Systems Chemistry

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An Allosteric Receptor by Simultaneous "Casting" and "Molding" in a Dynamic Combinatorial Library**

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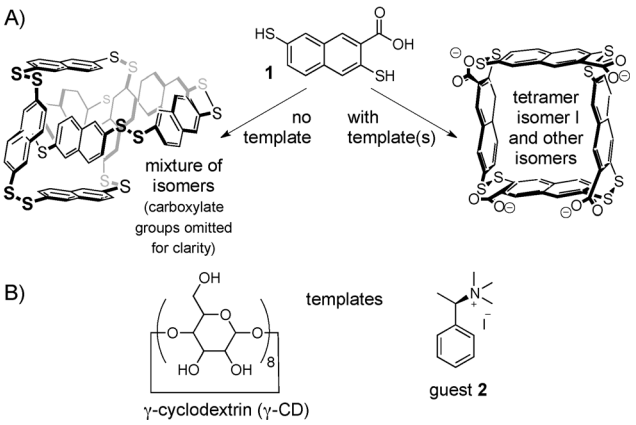
Abstract: *Allosteric synthetic receptors are difficult to access by design. Herein we report a dynamic combinatorial strategy towards such systems based on the simultaneous use of two different templates. Through a process of simultaneous casting (the assembly of a library member around a template) and molding (the assembly of a library member inside the binding pocket of a template), a Russian-doll-like termolecular complex was obtained with remarkable selectivity. Analysis of the stepwise formation of the complex indicates that binding of the two partners by the central macrocycle exhibits significant positive cooperativity. Such allosteric systems represent hubs that may have considerable potential in systems chemistry.*

The development of synthetic molecules that are able to bind to other molecules with high affinity and selectivity remains a considerable challenge, even for the simplest case of 1:1 host–guest complexes.[1] The challenge becomes even greater when aiming to develop molecules that can bind two or more partners simultaneously. Yet such systems are important stepping stones for building more complex functional systems, as they may act as hubs that control functional behavior. Prime examples are allosteric receptors (i.e. molecules that can bind two or more partners)[2] where binding occurs cooperatively,[3] that is, binding of the first partner affects the affinity with which subsequent partners bind and vice versa. Developing such systems by rational design, although not without success,[4] is often an iterative and time-consuming process, prompting us to explore an alternative selection approach using dynamic combinatorial libraries (DCLs).[5]

In dynamic combinatorial chemistry relatively simple building blocks are linked through a reversible reaction, giving rise to a mixture of interconverting library members that is typically under thermodynamic control. It is now well established that molecular recognition may shift the library composition, maximizing the noncovalent interactions in the system, thus amplifying the concentration of the molecules engaged in molecular recognition. Thus, the addition of small-molecule templates may result in amplification of synthetic receptors[5g,6] that are formed around the template (casting).[7] Alternatively, when using biomolecules as templates, small-molecule ligands may be amplified that form inside binding pockets[5j,8] (molding).[7]

We reasoned that by the simultaneous use of two templates we may be able to access allosteric receptors that are formed through a process of simultaneous casting and molding. Herein we report the first successful implementation of this strategy, which resulted in the formation of a synthetic receptor exhibiting positive allosteric cooperativity.

The design of our system is based on two established interaction motifs: the ability of γ-cyclodextrin (γ-CD) to bind up to two naphthalene rings in its hydrophobic cavity[9] and the ability of naphthalene building block **1** (Scheme 1) to



Scheme 1. A) Dynamic combinatorial libraries of building block **1** without or with different templates. B) Structures of the template molecules.

form dynamic combinatorial libraries containing receptors for hydrophobic ammonium ions.[10] Thus, γ-CD may exert a template effect through casting, whereas ammonium ions may induce the formation of a receptor around them through molding. We first investigated the response of the small dynamic combinatorial system made from dithiol **1** to the introduction of γ-CD or ammonium salt **2**. Thus, a solution of **1** (2.0 mM) in aqueous borate buffer (50 mM, pH 8.2) was allowed to oxidize in air and equilibrate for 7 days. Disulfide exchange takes place by reaction of residual thiolates with disulfides, as described previously.[11] Figure 1 A shows the composition of the library in the absence of any template, when the mixture is dominated by a series of isomeric catenanes, as described previously.[10] Figure 1 B shows that introducing γ-CD as a template leads to the amplification, through casting, of two isomers of the tetramer of **1** (isomers I

