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DYNAMIC COMBINATORIAL CHEMISTRY AND SYNTHETIC RECEPTORS

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Abstract

Dynamic combinatorial chemistry is a discipline that combines features of self-organization and adaptation of complex systems to external perturbations. Reversible chemistry is used to prepare dynamic combinatorial libraries that can respond to the presence of template molecules. Ideally the libraries respond by increasing the concentration of the receptors that is preferred by the template. In this article we illustrate the potential of the dynamic combinatorial chemistry approach for the discovery of synthetic receptors.

Key words: dynamic combinatorial chemistry, thermodynamics, amplification.

Resumen

Química combinatoria dinámica y receptores sintéticos. La química combinatoria dinámica es una disciplina en la cual se combinan características de auto-organización de la materia a nivel molecular y de adaptación de los sistemas complejos frente a perturbaciones externas. En esta estrategia se utiliza química reversible para preparar colecciones combinatorias dinámicas que son capaces de responder al agregado de una molécula molde. La respuesta ideal es la amplificación molecular del receptor preferido por el molde dentro de la colección. En este trabajo resaltamos los fundamentos de la química combinatoria dinámica e ilustramos su potencial para el desarrollo de receptores.

Palabras clave: química combinatoria dinámica, termodinámica, amplificación.

Dynamic Combinatorial Chemistry

The concept of dynamic combinatorial chemistry has been coined principally by Sanders [1-4] and Lehn [5-8] envisioning combinatorial chemistry under thermodynamic control, a key feature extracted from supramolecular chemistry [9]. This strategy is based on design, generation and study of dynamic combinatorial libraries (DCLs). A DCL is a mixture of compounds constructed from sets of building blocks that are connected using reversible chemistry. Therefore, in a DCL all the library members continuously interconvert into one another by recombination of building blocks (Figure 1).

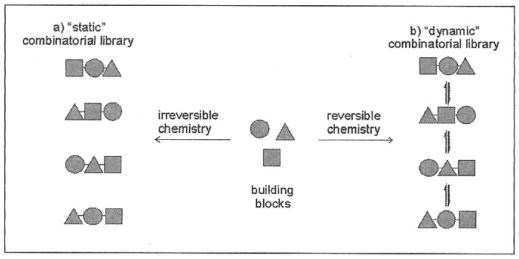

Fig. 1. Comparison between a) static and b) dynamic libraries.

Under appropriate reaction conditions, reversibility leads the DCL to an equilibrium state. At equilibrium, library composition depends on the Gibbs energy of each library member; consequently, external perturbations that affect the relative stability of library members - e.g. a change in temperature, solvents or pH- will shift the equilibrium altering the final product distribution. A particularly interesting perturbation is the addition of an external molecule to the DCL. If the added molecule binds at least one library member it can act as a template. The interaction with the template can selectively stabilize the selected library member, increasing its

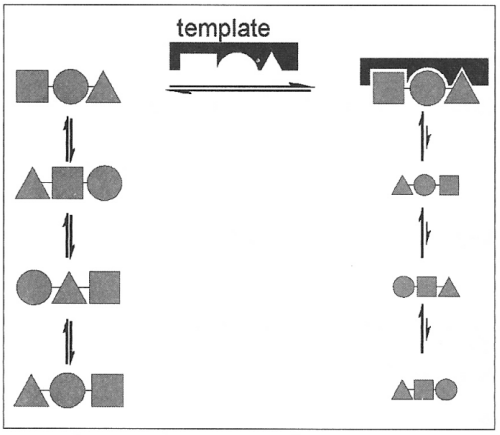

Fig.2. Template-induced molecular amplification in a DCL.

concentration at the expense of the rest of the DCL members. This effect is denominated molecular amplification (Figure 2).

