Competition between Receptors in Dynamic Combinatorial Libraries

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Published in:
Journal of the American Chemical Society

DOI:
10.1021/ja0509026

Publication date:
2005

J. Am. Chem. Soc.

Supporting Information

Competition between receptors in dynamic combinatorial libraries: amplification of the fittest?

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ADDITIONAL DATA

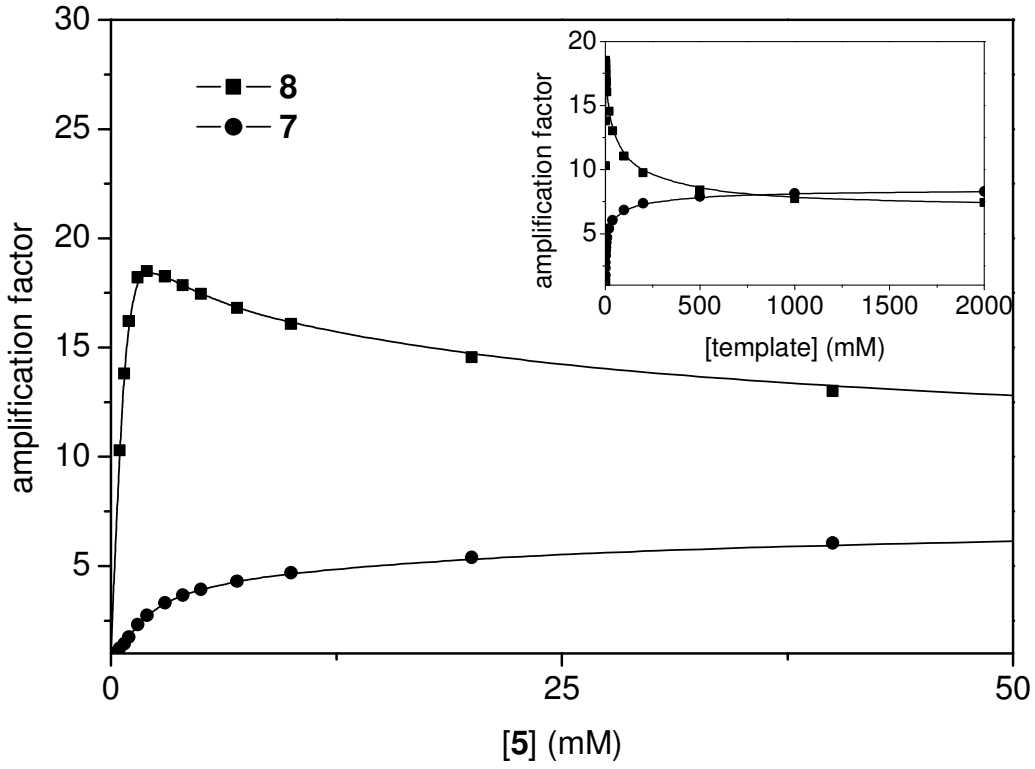

Figure S1. *Simulation results showing amplification factors of* ***7*** *(major diastereomer) and* ***8*** *(major diastereomer) as a function of the concentration of guest* ***5*** *for the library in Figure 1c. The inset shows the amplification factors at higher guest concentrations.*

