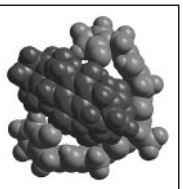
Applying biological principles to the assembly and selection of synthetic superstructures



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It has become clear over the past two decades that, in order to create functional synthetic nanoscale structures, the chemist must exploit a fundamental understanding of the self-assembly of large-scale biological structures, which exist and function at and beyond the nanoscale. This mode of construction of nanoscale structures and nanosystems represents the so-called 'bottom-up' or 'engineering-up' approach to fabrication. Significant progress has been made in the development of nanoscience by transferring concepts found in the biological world into the chemical arena. The development of simple chemical systems that are capable of instructing their own organisation into large aggregates of molecules through their mutual recognition properties has been central to this success. By utilising a diverse array of intermolecular interactions as the information source for assembly processes, chemists have successfully applied biological concepts in the construction of complex nanoscale structures and superstructures with a variety of forms and functions. More recently, the utility of assembly processes has been extended through the realisation that recognition processes can be used to select a single structure from a library of equilibrating structures. These developments open the way for the design and implementation of artificial assembly processes that are capable of adapting themselves to the local environment in which they are conducted.

1 Context

The possibility of creating a functional device or superstructure by 'engineering up' from a single molecule has become[1] an increasingly attractive prospect over the last 15 years. The activities associated with this kind of molecular engineering have become synonymous with the rapidly expanding area[2] of self-assembly. This expansion has been driven by a number of different potential user communities. Firstly, the drive for miniaturisation of integrated circuits, coupled with the growing realisation that the current 'top-down' approach employed by the electronics industry may eventually reach[3] a physical limit, has necessitated the development of an alternative approach to device fabrication. Secondly, the realisation that chemical syntheses based on the stepwise, kinetic approaches used traditionally to construct large molecules, such as palytoxin and vitamin B_{12}, are very demanding in terms of both time and resources has lead chemists to appeal to the highly convergent, and therefore highly efficient, nature of self-assembly in order to achieve the rapid syntheses of large structures. Thirdly, the realisation that biological systems exploit highly complex self-assembly processes routinely in the construction of large, regular superstructures from small, simple subunits has lead chemists to seek an understanding of the basic principles of biological self-assembling systems. Through this understanding, chemists may be able to emulate natural systems, for

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example, in the development of synthetic enzymes, efficient gene-therapy or drug delivery systems.

2 Self-assembly in biological systems

Chemists have turned to biological systems in search of viable paradigms for the construction of large assemblies. Biological systems display a diverse array of functional nanoscale structures and devices which range in size from 1 nm to the macroscopic level. The processes of self-organisation and self-assembly of molecular components, which interact chemically and physically in certain well-defined ways, are used to construct these superstructures and arrays. The specificity and precision displayed by biological systems are derived from the highly-directed mutual recognition displayed by the constituent components of a structure. Clearly, the ultimate goal for the chemist seeking to match the achievements of biological systems in creating and maintaining nanoscale structures is to control the assembly and function of synthetic structures from components with the same precision as that displayed by Nature. This objective can only be achieved by firstly understanding[2] the principles behind the self-organisation, self-assembly, and self-synthesis processes exhibited by biological systems. There are several important features common to all natural self-assembling systems which make them a desirable target for chemists to study. These features can be illustrated most easily by considering some examples.

Proteins are involved in almost all biological processes and are employed in a wide range of different roles. They are synthesised as linear chains of amino acids in a defined order dictated by cellular DNA. Knowledge of their amino acid sequence is essential for an understanding of their mechanism of action, however, proteins do not function correctly until they are folded into a specific three-dimensional structure which is also determined by the primary amino acid sequence. This folding process[4] relies on the formation of a large number of non-covalent bonding interactions and is not yet fully understood. However, it is clear that the protein must fold rapidly and reliably into the correct structure from the huge number of possible structures, since the consequences[5] of incorrect folding can be catastrophic, *e.g.* amyloid diseases, such as Alzheimer's, and prion diseases. Further, all of the information necessary to ensure that the correct fold is reached must somehow be encoded in the non-covalent interactions implicit within the primary sequence. The folding pathway may have many different routes to the final folded structure and incorrect structures are undoubtedly formed as transient species. However, all the interactions involved in the folding process are formed reversibly, and only the stable native state will persist under normal conditions. Thus, the folding process could be regarded as the recognition-directed selection of a single tertiary structure from a large library of tertiary structures encoded by the primary sequence.

Viruses represent some of the best natural examples of self-assembly from which chemists can learn,[6] and the tobacco mosaic virus[7] (TMV) (Fig. 1) is perhaps one of the best understood. TMV contains 2130 protein subunits (Fig. 1a), all of which are identical, thus minimising the amount of genetic information required to encode the final helical structure assembled around a single strand of RNA. All of this information is contained within the constituent parts of the virus and no additional factors are required for the assembly. However, although all of the protein subunits are bound to each other in the same manner, thus creating the symmetrical helical structure, the assembly process is by no means as straightforward as it might first appear. A two-layer disc—a sub-assembly—is constructed first (Fig. 1b), and this combines with a specific part of the TMV RNA located about 1000 base pairs

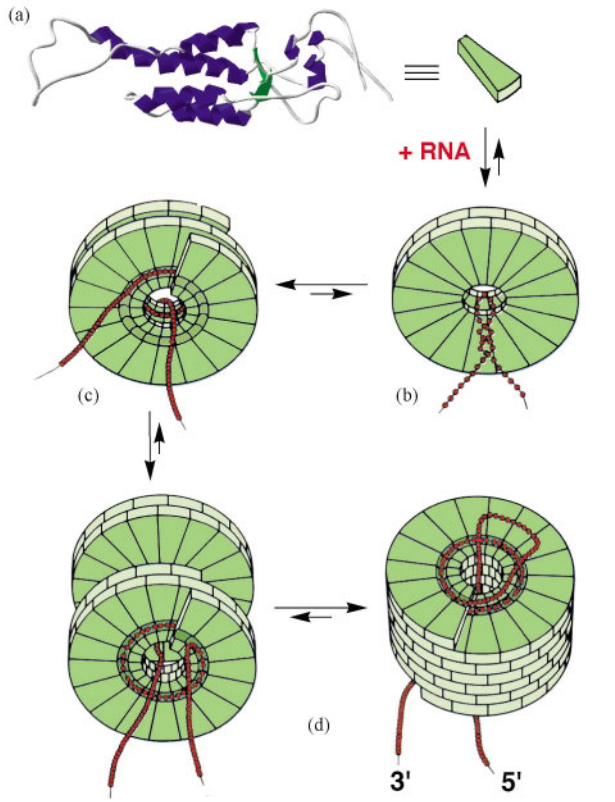


Fig. 1 The assembly of the tobacco mosaic virus. Protein sub-units (a) associate with viral RNA to form a double disk structure (b). Conformational change results in the formation of a slipped double disk (c) and initiates viral assembly (d). Adapted from ref. 13.

from the 3′ end. This priming, or nucleation, process (Fig. 1c) is followed by helix propagation (Fig. 1d) which gives rise to the intact viral particle. The self-assembly uses only reversible, non-covalent interactions, so the process is in dynamic equilibrium. The advantages of helix formation *via* this sub-assembly are clear. Firstly, the assembly is highly specific, as the nucleating region of the RNA must interact with up to 17 protein subunits, and foreign RNA is therefore recognised and rejected. Secondly, helix growth from the sub-assembly allows for the monitoring of protein subunits, so that the helix is efficiently and correctly assembled. Finally, the co-operative addition of 34 protein subunits more readily overcomes the entropic disadvantage of assembly.

The T4 bacteriophage virus is a much more complex virus than TMV in a structural sense, consisting of a head, a tail and six tail fibres. The assembly process[8] (Fig. 2) is more involved, occurring in a precise sequence, however, it is also highly convergent and only a small set of different interactions and reactions are employed. This means that a relatively small amount of information is required to encode for the whole assembly. Indeed, only 53 out of a total of 135 genes contain information directly related to the virus construction. The assembly occurs in a strict order meaning that only correct, complete virus particles are formed. T4 does not form using strict self-assembly alone, rather by a combination of self-assembly, scaffold-assisted assembly and enzyme-directed assembly. This paradigm suggests that strict self-assembly can be combined with conventional covalent chemistry to provide a sufficient level of sophistication to create complex structures readily.

These examples illustrate the basic principles of self-assembling systems that can be exploited by the synthetic chemist. 1) Self-assembly processes are highly convergent, and therefore, potentially, extremely efficient. 2) Simple, and often identical, subunits can be used to easily and accurately assemble large, functionally complex supermolecules. 3) The amount of information required to describe a structure will be small if the

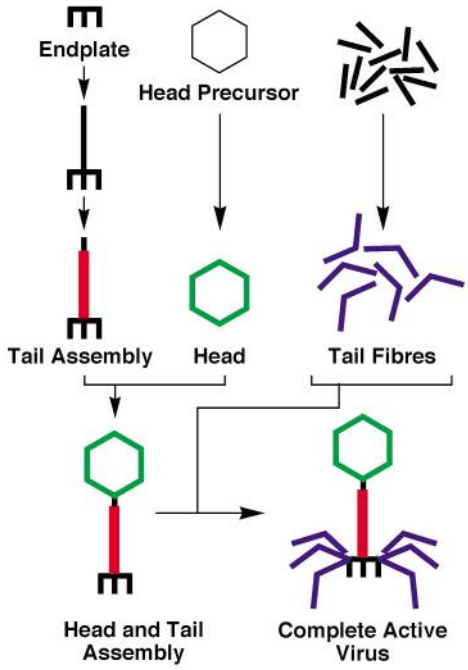


Fig. 2 The assembly pathway for the construction of the T4 bacteriophage.

number of different interactions required to construct it is small. 4) Self-assembly processes generally use reversible interactions or reactions. Therefore, the assembly process is always at, or close to, dynamic equilibrium. In this situation, the synthetic pathway will be error-checking.

It is clear that biological systems construct and utilise nanoscale structures with a high degree of accuracy and efficiency. Thus, self-assembly has become the paradigm of choice for synthetic chemists wishing to construct nanoscale assemblies. Understanding the interactions between non-covalently bonded molecules is clearly crucial to the successful design and synthesis of self-assembling wholly-synthetic structures. Thus, the science of supramolecular chemistry,[9] has contributed significantly to the dramatic expansion of activity in the area of synthetic self-assembling systems in the last ten years. This review will consider some important developments in the study of self-assembling systems that have been published since 1996. However, it does not set out to provide comprehensive coverage of the literature and self-assembled monolayers (SAMs) and self-assembled quantum dots, which account for a significant proportion of the literature on self-assembly, are beyond the scope of this review.

