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Supramolecular Interactions between Library Members Modulate the Behavior of Dynamic Combinatorial Libraries

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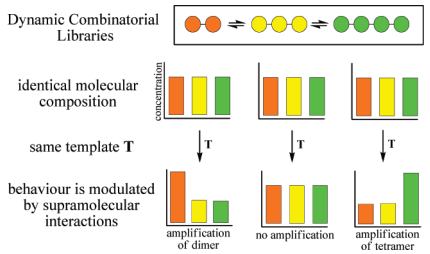

The presence of a supramolecular network of interactions between library members can lead to very different responses when libraries with identical molecular composition are exposed to the same template. Numerical simulations demonstrate that supramolecular interactions between library members of covalent dynamic combinatorial libraries (DCLs) can affect both degree and selectivity of the response of the library when a template molecule is added.

The conception of the dynamic combinatorial chemistry[1] was embedded in the generation of molecular diversity (combinatorial chemistry) and the use of noncovalent interactions (supramolecular chemistry). In the past decade, the discipline has emerged as a valuable strategy for the discovery of molecules with interesting molecular recognition properties including small molecule ligands for biomolecules,[2] and synthetic receptors.[3] The power of dynamic combinatorial chemistry for the discovery of new synthetic receptors depends on the intrinsic aptitude of a DCL to amplify the best hosts upon the addition of a guest. Despite the various examples of molecular amplification of receptors described to date, the "amplification of the fittest" concept was never unquestionably proved. On the contrary, investigations by Severin[4] and Sanders and Otto[5] have demonstrated that "amplification of the fittest" is not always the case. The correlation between binding and amplification can break down when two or more library members bind a template that is in excess and 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, smaller oligomers are favored over higher oligomers, and hetero oligomers over homo oligomers. To ensure selective amplification of the best receptor it is important that the concentration of template molecules does not demand more molecules of the preferred building blocks than the system can provide. This can be achieved by using a low concentration of template. Noncovalent exchange processes involving hydrogen bonds[6] and metal−ligand coordination[7] have been used extensively in the preparation of DCLs of potential receptors and several examples of receptor amplification have been reported.[8] However, isolation and reuse of the amplified receptors is problematic because of the labile connections between building blocks. To overcome this problem reversible covalent chemistry can be applied to connect building blocks.[9] We will focus on DCLs based on reversible covalent bonds where noncovalent

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(supramolecular) interactions between library members are operational as well. Several covalent dynamic libraries have been described where noncovalent interactions between library members exist.[10] In those DCLs, one library member interacts selectively with itself leading to self-selection; however, it may be possible that interactions between library members produce changes in library composition that are less evident. Herein we investigated the effect of supramolecular interactions between library members on the amplification factor produced by a template in simulated dynamic libraries (SDLs) of identical molecular composition. Previously,[10d] a model of a dynamic system considering covalent and noncovalent reversible bonds was reported wherein selected supra(di)molecular interactions were sufficient to account for the experimental observations. In line with this, we investigated a simple model as a representation of a dynamic library where monomer A is in equilibrium with dimer A_2 and trimer A_3. The library members A_2 and A_3 are successively formed by recruitment and assembly of building block A through covalent connections (eqs 1 and 2).

$$A + A \overset{Kf_{1\text{-}2}}{\rightleftharpoons} A_2 \quad (1)$$

$$A_2 + A \overset{Kf_{2\text{-}3}}{\rightleftharpoons} A_3 \quad (2)$$

The initial monomer concentration and the association constants were set to lead to the formation of equal amounts of the two receptors ($[A_2] = [A_3] = 4$ mM) together with a very small amount of the monomer A remaining in its free form ([A] = 0.05 mM). Once A_2 and A_3 have been assembled, noncovalent interactions between them are considered with the addition of all the possible bimolecular homophilic and heterophilic complexing reactions (eqs 3−5).

