Supramolecular Chemistry

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Dynamic Formation of Hybrid Peptidic Capsules by Chiral Self-Sorting and Self-Assembly**

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Abstract: *Owing to their versatility and biocompatibility, peptide-based self-assembled structures constitute valuable targets for complex functional designs. It is now shown that artificial capsules based on β-barrel binding motifs can be obtained by means of dynamic covalent chemistry (DCC) and self-assembly. Short peptides (up to tetrapeptides) are reversibly attached to resorcinarene scaffolds. Peptidic capsules are thus selectively formed in either a heterochiral or a homochiral way by simultaneous and spontaneous processes, involving chiral sorting, tautomerization, diastereoselective induction of inherent chirality, and chiral self-assembly. Self-assembly is shown to direct the regioselectivity of reversible chemical reactions. It is also responsible for shifting the tautomeric equilibrium for one of the homochiral capsules. Two different tautomers (keto-enamine hemisphere and enol-imine hemisphere) are observed in this capsule, allowing the structure to adapt for self-assembly.*

Self-sorting processes, which involve the formation of well-defined subsystems from mixtures containing many different components and facilitate the synthesis/organization of well-defined functional structures, are universal in Nature. In chemistry, the applicability of self-sorting is restricted by requirements of high relative thermodynamic stability of particular products and reversibility of their formation.[1] In this regard, a combination of dynamic covalent chemistry (DCC), which exploits reversible chemical reactions,[2] and self-assembly, which is able to provide substantial stabilization, has been recognized as a very useful method for designing a variety of synthetic self-sorting systems.

Peptides, with their natural tendency to form specific secondary structures and their capability to produce novel biocompatible materials,[3] are valuable components of self-sorting mixtures that are driven by self-assembly.[4] However, short peptides remain challenging building blocks owing to their inherent flexibility, which makes precise complementarity between interacting molecules (required to keep the entropic costs of ordering as low as possible) difficult to achieve. Nevertheless, short peptides can still become structure-ordering and biocompatible fragments of self-sorting systems when their ordering is reinforced by interactions provided by additional groups. Examples of such hybrid designs have been illustrated by the groups of Otto[5] and Ulijn.[6] Among the possible peptide structural motifs, β-sheets are recognized as the most reliable secondary structure, and they are therefore predominately used in dynamic self-sorting systems, producing various fibrous structures.[3e,6,7] The formation of discrete, well-defined peptidic structures, such as virus-like cages, is more demanding and usually requires either the use of larger protein motifs[8] with oligomerization properties programmed by genetic methods[9] or the binding of small chemical molecules[10] or metal-mediated assembly.[11] Although the construction of discrete self-assembled structures using short peptides has already been reported,[12] their formation by means of dynamic covalent chemistry remains an unexplored area. Herein, we report the results of our efforts to create discrete capsules by self-assembly and self-sorting of short peptides at the macrocyclic scaffold.

Tetraformylresorcin[4]arene **1** was used as a macrocyclic scaffold as it possesses a proper vase-like shape and easily reacts with aliphatic and aromatic primary amines to form imines in a reversible process.[13] It has been reported that the resulting imines quantitatively tautomerize to their keto-enamine forms (Scheme 1a,b). Owing to the isomerism of the double bond and the inherent chirality of the bowl-shaped molecule, for an optically pure chiral amine, the formation of six different isomers is possible (Table S1). However, a network of intramolecular hydrogen bonds drives the equilibrium towards two diastereoisomers (with C_4 symmetry and either *M* or *P* inherent chirality, Scheme 1b), albeit with very low diastereoselectivity.[13]

Currently, we used the short peptides **2a**–**5** containing L- or D-amino acids as the amine components of the imine forming reaction. Sequences with alternating hydrophobic (Phe) and hydrophilic (Gly) amino acids were chosen inspired by natural β-barrels. It should be noted that attempts have previously been made to construct self-assembled capsules based on peptides covalently attached to calixarene-type skeletons.[14] Although many interesting applications have been reported for these products (e.g., effective cell transfection),[15] self-assembly was only detected in rare cases and characterized by moderate association constants.[12c] None of