3 Chemical systems—non-covalent self-assembly

Metal centres have been used extensively in self-assembly in the construction of a wide variety of structures including macrocycles,[10] linear[11] and circular[12] helicates, catenates,[13] grids,[14] cages,[15] ladders[16] and dendrimers.[17] Metal–ligand interactions are stronger than other non-covalent bonds and are highly directional. The use of metal ions in self-assembly could potentially lead to the generation of structures with interesting properties as a result of the redox or magnetic properties of the metal. These well-defined assemblies may be useful as models for the study of biological charge transport systems.[18] Furthermore, the preference of certain metals for specific coordination geometries provides the chemist with a basis for a more rational design approach to the synthesis[2] of particular architectures. Lehn and co-workers have recently constructed[19] rectangular grids (Fig. 3) using a mixture of a bidentate **1** and a tridentate ligand **2** which complex Ag^+ ions. If the three possible

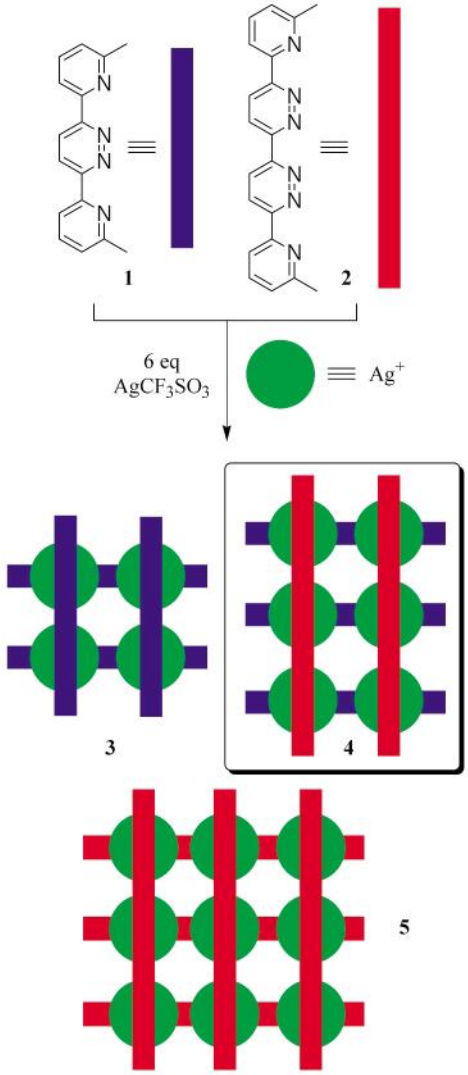


Fig. 3 The Ag^+-mediated self-assembly of a 2 × 3 grid.

grid structures, the 2 × 2 grid **3**, 2 × 3 grid **4** and the 3 × 3 grid **5**, form statistically, the expected ratio of **3**:**4**:**5** should be 36:46:16. However, the experimental ratio is 8:90:2. The overwhelming bias in preference of the heteroleptic grid **4** may possibly result from an active avoidance of the 3 × 3 grid **5** which requires the formation of the least favoured metal–ligand coordination interaction, *i.e.* the central $Ag(pyridazine)_4$ unit. It would therefore appear that information provided by both the ligand structure and the metal ion geometry are responsible for the formation of a single structure.

Lehn and co-workers have also investigated[20] the outcome of the integration of opposing programs, or subroutines—*i.e.* does the resulting architecture arise from the linear combination of the two subroutines, or from a more complex convolution of instructions, each subroutine clashing with the other? In order to answer this question, they designed the ligand **6** (Fig. 4). This ligand contains both a bis(bipyridine) ligand (red, Fig. 4) and a bis(pyridylpyridazine) ligand (blue, Fig. 4). In isolation, the bis(bipyridine) ligand forms a double helical structure (Fig. 4a) with Cu(I) cations and the bis(pyridylpyridazine) ligand a square 2 × 2 grid (Fig. 4b). Complete solution phase characterisation of the product of a copper(I) mediated self-assembly process involving four molecules of ligand **6** and twelve Cu(I) cations suggests the complex produced has either geometry **A** or **B** (Fig. 4c). Structure **A** would be the expected product since it is the result of a linear combination of the two subroutines. Structure **B** would be the result of a more complicated combination of possible coordination geometries. Crystal structure solution demonstrated that the assembly

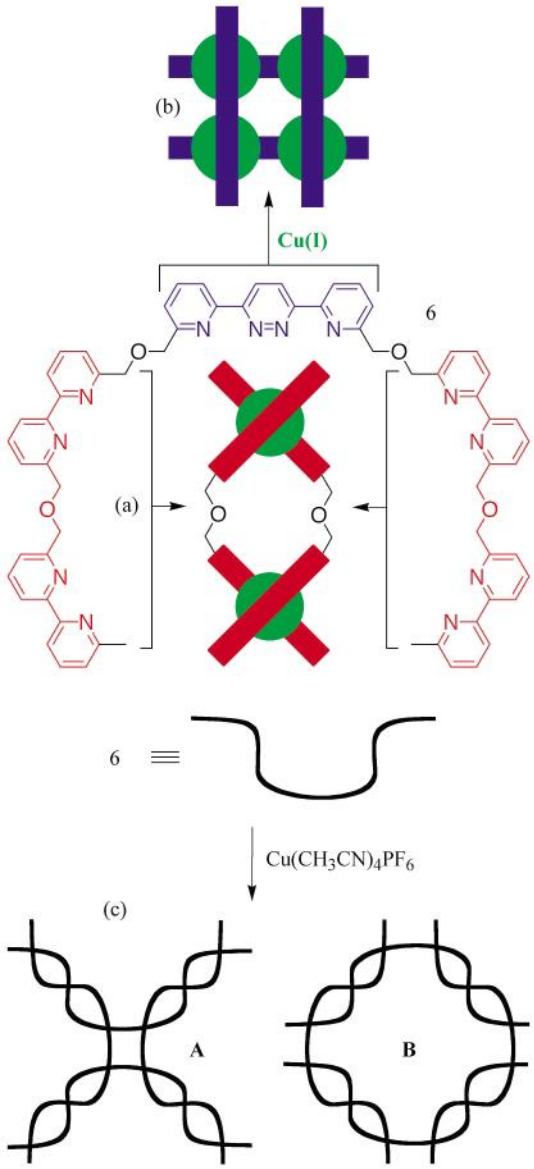


Fig. 4 The red bipyridine ligands encode a helical structure (a) and the blue pyridylpyridazine ligands encode a grid structure (b). (c) Combination of these two subroutines can give either structure **A** (linear combination) or structure **B** (more complex convolution).

process had, in fact, afforded structure B. Thus, for this case at least, the combination of the two different sub-routines produces a product which arises from a convoluted interaction between the two instruction sets and, hence, forms a novel structure.

Self-assembly processes generally result in the formation of the thermodynamically most stable product. However, interesting recent work by Lehn and co-workers has shown[21] that the kinetic products may be formed as 'intermediate' species on the pathway to the final assembly. The tris(bipyridine) ligand **7** (Fig. 5) forms[22] a circular helicate **8** on the addition of Fe^{2+} cations. However, the same ligand forms a linear triple helicate **9** when it complexes Ni^{2+} cations, despite the fact that both these metals favour the same coordination geometry. However, when the formation of the Fe(II) complex is monitored by NMR spectroscopy, the formation of the Fe(II) analogue of the linear triple helicate **9** can be detected. Indeed, this species, which is the kinetic product, formed fully in less than one minute. This assembly process is reversible, and a partial disassembly allows the formation of the thermodynamic product—the circular helicate **8**. From these results, it appears that the linear triple helicate may represent a local minimum[23] on the energy hypersurface for the reaction when Fe(II) cations are used, although it is not immediately obvious why the circular helicate should be the thermodynamic product in this case. A plausible explanation, however, is that it is stabilised by the electrostatic interaction with the bound counter anion (Cl^-).

Many groups have studied[24] the self-assembly of macrocyclic receptors which employ metal cations to direct the assembly. Macrocyclisation under kinetic control is an unfavourable process, but the construction[25] of products of this kind is possible under thermodynamic control. These receptors may show new and interesting properties due to the inclusion of the metal ions in the structure. Fujita and co-workers have demonstrated (Fig. 6) the assembly of square Pd- or Pt-linked complexes through use of appropriately protected Pd or Pt building blocks and ligands with a linear geometry. They note[26] that an equilibrium exists between a square and a triangular complex. Interestingly, Hong *et al*. found[27] it is possible to bias the equilibrium in favour of either complex by the addition of an appropriate guest. A large guest favours the square complex and a small guest the triangular complex.

Fujita and co-workers have also demonstrated[28] an interesting guest-templated self-assembly of a receptor molecule (Fig. 7a). Ligand **10** and metal complex **11** react in the presence of the sodium salt of adamantanecarboxylic acid **12**, a large guest, to form a molecular cage **13** containing ten separate molecular components—four tridentate ligands and six metal centres. When the metal centre is Pd(II), the nanocage forms readily and reversibly. However, when Pt(II) is used, formation

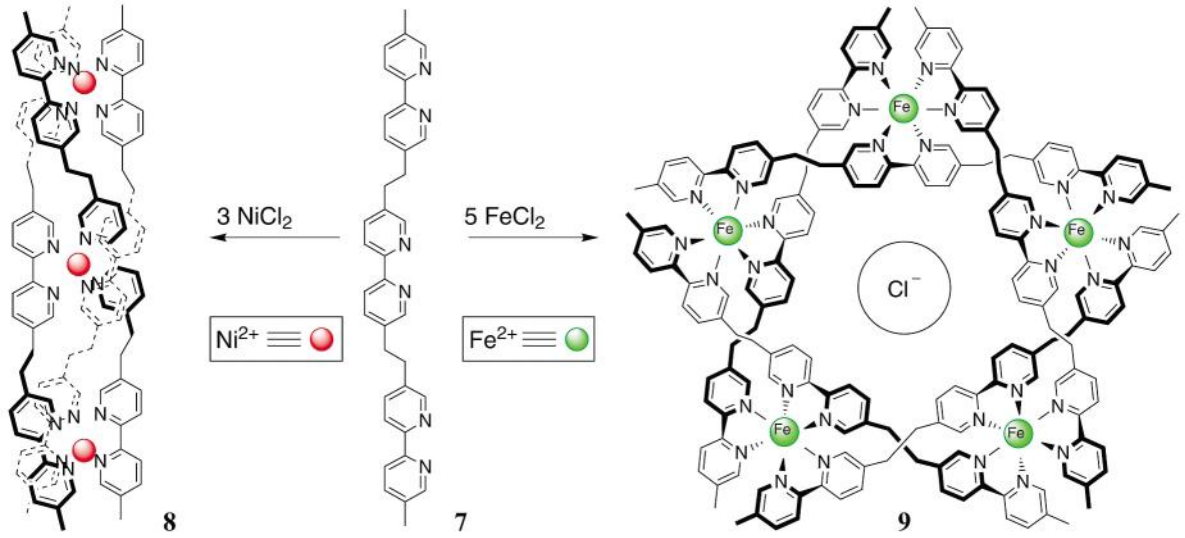


Fig. 5 Circular helicate **8** is the thermodynamic product of an assembly process which involves linear helicate **9** as an intermediate.