Molecular amplification can be used as a tool for screening of DCLs for molecules with interesting recognition properties. DCLs of potential receptors can be prepared and a given molecule can be used to select, stabilize and amplify its preferred receptor. Ideally, building blocks are selected in order to obtain a DCL whose composition covers a broad "space" of potential hosts. Once a template has induced a change in the composition, amplification of a desired receptor can be optimized adjusting the composition to favor their formation. Biased libraries containing only those building blocks that were selected by the template can be generated to favor the high yield preparation of the selected receptor [10].

An attractive feature of dynamic combinatorial chemistry is that detailed receptor design is not necessary. The chemist designs and synthesizes building blocks equipped with potential recognition groups for the template. During the experiment, the molecules constructs themselves, adopting the optimum arrangement of subunits to give the receptor more globally favored in an increased -or amplified- amount.

Moving from receptor design to library design permits the incorporation of different recognition groups, shapes as well as levels of rigidity in the set of building blocks to be combined. It becomes then unnecessary to know in detail the exact combination of those variables that will produce a good receptor, as long as such combination is present within the library.

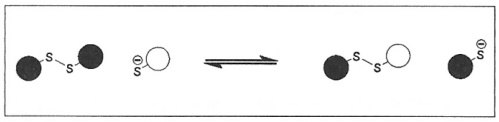

Fig. 3. Disulfide exchange as reversible connection to prepare DCLs.

Reversible Chemistry

Key to the success of dynamic combinatorial chemistry is the reversible chemistry involved in the preparation of the libraries. Although there is a long list of requirements for the reversible reaction to be used [9], two requirements are crucial for the development of receptors: (a) the exchange should be active in a reasonable timescale under appropriate reaction conditions. This determines the time that is necessary for the DCL to respond to the addition of a template molecule. Appropriate reaction conditions are those that are compatible with the structure of building blocks and template, particularly with the recognition groups involved in the intermolecular interactions that drive molecular amplification. (b) It should be possible to stop the exchange so that the amplified library members can be isolated and characterized. Ideally the changes in the receptor and/or template structures, produced by the experimental operations necessary to stop the exchange, should not affect the binding between the amplified host and the guest used as template.

Two of the most productive reversible chemistries used in DCC are the exchange of hydrazones and the exchange of disulfides. Disulfide exchange was one of the first reactions used for the preparation of receptors under reversible conditions [11] (Figure 3). Thiols oxidize to disulfides in aqueous solution upon exposure to air and thiol/disulfide exchange takes place under mild conditions [12, 13]. In practice, disulfides DCLs are often prepared in water from thiol building blocks, which are allowed to oxidize to the desired disulfides. At the same time that oxidation occurs the reaction goes through a phase where thiol and disulfide coexist, allowing the reversible exchange process to take place through the nucleophilic attack of thiolate anion on the disulfide bond producing another thiolate [14, 15]. The exchange can be switched off either through protonation of the thiolate nucleophile or by complete oxidation of the thiolate. Disulfide exchange can also take place in organic solvents like chloroform or methanol in the presence of bases such as TEA or DBU [11, 16, 17]. Recently,

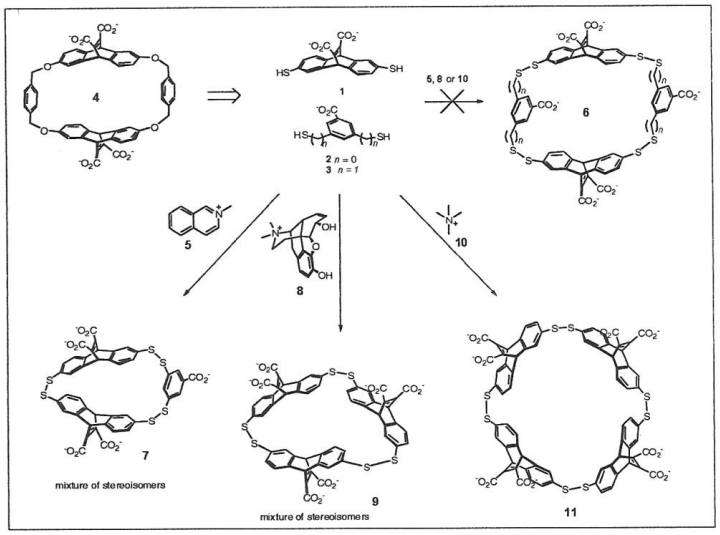

Fig. 4. Receptors for ammonium cations from DCLs based on disulfide exchange.

a small DCL was prepared using disulfide exchange in a two phase system, which increases the possibilities and the scope of dynamic combinatorial chemistry by facilitating the combination of otherwise incompatible building blocks [18].

