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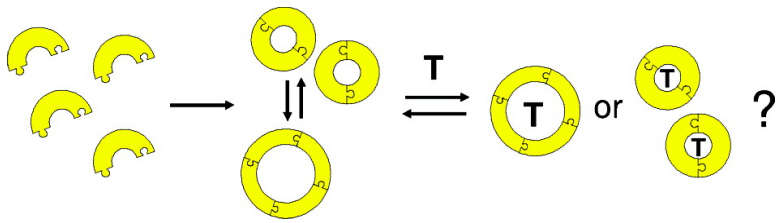

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Competition between Receptors in Dynamic Combinatorial Libraries: Amplification of the Fittest?

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The strong binders survive and are amplified, while the weak perish. This principle is the foundation of dynamic combinatorial chemistry.[1] In dynamic combinatorial libraries (DCLs), all constituents are in equilibrium through reversible reactions that exchange the building blocks from which the different library members are assembled. Adding to a DCL a "template" which binds to a subset of the library members causes the equilibrium to shift, increasing the amount of strong binders at the expense of the weak binders.[2] Ideally, the "fittest"[3] will be most amplified, or, in more general terms, the efficiency of amplification will correlate with the strength of binding. However, recent theoretical studies by Severin[4] and ourselves[5] suggest that, while the correlation between binding efficiency and amplification is often satisfactory, special cases occur where the correlation breaks down. We now report the first quantitative experimental evidence showing how, in such special cases, weaker binders can indeed beat stronger binders.[6] We also demonstrate how a simple adjustment of library conditions ensures that the best binder is most amplified.

The breakdown of the correlation between amplification and binding efficiency occurs as a result of the tendency of DCLs to maximize the binding interactions in the entire library.[4,5] When there is a choice between producing a large number of small moderate binders and producing a small number of larger, stronger binders, the latter option will not necessarily be preferred. Thus, when there is competition between several binders, the best binder *may* go unnoticed during library screening because it is not sufficiently amplified. More specifically, suppression of the best binder by "inferior" competitors may occur when (i) a homo-oligomer (a library member built up from a number of identical building blocks) competes with a hetero-oligomer (a compound containing several different building blocks); or (ii) a large oligomer competes with one containing a smaller number of building blocks. In the presence of an excess template, hetero-oligomers can suppress homo-oligomers and small oligomers can suppress large oligomers, simply because, with a fixed amount of building block, the library can produce more molecules of hetero-oligomers than homo-oligomers and more small oligomers than large oligomers.

We have previously reported the efficient amplification of a series of micromolar receptors for ammonium ions from DCLs of macrocyclic disulfides in water.[2a−c] These receptors are assembled from building blocks **1** and **2** and include a heterotrimer (**7**), a homotrimer (**8**), and a tetramer (**9**).[7] In a DCL containing these receptors, competition for building block **1** is inevitable; the tetramer has to compete with the smaller trimer **8**, and in turn, in the presence of building block **2**, the homotrimer **8** has to compete with the heterotrimer **7**. These are exactly the two scenarios for which theory predicts that the correlation between amplification efficiency and binding affinity can break down. However, previously, we have observed no such breakdown.[2a−c] Figure 1 shows that amplification can be well-behaved and selective for the best binders. When we use an excess of guest **4** in a library made from building blocks

