ORGANIC
LETTERS

2010
Vol. 12, No. 9
1920−1923

A Simple Network of Synthetic Replicators Can Perform the Logical OR Operation

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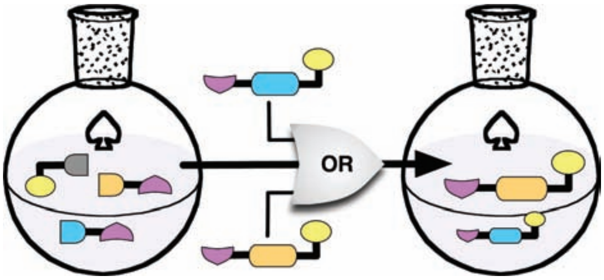
Received February 17, 2010

ABSTRACT



A small network of synthetic replicators is capable of responding to instructional inputs such that the output of the network is an excess of one of the replicators whenever the input contains either or both of the replicators, mirroring the OR boolean logic operation.

We have become interested in the exploitation of synthetic strategies that encompass replication processes[1] for facilitating the fabrication of molecular architectures at the nanometer scale. In order to evolve synthetic machinery that is capable of directing its own synthesis and cooperating with other similar systems to create[2] an organized hierarchy, it is important to develop a fundamental understanding of recognition-mediated processes that allow molecules to function as specific and efficient templates for the formation of themselves (autocatalysis) and other templates (cross catalysis). Such an understanding should permit the development of efficient protocols that allow us to establish and manage replication, organization, and evolution within synthetic molecular and supramolecular assemblies, so-called[3] "systems chemistry". Ultimately, the creation of large molecular and supramolecular assemblies can be programmed by exploiting[4] selection and amplification through emergent system behavior.

As an initial step toward this ambitious goal, we explored a system in which a small network of interdependent replicating systems cooperated to exhibit defined emergent behavior. In order to achieve this aim, we must first identify a family of structurally similar self-replicating templates that possess the correct steric and electronic properties to allow them to interact with each other. Previously, we described[5] the kinetic behavior of template $\mathbf{T_1}$, which was constructed from nitrone **A** and maleimide **B** (Figure 1). This template is the major product of the reaction between **A** and **B** and was shown to be capable of increasing the rate of its own formation by around 3-fold, at the expense of its catalytically

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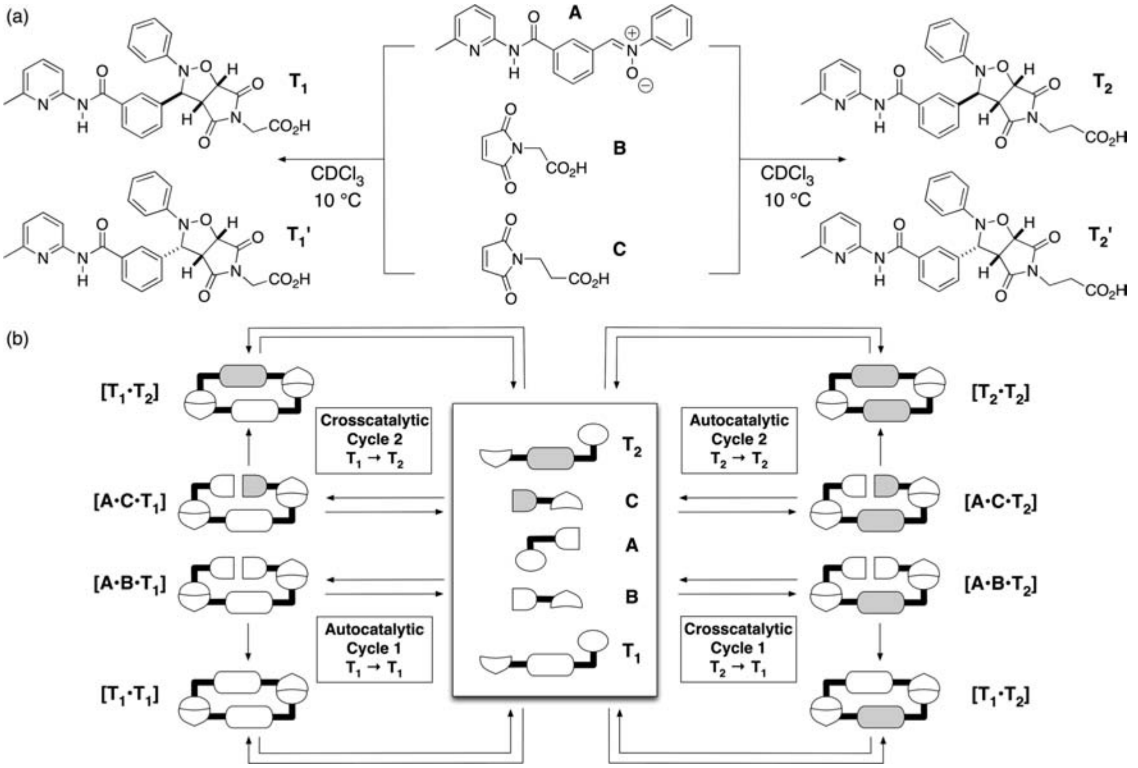