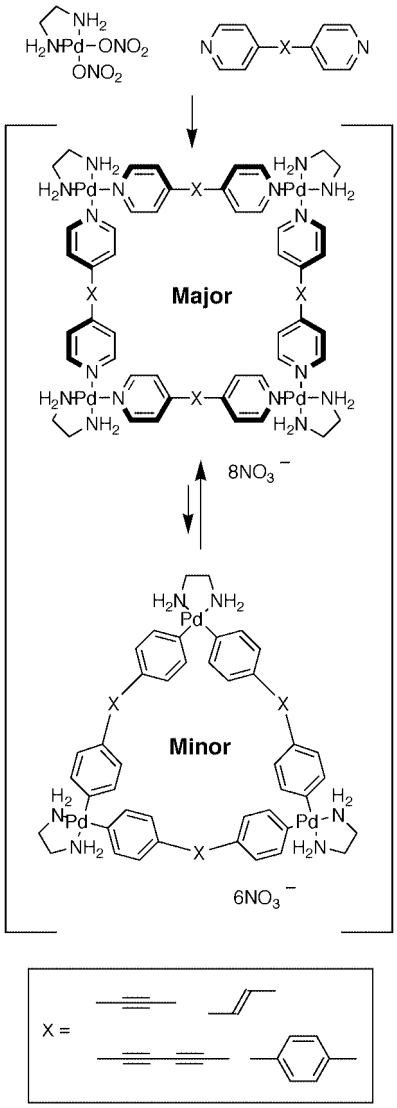


Fig. 6 The coordination geometry around the Pd centre encodes either a square (major product) or a triangular (minor product) complex.

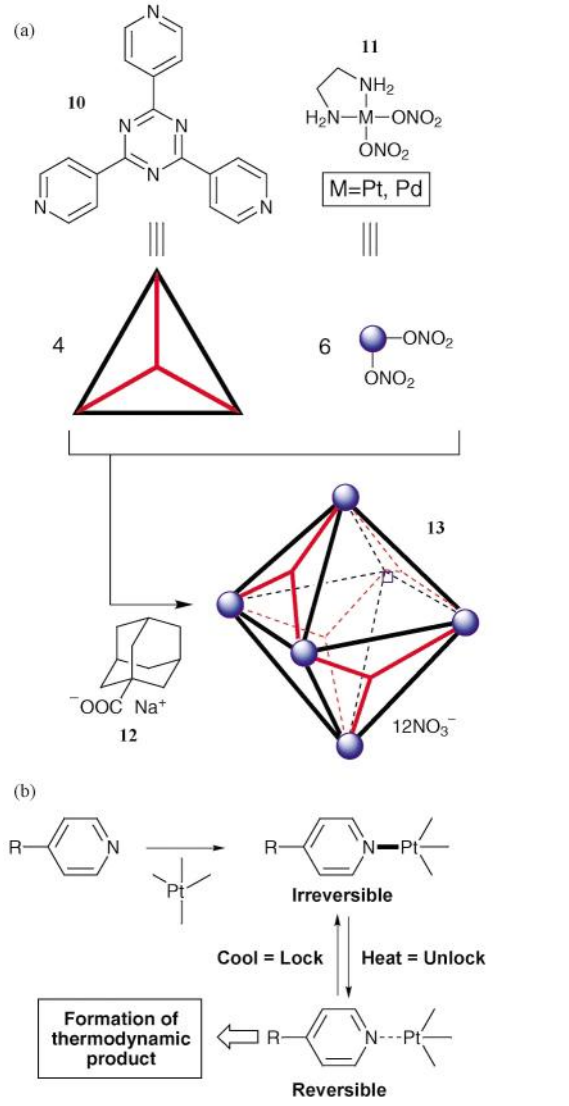


Fig. 7 (a) A combination of tridentate ligand **10** and a square planar metal centre **11** affords the octahedral cage **13**. (b) The formation of the thermodynamic product can be assured by using heating and cooling as a method to unlock and lock the Pt–N coordinate bond.

of the nanocage is hampered by the extremely slow exchange of the ligand sphere around the metal centre. Nonetheless, this slow exchange of the Pt(II)–pyridine coordinate bond can be used as a switchable lock (Fig. 7b). Usually kinetically inert, the Pt–N bond becomes labile when heated to 100 °C, and formation of the cage molecule can then be templated by sodium adamantanecarboxylate. However, unlike many other templated receptors, once the heat source is removed the template could also be removed without destroying the receptor, since the inert Pt–pyridine bonds lock the cage molecule. The nanocage constructed in this manner is extremely stable. The cage remains intact from pH 1 to pH 11 at room temperature and acid (HNO_3), base (K_2CO_3) and even a good nucleophile (NEt_3) can be added to a solution containing the cage with no ill effects to the cage structure. Fujita and co-workers have also used the same metal complex with different pyridine-based ligands to demonstrate[29] that the equilibration between a series of similar receptors can be biased by addition of appropriate templates that stabilise a particular receptor.

Hydrogen bonding interactions, with their strength, specificity and directionality, are also widely used in self-assembly of complex structures[30] from 'programmed' sub-units. In 1996, Zimmerman and co-workers described[31] the use of the hydrogen-bonded carboxylic acid dimer motif in the self-assembly (Fig. 8) of hexameric dendritic structures. The self-assembly of dendrimers represents a way of synthetically generating species with molecular weights approaching those of proteins. Covalent synthesis under kinetic control rarely results in the generation of materials with nanoscale dimensions. The monomer unit **14** contains two isophthalic acid groups connected by a rigid aromatic spacer, on to which is also attached a poly-(phenylether) dendritic wedge. On assembly, the monomer can form a cyclic hexamer **15** or a range of linear oligomers **16**, which are expected to be disfavoured enthalpically since they do not contain the maximum number of hydrogen bonds possible. ^{1}H NMR spectroscopy in apolar solvents shows broad signals due to aggregation, and size exclusion chromatography (SEC) suggests the formation of a discrete cyclic hexamer. In polar solvents, which disrupt the hydrogen bonding, NMR and SEC both give evidence for the existence of monomers. The stability of the hexamer appears to be generation dependent, *i.e.* smaller monomers (those with lower generation dendrimers attached) are more likely to exist as linear oligomers. The distance between adjacent dendrimer attachment points is smaller in the linear oligomers than in the cyclic hexamer and larger, rigid dendrimer substituents cannot be accommodated. This suggestion is backed up by the observation that a more flexible dendrimer substituent, although a similar size to the larger rigid dendrimers, does not form the cyclic hexamer in preference to linear oligomers. Molecular modelling suggests that several aromatic rings on adjacent dendrimer units are very close. It seems that there is a subtle and unpredictable balance

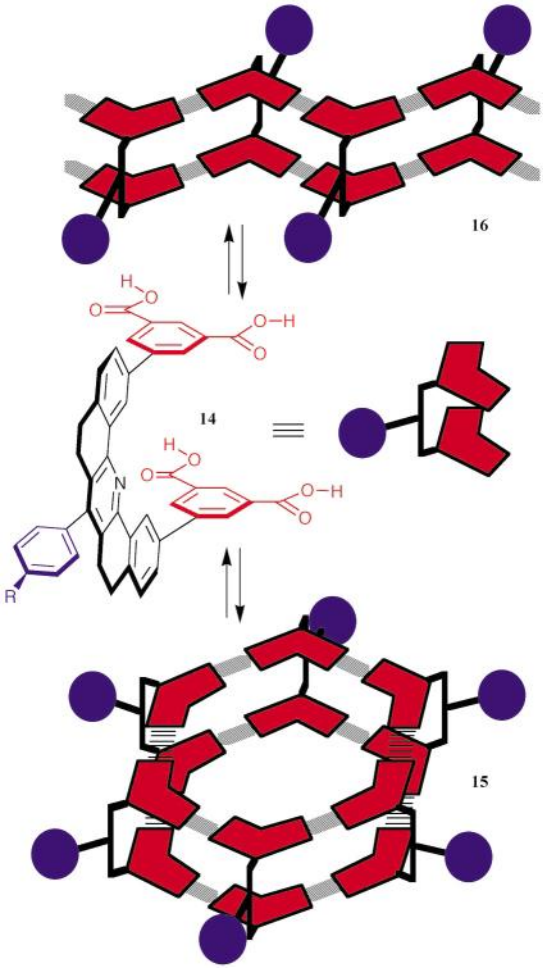


Fig. 8 Monomer **14** can self-assemble through the formation of hydrogen-bonded carboxylic acid dimers into either a linear **16** or a hexagonal superstructure **15**. The substituent R is an aromatic ether dendritic wedge and has been omitted for clarity.