$$A_2 + A_2 \overset{K_{22}}{\rightleftharpoons} A_2 : A_2 \quad (3)$$

$$A_3 + A_3 \overset{K_{33}}{\rightleftharpoons} A_3 : A_3 \quad (4)$$

$$A_2 + A_3 \overset{K_{23}}{\rightleftharpoons} A_2 : A_3 \quad (5)$$

A series of SDLs were generated by selecting sets of binding constants (K_{22}, K_{33}, and K_{23}) for the complexes between library members in a way that (a) the equilibrium total concentration of A_2 and A_3 is the same along the series (≈4 mM) and (b) the addition of the change of free energies, $\sum\Delta G^\circ_{ij}$, associated with the reactions described in eqs 3−5 is different for each library, where ΔG°_{ij} denotes the change of free energy associated with the binding between library members A_i and A_j ($\sum\Delta G^\circ_{ij} = \Delta G^\circ_{22} + \Delta G^\circ_{33} + \Delta G^\circ_{23}$). The effect of three templates T1, T2, and T3, added separately, on the amplification factor for receptor A_2 (f_{A2}) was

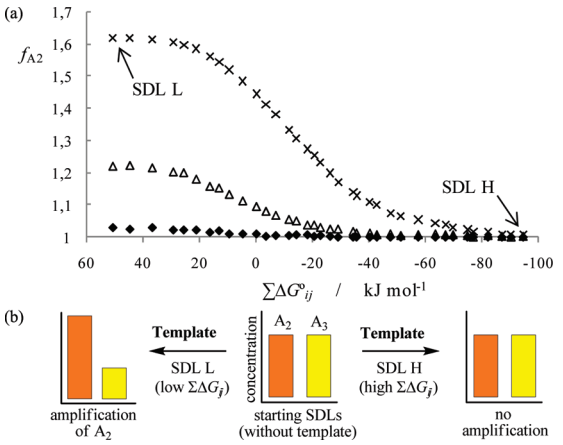

FIGURE 1. (a) Amplification factor of A_2 (f_{A2}) induced by the individual addition of 5 mM of templates T1, T2, and T3 with binding affinities (mM^{-1}) $K_{2T1} = 0.01$ (♦), $K_{2T2} = 0.1$ (Δ), and $K_{2T3} = 1$ (×) in a set of SDLs with increasing $\sum\Delta G^\circ_{ij}$. (b) Concentration of library members, of two selected SDLs before and after the addition of T3.

determined for each SDL.[11] T1, T2, and T3 were arbitrarily set to bind only to the free dimer with affinities $K_{2T1} = 0.01$ mM^{-1}, $K_{2T2} = 0.1$ mM^{-1}, and $K_{2T3} = 1$ mM^{-1}, respectively. Figure 1 shows the effect of supramolecular interactions between library members, $\sum\Delta G^\circ_{ij}$, on f_{A2} along the series of SDLs. As expected, the achieved amplification depends on the affinity between A_2 and the template; however, for any template, f_{A2} decreases with the increase in $\sum\Delta G^\circ_{ij}$. Therefore, a low affinity receptor can be amplified from a SDL with a weak supramolecular network: in libraries with $\sum\Delta G^\circ_{ij} > 25.34$ kJ mol^{-1}, $f_{A2} > 1.2$ is produced by template T2 ($K_{2T2} = 0.1$ mM^{-1}). In contrast, a better receptor could pass undetected in a library with a stronger supramolecular network: if $\sum\Delta G^\circ_{ij} < -63.71$ kJ mol^{-1}, $f_{A2} < 1.05$ with template T3 ($K_{2T3} = 1$ mM^{-1}) (Figure 1b).