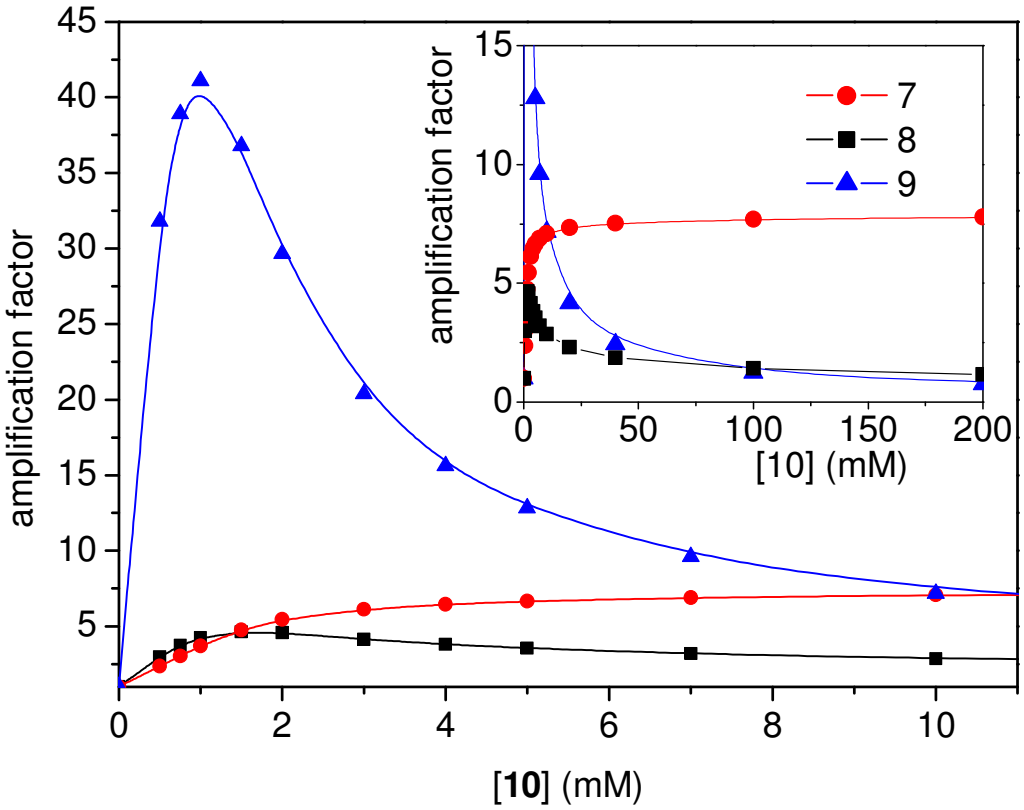

Figure S2. *Simulation results showing amplification factors of* ***7*** *(major diastereomer),* ***8*** *(major diastereomer) and* ***9*** *as a function of the concentration of guest* ***10*** *in the DCL made from rac****1*** *(3.33mM) and* ***2*** *(1.67mM). The inset shows the amplification factors at higher guest concentrations. Note that at high template concentrations the amplification factors are in the order* ***7*** *>* ***8*** *>* ***9*** *whereas the binding constants are in the order of* ***9*** *>* ***8*** *>* ***7****.*

Table S1. *Simulated concentrations of **7** (major diastereomer), **8** (major diastereomer) and **9** in the DCL made from rac**1** (3.33mM) and **2** (1.67mM) as a function of the concentration of guest **10**.*

[**10**] (mM)	[**9**] (μM)	[**8**] (μM)	[**7**] (μM)
0	0.17	30	103
0.50	5.50	90	242
0.75	6.73	113	312
1.0	7.12	128	378
1.5	6.37	141	487
2.0	5.13	138	558
3.0	3.52	126	629
4.0	2.70	115	662
5.0	2.22	108	682
7.0	1.66	97	706
10	1.24	87	725
20	0.72	70	753
40	0.42	56	771
100	0.21	43	789
200	0.13	35	797
500	0.07	28	806
1000	0.05	25	809
2000	0.03	22	812

EXPERIMENTAL DETAILS

Materials

The synthesis of building blocks **1**[2b], **2**[2b] and **3**[S1] and receptors **7**[2b], **8**[2b] and **9**[2a] and guests **4**[2b], **5**[2b] have been described previously.

Dynamic combinatorial libraries

DLCs were constructed by dissolving the required building blocks **1**-**3** in water, and adjusting the pH to 8 using NaOH and HCl. The resulting solutions (5mM or 10mM) were allowed to equilibrate for at least 2 weeks, either in the absence or presence of the guest, by stirring as 1mL aliquots in closed 2mL HPLC vials.

HPLC conditions for analysis of the DCLs in Figures 1-4.