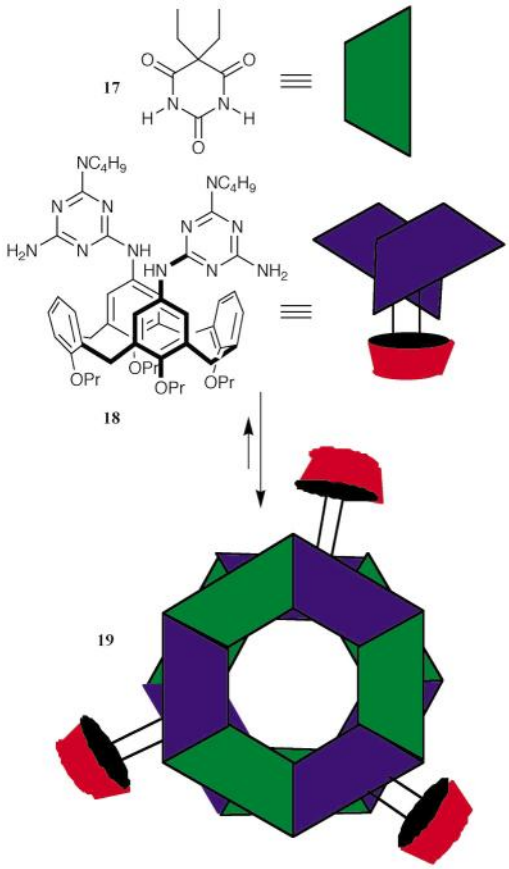


Fig. 9 Self-assembly of a double rosette superstructure **19** through the interaction, mediated by hydrogen bonds, of barbiturate **17** with melamine derivative **18**.

between the influence of favourable van der Waals contacts and hydrogen bonds, and unfavourable steric repulsions. Later work by Zimmerman and Kolotuchin[32] on the self-assembly of dendrimers, employs the complementary triple hydrogen bond motifs DDA and AAD. Self-assembly results in the formation of a highly stable, hexameric dendritic aggregate, demonstrating the unambiguous information content of this motif.

The network of complementary hydrogen bonds formed between melamine and cyanuric acid has been exploited by Reinhoudt and co-workers (Fig. 9) to construct[33] self-assembling molecular boxes based on calix[4]arenes. In $CDCl_3$ solution, diethylbarbiturate **17** assembles with the calix[4]arene **18** bearing two melamine residues. The formation of the classic[34] melamine–barbiturate rosette structure affords the molecular box **19**. This assembly possesses a double layer rosette structure with the two layers spaced apart by the calix[4]arene bowls. It is these calix[4]arenes which serve to preorganise the hydrogen bonding groups facilitating the formation of the final assembly. This methodology has been extended to allow[35] covalent capture of the assemblies and the construction[36] of rod-like hydrogen-bonded nanostructures.

Recently, reports concerning self-assembling capsules that can bind solvent or other guests inside a cavity have begun to appear. Rebek and co-workers have synthesised molecules that are capable of dimerisation through hydrogen bonding to form capsules.[37] The capsule formation may be templated by the incorporation of solvent molecules in the cavity.[38] Enclathration of a single large guest molecule (*e.g.* adamantane or ferrocene derivatives), however, is entropically favoured since it results in the release of several solvent molecules from the capsule.[39] The monomer **20** (Fig. 10) is self-complementary and will form a hydrogen-bonded dimer [**20**·**20**], termed a softball, in solution. It was noted that the capsule readily encapsulates two molecules of benzene, and this raised the possibility of using the capsule as a chamber for a bimolecular reaction. Using these methods it is possible to construct systems which are capable of binding multiple guests and a reaction between the included guests may be accelerated within the capsule. Thus, Rebek and co-workers have used a self-assembled capsule to bind *p*-benzoquinone and cyclohexadiene and observed[40] significant rate enhancement, but not true catalysis, of the Diels–Alder reaction between them. In later work, it was envisaged[41] that the use of 2,5-dimethylthiophene *S*,*S*-dioxide **21** as the diene would permit turnover through the aromatisation and loss of SO_2 from the cycloadduct formed between **21** and *p*-benzoquinone **22**. The softball does indeed catalyse the reaction between **21** and **22**, but for quite different reasons. The softball [**20**·**20**] shows relatively high affinity for quinone **22**, but no measurable affinity for the diene **21**. This observation led Rebek and co-workers to propose the catalytic cycle for the reaction shown in Fig. 10. In the resting state, [**20**·**20**] contains two molecules of *p*-benzoquinone. Occasionally, one of the molecules of **22** is displaced by **21** to give the catalytically active complex. A moderately accelerated cycloaddition between **21** and **22** is followed by the ejection of the cycloadduct from the cavity of [**20**·**20**] and regeneration of the resting state complex. Analysis of kinetic data indicates that the reaction between **21** and **22** is accelerated by around an order of magnitude within the cavity of [**20**·**20**].

Fujita and co-workers have also reported[42] the use of a molecular capsule in the synthesis (Fig. 11) of otherwise highly-labile cyclic trisilanol trimers and tetramers. Polycondensation of trialkoxysilanes, such as phenyltrimethoxysilane **23**, usually generates cyclic trimer **24** as a short-lived intermediate which transforms rapidly[43] into more thermodynamically stable

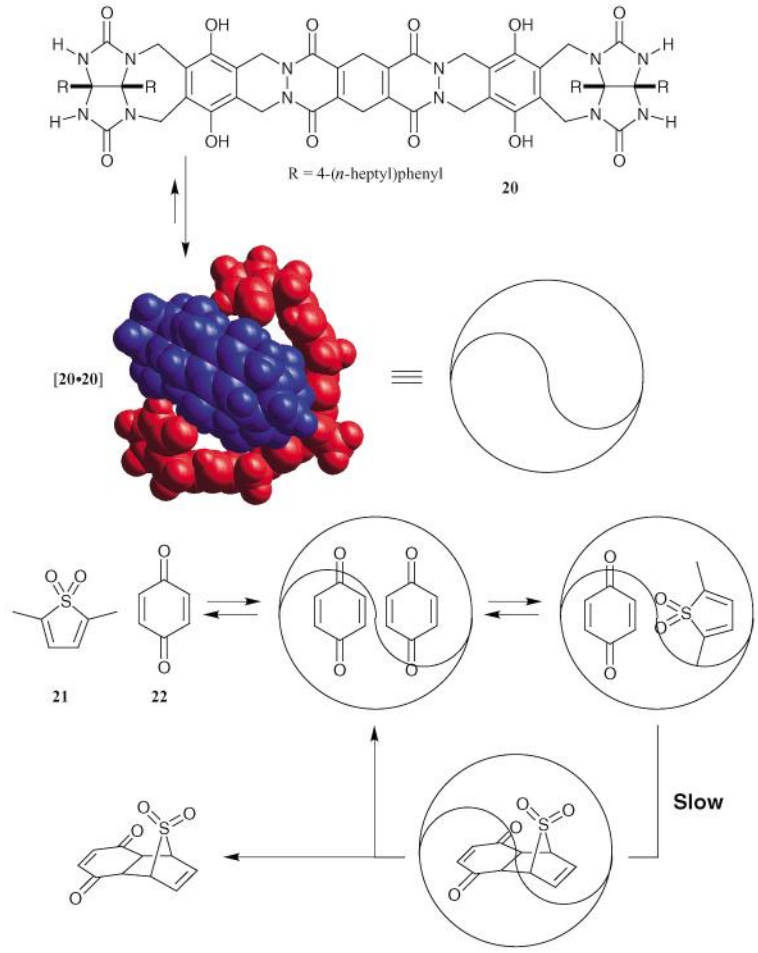


Fig. 10 Self-association of the cyclic bisurea derivative **20** through eight hydrogen bonds gives a structure which resembles a softball. The capsule is capable of catalysing the Diels–Alder reaction between **21** and **22**.

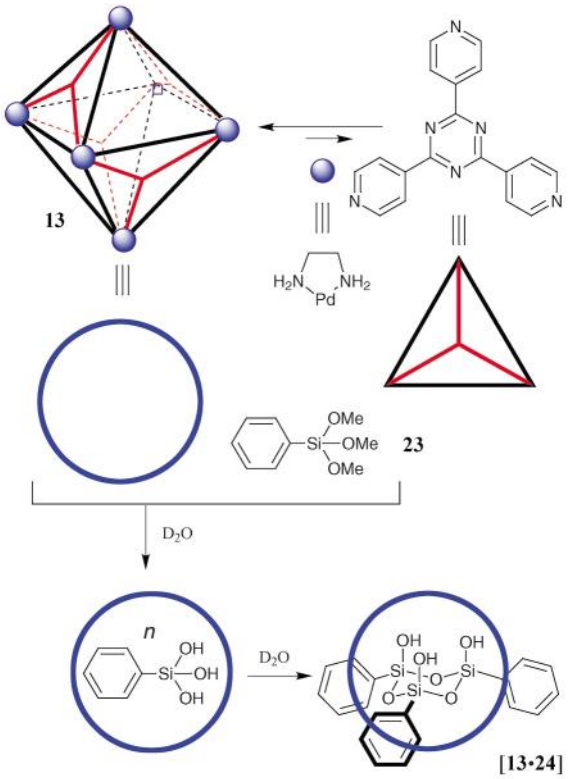


Fig. 11 Stereoselective synthesis of the unstable trisilanol **24** occurs inside the cavity of the octahedral cage **13**.

oligomers. However, when the condensation reaction is carried out in the presence[44] of the self-assembled nanocage **13** (Fig. 11) the pure hepta-hydrated complex [**13**·**24**] is readily isolated and found to be extremely stable. Furthermore, the reaction exhibits strong stereochemical control, with only the all-*cis* isomer being formed. This synthesis has been described[45] as 'ship-in-a-bottle' synthesis, since the trialkoxysilanes can readily enter the capsule, but cannot exit from the capsule once reacted to form the larger trimer. Further work investigated the efficacy of the same self-assembled nanocage **13** as a phase transfer catalyst. Nanocage **13** is highly soluble in water and also binds organic molecules well. It has been shown[46] that, with a Pd(II) co-catalyst, the nanocage catalyses the aerobic, aqueous oxidation of styrene to acetophenone, a reaction which does not occur in the absence of the cage.

Several elegant examples of self-assembling capsules have also been reported[47] by Böhmer and co-workers. These capsules are based on calix[4]arene skeletons with urea groups on their upper rims. Self-recognition of the ureas then drives the formation of capsule dimers in non-polar solvents. Extensive NMR studies have probed the reversible dimerisation of these calix[4]arenes and the exchange of the small guests, such as benzene, included within the capsule cavities has been studied. A similar approach has led to the synthesis[48] of larger calixarene-based capsule by de Mendoza and co-workers.