According to these results, the interactions between library members can affect in a significant degree the amplification factor produced by the template in libraries where only one receptor binds the template. A similar situation can be expected in SDLs where various library members bind the template. Therefore, a logical extension of the previously described model was employed in the generation of SDLs constituted by receptors A_2, A_3, and A_4, with an equimolar equilibrium concentration (≈4 mM of each one). Again, sets of binding constants for the supramolecular interactions between library members were selected in such a way that, in the absence of a template, the total concentration of library members is the same but the addition of the binding constants between library members is different for each SDL. Addition of a template T which binds A_2, A_3, and A_4 ($K_{2T} = 10$ mM^{-1}, $K_{3T} = 100$ mM^{-1}, and $K_{4T} = 1\,000$ mM^{-1}, respectively) induced different responses in each SDL.[12] The best receptor (A_4) is amplified

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(11) The amplification factor is the ratio of the total concentration of host A_i (complexed and free) present in the templated library and the corresponding concentration in the nontemplated library. See refs 4b, 5a, and 5d.

(12) Some RNA structures exhibit a strong correlation between size and binding affinity, reflecting the fact that larger structures have more potential information content. See: Carothers, J. M.; Oestreich, S. C.; Davis, J. H.; Szostak, J. W. *J. Am. Chem. Soc.* **2004**, *126*, 5130. In order to resemble this evidence we assigned a higher K_{iT} to the largest oligomers. Although large oligomers in dynamic systems tend to be more sensitive to disruption of amplification by relatively weak interactions involving smaller oligomers, larger species are potentially more interesting from a functional point of view.

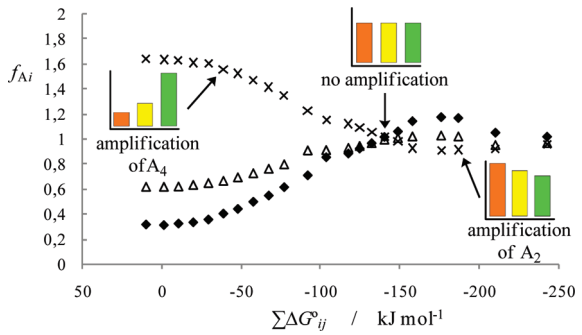

FIGURE 2. Amplification factor for A_2 (♦), A_3 (Δ), and A_4 (×) induced by a template T (10 mM) in a set of SDLs with increasing $\sum\Delta G^{\circ}_{ij}$. K_{2T} = 10 mM^{-1}, K_{3T} = 100 mM^{-1}, and K_{4T} = 1 000 mM^{-1}. Inserts show the SDL composition after templating in three representative SDLs.

only when $\sum\Delta G^{\circ}_{ij}$ values do not exceed a critical value where no amplification is observed (Figure 2). For lower $\sum\Delta G^{\circ}_{ij}$, the amplification selectivity is reverted and A_2 becomes the thermodynamically favored receptor. Exposure of the SDLs to templates with higher binding constants does not avoid "incorrect" amplifications. If the affinities of A_2, A_3, and A_4 for the template are ten times stronger (K_{2T} = 100 mM^{-1}, K_{3T} = 1 000 mM^{-1}, and K_{4T} = 10 000 mM^{-1}) or 100 times stronger (K_{2T} = 1 000 mM^{-1}, K_{3T} = 10 000 mM^{-1} and K_{4T} = 100 000 mM^{-1}), amplification of the worst receptor A_2 is stronger (Figure S1, Supporting Information).

Hence, the presence of a supramolecular network of interactions between library members can lead to very different responses when libraries are exposed to the same template, even in DCLs with the same molecular composition. As a consequence when three SDLs that contain the same receptors (A_2, A_3, and A_4) in the same concentration are exposed to the same template, depending on the strength of the interactions between library members, the response of the DCL could be (a) amplification of the best binder A_4, (b) no amplification, or (c) amplification of the worst binder A_2.

In a previous study,[4c] Severin observed that sometimes the concentration of template-bound complexes could be a better indicator than the amplification factor to spot good binders in DCLs. As observed for the amplification factor along the series, the concentration of the tetramer:template complex, [A_4:T], is highest only if $\sum\Delta G^{\circ}_{ij}$ values exceed the "no amplification" value. For lower $\sum\Delta G^{\circ}_{ij}$, the relative concentration of complexes is reverted and the concentration of A_2:T is highest (Figure 3).