Conditions for Figure 1a-c are described in ref. 2b. The remaining HPLC analyses were carried out on Hewlett Packard 1050 and 1100 systems coupled to a UV analyzer, set to 320nm. The data were processed using HP Chemstation software. Separations were achieved using a Waters Symmetry C_{18} column (25.0 cm × 4.6 mm, 5 μm particle size).

For Fig. 1d,e and Fig. 2c,d the following gradient was used at a flow rate of 1mL/min, at 45°C, with 2µl injections:

time (min)	% H_2O (0.1% TFA)	% MeCN (0.1% TFA)	% IPA (0.1% TFA)
0	95	4.2	0.8
30	5	79.8	15.2
34	5	79.8	15.2
35	95	4.2	0.8
50	95	4.2	0.8

For Fig 2a,b and 3b the following gradient was used at 1mL/min, 45°C, with 2µl injections:

Time (min)	% H_2O (0.1% TFA)	% MeCN (0.1% TFA)	% IPA (0.1% TFA)
0	50	38	12
30	50	38	12
31	0	76	24
35	0	76	24
36	50	38	12
50	50	38	12

For the data in Fig. 4b an isocratic mobile phase was used at 1mL/min, consisting of acetonitrile, water and trifluoroacetic acid in the ratio of 55:45:0.1. The analysis was performed at room temperature. An injection volume of 2µl was used.

Equilibrium Simulations

Equilibrium calculations were performed using DCLSim 1.1, using the modules that support the calculation of user-specified equilibria. The algorithms used in DCLSim 1.1 are unchanged from DCLSim 1.0 – however, they have been re-coded in C to increase the speed of the simulations. Further details are provided in the supporting information to ref. 5. Please contact the authors for the availability of the software.

The equilibrium models used represent an idealized versions of the dynamic combinatorial libraries studied – the only library members that were given affinities for the template were the various stereoisomers of **7**, **8**, and **9**. All other library members were assigned zero Gibbs energies of binding. The library distributions have not been fitted to experimental peak areas. One adjustment has been made: dimers **1.2** and **2.2** have not been included. This is consistent with the observed experimental behavior of the library, in which those dimers are not formed in significant quantities. Control simulations in which **1.2** and **2.2** were allowed to form gave similar overall results.

The various stereoisomers of **7** are explicitly considered, assuming equilibrium constants for guest binding for all stereoisomers to be equal to the experimental value for the major diastereomer. The same assumptions were made for **8**. Control simulations in which the binding constants the minor stereoisomers of **7** and **8** were varied resulted in variation of

the absolute values of the amplification factors, but did not change the overall shape of the curves.
The stereochemistry of the major stereoisomer of **7** is still under investigation. However in the simulations the amplification factors of meso(*R***1**.*S***1**.**2**) and rac(*R***1**.*R***1**.**2**) are identical. The amplification factors for the major diastereomer of **8** (as a racemic mixture) were determined using the following equation:

$$\text{AF}(\mathbf{8}) = ([R\mathbf{1}.S\mathbf{1}.S\mathbf{1}]_{\text{templ.}} + [S\mathbf{1}.R\mathbf{1}.R\mathbf{1}]_{\text{templ.}}) \;/\; ([R\mathbf{1}.S\mathbf{1}.S\mathbf{1}]_{\text{untempl.}} + [S\mathbf{1}.R\mathbf{1}.R\mathbf{1}]_{\text{untempl.}})$$

Simulations using a highly simplified model completely ignoring stereoisomerism gave very comparable curves although the absolute values for the amplification factors are somewhat different. For example, Figure S3 shows the library of Figure 4a when simulated without considering stereoisomerism (i.e. assuming only one dimer, one trimer and one tetramer can be formed).