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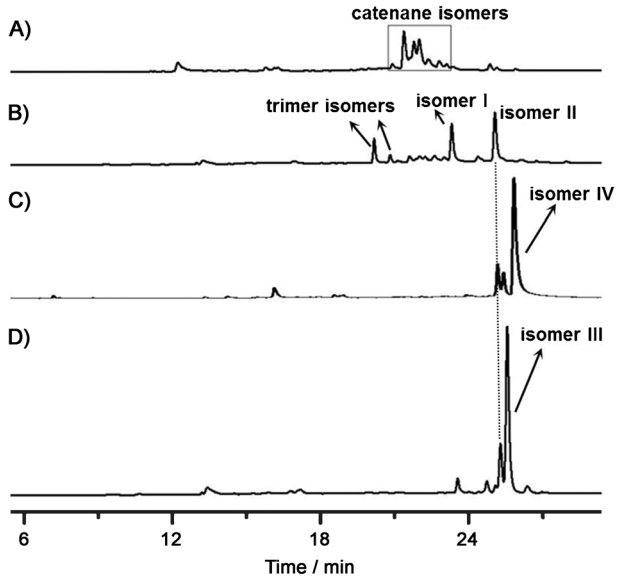


Figure 1. HPLC–MS analysis of DCLs made from a solution of building block **1** (2.0 mM) in aqueous borate buffer (50 mM, pH 8.2) A) without any added template; B) with template γ-CD (8.0 mM); C) with guest **2** (8.0 mM); and D) with both γ-CD (8.0 mM) and **2** (4.0 mM).

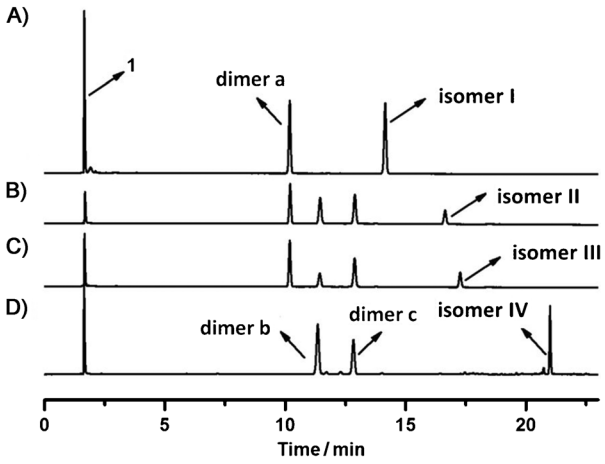
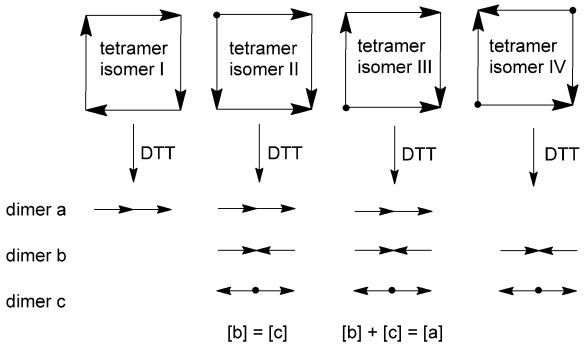


Figure 2. HPLC–MS analysis of the product distribution obtained upon partial reduction of A) isomer I, B) isomer II, C) isomer III, and D) isomer IV in borate buffer (50 mM, pH 8.2), through the addition of 0.3 equivalents of dithiothreitol per disulfide linkage.



Scheme 2. Partial reduction of the four tetramer isomers results in the formation of different mixtures of dimer isomers. A single building block is denoted by one arrow, with different tetramer and dimer configurations represented by different arrow arrangements.

and II; nomenclature based on the order of elution), alongside minor quantities of two isomeric trimer macrocycles. In contrast, ammonium template **2** induces, through molding, the formation of a different tetramer (isomer IV; Figure 1 C). Interestingly, when both templates were added together the library responded in a way that could not be predicted based on the response to the two templates in isolation, that is, the system showed emergent behavior. In this case, a fourth tetramer (isomer III) was amplified with remarkable selectivity, even though formation of this species was disfavored when only one of the two templates was present (Figure 1 D).