In an attempt to evaluate the selectivity of the amplification, a series of amplification experiments with different template concentrations were carried out in a SDL where a little amplification of A_4 was observed. High template concentration produces incorrect amplification of A_2, whereas a low concentration produces the right amplification of A_4. For this system, the correction is produced for a [T] = 15 mM (Figure 4a). Below 15 mM the amplification factor for the A_4 ranges between 1.00 and 1.05. Although the best binder is amplified, the change in concentration is probably too small to be experimentally detected in a reliable way. A better relative difference in concentration and an expanded range of template concentration favoring the amplification of A_4 (19 mM) is observed when the concentration of complexes with the template is measured (Figure 4b).

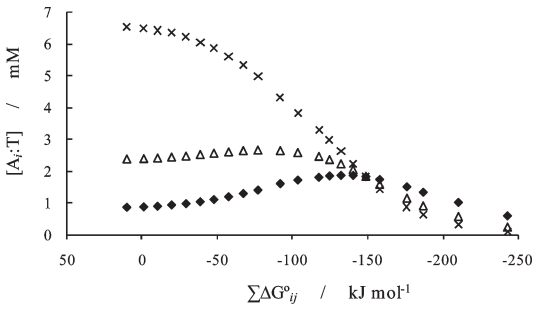

FIGURE 3. Concentration of complexes between T and A_2, A_3, or A_4 in SDLs with increasing $\sum\Delta G^{\circ}_{ij}$. K_{2T} = 10 mM^{-1}, K_{3T} = 100 mM^{-1}, and K_{4T} = 1 000 mM^{-1}. [T] = 10 mM. [A_2:T] (♦), [A_3:T] (Δ), [A_4:T] (×).

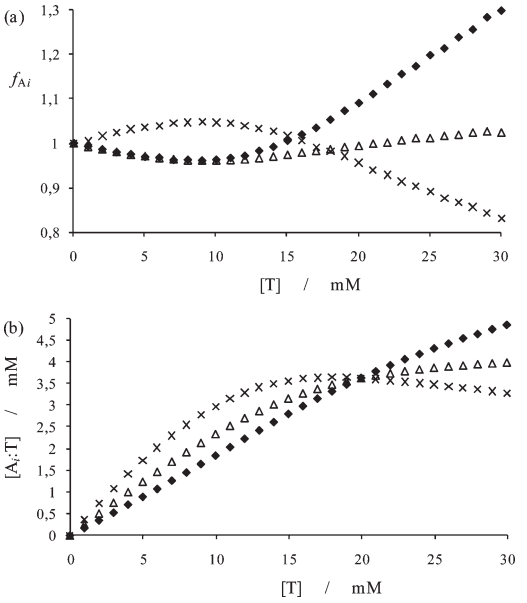

FIGURE 4. Amplification factor (a) and concentration of complexes between library members and the template T (b) for A_2 (♦), A_3 (Δ), and A_4 (×) produced by addition of different concentrations of T in a SDL with $\sum\Delta G^{\circ}_{ij}$ = −140.55 kJ mol^{-1}. K_{2T} = 10 mM^{-1}, K_{3T} = 100 mM^{-1}, and K_{4T} = 1 000 mM^{-1}.

The small amplification factor of the best binder does not improve for sets of templates with increasing affinity. The maximum f_{A4} is 1.05 for templates with binding constants 10 times higher or 100 times higher (Figures S2 and S3, Supporting Information).