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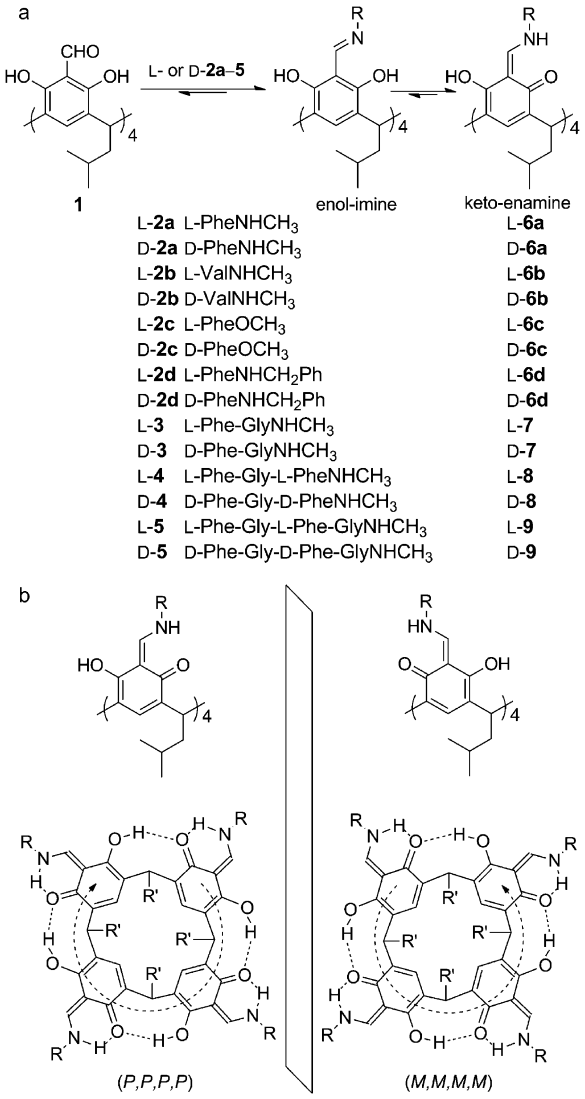

Scheme 1. a) Reversible imine formation with **1** and with various peptides and reversible tautomerization. b) C_4-symmetric diastereoisomers.

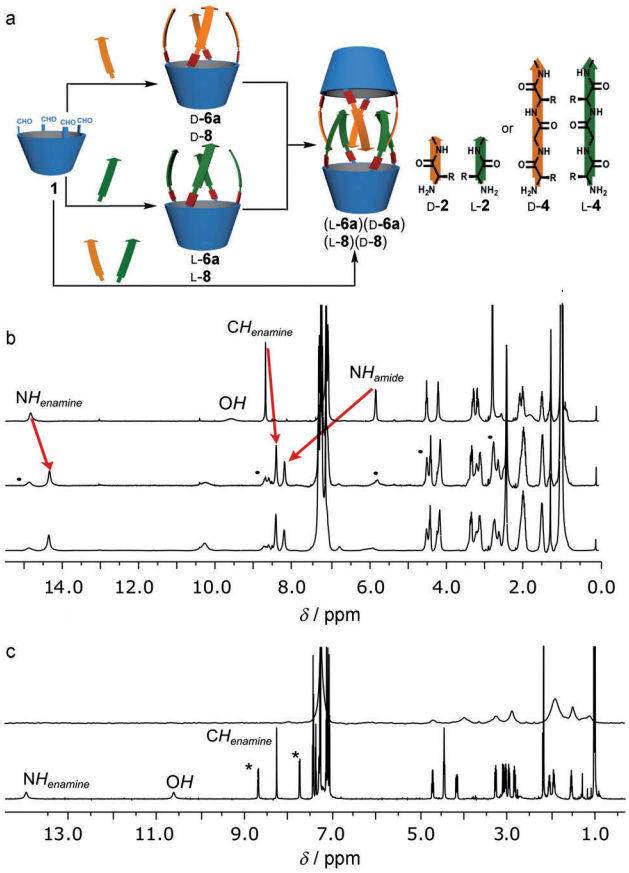

Figure 1. Heterochiral capsules obtained by self-sorting. a) Formation of capsules by self-assembly of pre-synthesized hemispheres (top) or by self-sorting (bottom). b) ^{1}H NMR spectra of L-**6a** (top), a mixture containing L-**6a** and D-**6a** (middle), and of the reaction mixture of the self-sorting process leading to (L-**6a**)(D-**6a**) (bottom). ●: Peaks that are due to the non-associated hemisphere. Spectra recorded in $CDCl_3$ at 298 K and 400 MHz. c) ^{1}H NMR spectra of L-**8** (top) and of the reaction mixture of the self-sorting process leading to (L-**8**)(D-**8**) (bottom). Spectra recorded in $CDCl_3$ at 298 K and 600 MHz. *: $-NH_{amide}$.

these systems has been shown to assemble in a dynamic covalent way.