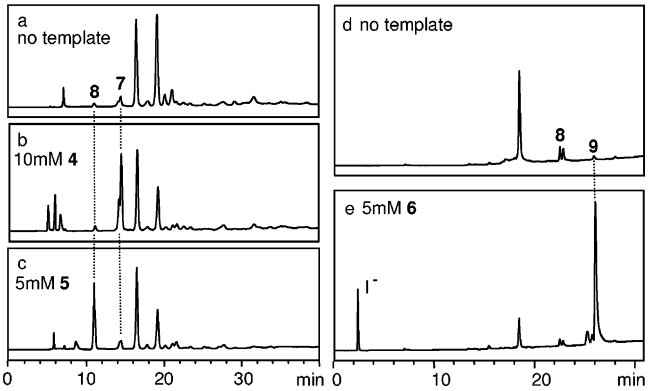

Figure 1. Left: HPLC analysis of a DCL made from dithiols **1**−**3** (3.33 mM each) in water at pH 8−9 in the absence of template (a) and in the presence of 10 mM of guest **4** (b) and 5 mM of guest **5** (c).[2b] Right: HPLC analysis of a model DCL made from **1** (5 mM) in the absence of template (d) and in the presence of 5 mM of guest **6** (e).[2a]

1−**3**, we selectively amplify host **7** (Figure 1b).[2b] Similarly, morphine derivative **5** gives selective amplification of host **8** (Figure 1c).[2b,8] Also, tetramer **9** can be formed without any simultaneous amplification of trimer **8** when guest **6** is used (Figure 1e).[2a] For this series of guests, the equilibrium constant for binding to the best receptor is apparently sufficiently larger than that for the next-best receptor, so that amplification is selective for the best binder. The binding constants for the relevant host−guest combinations are shown in Table 1. Note that guest **5** selectively amplifies homotrimer **8**, as a result of binding it with micromolar affinity, despite the fact that its affinity for heterotrimer **7** is only a factor 22 less.[8] The fact that under the conditions of the above experiments amplification is selective for the best receptor illustrates that dynamic combinatorial chemistry can give the "right answers",[3] even in competition situations where theory warns of a possible breakdown of the correlation between binding and amplification efficiency. While this is comforting, we wanted to know what it would take before such breakdown would actually happen. For this purpose, we focused on guest **10**, which binds the various receptors in the order of: tetramer ≫ homotrimer > heterotrimer (see Table 1). Note that the tetramer has to compete with two different trimers, one of which is a hetero-compound.

We have prepared a DCL from building blocks **1** and **2** in a 2:1 ratio with a total building block concentration of 5.0 mM. Figure 2a,b shows the HPLC analysis of this library in the absence of any guest and in the presence of 10 mM of **10**, respectively. Whereas under these conditions clear amplification of both trimers was observed, the weaker binding heterotrimer was more amplified than the stronger binding homotrimer (the amplification factors[9] are 7.4 versus 6.2, respectively); that is, the library appears to give the "wrong"[3] answer.

The simulations of DCLs predict that the template concentration is an important parameter in the correlation between amplification factors and binding affinities, with correlations improving as the

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Table 1. Equilibrium Constants K (M^{-1}) and Gibbs Energies of Binding $\Delta G°$ ($kJ \cdot mol^{-1}$) of Guests **4**, **5**, **6**, and **10** to Hosts **7**−**9**

	7[7]		**8**[7]		**9**	
	K[a]	$\Delta G°$	K[a]	$\Delta G°$	K[a]	$\Delta G°$
4	2.0×10^5	−30.2	4.4×10^4	−26.5		
5	2.5×10^4	−25.1	5.4×10^5	−32.7		
6			8.0×10^2	−16.6	4.0×10^6	−37.7
10	5.0×10^4	−26.8	7.9×10^4	−28.0	1.3×10^6	−34.8

[a] Equilibrium constants were determined by isothermal titration calorimetry in borate buffer (10 mM pH 9.0) at 298 K.