In conclusion, supramolecular interactions between members of DCLs can produce significant changes in the behavior induced by a template molecule. Such interactions can lead to different degrees of amplification of the best binder or they can lead to incorrect amplification of poor binders. In SDLs with strong interactions between library members, correct amplifications can be obtained by measuring concentration of complexes and using low template concentration. An important proportion of the receptors developed to date using dynamic combinatorial chemistry have been inspired by receptors previously developed by design. In these cases dynamic combinatorial chemistry could be considered more as an optimizing tool than as a source of novel host structures. To develop truly novel receptors it is

necessary to improve library diversity and to identify rules to guide the design of libraries to target specific guests.

The structural diversity of most covalent DCLs of receptors described to date is modest: the majority of the libraries have been prepared from one to three building blocks which contain very similar recognition groups. Although a significant number of potential receptors with different shapes may be obtained with such a simple setup, it is clear that the properties of complex dynamic mixtures of receptors with significant differences in recognition groups have not been explored yet. The use of diverse recognition groups within one DCL could be hampered by the appearance of supramolecular interactions between library members. Such interactions can affect the response of the library to the addition of templates, compromising the utility of the amplification factor as an indicator to spot good binders within a DCL. The generality and impact of the effect in real systems,[13] how the effect can be minimized through library design, and what is the influence of the type of species involved in supramolecular interactions on the amplification factor[14] will demand further studies.

In this context, the use of resin bound templates[15] that allow quantification of complexes with the template and the use of resin bound dynamic combinatorial libraries[16] that prevent interaction between library members may be useful tools to detect the best binders.

Experimental Section

Numerical Simulations. The equilibrium concentrations of SDLs were calculated with the default setting of the steady state method implemented in the program CoPaSi.[17]

The equimolar concentration of A_2 and A_3 was obtained holding as constants the following parameters of eqs 1 and 2: initial monomer concentration $[A]_i = 20$ mM, $Kf_{1-2} = 1\,600$ mM^{-1}, and $Kf_{2-3} = 20$ mM^{-1}.[4b] A heuristics-based approach was employed in the fine-tuned selection of supramolecular constants K_{ij}, in order to obtain SDLs with the same total (free + complexed) concentration of library members. K_{ij} denotes the binding constant between receptors A_i and A_j. The initial concentrations of members used in the simulations range between 3.99 and 4.03 mM for A_2 and between 3.98 and 4.01 mM for A_3. When the equilibriums between A_2, A_3, and A_4 were considered, A_4 was formed from A_3 and A, following the increasing stoichoimetry order shown in eqs 1 and 2. In this case, the following fixed parameters were used: $[A]_i = 36$ mM, $Kf_{1-2} = 1\,600$ mM^{-1}, and $Kf_{2-3} = Kf_{3-4} = 20$ mM^{-1}. Kf_{3-4} denotes the association constant of A_4. All the space of possible supra(di)molecular interactions between A_2, A_3, and A_4 was taken into account. Thus, $\sum\Delta G^\circ_{ij}$ is calculated as follows: $\sum\Delta G^\circ_{ij} = \Delta G^\circ_{22} + \Delta G^\circ_{33} + \Delta G^\circ_{44} + \Delta G^\circ_{23} + \Delta G^\circ_{34} + \Delta G^\circ_{24}$.

Amplification Factor. The amplification factor of A_i was calculated dividing the total concentration of molecules of A_i complexed ($[A_i{:}X]$) and free ($[A_i]_f$) after the addition of a template by to the corresponding concentrations before the addition of a template. For example, the amplification factor of A_2 is calculated as follows: $f_{A2} = ([A_2]_f + 2[A_2{:}A_2] + [A_2{:}A_3] + [A_2{:}T])_{Templated}/([A_2]_f + 2[A_2{:}A_2] + [A_2{:}A_3])_{nonTemplated}$.

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Supporting Information Available: Figures S1−S3 and raw data of the SDLs. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) It could be expected that the distribution of the interactions between library members will affect amplification in different degrees. For example, it is expected that a given affinity between dimers will have a different effect on the amplification than the same affinity between tetramers. Also, it is expected that the size of the DCL will affect the influence of supramolecular interactions on the amplification factor.

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