The reaction of **1** with amino acid derivative L-**2a**, which contains a methylamide-functionalized C terminus, yielded a single diastereoisomer, namely L-**6a** (Scheme 1a). Thus, the reaction proceeded with high regioselectivity and also high diastereoselectivity (*de* > 95%, based on NMR analysis). Upon mixing of L-**6a** with its enantiomer D-**6a** in $CDCl_3$, a new set of signals emerged in the NMR spectrum in addition to the original signals, indicating the formation of new, heterochiral species (Figure 1b). In solution, they exhibited average S_8 symmetry with a substantially upfield-shifted amide NH signal. ROESY and DOSY spectra (see the Supporting Information, Figures S9 and S10, Table S2) indicate that the heterochiral species are capsular hydrogen-bonded dimers of the composition (L-**6a**)(D-**6a**) with $K_{dim} \approx 180\,M^{-1}$ (calculated from the NMR spectrum; Figure S8). X-ray analysis confirmed that the dimer (L-**6a**)(D-**6a**) had been formed by interdigitation of the peptide backbones and was sealed by a seam of hydrogen bonds (Figure 3a) as also postulated for the species in solution. In the solid state, the seam of hydrogen bonds is partially disturbed by the protruding guest molecules (toluene).

To explore the self-sorting ability, we carried out a one-pot reaction between **1** and a racemic mixture of L-**2a** and D-**2a** (Figure 1a). It should be noted that the statistical yield of heterodimer (L-**6a**)(D-**6a**) was expected to be $(2/2^8) \times 100\% \approx 0.78\%$. However, in this case, the reaction gave a mixture that was identical to that obtained in the self-assembly experiment (as demonstrated by the identical ^{1}H and ^{13}C NMR spectra, Figure 1b and Figure S7), indicating social chiral self-sorting ("non-self", i.e., occurring between different species).[1b] To confirm that this chiral self-sorting process was driven by self-assembly, control experiments were carried out with amino acid derivatives **2c** or **2d**, which, upon reaction with **1**, gave products incapable of self-assembling. In both cases, complicated product mixtures

were obtained, indicating that no chiral sorting had occurred in the absence of self-assembly (Figure S17 and S18). Additional control experiments were also performed to demonstrate the reversibility of the imine formation reactions (scrambling experiments, Figure S19).

In the case of dipeptides L-**3** and D-**3**, the reaction of **1** with either enatiomerically pure L-**3** or with a racemic mixture of L-**3** and D-**3** led to products having identical NMR spectra (Figure 2a, b, Figures S22, S23). 2D NMR spectra indicated that capsular homochiral self-assembled dimers were formed exclusively in both reactions [i.e., (L-**7**)(L-**7′**) for enantiomerically pure L-**3**]. Therefore, the reaction proceeded with complete chiral narcissistic self-sorting ("self", occurring between the same species)[1b] and with a high dimerization constant as no monomeric products were detected. Even though the two hemispheres of dimer (L-**7**)(L-**7′**) were chemically equivalent, they were not identical, as the imine linkers were present in different tautomeric forms. One of the hemispheres contains four linkers in the typical keto-enamine forms. This hemisphere exhibits a ^{15}N NMR signal at −205 ppm (characteristic of an enamine) and a set of ^{13}C NMR signals characteristic of a ketone (δ 170.6 ppm for the ketone C atom). On the other hand, the opposite hemisphere features linkers in their enol-imine forms, an unknown occurrence for compounds of this type. The enol-imine hemisphere exhibits a ^{15}N NMR signal at −103 ppm (typical for an imine) and ^{13}C NMR signals at typical values for an enol (Figure S20–S25).