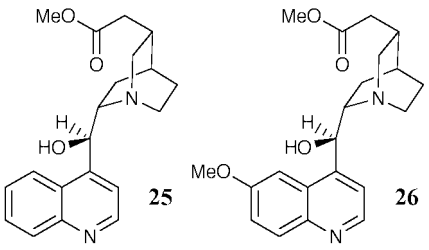
4 Self-assembly using covalent bonds

One major disadvantage of the use of non-covalent bonds in self-assembly is that non-covalent interactions are relatively

weak. They are used to direct the formation of the structure, but also to stabilise the final product structure, and, hence, many interactions are needed to offset the unfavourable entropy arising from the association of several molecules. The prototypical example of this phenomenon is the formation[49] of the DNA double helix. This process proceeds by an essentially 'all-or-nothing' mechanism and the process consists of two phases—nucleation and propagation. The nucleus is the shortest double strand for which the free energy of base pairing is negative. Formation of these first four base pairs is entropically disfavoured, but this unfavourable step is overcome by the negative free energy contribution of the many subsequent base-pairing interactions.

This problem can potentially be overcome by the use of covalent bonds to direct the assembly. Covalent interactions are significantly stronger than non-covalent bonds, so fewer stable bonds are required to offset the unfavourable entropy of self-assembly. In order to retain all the advantages of a non-covalent self-assembly process, however, we still require that the covalent bonds should form under thermodynamic control, *i.e.* their formation must be reversible. Covalent self-assembly has, so far, been relatively underexploited, probably since examples of covalent bonds whose formation is reversible, which include imine, disulfide, alkene, carboxylate ester and borate ester linkages, are somewhat limited.

Reports of systems designed to operate through the reversible formation of covalent bonds began to appear in the mid 1990s. Sanders and co-workers have investigated[50] the use of trans-esterification as the source of reversible covalent chemistry in the synthesis of macrocycles from the methyl esters of quinine and cinchonidine alkaloids (**25** and **26**). The macrocyclisation, under thermodynamic conditions, of suitable monomers affords almost exclusively cyclic trimers. These results contradict theoretical predictions which suggest[51] a wide range of oligomers should be observed. It can be demonstrated that the reaction is indeed under thermodynamic control by re-subjecting a single pure cyclic oligomer to transesterification. Within minutes, the original distribution of products has been re-established. Cyclisations of synthetic linear oligomers were also carried out[52] under kinetic conditions, in order to prove that there was no kinetic barrier to the formation of cyclic oligomers other than the trimer.



NMR spectroscopic studies show that the conformations of the monomers are altered on cyclisation, suggesting that the monomer is not preorganised to form the trimer, rather that it is predisposed. Constable first used[53] the term 'predisposed', in describing ligands that adopt[54] a helical conformation on complexation to a metal centre, but which are not necessarily helical in the free state. This is distinguished from 'preorganisation' which he uses to describe ligands[55] which are already in a helical conformation. An simple example of predisposition can be found in a recognition-based approach[56] to the acceleration and facilitation of cycloaddition reactions. 2-Phenylfuran derivatives are extremely poor dienes since they possess a high level of conjugation that is destroyed during a Diels–Alder reaction. However, the location[57] of complementary recognition sites on the 2-phenylfuran derivative **27** and maleimide **28** (Fig. 12) allows the formation of a [**27**·**28**] complex, rendering the reaction between **27** and **28** pseudointramolecular. Although

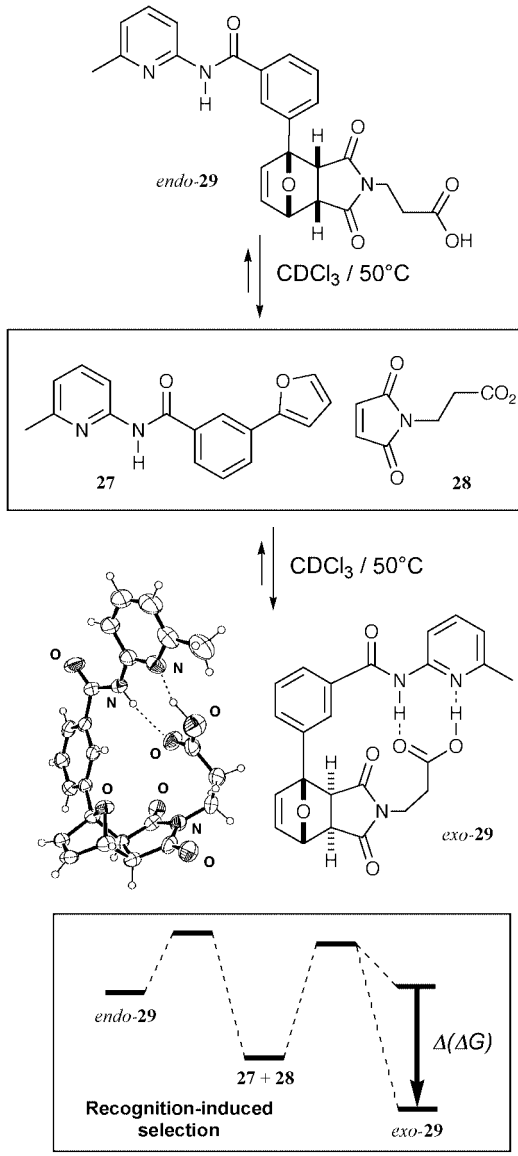


Fig. 12 The formation of two intramolecular hydrogen bonds in *exo*-**29** predisposes the system to form this cycloadduct exclusively. The presence of these intramolecular hydrogen bonds is confirmed by X-ray crystallography.

the reaction within this complex is accelerated[58] with respect to the bimolecular process, a more important effect is selective stabilisation of the *exo*-**29** product ground state. The two intramolecular hydrogen bonds that are present in *exo*-**29**, but not in *endo*-**29** predispose the system to form only *exo*-**29**. The importance of the intramolecular hydrogen bonds [$\Delta(\Delta G)$, Fig. 12] to the overall stability in this system can be readily demonstrated by the fact that the retro Diels–Alder reaction is rapid in d_6-DMSO, which disrupts the hydrogen bonds in *exo*-**29**. This system is an example of predisposition, which can best be described as a strong conformational or structural preference expressed by the product of a reaction and is therefore a thermodynamic effect, as opposed to preorganisation, which is a kinetic effect.

Sanders and co-workers have also synthesised[59] a xanthene monomer **30** (Fig. 13) which is extremely rigid and is strongly preorganised to give only the cyclic dimer. When the xanthene monomer **30** and cinchonidine monomer **25** are mixed and subjected to transesterification conditions, self-sorting is observed. That is, predominantly the cinchonidine trimer **26** and the xanthene dimer **31** are observed, although there is apparently no kinetic barrier to the formation of hetero-products, such as **32**. This process is essentially a covalent

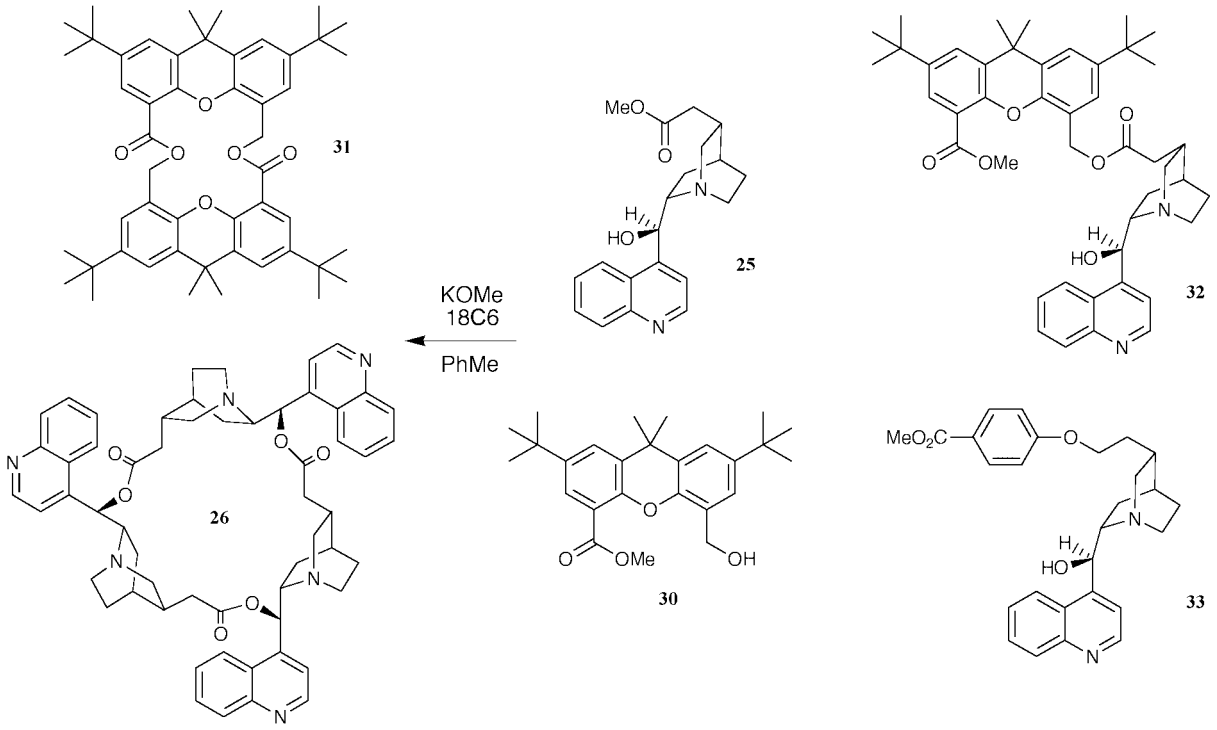


Fig. 13 Monomers **25** and **30** self-sort under transesterification conditions affording the cyclic trimer **26** and the cyclic dimer **31** respectively.

analogue of the self-sorting metal-based double and triple helices reported[60] by Lehn. The synthesis[61] of an extended monomer **33**, which differs from the original cinchonidine monomer **25** only by the addition of a spacer group, is aimed at the creation of a thermodynamic combinatorial library, which will be discussed in more detail later. The extended monomer **33** is more flexible and this leads to a relaxation of predisposition on cyclisation. Similar experiments can be carried out[62] using cholate monomers, which are more flexible than the cinchonidine monomers. In this case, the cyclisation is complete after 10 minutes and the product distribution is wider than for the cinchonidine monomer—still containing mainly trimer, but also tetramer and a small amount of dimer and pentamer. This distribution can be biased[63] by the addition of sodium ions, which cause a 100% increase in the amount of tetramer obtained.