Although we previously described the formation of the tetramers of **1** using ammonium ions,[10] the amplification was never very selective, so we did not assign the four tetramer isomers. The present results prompted us to undertake such assignment. Thus, we isolated the four different tetrameric isomers by preparative HPLC. Inspection of the ^{1}H NMR spectra of these compounds was not informative (see Supporting Information) and we failed to obtain crystals suitable for X-ray diffraction. However, we succeeded in assigning the four isomers based on their fragmentation products upon treating them with a substoichiometric amount of reducing agent (dithiothreitol; DTT). The results of these experiments are shown in Figure 2. For all isomers we obtained only building block **1** and its dimers as fragmentation products, whereas we did not detect any linear trimer and tetramer. Molecular dynamics (MD) simulations suggest that most of the linear tetramers fold into a conformation in which the central disulfide bond is more exposed than the other two disulfides, which may enhance the reactivity of the central disulfide (see Supporting Information for details). Reduction of isomer I only produced one dimer (dimer a), so it must correspond to the tetramer in which all building blocks are arranged in a head-to-tail fashion (Scheme 2). Fragmentation of isomer IV yielded the two other dimers (dimers b and c), consistent with a structure in which no head-to-tail arrangements exist (i.e. no dimer a is produced). Isomers II and III fragmented to give all three dimers, but in different relative quantities. It can be shown (see Supporting Information) that fragmentation of isomer II should give equal amounts of dimers b and c ([dimer b] = [dimer c]), whereas for isomer III we expect [dimer a] = [dimer b] + [dimer c]. These relationships are indeed in good agreement with the detected product ratios. To further confirm the assignment we recorded a ^{13}C NMR spectrum of isomer III, which showed the number of signals attributable to carbonyl group expected for this least symmetrical of all isomers (see Figure S20 in the Supporting Information).

Having assigned the isomers, we proceeded to record the stepwise equilibrium constants for the formation of the termolecular complex between isomer III, γ-CD, and ammonium guest **2** using UV/Vis absorption titrations (see Supporting Information). Starting from isomer III we performed

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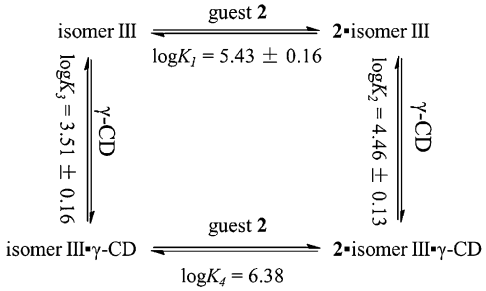
the titrations following two different orders of addition: first γ-CD followed by ammonium guest **2**, or vice versa. The titration of **2** to the preformed cyclodextrin–tetramer complex showed very tight binding which hampered the direct determination of the binding affinity. However, since we were able to obtain binding constants for the other three legs of the thermodynamic cycle (shown in Scheme 3), we could



Scheme 3. Equilibrium constants for the stepwise binding of tetramer isomer III to γ-cyclodextrin and guest **2**. The value of K_4 was calculated from the values of K_1–K_3. See Supporting Information for details.

calculate the last remaining binding constant. Note that all individual binding isotherms fitted well to a 1:1 binding model, while 2:1 binding models gave significantly poorer fits (see Supporting Information). The resulting binding constants are shown in Scheme 3 and reveal that isomer III acts as an allosteric binder. Affinity for guest **2** is enhanced by an order of magnitude when γ-CD is bound to the tetramer. Similarly, the affinity of the tetramer for γ-CD is enhanced by the same amount when guest **2** is bound.

To understand the molecular basis of the cooperative behavior of casting and molding, we modelled the systems shown in Scheme 3 using molecular dynamics in explicit water using the Amber 11 program package.[12] Isomer III and guest **2** were described with the general Amber force field,[13] and GLYCAM04[14] was used for γ-CD.