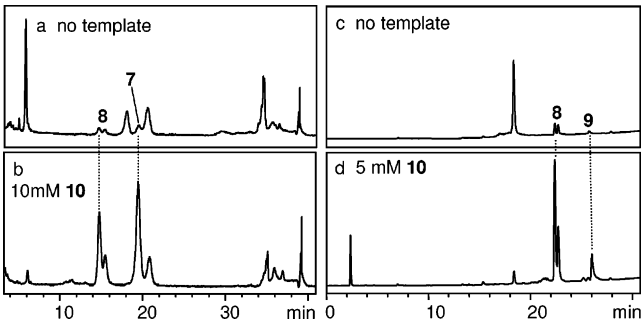
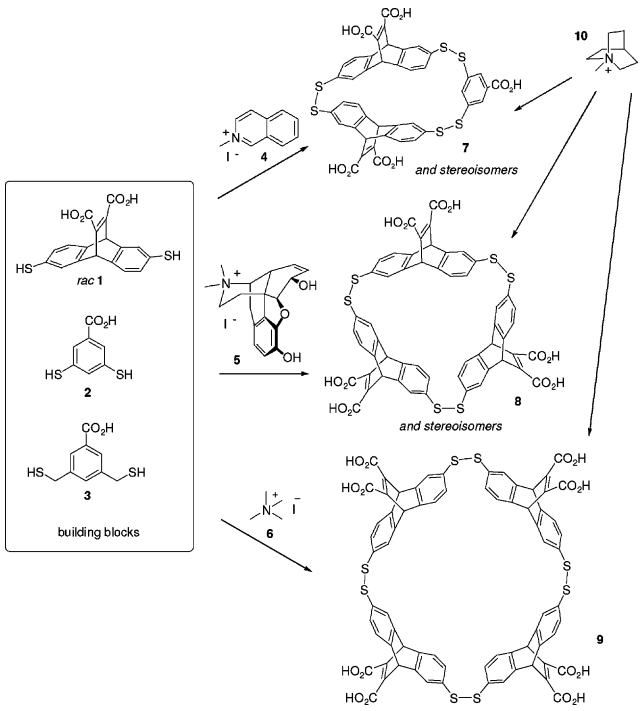


Figure 2. Left: HPLC analysis of a DCL made from dithiols **1** (3.33 mM) and **2** (1.67 mM) in water at pH 8−9 in the absence of template (a) and in the presence of 10 mM of guest **10** (b). Right: HPLC analysis of a DCL made from dithiol **1** (5 mM) in the absence of template (c) and in the presence of 5 mM of guest **10** (d).

Scheme 1. Guest-Induced Receptor Amplification



template concentration is reduced.[4,5] We have performed simple computer simulations[10] of the distribution of the library in Figure 2b at different template concentrations, using the binding constants in Table 1 and assuming that receptors **7**−**9** are the only library members that have any affinity for the guest. The results of these simulations are shown in Figure 3a and suggest that when the supply of template is limited, producing a large number of moderate binders is no longer beneficial and the library will revert to preferentially producing the highest affinity host−guest complexes.[4,5]

We then analyzed experimentally the amplification of both trimers as a function of the guest concentration. The results are

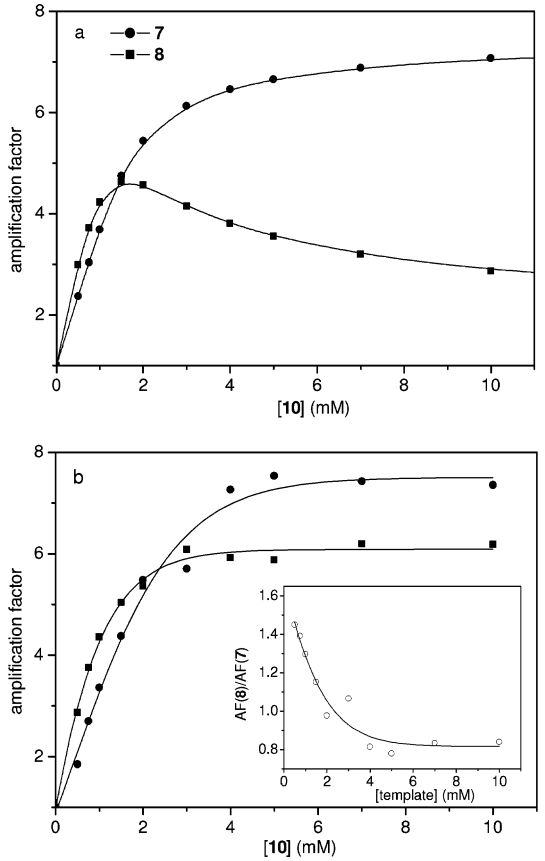


Figure 3. Simulated (a) and experimentally observed (b) amplification factors[9] for hosts **7** (●) and **8** (■) as a function of the concentration of template **10**. The inset shows the ratio of experimentally observed amplification factors (AFs) for **7** and **8** as a function of template concentration. The lines are purely for visual guidance.