Figure 1. (a) Nitrone **A** and maleimides **B** and **C** are combined to create two self-replicating templates $\mathbf{T_1}$ and $\mathbf{T_2}$. The corresponding diastereoisomeric templates, $\mathbf{T_1'}$ and $\mathbf{T_2'}$, are catalytically inert. (b) Templates $\mathbf{T_1}$ and $\mathbf{T_2}$ cooperate through a network of two autocatalytic and two cross-catalytic cycles.

inactive diastereoisomer $\mathbf{T_1'}$. Building on this discovery, we identified template $\mathbf{T_2}$, and its diastereoisomer $\mathbf{T_2'}$, as suitable candidates from which to construct the simple network of interdependent catalytic cycles shown in Figure 1. Electronic structure calculations led us to expect that, in common with template $\mathbf{T_1'}$, $\mathbf{T_2'}$ would be catalytically inactive. Therefore, considering this network, there are two autocatalytic cycles in which each template, $\mathbf{T_1}$ and $\mathbf{T_2}$, directs its own formation. Additionally, because $\mathbf{T_1}$ and $\mathbf{T_2}$ differ by only one CH_2 group, we might expect that two cross-catalytic cycles would also exist in which $\mathbf{T_1}$ directs the formation of $\mathbf{T_2}$ and vice versa. Therefore, the products of a reaction between **A**−**C** should depend on the relative efficiencies of each of these four cycles.

Initially, it was important to demonstrate that template $\mathbf{T_2}$ is capable of directing its own formation. We have described[6] protocols for accomplishing this task previously, and these methods were applied to this system. All reactions were performed from a starting concentration of 25 mM of each reagent at 10 °C in $CDCl_3$ and were monitored by 400 MHz ^1H NMR spectroscopy. The control reaction between **A** and the methyl ester of **C** affords a 3.8:1 ratio of the methyl esters of $\mathbf{T_2}$ and $\mathbf{T_2'}$ at 11% conversion after 10 h. This ratio and conversion represent the baseline behavior for this system in the absence of any recognition-mediated reactions. The reaction of **A** with **C** affords a 11.4:1 ratio of $\mathbf{T_2}/\mathbf{T_2'}$ at 42% conversion after 10 h. The important role of recognition in this system was demonstrated by the reaction of **A** with **C** in the presence of the competitive inhibitor benzoic acid (8.4:1 ratio of $\mathbf{T_2}/\mathbf{T_2'}$; 23% conversion after 10 h). Finally, we demonstrated that $\mathbf{T_2}$ is a template for its own formation through an experiment in which 40 mol % of $\mathbf{T_2}$ was added at the start of the reaction between **A** and **C**. Under these conditions, the rate of formation of $\mathbf{T_2}$ is enhanced significantly (1.6-fold) and the ratio of $\mathbf{T_2}/\mathbf{T_2'}$ is 16.8:1 at 54% conversion after 10 h. The template $\mathbf{T_2'}$ does not show any recognition-mediated behavior in these reactions.

Having demonstrated that template $\mathbf{T_2}$ is capable of directing its own formation, we set about establishing the cross-catalytic behavior of these templates. In these experiments, the building blocks required to construct one template, either $\mathbf{T_1}$ or $\mathbf{T_2}$, were dissolved in $CDCl_3$ at 25 mM. This reaction mixture was then doped with 40 mol % of one of the templates, either $\mathbf{T_1}$ or $\mathbf{T_2}$. Each reaction mixture was then incubated at 10 °C for 16 h, and the progress of the reaction was followed by 400 MHz ^1H NMR spectroscopy. The results of this study are presented in Figure 2.

As expected, the results confirm that each template is capable of directing its own formation. The addition of $\mathbf{T_1}$ engenders a 1.6 times enhancement in the maximum rate[7]

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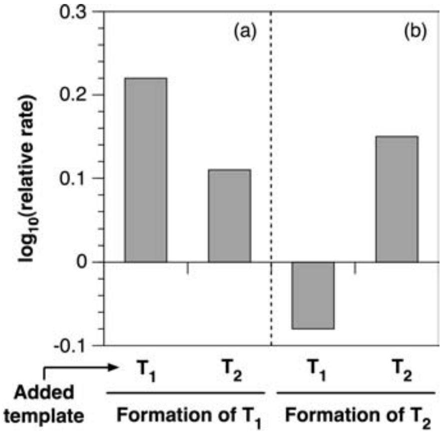

Figure 2. Effect of the addition of $\mathbf{T_1}$ and $\mathbf{T_2}$ on (a) the formation of $\mathbf{T_1}$ and (b) the formation of $\mathbf{T_2}$. The effect of the added template (40 mol % added at the start of the reaction) is measured by log(relative rate). This measure is calculated by taking the logarithm of the ratio of the maximum rates of the reactions in the experiments with and without added template.

of formation of itself. $\mathbf{T_2}$ is a somewhat poorer template for its own formation, engendering a 1.3 times increase in the maximum rate for its formation. It is clear that the behavior of the templates toward each other is markedly different. Template $\mathbf{T_2}$ also directs the formation of $\mathbf{T_1}$ (1.4 times increase in the maximum rate of $\mathbf{T_1}$ production), whereas $\mathbf{T_1}$ inhibits the formation of $\mathbf{T_2}$ slightly (the maximum rate 0.8 times that in the absence of added template). This behavior implies that, although cross-catalytic cycle 1 (Figure 1b) operates efficiently, cross-catalytic cycle 2 does not.

