guests inside at the concentrations used.

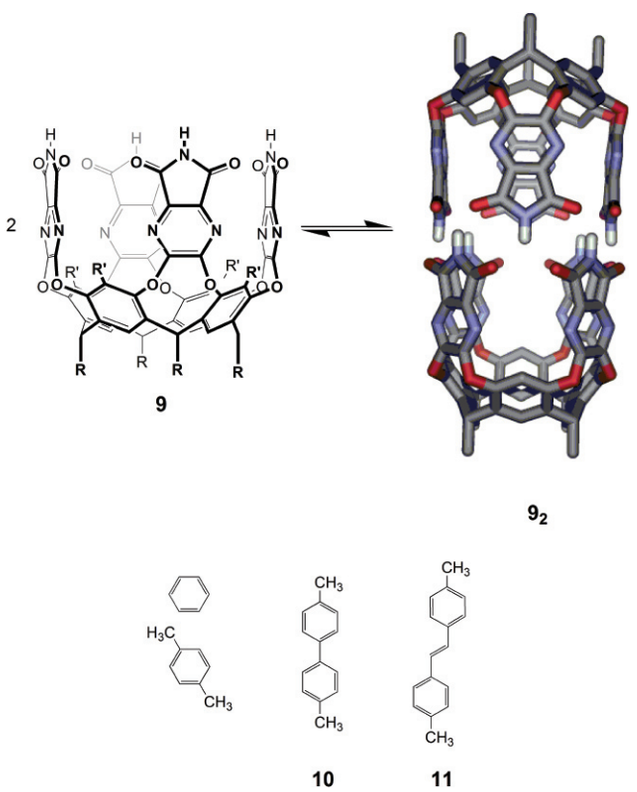

Fig. 15 Structure of the dimeric cylindrical capsule and selected guests. Alkyl chains (R = $C_{11}H_{23}$) have been truncated for clarity.

The lifetime of the capsule is ~0.5 s and exchange of guests in and out of the capsule is slow at ambient temperature. The guest exchange mechanism depends on the properties of the guest and solvent. The capsule can accommodate between one and three guests depending on their size.

In a solution of **9** in *p*-xylene-d_{10} with benzene, the resulting capsule shows an optimal filling of guests: encapsulation of a single deuterated xylene and a single benzene (Fig. 16a). A magnetization transfer study was used to observe exchange in such a capsule at 335 K.[37] Exchange is linear with the concentration of benzene, but two different processes can be extracted: (1) a concentration-dependent exchange that is first order in benzene and (2) a separate process with second-order rate constant that is independent of benzene concentration. The first-order process may result from a solvent-assisted mechanism, whereby external *p*-xylene helps to purge the capsule of benzene before reentry of a new benzene molecule. The second-order process requires the capsule flaps to open to allow exchange without dissociation of the capsule components (see below). No magnetization transfer was observed between capsule halves, demonstrating that the capsule remains intact over the timescale of the NMR experiment. Since the capsule halves are not "scrambled", the incoming benzene must displace outgoing benzene with no movement of *p*-xylene into the "benzene" half of the capsule.

A different and more complicated mechanism appears in the presence of large guests (Fig. 16b). This case was investigated using displacement of encapsulated 4,4′-dimethylbiphenyl (**10**) by 4,4′-dimethylstilbene (**11**).[37] The observed kinetics are linear with the concentration of incoming guest **11**. At high concentrations of **11**, any formation of the reactive intermediate leads directly to product, and saturation kinetics are observed at even higher concentrations. The rate of exchange was inversely proportional to the concentration of the leaving guest **11**. This exchange occurs without dissociation of the capsule and without necessarily forming a completely vacant capsule. The *n*-alkanes of eight to ten carbons are singly encapsulated by $\mathbf{9_2}$ in their extended conformations. However, broadening on the ^{1}H NMR shift timescale (600 MHz) indicates poor guest–host complementarity and probably represents a composite of in–out exchange and guest motion within the capsule. Longer alkanes like *n*-undecane twist to maximize host–guest interactions and to minimize void spaces.[38] This guest, which should have slower guest exchange, still has some broadening, possibly due to unsymmetrical coiling at the capsule ends. The longest accommodated guest, *n*-tetradecane, coils into a tight helical conformation and shows very sharp resonances with well-resolved splitting at the guest ends, as expected for rapid rotation around the terminal C–C bonds. Helices are chiral structures and each methylene is diastereotopic. However, the geminal coupling is conspicuously absent at the penultimate methylenes, suggesting rapid helix–helix interconversion (racemization) on the NMR timescale. Since the guest is too long to take a fully extended conformation, the racemization may proceed through propagation of short-range uncoiling.