shown in Figure 3b and are in agreement with the theoretical predictions; the best binder is the most amplified at lower template concentrations, whereas a relatively small excess of template allows the amplification factor of the heterotrimer to overtake that of the homotrimer. The inset in Figure 3b shows the ratio of amplification factors as a function of template concentration, indicating that amplification is most selective at low template concentration. Although the absolute values of the amplification factors deviate somewhat from the simulated data, the simulations reproduce the overall behavior reasonably well. This confirms that simulations are a very rapid and practical way of exploring the behavior of dynamic libraries and are a powerful tool to guide the design of DCL experiments.

We have attempted to study the amplification behavior of the tetramer **9** in the library made from **1** and **2**, but unfortunately, in the HPLC analysis, the peak due to the tetramer could not be separated from the other library members, so that no accurate quantitative data could be obtained. Moreover, our simulations predict that the tetramer constitutes 0.014% of the library in the absence of template, and while its amplification factor in the presence of template can be as high as 42 (see Supporting Information), the amount of tetramer remains less than 0.6% of the total library material. We therefore shifted our attention to a simpler "library" made exclusively from building block **1**. Figure 2c,d shows the amplification of the competing homotrimeric and tetrameric receptors **8** and **9** upon introducing guest **10** (at 5 mM concentration). The stronger binding tetramer was amplified 30 times, whereas the trimer was amplified only 8 times. That the peak due to the trimer is nevertheless larger than that corresponding to

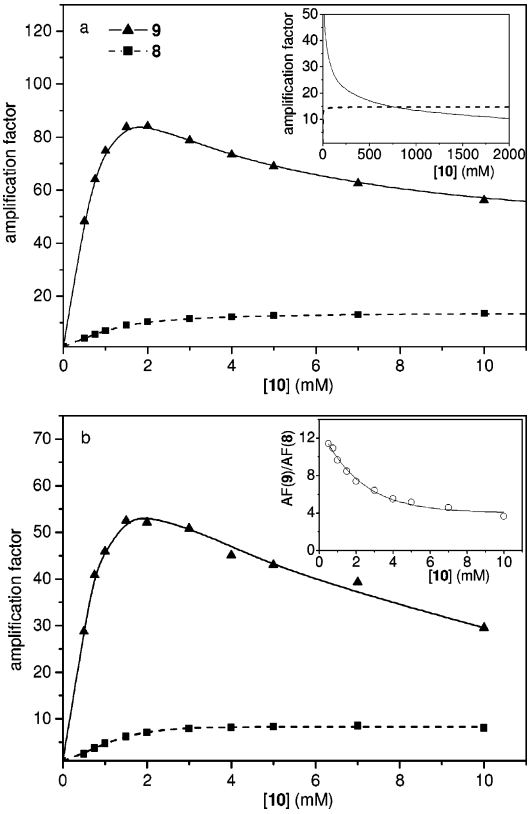


Figure 4. Simulated (a) and experimentally observed (b) amplification factors[9] for hosts **9** (▲) and **8** (■) as a function of the concentration of template **10**. The inset in (a) represents the simulated amplification factors for hosts **9** (solid line) and **8** (dashed line) at higher template concentrations. The inset in (b) shows the ratio of experimentally observed amplification factors for **7** and **8** as a function of template concentration. The lines are purely for visual guidance.

the tetramer is due to the fact that the trimer starts off at a higher concentration than the tetramer in the absence of template.