Ipaktschi and co-workers have developed[64] a method for the synthesis of large macrocycles based on the covalent self-assembly of quinodimethane derivatives. The monomer unit spontaneously self-assembles to form a spherical, cyclic tetramer. The macrocyclisation process is driven by the reversible formation of four weak carbon–carbon bonds. Any errors in the cyclisation process can be reversed and corrected. They also report the synthesis of further functionalised quinodimethane derivatives, which can be utilised in the preparation of a self-assembling dendrimer and a macrocycle with ferrocene units at the periphery of the molecule. The addition of such large substituents did not hinder the cyclisation process.

Protein structures are often stabilised through the reversible formation of a disulfide bridge and this thiol–disulfide interconversion involves forming strong, covalent S–S bonds at room temperature. Chang and co-workers have exploited[65] this principle to improve the thermodynamic stability of a tris(disulfide). The monomer thiol groups are preorganised in a conformation that resembles those adopted by the disulfides in the dimer, thus reducing the unfavourable entropy change on dimerisation.

We have described[66] the use of the bis(borazaaromatic) **34** in the covalent self-assembly (Fig. 14) of a dimeric macrocycle **35**. Hydroxyborazaaromatic compounds undergo[67] reversible hom-

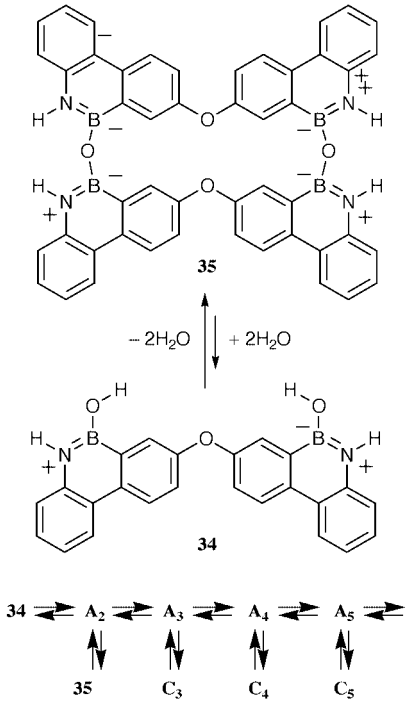


Fig. 14 Although many products are formed initially from the dehydration of **34**, the reversible formation of the borazaromatic anhydride ensures that, ultimately, only **35** is present in the reaction mixture.

oanhydride formation under appropriate conditions. Monomer **34** can spontaneously dehydrate in acetone solution to form both linear (A_n) and cyclic (C_n) oligomers. When a sample of **34** is dissolved in acetone, MALDI-TOF mass spectrometry indicates that, after thirty minutes, only the acyclic and cyclic dimer, A_2 and **35** respectively, are present. However, if the evolution of the species present in solution is monitored over time the picture changes significantly. After eight hours, higher oligomers, up to A_6 and C_6 are observed. After four days, the concentration of higher oligomers had diminished and the amount of cyclic dimer **35** present had increased significantly.

Finally, after 12 days, the only species present in solution is the cyclic dimer **35**. This behaviour is rationalised by considering the process as a dynamic equilibrium. The first species formed is the acyclic dimer A_2, and the subsequent formation of the cyclic dimer is expected to be favoured because it is intramolecular. However, if the effective molarity for this process is relatively low, then some A_2 will be diverted into the formation of higher oligomers, both cyclic and acyclic. However, since the cyclic dimer **35** is the thermodynamic product of the reaction, and all the bond-forming reactions are reversible, all of the material in the reaction will eventually be recycled back through the equilibrium manifold to become cyclic dimer. Thus, the conformational space accessible to the monomer **34** predisposes the monomer to the formation of the cyclic dimer **35**.

5 Dynamic combinatorial libraries

Combinatorial chemistry[68] is a rapidly growing field allowing the fast synthesis of a large number of potentially useful compounds, followed by separate screening for active compounds. The library is prepared using irreversible covalent chemistry, and a method for the screening of each component of the library must be developed. The generation[69] of dynamic combinatorial libraries (DCLs), utilising reversible covalent formation or non-covalent bonds, is a more recent development that represents a logical extension of the chemistry described in the previous section. Reaction of an initial component set potentially leads to the creation of a highly diverse array of products. However, since all these reactions are under thermodynamic control, the final product distribution will be influenced by the relative thermodynamic stability of each product. Whereas static combinatorial chemistry does not allow for any post-synthetic transformations, a dynamic combinatorial library can be biased by the addition of different receptors or templates.[70] Indeed, some components of the library may not exist in significant amounts in the absence of a suitable binding partner[71], hence the description[72] of this process as a virtual combinatorial library. Once a receptor is bound to a particular library member, the equilibrium will tend to shift in favour of that component in order to maintain the equilibrium. Thus, it is possible to select from the library the structure most suitable for the task for which it is required. Theoretically, it may even be possible to reduce the library to a single component, making isolation of the active species simple. Furthermore, the equilibrium population may be fixed by performing an irreversible covalent conversion. Dynamic combinatorial chemistry has also been described in the literature as molecular evolution, targeted equilibrium shifting, thermodynamic templating, receptor-driven ligand evolution and adaptive chemistry. The relevance of this approach to the efficient generation of receptors, substrates, catalysts and other substrate-specific reagents has already been demonstrated. The target-driven assembly of and selection from a virtual combinatorial library may proceed along one of two courses—molding or casting (Fig. 15). Both of these processes employ the reversible combination of a constituent set, generating a potentially large library, followed by the recognition-led selection of a particular product. However, they differ in the nature of the template used. Molding describes the directed assembly of the best receptor for a target substrate—using the substrate as an *exo*-receptor. Casting utilises a target receptor to direct the assembly of the optimal substrate from the virtual substrate library—using the target as an *endo*-receptor.

Eliseev and Nelen reported[73] one of the first examples of targeted equilibrium shifting in 1997. Their system, designed to select an anionic receptor for arginine, was based on the photochemical interconversion of the three isomeric forms of an

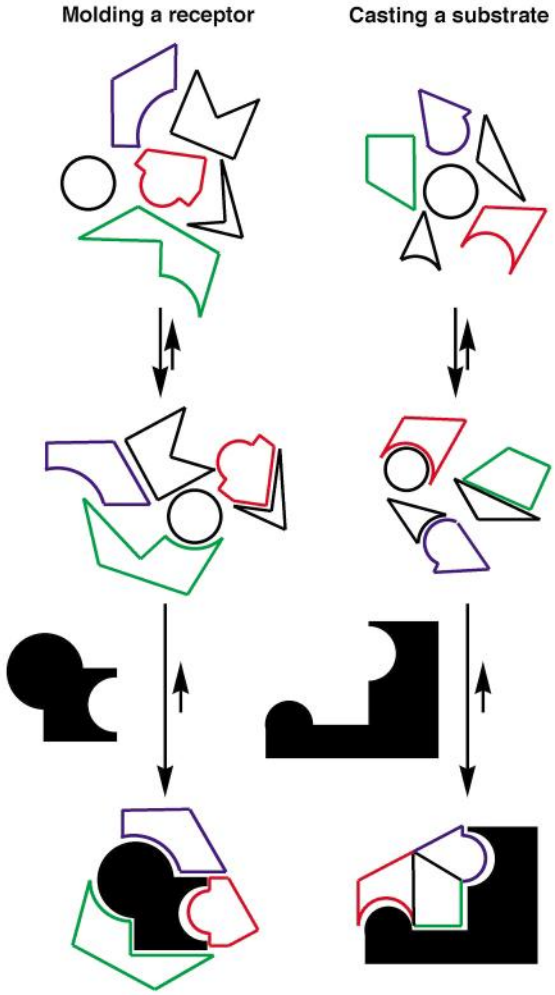
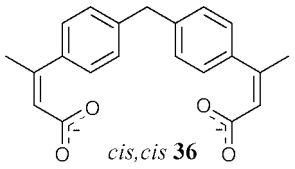


Fig. 15 Applications of dynamic combinatorial libraries—molding a receptor and casting a substrate.

unsaturated dicarboxylate **36**. Binding constant determination in ethanol showed that, as expected, the *cis*,*cis* isomer was ideal for binding methylguanidinium hydrochloride, whereas the *cis*,*trans* and *trans*,*trans* isomers showed much weaker binding. Execution of the equilibration and binding experiment was effected using a two-chamber system. Ultra-violet irradiation of the receptor results in the formation of a distribution of isomers (*cis*,*cis*:*cis*,*trans*:*trans*,*trans* = 3:8:89) which is then passed through an affinity column consisting of the arginine substrate bound to a silica gel support. The *cis*,*cis* isomer binds to the arginine and is retained on the column. The remaining mixture, which is now depleted in the *cis*,*cis* isomer, is then passed back through the irradiation chamber, in order to regenerate the initial distribution of isomers. After 30 mutation and selection cycles the bound receptor is washed from the affinity column. The ratio of *cis*,*cis*:*cis*,*trans*:*trans*,*trans* is now 85:3:2. Eliseev believes[73] that the physical separation of the equilibrium and binding sites will eliminate any problems associated with possible side-reactions/binding events when the target compound is also present in the mixture. This example, as for many other early examples[74] of dynamic combinatorial chemistry, relied on prior identification of a suitable receptor for a specific target, followed by the generation of a very small set of components. More recent illustrations of chemical evolution[75] show the practical potential with larger reactant libraries.

In 1997, Lehn and Huc demonstrated[76] a practical application of the casting approach in dynamic combinatorial chemistry, targeted at the identification of an inhibitor for carbonic anhydrase II (CA). The formation of imines by the reversible reaction of aldehydes and amines was chosen as a suitable reaction for generating the library, since it is fast and takes place



cis,cis **36**

at physiological pH and temperature. Furthermore, reduction of the imines with $NaBH_3CN$ allows the products to be fixed readily for isolation and analysis. In the presence of CA, it is expected that the distribution of imines formed will mirror their strength of binding to the enzyme active site. In the absence of CA, 15 reduction products and 4 starting amines arising from the dynamic library were isolated. In the presence of CA, the products from the dynamic library were biased in favour of those with the highest affinity for the CA active site.