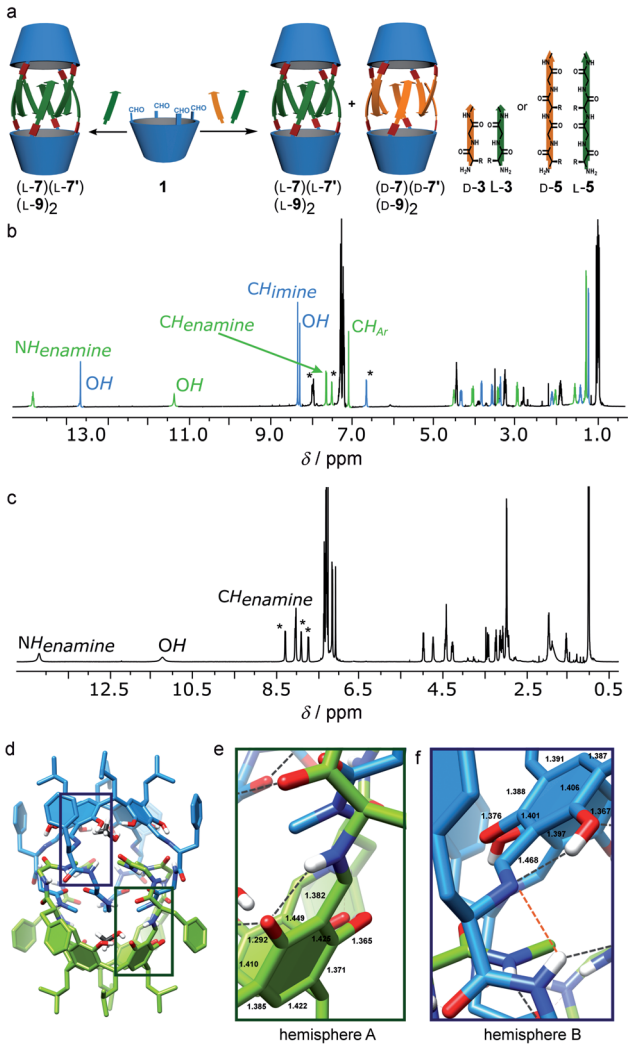

Figure 2. Homochiral capsules obtained by self-sorting. a) Formation of capsules by self-assembly of hemispheres (left) or by self-sorting (right). b) ^{1}H NMR spectrum of (L-**7**)(L-**7′**). Blue: signals of the enol-imine form; green: signals of the keto-enamine form; black: overlapping signals. *: $-NH_{amide}$. Spectra recorded in $CDCl_3$ at 298 K and 600 MHz. c) ^{1}H NMR spectrum of (L-**9**)$_2$ ($CDCl_3$, 298 K, 600 MHz). *: $-NH_{amide}$. d) X-ray structure of (D-**7**)(D-**7′**). e) Enlarged structure of the keto-enamine part of (D-**7**)(D-**7′**). f) Enlarged structure of the enol-imine part of (D-**7**)(D-**7′**). Gray dashed lines: hydrogen bonds; orange dashed line: short non-covalent interactions.

The structure of the capsular dimer (L-**7**)(L-**7′**) was also confirmed by X-ray analysis (Figures 2d–f, 3b). The sample was crystallized from a reaction mixture containing **1** and racemic dipeptide (L-**3** and D-**3**). The crystals of the product exhibited the centrosymmetric space group *P*4/*n* meaning that both enantiomers are present in the structure. However, each single capsule consists of eight dipeptides of the same chirality. This observation corroborates that narcissistic chiral self-sorting has taken place. The dimer is sealed by a seam of twelve hydrogen bonds between the peptide backbones. Comparative analysis of the bond lengths, torsion angles, and non-covalent interactions indicates that the two hemispheres indeed have different tautomeric forms. The keto-enamine hemisphere (A, Figure 2e) was characterized by four of the C_{ar}–O bonds being substantially shorter than the remaining four, an asymmetric bond-length distribution for the dearomatized rings, and a co-planar arrangement of the rings with respect to the enamine moiety. The enol-imine hemisphere (B, Figure 2f) displays C_{ar}–O bonds that are all of the same length and considerably twisted arrangements of the aromatic rings and the imine moieties. Thus, the X-ray structure confirms the homochiral arrangement of (D-**7**)(D-**7′**) with one of the hemispheres assuming a more flexible, albeit less preferred, enol-imine tautomeric form to adapt for self-assembly.

The self-sorting experiments were also carried out for tri- and tetrapeptides. For the tripeptide, the reaction between **1** and enatiomericaly pure L-**4** or D-**4** proceeded (as indicated by TLC), but the homochiral products were not soluble in $CDCl_3$, presumably owing to non-specific aggregation. On the contrary, the reaction between **1** and a racemic mixture of L-**4** and D-**4** gave a single product, which was was identified by ROESY and DOSY spectroscopy as heterochiral dimer (L-**8**)(D-**8**) (Figure 1c; see also Figures S32 and S34 and Table S2). The capsular structure of (L-**8**)(D-**8**) was also confirmed by X-ray crystallography (Figure 3c). In the solid state, the binding motif involves the formation of 24 hydrogen bonds. The structure is self-complementary, that is, all possible hydrogen bond donors and acceptors are involved in interactions with neighboring peptide chains. A similar binding motif was also postulated in solution based on ROESY spectroscopy (Figure S32). In contrast to the previous capsules, the internal cavity of the (L-**8**)(D-**8**) capsule is