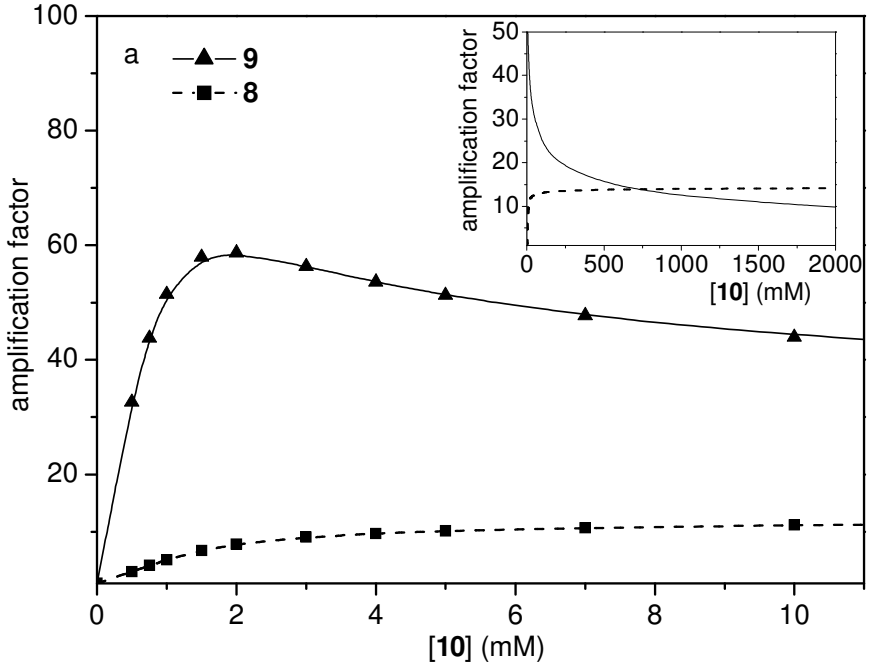

Figure S3. *Simulated amplification factors for hosts* ***9*** *(▲) and* ***8*** *(■) as a function of the concentration of template* ***10****, without considering stereoisomerism. The inset represents the simulated amplification factors for hosts* ***9*** *(solid line) and* ***8*** *(dashed line) at higher template concentrations. The lines are purely for visual guidance.*

The listings below represent the library compositions and equilibrium constants for the formation of the library members from the building blocks (K) and the experimental Gibbs energy of binding of the library members to the guest ($\Delta G°$), used as input for the simulations. The equilibrium constants of formation are set such that a statistical distribution of library members is produced in the absence of guest.

Simulated DCL for Fig. 3a and Fig. S2:

Building Block	Total concentration (M)
***R*1**	0.00167
***S*1**	0.00167
2	0.00167

Library Member	*K*	ΔG°
9 (***R*1.*S*1.*R*1.*S*1**)	2	-34.8
8 (***R*1.*R*1.*R*1**)	1	-28.0
8 (***S*1.*S*1.*S*1**)	1	-28.0
8 (***S*1.*R*1.*R*1**)	3	-28.0
8 (***S*1.*S*1.*R*1**)	3	-28.0
7 (***S*1.*S*1.2**)	3	-26.8
7 (***R*1.*R*1.2**)	3	-26.8
7 (***S*1.*R*1.2**)	6	-26.8
***R*1.2.2**	3	0
***S*1.2.2**	3	0
2.2.2	1	0
***R*1.*R*1**	1	0
***S*1.*S*1**	1	0
***R*1.*S*1**	2	0
***R*1.*R*1.*R*1.*R*1**	1	0
***R*1.*R*1.*R*1.*S*1**	4	0
***R*1.*R*1.*R*1.2**	4	0
***R*1.*R*1.*S*1.*S*1**	4	0
***R*1.*R*1.*S*1.2**	12	0
***R*1.*R*1.2.2**	6	0
***R*1.*S*1.*S*1.*S*1**	4	0
***R*1.*S*1.*S*1.2**	12	0
***R*1.*S*1.2.2**	12	0
***R*1.2.2.2**	4	0
***S*1.*S*1.*S*1.*S*1**	1	0
***S*1.*S*1.*S*1.2**	4	0
***S*1.*S*1.2.2**	6	0
***S*1.2.2.2**	4	0
2.2.2.2	1	0

Simulated DCL for Fig. 4a:

Building Block	Total concentration (M)
***R*1**	0.0025
***S*1**	0.0025

Library Member	*K*	Δ*G*°
9 (***R*1.*S*1.*R*1.*S*1**)	2	-34.8
8 (***R*1.*R*1.*R*1**)	1	-28.0
8 (***S*1.*S*1.*S*1**)	1	-28.0
8 (***S*1.*R*1.*R*1**)	3	-28.0
8 (***S*1.*S*1.*R*1**)	3	-28.0
***R*1.*R*1**	1	0
***S*1.*S*1**	1	0
***R*1.*S*1**	2	0
***R*1.*R*1.*R*1.*R*1**	1	0
***R*1.*R*1.*R*1.*S*1**	4	0
***R*1.*R*1.*S*1.*S*1**	4	0
***R*1.*S*1.*S*1.*S*1**	4	0

Simulated DCL for Fig. S3:

Building Block	Total concentration (M)
1	0.005

Library Member	*K*	Δ*G*°
9 (**1.1.1.1**)	1.0	-34.8
8 (**1.1.1**)	1.0	-28.0
1.1	1.0	0.0

Simulated DCL for Fig. S1:

Building Block	Total concentration (M)
***R*1**	0.00167
***S*1**	0.00167
2	0.00333
3	0.00333

Library Member	*K*	ΔG°
8 (*R*1.*R*1.*R*1)	1	-32.7
8 (*S*1.*S*1.*S*1)	1	-32.7
8 (*R*1.*R*1.*S*1)	3	-32.7
8 (*R*1.*S*1.*S*1)	3	-32.7
7 (*R*1.*R*1.**2**)	3	-25.1
7 (*R*1.*S*1.**2**)	6	-25.1
7 (*S*1.*S*1.**2**)	3	-25.1
*R*1.*R*1.**3**	3	0
*R*1.*S*1.**3**	6	0
*R*1.**2.2**	3	0
*R*1.**2.3**	6	0
R1.3.3	3	0
*S*1.*S*1.**3**	3	0
*S*1.**2.2**	3	0
*S*1.**2.3**	6	0
*S*1.**3.3**	3	0
2.2.2	1	0
2.2.3	3	0
2.3.3	3	0
3.3.3	1	0
*R*1.*R*1	1	0
*S*1.*S*1	1	0
*R*1.*S*1	2	0
2.3	2	0
3.3	1	0
*R*1.*R*1.*R*1.*R*1	1	0
*R*1.*R*1.*R*1.*S*1	4	0
*R*1.*R*1.*R*1.**2**	4	0
*R*1.*R*1.*R*1.**3**	4	0
*R*1.*R*1.*S*1.*S*1	6	0
*R*1.*R*1.*S*1.**2**	12	0
*R*1.*R*1.*S*1.**3**	12	0
*R*1.*R*1.**2.2**	6	0
*R*1.*R*1.**2.3**	12	0
*R*1.*R*1.**3.3**	6	0
*R*1.*S*1.*S*1.*S*1	4	0
*R*1.*S*1.*S*1.**2**	12	0
*R*1.*S*1.*S*1.**3**	12	0
*R*1.*S*1.**2.2**	12	0
*R*1.*S*1.**2.3**	24	0
*R*1.*S*1.**3.3**	12	0
*R*1.**2.2.2**	4	0

***R*1.2.2.3**	12	0
***R*1.2.3.3**	12	0
***R*1.3.3.3**	4	0
***S*1.*S*1.*S*1.*S*1**	1	0
***S*1.*S*1.*S*1.2**	4	0
***S*1.*S*1.*S*1.3**	4	0
***S*1.*S*1.2.2**	6	0
***S*1.*S*1.2.3**	12	0
***S*1.*S*1.3.3**	6	0
***S*1.2.2.2**	4	0
***S*1.2.2.3**	12	0
***S*1.2.3.3**	12	0
***S*1.3.3.3**	4	0
2.2.2.2	1	0
2.2.2.3	4	0
2.2.3.3	6	0
2.3.3.3	4	0
3.3.3.3	1	0

REFERENCES

(S1) Otto, S.; Furlan, R.L.E.; Sanders, J.K.M. *J. Am. Chem. Soc.* **2000**, *122*, 12063.