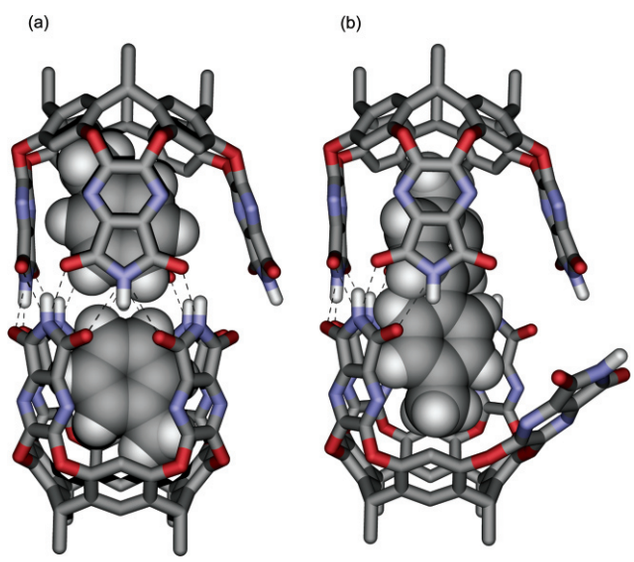

Fig. 16 Small guests like toluene can escape by opening of a single flap, left. Larger guests like dimethylbiphenyl require opening of two or more flaps, right.

With a good guest like **11**, the cylindrical capsule can also assemble even in the presence of large excesses of protic solvents (*e.g.*, 2500 equiv. methanol).[39] As with some other capsules,[40,41] the guest-binding process is entropically driven, as shown by both NMR and isothermal titration calorimetry (ITC). More germane to this review were the dissociation kinetics as determined by 2D EXSY studies with 20, 30, and 40% methanol (v/v in mesitylene-d_{12}). The rate constant doubles with each increment, corresponding to a lowering of the activation energy by ~0.5 kcal mol^{-1} with each addition. It is not surprising that methanol would destabilize the capsule; what is surprising is its effect on guest exchange. With guest **11** and 12% methanol, the

rate constants of capsule dissociation and guest exchange are nearly identical (k_{diss} = 0.16 s^{-1}). Contrasting the mechanism in apolar media, the presence of methanol seems to cause large guest exchange by complete dissociation of the capsule subunits. Complexes of small guests are not stable under these conditions.

Hexameric capsule exchange

Our most recent efforts to study exchange have focused on the hexamer formed from resorcinarene **1**—the same compound used to prepare cavitands and the dimeric cylindrical capsule (Fig. 17). The larger cavity (1375 Å^3) permits encapsulation of more and larger guests compared to most of the other capsular assemblies—eight benzenes, three biphenyls, and even bulky tetraalkylammonium salts. In the solid state, the hexamer is assembled as a snub cube.[42] Capsule formation in both the solid state[42] and in solution[43] is assisted by eight waters. These occupy the corners of the cube while the resorcinarenes are the sides. On the table with the tennis ball and softball, this assembly resembles a volleyball.

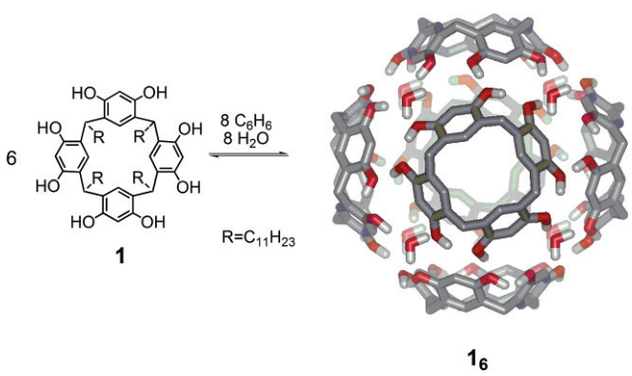

Fig. 17 Structure of hexameric resorcinarene capsule. Alkyl chains (R) have been omitted for clarity.