To date, a good number of the receptors developed using dynamic combinatorial chemistry have been discovered from DCLs designed to include compounds that emulate known designed receptors. Such is the case of a series of macrocyclic receptors that bind ammonium ions in water developed by Otto and Sanders using DCLs prepared from building blocks **1-3** (Figure 4) [10, 19]. The design of the dithiol building blocks was inspired by the Dougherty cyclophane receptor **4** [20].

Initially guest **5**, which is one of the best binders to the original Dougherty host, was used as the template expecting molecular amplification of macrocycle **6** analogous to the cyclophane **4**. Unexpectedly, macrocycle **7** was amplified instead.

The lack of amplification of **6** may be due to the differences between the -S-S- units in the disulfide macrocycles as compared to the -CH_2-O- units in **4**: -S-S- units are more rigid, they have increased length and different bond angles. These differences are important since it is known that host **4** binds guest **5** in a partially collapsed conformation [21-25]. Probably, a similarly collapsed conformation cannot be adopted by the more rigid disulfides analogous **6**.

Exposure of the same library to the morphine based template **8**, which is significantly larger than guest **5**, did not result in the amplification of the expected macrocycle **6** either. This time a cyclic trimer of building block **9** was amplified [10].

Even more surprisingly is the response of the library to the small template tetramethylammonium iodide **10**: the cyclic tetramer of dithiol **11** was amplified [19]. Given that building block **1** was used as a racemic mixture, four diastereomeric disulfides can in principle be formed. It turned out that only the diastereomer in which the four subunits have alternating chirality **11** was significantly amplified. Only this isomer can fold up in a way that effectively surrounds the small cationic guest **10**. In the examples described, the reversible chemistry also provided an efficient synthetic route to the selected receptors, which can be formed in up to 96% yield in the biased DCLs containing only the required building blocks in the proper ratio.

These results demonstrate that dynamic combinatorial chemistry is an effective method for identifying unpredictable receptors. Such unexpected receptors can be amplified even from DCLs that have been designed to contain members that resemble known receptors.

In order to take the dynamic combinatorial chemistry approach to a next step and pro-