Work by Sanders and co-workers towards the development of a thermodynamic library for the generation of macrocyclic cinchona alkaloid condensation products has already been discussed above. This system was not thought to be ideal, as the exchange reaction requires fairly harsh conditions (refluxing in toluene, with KOMe and 18-crown-6 as catalyst). Accordingly, they have developed libraries that operate under milder conditions, for example using trifluoroacetic acid as a catalyst for the generation[77] of a pseudo-peptide hydrazone DCL (Fig. 16) from monomer **37**. Electrospray mass spectrometry confirms the presence of 10 cyclic oligomers, including **38**, **39** and **40**, which, they reason, must interconvert *via* 10 or more related linear oligomers, although these are not detectable using mass spectrometric or HPLC analyses. However, under acidic conditions, the hydrazide functionality of these linear species will be protonated and therefore susceptible to binding by a crown ether compound. Addition of 18-crown-6 **41** to the equilibrated mixture causes a considerable equilibrium shift, and analysis by HPLC shows a decrease in the concentration of large macrocycles and the appearance of a new species that was not previously detected. Although this cannot be isolated, it is identified as the aldehyde monomer **42**. This result—the amplification of an initially trace product to a major product—demonstrates the large amplification possible using a DCL. This pseudopeptide methodology has been extended[78] to the selection of a receptor for *N*-methyl alkylammonium cations. Another example,[79] involving the templating of the palladium-catalysed transesterification of three porphyrin dimers, has also been described.

Further examples of the use of targeted equilibrium shifting include those reported[80] by the laboratories of Reinhoudt, Albrecht and Miller. Although there are some areas where

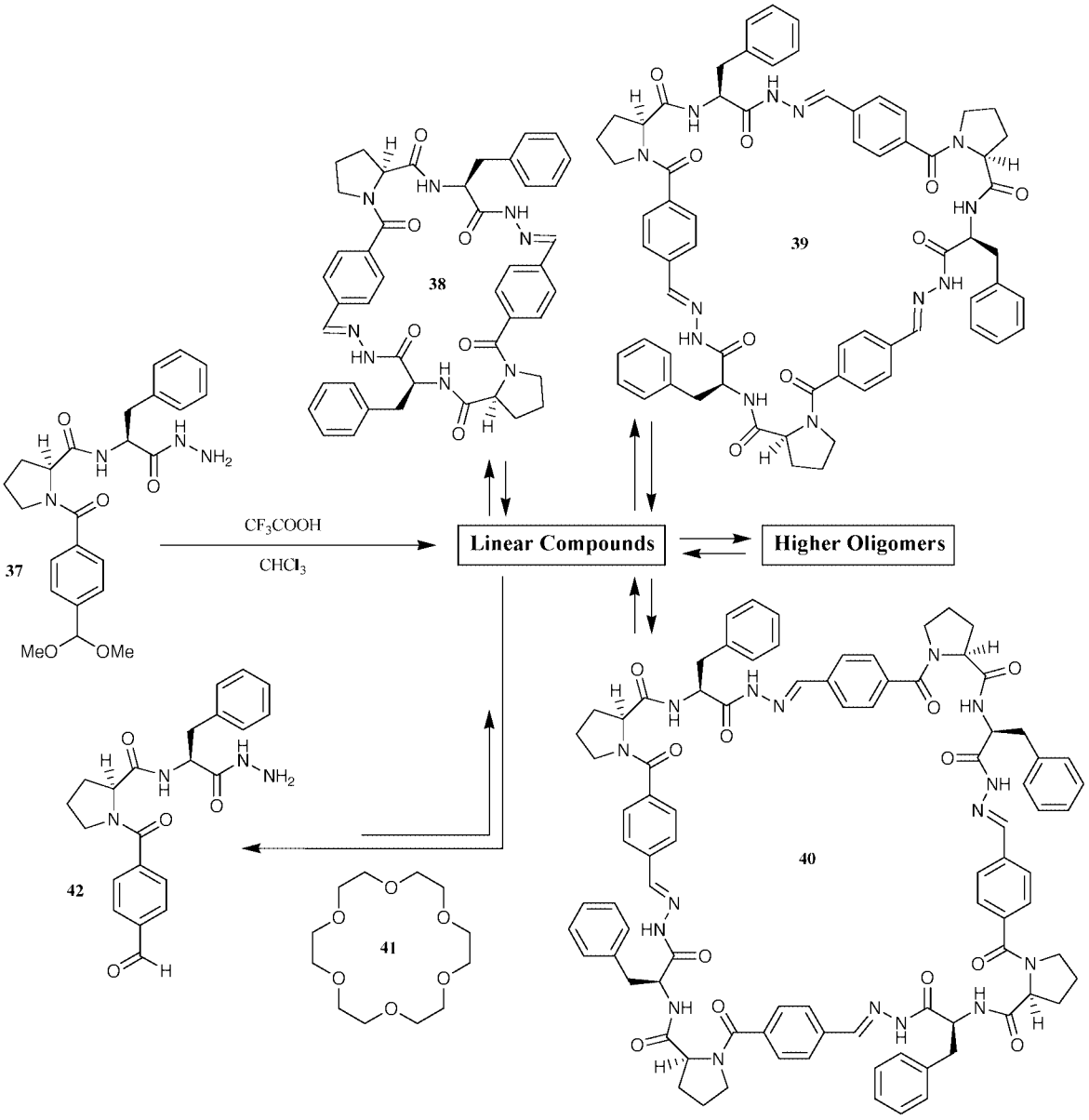


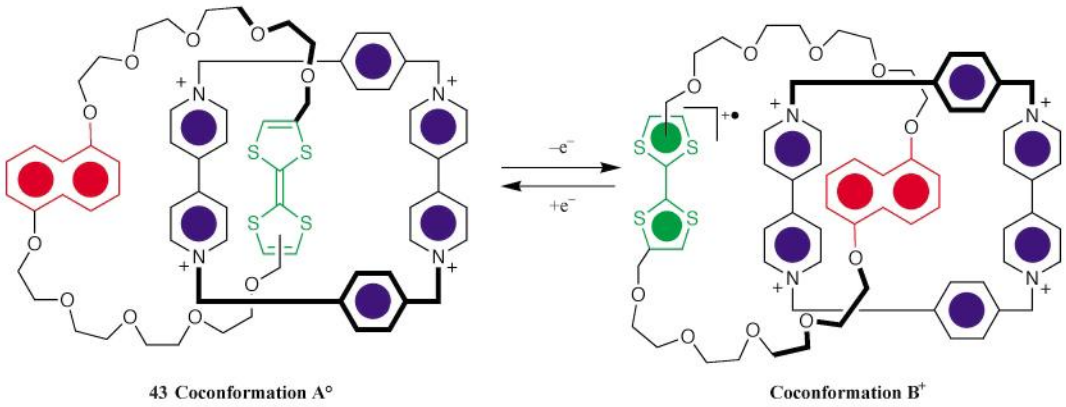
Fig. 16 The distribution of products formed by monomer **37** is influenced strongly by introduction of 18-crown-6 **41**.

dynamic combinatorial chemistry may not prove useful[81] these experiments demonstrate that dynamic combinatorial chemistry is a versatile approach with many possible applications.

6 Towards micro- and macroscopic self-assembling systems

In the 1960's Moore first observed that the size of transistors decreases by a factor of 2 every 18 months. However, the size limits of current photolithographic techniques are rapidly being approached[82], providing the impetus for the search for innovative methods of synthesising both nanoelectronic devices and materials with new magnetic, electronic or optical properties. There are two major approaches to this goal. The first is to use supramolecular chemistry to fabricate 'micromachines'[83] which mimic their biological counterpart. Indeed, Nature has long been using the 'bottom-up' approach to create[84] complex devices such as bacterial flagella, transmembrane ion channels and cell proton pumps. The second approach concentrates on the construction of components which are direct analogues of existing electronic devices, but which are synthesised using a 'bottom-up' approach. A further related tactic combines these approaches, using biological principles and/or interactions to direct the formation of 'non-biological' assemblies.

Peptide- and protein-based tube structures are prevalent in Nature. Cyclic peptide structures with alternating D- and L-amino acids form flat rings, which in turn stack[85] to give hydrogen-bonded tubes. Altering the amino acid side chains and the peptide ring size can vary the properties of the three-dimensional structures. Addition of hydrophobic side chains allows the nanotubes to cross into a lipid layer, so that it can function in a transmembrane channel. The final criterion is that the tubes must be positioned approximately perpendicular to the lipid membrane itself, in order that it should be traversable.



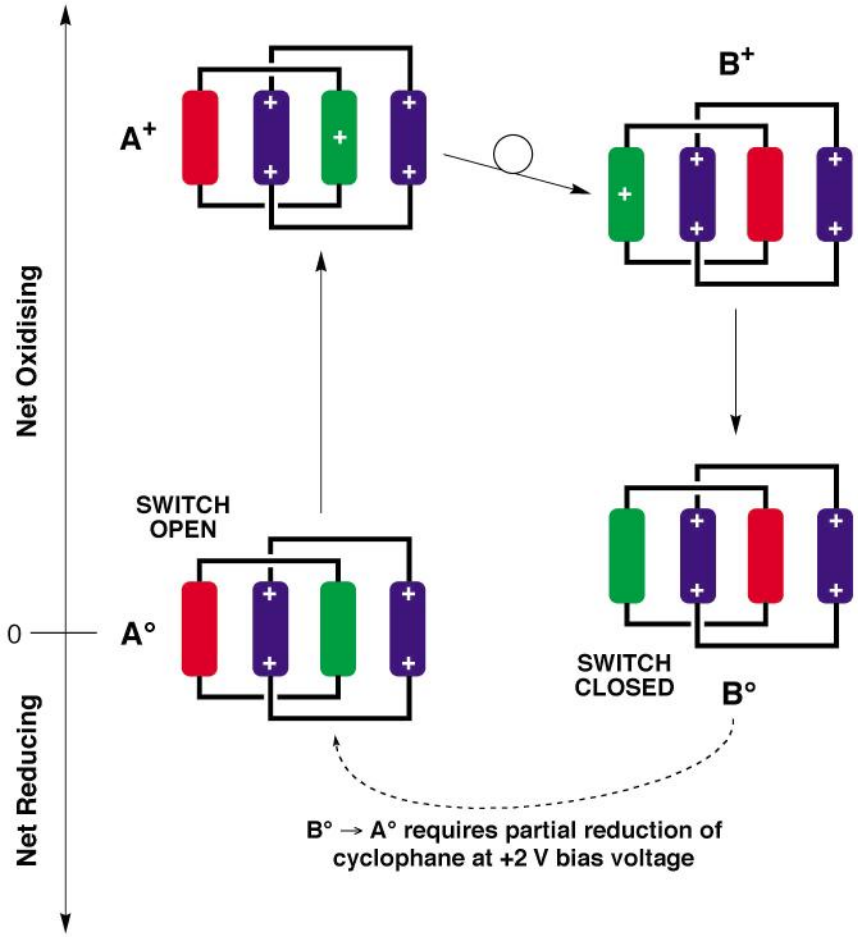


Fig. 17 An electronically reconfigurable electronic switch based on a [2]catenane.