Inspection of equilibrated trajectories showed that the interactions between the components of the Russian-doll-like complex[15] are driven mostly by the hydrophobic effect, as shown in Figure 3. Isomer III remained in a closed conformation both in its free form and when bound with the cyclodextrin. On the other hand, the tetramer assumes a rhomboidal conformation when binding guest **2**. Orientation of the carboxylate groups did not seem important for the binding between γ-CD and isomer III in the bimolecular complex, as we were able to obtain stable trajectories with all charged groups pointing outward as well as with one of them inside the hydrophobic pocket of the cyclodextrin. In contrast, we could obtain only one stable trajectory of the ternary complex where all carboxylates are pointing out of the γ-CD; our attempts to simulate other configurations led to rapid (<0.5 ns) expulsion of guest **2** from the complex. This behavior suggests that the former arrangement is the preferred structure of the Russian-doll-like complex. Cooperativity is likely caused by a conformational change of isomer III upon binding of guest **2**, which makes it fit better into the toroidal cavity of γ-CD. This behavior is reflected in

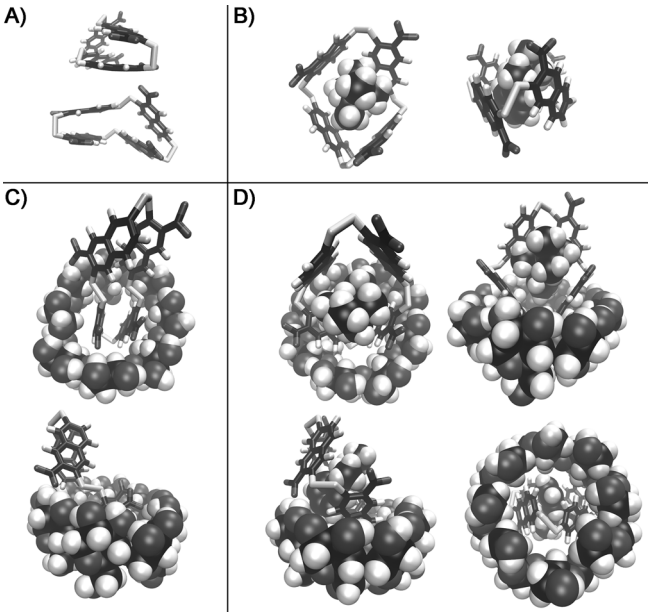


Figure 3. Representative structures obtained by MD simulations with the General AMBER Force Field in water. A) Isomer III, B) guest **2** inside isomer III (casting), C) isomer III inside γ-CD (molding), and D) a "Russian doll" composed of γ-CD, isomer III, and guest **2** (simultaneous casting and molding). Isomer III is drawn using a stick representation, γ-CD and **2** are represented by van der Waals spheres.

the shape changes of γ-CD: in the ternary complex it does not differ significantly from the native conformation whereas in the complex with only isomer III it is significantly distorted (Figure S24). We have previously shown similar effects in another cyclodextrin-based system, where the influence of tight alignment between cyclodextrins and their guests led to either positive or negative cooperativity.[16]

In conclusion, our data demonstrate for the first time that exposing a small dynamic combinatorial library simultaneously to two different templates may allow access to termolecular complexes that exhibit allosteric binding. The amplification of the resulting allosteric receptor came about through simultaneous casting and molding and showed an unusual selectivity for one of four closely related isomeric products. The absence of significant amplification of this isomer by any of the two templates acting in isolation indicates that the formation of the allosteric receptor is an example of emergent behavior that is difficult to predict on the basis of the pairwise interactions. Allosteric termolecular complexes of the type described herein may be used as hubs in even more complex molecular networks and constitute promising ingredients in systems chemistry.[5f,17]

Experimental Section

Building block **1** was prepared as reported previously.[10] Dynamic combinatorial libraries were prepared by dissolving building block **1** with or without template(s) γ-CD and template **2** in borate buffer (50 mM, pH 8.2) to obtain a specific concentration. The final pH value of the solution was adjusted to 8.2 by addition of a KOH solution (2.0 M). The four tetrameric isomers used for structure assignment

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were isolated by preparative HPLC. Procedures for HPLC and LC–MS analysis of the libraries, UV/Vis absorption titrations for evaluating the stepwise equilibrium constants for the formation of the termolecular complex, and details of molecular dynamics simulations are described in the Supporting Information. Mol2 files with partial charges for the naphthalene building block and template **2**, solvent-stripped trajectories, and representative PDB snapshots were deposited with figshare.[18]

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