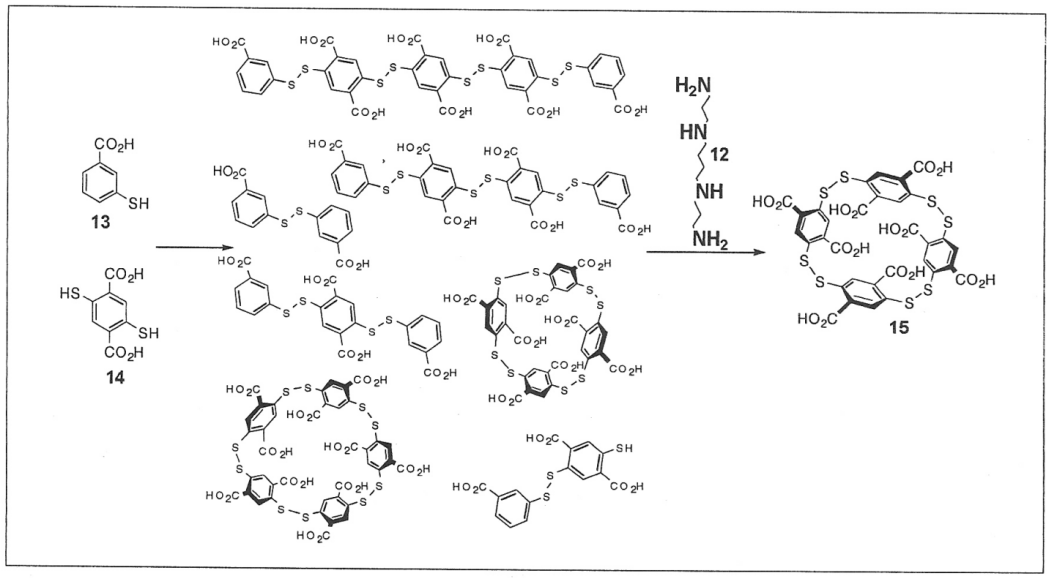

Fig. 5. Receptor for spermine developed from a DCL based on disulfide exchange.

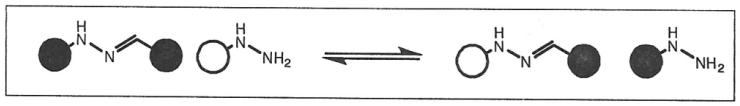

Fig. 6. Hydrazone exchange as reversible connection to prepare DCLs.

duce novel receptors, it is important to identify rules to guide the design of DCLs to target novel guests. Otto *et al.* [26] have addressed this issue while developing their receptor for spermine **12**, a polyamine that plays an important role in numerous cellular processes including apoptosis and cancer. The design of the building blocks for the recognition of spermine started with identifying carboxylate–amine interactions as a potential mode of recognition. Since it was not clear whether the DCL should include linear or cyclic compounds, monothiol building block **13** and dithiol **14** were used to generate both types of structures (Figure 5). When the DCL from those building blocks was exposed to spermine, the cyclic tetramer **15** was amplified (Figure 5).

This receptor turned out to have a remarkably high affinity for spermine ($K = 4.5 \times 10^7\ M^{-1}$ in 3 mM TRIS buffer pH 7.4) and binds it by forming a pseudorotaxane-type complex. Binding is strong enough to enable the receptor to sequester spermine from one of its natural hosts, DNA. Developing a synthetic receptor with sufficient affinity to bind spermine in a biological system was the first step to developing a spermine sensor that can assist in elucidating the exact biological role of the polycation.

Hydrazone exchange is somehow complementary to disulfide exchange (Figure 6). It requires acidic conditions (typically TFA in $CHCl_3$, or pH 4-5 in aqueous solutions), although for some substrates containing strongly electron withdrawing groups, exchange is feasible under neutral conditions [27].

The hydrazone exchange reaction was first used for the preparation of DCLs of potential receptors by the Sanders group, who developed a family of amino acid-based bifunctional building blocks featuring one protected aldehyde unit and one acylhydrazide unit [28]. A series of molecular amplification examples observed with these DCLs have demonstrated the compatibility of the reaction conditions with binding of metal and organic guests [29-34].

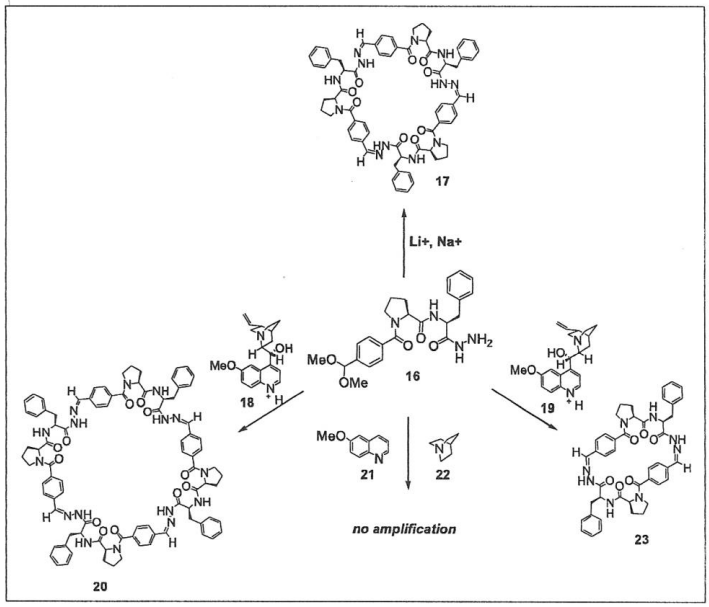

Fig. 7. Receptors for ammonium and metal cations from DCLs based on hydrazone exchange.