Aoyama and coworkers showed that a wide variety of polar guests are bound to resorcinarenes in slow exchange between free and bound states.[44] Using recently popularized NMR techniques, we have shown that some of these slowly exchanging processes are the result of guest encapsulation within the hexamer.[45]

Large tetraalkylammonium halides (R_4NX, where R = propyl–octyl) are encapsulated without requiring water for the assembly.[46] As the size of the ammonium guest increases, binding affinity decreases up to the largest guest—tetraoctylammonium bromide. Presumably large guests cause more steric crowding within the confines of the capsule interior. 2D EXSY studies showed guest-release barriers that *increase* from 17 kcal mol^{-1} for tetrapropylammonium bromide to 21 kcal mol^{-1} for tetraoctylammonium bromide. By analogy to the earlier capsules, each resorcinarene monomer corresponds to a "flap": one subunit must dissociate as the first step of guest exchange (Fig. 18). The resulting portal limits the size of species exchanging, so larger guests must fold to be able to fit through the aperture. As with other capsular assemblies, conditions that disrupt hydrogen bonds—heat, competitive solvents, and excess salts—also denature the assembly. With an excess of tetrabutylammonium bromide (4 equiv. per resorcinarene), both capsular and monomeric resorcinarene species is observed in equilibrium at 303 K.

Alternatively, one hexameric capsule can bind three copies of smaller onium halides (Et_3NHX, Et_4NX, Et_4PX).[45] 2D EXSY analysis on a solution of **1** and Et_3NHBr (1.5 equiv) showed that the guest is released from the assembly with a rate constant of *ca.* 0.5 s^{-1} at 303 K. This is slower than the release rates for larger tetraalkylammonium cations and probably reflects a combination of guest–guest and guest–host hydrogen bonding. NMR

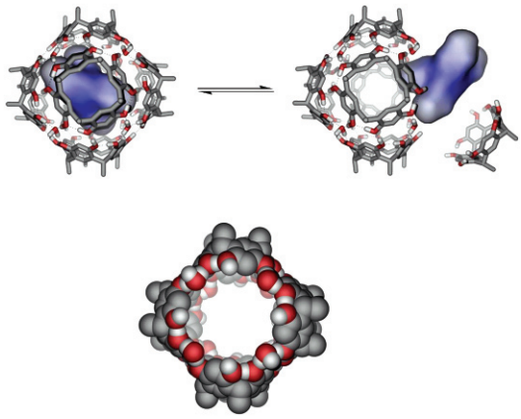

Fig. 18 Top: proposed mechanism for guest release. The tetrabutylammonium cation is depicted by its van der Waals surface. The counterion and co-encapsulated solvent molecules are omitted for clarity. Bottom: CPK figure showing the size of the portal in the pentameric transition state. A pair of opposite subunits was cut away to allow visualization the size of the opening. (Figure adapted from ref. 40.)

and ITC studies both show that ammonium binding within the large hexamer is entropically driven.

A particularly good guest was found in 1,2-*cis*-cyclohexanediol. However, we found that excess of this guest (*ca.* 7 equiv) induces partial melting of the capsule, to give a mixture of hexamer and unassembled resorcinarene in slow exchange. Variable-temperature NMR showed coalescence at approximately 50 °C, corresponding to an activation barrier of about 16 kcal mol^{-1}. A separate 2D EXSY experiment showed the in–out guest exchange rate constant for 12 to be 0.36 s^{-1},[12] indicating an activation barrier of 20 kcal mol^{-1}. This 4 kcal mol^{-1} higher guest exchange barrier must be related to some combination of guest–guest or guest–host interactions in addition to capsule opening.

Based on the earlier calixarene studies, the effect of a guest's polarity and hydrogen-bonding capacity are likely to affect the rate of capsule exchange. However, the phenomenon is particularly dramatic in the present system. Neutral tetrabutylantimony bromide exchanges 85-fold slower than the nearly isosteric tetrabutylammonium bromide at 323 K.[40] Apparently, the charged salts contribute significantly to kinetic destabilization of the hydrogen-bonded hexamer.