Amplification of trimer and tetramer was studied at different template concentrations in silico as well as experimentally. The results of both studies are summarized in Figure 4a,b. Both simulation and experiment show that the amount of tetramer reaches a maximum at a guest concentration of approximately 1.5 mM. Increasing the concentration of guest beyond this value *reduces* the amount of the best binder. The weaker binding trimer is gradually suppressing the stronger binding tetramer. Whereas within our experimental window the latter remains the most amplified compound, the simulations predict that the amplification factor of the trimer will eventually be larger than that of the tetramer, but only at template concentrations in excess of 500 mM (see inset in Figure 4a).

In conclusion, our results represent a first quantitative experimental glimpse of the intriguing behavior of complex equilibrium systems, confirming that the competition between receptors within a dynamic library need not always be won by the best receptor. However, in the systems we have investigated, such behavior was only observed when the affinities of the competing receptors are similar[11] or when large amounts of template are used. Reassuringly, in all instances, dropping the amount of template to close to stoichiometric concentrations ensures amplification of the fittest without any serious loss of templating efficiency.

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Supporting Information Available: Simulation results for amplification as a function of template concentrations for the library in Figure 1c; analogous data for the amplification of **9** in the library in Figure 2b; experimental and simulation procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For reviews, see: (a) Otto, S. *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 509−520. (b) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898−952. (c) Ramström, O.; Bunyapaiboonsri, T.; Lohmann, S.; Lehn, J.-M. *Biochim. Biophys. Acta* **2002**, *1572*, 178−186.

(2) For examples of amplification in DCLs, see: (a) Corbett, P. T.; Tong, L. H.; Sanders, J. K. M.; Otto, S. J. *J. Am. Chem. Soc.* **2005**, *127*, in press. (b) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Science* **2002**, *297*, 590−593. (c) Brisig, B.; Sanders, J. K. M.; Otto, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1270−1273. (d) Otto, S.; Kubik, S. *J. Am. Chem. Soc.* **2003**, *125*, 7804−7805. (e) Roberts, S. L.; Furlan, R. L. E.; Otto, S.; Sanders, J. K. M. *Org. Biomol. Chem.* **2003**, *1*, 1625−1633. (f) Furlan, R. L. E.; Ng, Y. F.; Cousins, G. R. L.; Redman, J. E.; Sanders, J. K. M. *Tetrahedron* **2002**, *58*, 771−778. (g) Kieran, A. L.; Bond, A. D.; Belenguer, A. M.; Sanders, J. K. M. *Chem. Commun.* **2003**, 2674−2675. (h) Storm, O.; Lüning, U. *Chem.—Eur. J.* **2002**, *8*, 793. (i) Lüning, U. *J. Incl. Phenom. Macrocycl. Chem.* **2004**, *49*, 81−84. (j) Fuchs, B.; Nelson, A.; Star, A.; Stoddart, J. F.; Vidal, S. B. *Angew. Chem., Int. Ed.* **2003**, *42*, 4220−4224. (k) Cai, M. M.; Shi, X. D.; Sidorov, V.; Fabris, D.; Lam, Y. F.; Davis, J. T. *Tetrahedron* **2002**, *58*, 661−671. (l) Hochgurtel, M.; Kroth, H.; Piecha, D.; Hofmann, M. W.; Nicolau, C.; Krause, S.; Schaaf, O.; Sonnenmoser, G.; Eliseev, A. V. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3382−3387. (m) Hochgurtel, M.; Biesinger, R.; Kroth, H.; Piecha, D.; Hofmann, M. W.; Krause, S.; Schaaf, O.; Nicolau, C.; Eliseev, A. V. *J. Med. Chem.* **2003**, *46*, 356−358. (n) Kubota, Y.; Sakamoto, S.; Yamaguchi, K.; Fujita, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4854−4856. (o) Lins, R. J.; Flitsch, S. L.; Turner, N. J.; Irving, E.; Brown, S. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 3405−3507. (p) Lins, R. J.; Flitsch, S. L.; Turner, N. J.; Irving, E.; Brown, S. A. *Tetrahedron* **2004**, *60*, 771−780. (q) Congreve, M. S.; Davis, D. J.; Devine, L.; Granata, C.; O'Reilly, M.; Wyatt, P. G.; Jhoti, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4479−4482. (r) Albrecht, M.; Janser, I.; Runsink, J.; Raabe, G.; Weis, P.; Frohlich, R. *Angew. Chem., Int. Ed.* **2004**, *43*, 6662−6666. (s) Telfer, S. G.; Yang, X. J.; Williams, A. F. *Dalton Trans.* **2004**, 699−705. (t) Gonzalez-Alvarez, A.; Alfonso, I.; Lopez-Ortiz, F.; Aguirre, A.; Garcia-Granda, S.; Gotor, V. *Eur. J. Org. Chem.* **2004**, 1117−1127. (u) Zameo, S.; Vauzeilles, B.; Beau, J.-M. *Angew. Chem., Int. Ed.* **2005**, *44*, 965−969. (v) Lam, R. T. S.; Belenguer, A.; Roberts, S. L.; Naumann, C.; Jarrosson, T.; Otto, S.; Sanders, J. K. M. *Science*, **2005**, *308*, 667−669. For a theoretical discussion on the influence of library size on amplification, see: (w) Moore, J. S.; Zimmerman, N. W. *Org. Lett.* **2000**, *2*, 915−918. (x) Corbett, P. T.; Otto, S.; Sanders, J. K. M. *Org. Lett.* **2004**, *6*, 1825−1827.