A variety of receptors for cations have been discovered using dynamic combinatorial libraries based on hydrazone exchange. Some of those receptors were inspired in known receptors [30, 33], whereas other receptors have been discovered by chance. Such is the case of the responses induced by different cation templates on the DCL prepared from building block **16** based on the dipeptide L-Pro-L-Phe (Figure 7). The small DCL prepared from **16** was affected by the presence of Li^+ and Na^+ [32, 35]. When the library was exposed to these templates, the cyclic trimer **17** was amplified representing 98% of the library material and decreasing the concentration of the other macrocycles. This DCL also responded to the presence of the ammonium ions of cinchona alkaloids quinine **18** and quinidine **19** [29]. When the library was exposed to **18** a significant shift in the product distribution toward the cyclic tetramer **20** at the expense of the other macrocycles was observed. The template molecule quinine can be regarded as a quinoline moiety **21** attached to one quinuclidine **22** moiety by a one-carbon bridge.

In an attempt to determine which of those moieties was responsible for the molecular amplification observed, **21** and **22** were tested, individually and simultaneously, as potential templates. The lack of any amplification observed for either template or their combination suggests that the binding is driven by an interaction involving both moieties in the template. In order to test the importance of the relative positions of the moieties, templating by quinidine **19**, a diastereomer of quinine that possesses a different configuration in two of the four stereogenic centres, was tested. No amplification of the cyclic tetramer was observed; on the contrary, the concentration of this species along with other macrocycles in the mixture decreased to feed amplification of the cyclic dimer **23** that increased in abundance from 9% to 45% of the library material.

The same small DCL made from **16** was the origin of possibly one of the most impressive and surprising template effects observed to date: the molecular amplification of a catenane with the assistance of the small neurotransmitter acetylcholine [36]. Addition of acetylcholine hydrochloride to the reaction mixture slowly produces a change in the composition of the mixture by shifting the equilibrium to favor the formation of the catenane **24** (Figure 8).

The catenane is the result of assembling six molecules of **16** into two interlocked cyclic