Most recently, Avram and Cohen have used diffusion (DOSY) NMR experiments to observe formation of hexameric capsules containing monomers of different alkyl groups (namely, R = undecyl and R = isobutyl).[47] When mixed, the capsules "scramble" to give hetergeneous assemblies. While removal of a single monomer is relatively fast (*vide supra*), this scrambling appears to take longer—on the order of hours at room temperature.

Hartzell and coworkers investigated the four downfield resonances associated with the resorcinarene hydroxyls.[48] These four resonances coalesce to two at elevated temperature. The two sets of hydroxyl protons exchange with bulk water with activation energies of 9 and 10 kcal mol^{-1} respectively by 2D EXSY. The capsule assembly apparently polarizes the hydroxyl O–H bonds facilitating their exchange with free and bound waters. The role of water in the structure and dynamics of the capsule is still not completely understood.

Analogous tetrameric macrocycles have been prepared from pyrogallol. These hydroxylated-resorcinarenes form hexameric capsules in the solid state[49] and in solution (without the structural waters required for assembly of **1**).[50] While structurally similar, these hydroxyresorcinarenes do not form mixed capsules with the resorcinarene of type **1**.[47] The pyrogallol hexamer also binds many small molecules, but not ammonium cations.[47]

The hexameric capsule presents much more complex exchange possibilities than do the previous capsules. Namely,

we observe exchange of resorcinarene subunits, resorcinarene hydroxyls and water, and as many as three different types of guest molecules. The bringing of all these components together certainly comes at a significant entropic cost, as evidenced by the capsule's sensitivity to protic solvents, excess salts, and even excess diol guests.

Summary and outlook

Supramolecular systems, held together by weak bonds, may have much to teach us about noncovalent (loose) *versus* covalent (and tight) transition states. The studies of the dynamics of vancomycin antibiotics binding to their substrates by Williams,[51] the "structural memory" observed in Raymond's chiral capsules,[52] and slow dissociation of subunits from Reinhoudt's rosettes[53,54] signal an emerging interest in these phenomena; the field is dynamic, not static.

We are on the road to defining the sequence of events—the mechanism—of guest exchange. The earliest proposals that involved the complete dissociation of the assembly, exchange with solutes or solvents then recombination are unlikely to be correct.[55] This may be appropriate for the softball when two molecules of solvents can be found; what could be more economical than dissociating the system with one solvent molecule in each concave half? This has simplicity, but probably suffers from an economy of thought. In a system held together by many hydrogen bonds, the mechanism of complete dissociation, then recombination is probably the most expensive in energy. A biological counterpart of this mechanism, which underlines its problems, is provided by the HIV-1 protease, because it too is a dimer.[56] The active site is at the interface of the two subunits with structural "flaps" that comprise the top of a channel. The dynamics of this enzyme involve a complex interplay of the dimer dissociation and flap opening. For example, opening these flaps costs relatively little in energy, but provides access for substrates and exits for products at the active site while the dimers are still held together. Mutagensis studies confirmed that the flap regions mediate substrate binding and catalysis.[57] In the kinetics of drug design, inhibitors are sought that maximize k_{diss}—to maximize the time that the inhibitor is bound and minimize the time that it spends in solution where it can be degraded. Understanding the role of mutations on the flaps is key to the design of better inhibitors.

We now believe that the opening of flaps is likely to be the general mechanism in our systems. As to the substitution of guests, the term "nucleophilic" is probably not correct: the guest is the nucleus, the capsule is the "nucleophile". Besides, "nucleophilic" is already well established in other contexts. Instead, we propose SSg2 for "bimolecular guest substitution, supramolecular" for the guest exchange when the incoming guest appears in the rate expression and SSg1 for the case where flap opening or gating is the unimolecular, rate-determining step. For host exchange, such as disproportionation of calixarenes and the football exchange, we propose SSh1 for dissociation as rate-determining and SSh2 for the slower bimolecular equilibration involving the molecular "sieves."[58] It is hoped that these terms, unpronounceable as written, will not contribute to the further acronym decay of the chemical literature.

We find reversible encapsulation to be a natural outlet of our desire to understand molecular interactions. While not a model of any particular system, we hope that the exchange processes described herein inspire and encourage research projects in other types of assemblies.

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