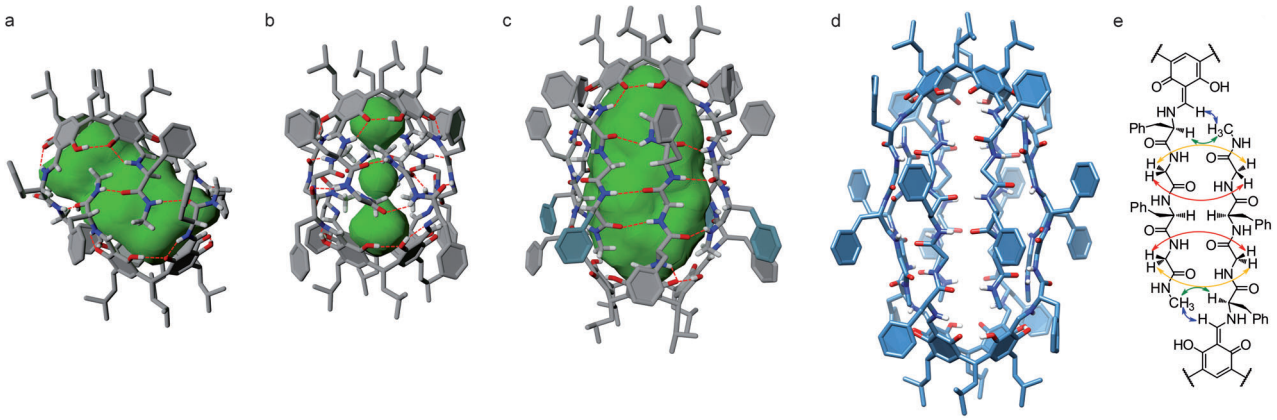

Figure 3. Structures of the peptidic capsules. X-ray structures of a) (L-**6a**)(D-**6a**), b) (D-**7**)(D-**7′**), and c) (L-**8**)(D-**8**). d) Modelled structure of (L-**9**)$_2$. e) Experimental NOE correlations for (L-**9**)$_2$. H atoms attached to C atoms omitted for clarity. Carbon gray or pale blue, hydrogen white, nitrogen blue, oxygen red. Green surfaces: molecular surfaces of the interior of the capsules calculated with the Chimera program using the MSMS method with a probe size of 1.4 Å.

quite spacious (883 Å^3), offering the possibility of accommodating suitable guest molecules.

Self-assembled capsular dimers were also formed by self-sorting using tetrapeptides L-**5** and D-**5**. Similarly to the dipeptides, tetrapeptides L-**5** and D-**5** also gave homochiral dimers, (L-**9**)$_2$ and (D-**9**)$_2$ (Figure 2a,c). Dimer (L-**9**)$_2$ has the lowest diffusion coefficient among all of the capsules presented herein (Table S2), in agreement with the largest radius of the species. The observed NOEs indicated deep interdigitation of the peptide chains and suggested a binding motif resembling the previous barrel structures (Figure 3d,e; see also Figure S39, Table S2). In the current homochiral structure, the change in the tautomeric form and the deep self-encapsulation of the terminal methyl groups, as observed for the smaller homochiral (L-**7**)(L-**7′**) dimer, were not observed. This can be attributed to the higher flexibility of a tetrapeptidic structure, which allows for adaptation without sacrificing its most stable tautomeric form.

The self-sorting phenomenon was observed not only between peptides of different chirality but also between peptides of different lengths (Figure S42). This observation underlines the importance of precise complementarity of binding motifs for effective self-sorting. Similarly, complementarity of the binding motifs for peptides with even and odd number of amino acids provides a tentative explanation for homochiral versus heterochiral selectivity (Figure S43).

In summary, we have designed and synthesized new self-assembled capsules based on short peptides of various chiralities. A great advantage of the approach, which combines dynamic covalent synthesis with self-assembly, is that through the use of very short sequences and a macrocyclic skeleton that provides additional preorganization, it is possible to induce self-organization of complex nanosized chiral molecular containers. The dynamic character of a covalent linkage offers unique possibilities for controlling integration–disintegration processes. Together with potential encapsulation properties, dynamic libraries of such peptidic capsules constitute a promising approach for the development of new materials with the prospect of gaining properties, such as controlled cargo-release or chirality-driven self-healing.

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