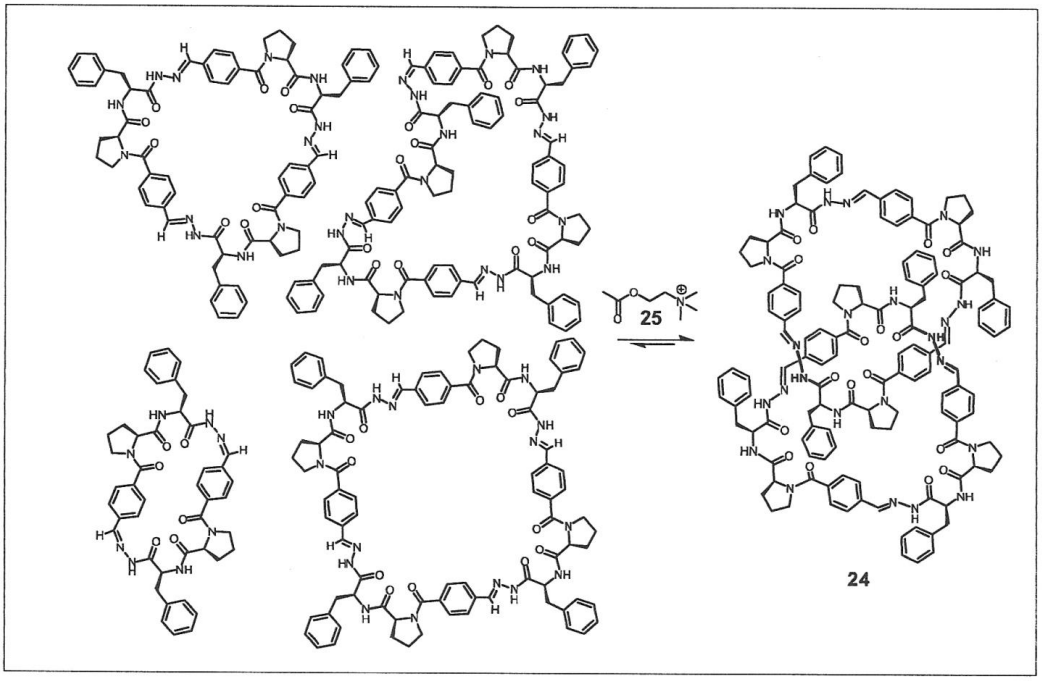

Fig. 8. Catenane receptor for acetylcholine developed by Sanders et al.

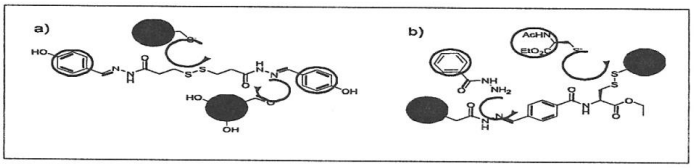

Fig. 9. Dynamic systems used to study the combination of disulfide exchange and hydrazone exchange in one single dynamic system in water (a) or chloroform (b).

trimers. With the assistance of the template and after 44 days of equilibration, this improbable product could be synthesized in one pot from **16** in 67% isolated yield. Although two stereoisomeric catenanes can be formed, formation of only one of them was observed. The catenane binds acetylcholine chloride with an exceptionally high affinity (1.4×10^7 M^{-1} in 95:5, $CHCl_3$:DMSO). Although the exact mode of binding has not been established in detail, several observations suggest that the trimethylammonium moiety of acetylcholine **25** is the primary recognition site. The catenane binds choline, acetylcholine and butyrylcholine in a similar way; however, replacing one methyl group in acetylcholine by ethyl reduces the binding constant by two orders of magnitude.

Discovery of this unpredictable and highly complex host through stereoselective amplification is a perhaps the most clear expression of the power of dynamic combinatorial chemistry as a tool for the development of new synthetic receptors.

The receptors shown above have been discovered from DCLs based on one reversible process (disulfide exchange or hydrazone exchange). The combination of different reversible reactions to connect building blocks in one dynamic combinatorial can enhance the level of diversity achieved. Two types of multilevel dynamic libraries have been described to date: "orthogonal", when the reversible processes operate independently, and "communicating", when the

processes cross over. Eliseev, Lehn *et al.* have reported the use of double-level "orthogonal" libraries where hydrazone exchange and ligand exchange around a cobalt ion can be addressed independently [37]. Otto, Sanders *et al.* reported that structural diversity within a DCL can be expanded by maintaining two exchange processes simultaneously generating a double-level "communicating" library based on exchange of disulfide and thioester linkages [38]. Recently two simple dynamic systems where reported where hydrazone exchange and disulfide exchange were combined.

Otto *et al.* studied the combination of both exchange processes in water (Figure 9a). By adjusting the pH of the solution from acidic to mildly basic it is possible to switch from exclusively hydrazone exchange to exclusively disulfide exchange, while at intermediate pH both reactions occur simultaneously [39].

Our group studied the exchange in a similar system in organic solvents (Figure 9b). Hydrazones were exchanged by acid catalysis in the presence of disulfide and a thiol group without interference; neutralization of the reaction medium turns off the exchange of hydrazones and, at the same time, activates thiolate–disulfide exchange [40].

These results show that two selectively addressable dynamic chemistries can be combined for the preparation of fully covalent double level dynamic combinatorial libraries from building blocks appropriately functionalized to participate in both exchange processes. This process can be carried out in both directions: disulfide exchange followed by hydrazone exchange and *viceversa*. Different product distributions could be obtained depending on the order in which the two exchange reactions are activated, so this sequential process is neither commutative [41] nor communicative [38].