(3) We use the term "fittest" for the strongest binding receptor in the library. Libraries in which the strongest binder is selectively amplified are said to give the "right" answer, whereas libraries in which this is not the case are giving the "wrong" answer.

(4) (a) Grote, Z.; Scopelliti, R.; Severin, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 3821−3825. (b) Severin, K. *Chem.—Eur. J.* **2004**, *10*, 2565−2580.

(5) Corbett, P. T.; Otto, S.; Sanders, J. K. M. *Chem.—Eur. J.* **2004**, *10*, 3139.

(6) During preparation of this manuscript, Severin and co-workers reported qualitative experimental evidence of a disruption of the correlation between binding strength and amplification efficiency. See: Saur, I.; Severin, K. *Chem. Commun.* **2005**, 1471−1473.

(7) Homo- and heterotrimers are amplified as mixtures of stereoisomers. In this paper, we will only discuss the behavior and binding data for the major diastereomers.

(8) Templating experiments were conducted using a total building block concentration of 10 mM and a guest concentration of 5 mM. Use of higher guest concentrations may, in theory, favor the heterotrimer. Unfortunately, the limited solubility of **5** does not allow experimental studies at much higher concentrations. However, computer simulations using the experimentally determined binding constants suggest that amplification remains selective for the best binder up to template concentrations of 750 mM (see Supporting Information).

(9) The amplification factor is defined as the concentration of a library member in the presence of the template divided by the corresponding concentration in the absence of the template.

(10) Simulations were performed with our software package *DCLSim* (see Supporting Information of ref 5 for details). Stereoisomers of **7** and **8** are considered explicitly, and all stereoisomeric compounds have been given identical binding constants. Simulations that allow binding constants of stereoisomers to differ by a factor of 3 (the maximum level of difference observed between diastereomers) produce very comparable results.

(11) When two members of a dynamic combinatorial library have similar binding constants, they tend to be amplified to similar extents. This behavior is probably desirable in most cases;[2c] however, when there is a need for very high selectivity, then pseudo-dynamic combinatorial chemistry is an attractive alternative approach. See: Corbett, A. R.; Cheeseman, J. D.; Kazlauskas, R. J.; Gleason, J. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 2432−2436.

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