Several routes have been investigated in the search for practical non-lithographic techniques for the construction[86] of electronic devices. The most successful route is that based on the use of interlocked molecules—rotaxanes and catenanes[87]—as molecular switches constructed by self-assembly processes. Several groups have successfully constructed interlocked molecular architectures that respond to external stimuli[88], including those of Sanders,[89] Sauvage[90], Leigh[91] and others.[92] However, the largest body of work in this area has emerged from the laboratories of Stoddart who has constructed a huge array of complex molecular and supramolecular structures based on recognition-mediated assembly processes. The reader is directed to the many excellent reviews[93] of this body of work for further details. More recently, this group has turned its attention to the construction of bistable molecular devices that are deposited on electrodes and act as true electronic devices. The [2]catenane **43** has a resting state[94] (A°, Fig. 17) in which the tetrathiafulvalene (TTF) ring system is encapsulated by the tetracationic cyclobis(paraquat-*p*-phenylene) ring. Oxidation of the TTF generates the corresponding radical cation and coulombic repulsion between the $TTF^{+\cdot}$ ring and the tetracation causes circumrotation of the rings with respect to each other and the formation of structure B^+. This voltage-driven bistable behaviour is exploited[95] as the basis of a solid state electronically reconfigurable switch. Using appropriate techniques, a device can be constructed based on this bistable behaviour. The active component of the device is a Langmuir–Blodgett film composed of the [2]catenane **43** and an anchor phospholipid. Coconformer A° represents the 'switch-open' state of the device. Application of a −2 V bias to the device results in the oxidation of the TTF unit (green, Fig. 17) in [2]catenane and structure A^+ is formed. The TTF unit then experiences a coulombic repulsion from the tetracation (blue, Fig. 17), resulting in circumrotation and formation of structure B^+. Reduction of the bias voltage to near zero results in reduction of the TTF unit to give structure B°. This condition represents the 'switch-closed' state of the device. The switch can only be opened, regenerating A°, by application of bias of +2 V. Analysis of the behaviour of the solid state device reveals that it operates through an almost identical mechanochemical mechanism to the solution phase.

Mann and co-workers have combined biotechnology and materials science to create[96] gold and silver nanoparticles coated with antibodies—specifically anti-dinitrophenyl (DNP) IgE or anti-biotin IgG (Fig. 18). Both a homo-Janus (DNP–DNP) **44** and a hetero-Janus (DNP–biotin) antigen **45** were also synthesised. Mixing the appropriate antigen with a solution of the appropriately antibody coated nanoparticle results in the formation of a cross-linked microscopic structure with the idealised structures shown in Fig. 18. Adding the hetero-Janus antigen to a mixture of both the IgE and the IgG coated nanoparticles gives even higher order gold filaments. It is believed that the increased flexibility of the hetero- over the homo-antigen encourages directional order. This work clearly benefits from the high specificity of the antibody–antigen recognition process. In certain cases, however, this high degree of specificity may not always be beneficial. In the synthesis of molecular tubes from protein sheets, Atkins and Dabrowski consider[97] that the specificity of biological macromolecules is a distinct disadvantage, and obtain more useful structures from proteins into which they have introduced a mutation.

Another example of the macroscopic expression of molecular recognition processes include those described[98] by Whitesides and co-workers. They have been investigating the use of polydimethylsiloxane (PDMS) blocks as millimetre-scale extensions of the principles of molecular self-assembly. PDMS is hydrophobic, but one face and some of the sides of the plate may be rendered hydrophilic by oxidation with O_2 plasma. The plates are allowed to float at a water–perfluorodecalin interface and agitated at a specific frequency. When hydrophobic sides of two plates come within a critical distance, they move into contact to form a 'capillary bond'. A range of complex hierarchical arrays (Fig. 19) can be formed in this way, simply by varying the relative position of the hydrophobic sides. The similarities between these macroscopic systems and molecular scale recognition and assembly are highlighted by an investigation into the hierarchy of interactions connecting the plates. Permanent ('covalent') interactions are made by physically gluing the plates together, strong non-covalent bonds are represented by the interaction between concave and convex hydrophobic surfaces, and weak non-covalent interactions are those made between two concave hydrophobic surfaces. The

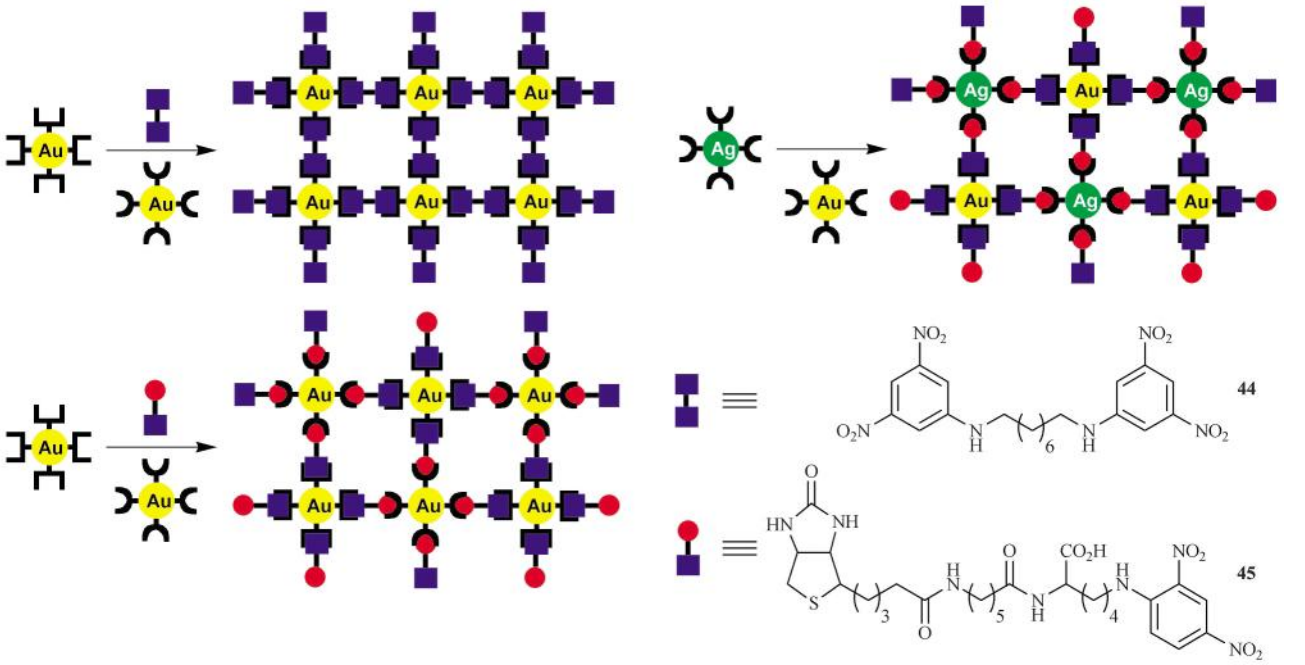


Fig. 18 Mixing antibody functionalised nanoparticles with appropriate antigens leads to the formation of networks. The networks are shown in an idealised form—in reality, the structures which form are highly disordered.

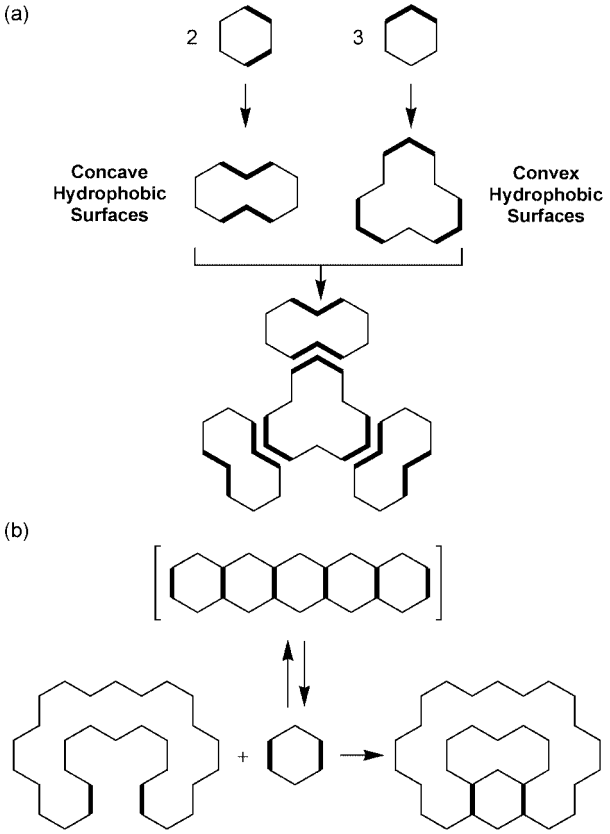


Fig. 19 (a) Hierarchical assembly using 'capillary' bonds. (b) Receptor-like behaviour in a system using 'capillary' bonds.

latter two types of interaction may give rise to a variety of assemblies, but the components can be designed to give specific arrays that maximise the favourable interactions. Furthermore, shape- and size-selective systems have been developed (Fig. 19b). However, there are some substantial differences between the interactions of polymer plates and the interactions of molecules. Firstly, collision frequencies are far lower for macroscopic than for molecular systems, and secondly, the relative energies of the polymer plates are likely to be similar, rather than being described by a Boltzmann distribution.

7 The future

Over the past ten years, the field of synthetic self-assembling systems has expanded dramatically both in terms of the volume of research output and the complexity of the systems described. This expansion has been driven by the development and application of a fundamental knowledge of how complex biological structures self-assemble and operate. There is now no doubt that self-assembly processes can be exploited by chemists to construct a wide range of synthetic structural types with ease.

To date, the establishment of the principles governing the development of efficient self-assembly processes has perhaps been more important than building function into these self-assembled structures. This approach can be justified on the basis that a 'bottom-up' approach to molecular-scale devices not only requires the construction of the devices themselves, but also the infrastructure to support them and to interface them with the outside world. The challenge for the future is to build designed function into self-assembled superstructures and to interface that function efficiently with the macroscopic world. Although the first steps in this direction have already been taken, the success of 'bottom-up' approaches will ultimately rely on the ability of chemists to exploit the interplay of construction methods based on self-assembly with techniques of selection and amplification which operate at a molecular level.

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