The adaptability of the system to a template molecule could be studied activating hydrazone exchange by acid. Under these reaction conditions, disulfide exchange stays turned off. On the other hand, hydrazone exchange can be turned off by neutralization of the reaction whereas the disulfide exchange is turned on. Finally, this second level of diversity can be turned off by complete oxidation of the thiols. These findings pave the way to future construction of more complex molecular architectures and chemical systems capable of being evolved by alternating use of the two reversible covalent chemistries.

Ensuring amplification of the fittest

The success of dynamic combinatorial chemistry for the discovery of new synthetic receptors stands on the original conception that the best hosts in a DCL will be most amplified upon addition of a guest. Some early results on amplification of receptors from simple model systems somehow supported that idea [11, 33]. However, despite the variety of examples of molecular amplification of receptors described during the last twenty years, the power of molecular amplification to spot the best receptor within a DCL was never conclusively proved.

On the other hand, research by Severin [42-44], Sanders and Otto [45-47] in the recent years, have demonstrated that "amplification of the fittest" is not always the case. It is now recognized that the correlation between binding and amplification can be broken when two or more library members: (a) bind a template that is in excess and (b) include in their structures different numbers of one building block that is in short supply. Under such conditions those library members constituted by a smaller number of the scarce building block have a competitive advantage over those members that contain larger numbers of that particular building block. Consequently, lower oligomers tend to have an advantage over higher oligomers, and hetero oligomers over homo oligomers.

When the template is added to a dynamic system, the system will readjust its composition in a way that the total gain in host–guest binding energy is highest. This total energy gain will depend on the individual energy gain produced by each binding event and the total number of binding events in the system [48].

In the presence of a modest amount of template, the number of possible binding events is limited by the number of template molecules. Thus, binding events that produce the highest energy gain are preferred, and the best host will be amplified. However, in the presence of an excess of template molecules the total number of binding events is limited by the number of hosts. Under such conditions the total binding energy gain may be increased by raising the number of binding events, even when the energy gain *per* event is lower. This can be achieved by converting higher oligomers into lower oligomers or homo oligomers into hetero oligomers. In this way, the system attains the maximum energy gain per molecule of the building block that is in short supply.

Consequently, to assure a good correlation between binding and amplification, care should be taken to guarantee that the concentration of the template in the DCL is always well below the concentration of building blocks, in particular those building blocks that are involved in binding.

Conclusions

A good receptor must have recognition groups which are of the appropriate electronic character to complement the binding sites of its guest, positioned in a way that they can interact with recognition groups from the guest when both receptor and guest are in the binding conformation. The required balance of all the variables involved in this recognition process makes the design of successful receptors a significant challenge.

Despite these difficulties, numerous receptors have been prepared to date that are able to bind guests with high affinities [49]; however, the rational design and synthesis of novel receptors can be both time-consuming and frustrating [50].

In a dynamic combinatorial strategy the preferred receptor is selected by the guest and amplified at the expense of the unselected compounds within a DCL [9]. The whole process can be regarded as the adaptation of the dynamic system to the presence of the guest; expectedly, this adaptation includes corrections in the original composition that leads to an increased concentration of the best receptors.

In this article, we have presented selected examples of synthetic receptors that have been discovered using this approach. They include some receptors with exceptionally high affinities for biologically relevant guests such as acetylcholine and spermine.

A frequent feature of receptors discovered to date from DCLs is their unexpected structures, even in cases where the library had been designed to include a member that resembles a known host for a given guest, and such guest was used as template. The DCC approach has also lead to the discovery of rather flexible receptors that are not preorganized. It is very unlikely that these receptors would have been discovered by more traditional design and synthesis strategies [48].

It is important to note that the same templated reversible chemistry used for screening of the library can also be used for the high yield preparation of the selected receptors. In this procedure, the number and relative concentration of building blocks used can be restricted to those required to prepare the desired product. These biased DCLs have proved to be a useful synthetic tool for the preparation of complex macrocyclic receptors.

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