We found these results surprising given the structural similarity of the templates. We therefore turned to electronic structure calculation of the transition states of the autocatalytic and cross-catalytic ternary complexes (Figure 3) in order to gain some insight into the subtle structural mismatch between the templates. Calculations were performed at the B3LYP/6-31G(d,p) level of theory, and transition states leading to the appropriate products were located successfully for each of the four ternary complexes.

The results of these calculations are revealing. For the autocatalytic ternary complexes [$\mathbf{A{\cdot}B{\cdot}T_1}$] and [$\mathbf{A{\cdot}C{\cdot}T_2}$], the transition states leading to [$\mathbf{T_1{\cdot}T_1}$] and [$\mathbf{T_2{\cdot}T_2}$] are supported on the preformed template without distortion and the transition states are bound to the preformed templates by four hydrogen bonds. The structure of [$\mathbf{A{\cdot}B{\cdot}T_1}$] (Figure 3a) is representative of the transition states where the templates are matched. In the case of the cross-catalytic ternary complex [$\mathbf{A{\cdot}B{\cdot}T_2}$], the transition state leading to [$\mathbf{T_1{\cdot}T_2}$] is also supported on the preformed template without distortion by four hydrogen bonds. However, the calculated structure of the transition state accessed by the cross-catalytic ternary complex [$\mathbf{A{\cdot}C{\cdot}T_1}$] shows significant distortion (Figure 3b) and forces maleimide **C** to adopt an unfavorable conformation (starred bond, Figure 3b) in order to access the transition state. This distortion is a result of the shorter overall length of the $\mathbf{T_1}$ template. Although the $\mathbf{T_2}$ template can compress itself to accommodate the transition state leading to $\mathbf{T_1}$ within [$\mathbf{A{\cdot}B{\cdot}T_2}$], the shorter and more rigid structure of $\mathbf{T_1}$ forces compression of the $\mathbf{T_2}$ transition state when accessed from [$\mathbf{A{\cdot}C{\cdot}T_1}$]. These results are in complete accord with those observed experimentally and identify the small structural mismatch that gives rise to the observed behavior.

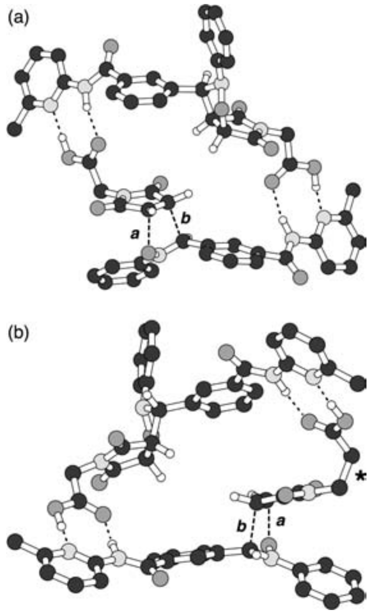

Figure 3. Calculated (B3LYP/6-31G(d,p)) transition-state structures for (a) forming $\mathbf{T_1}$ on a $\mathbf{T_1}$ template ($a = 2.11$ Å; $b = 2.12$ Å) and (b) forming $\mathbf{T_2}$ on a $\mathbf{T_1}$ template ($a = 2.07$ Å; $b = 2.16$ Å). The dihedral angle about the starred bond is 61°. Atom types are represented by shading dark to light in the order carbon, oxygen, nitrogen, and hydrogen. Hydrogen bonds are represented by dotted lines and partial bonds in the transition states by dashed lines. Most hydrogen atoms have been removed for clarity.

It is therefore clear that, in a reaction mixture containing **A**−**C**, only three of the four possible catalytic cycles will be operative. Hence, we might expect that this network will display emergent behavior analogous to the logical OR operation. If we imagine that system inputs are the identities of the templates added and a reaction mixture containing **A**, **B** and **C** and the system output is the $\mathbf{T_1}$/$\mathbf{T_2}$ ratio, we can use our knowledge of the kinetic behavior of the templates in isolation to predict the overall network topology. In the absence of any added template, the replicators are of similar efficiency and should therefore coexist. In the presence of added template $\mathbf{T_1}$, $\mathbf{T_1}$ will be amplified and $\mathbf{T_2}$ suppressed since $\mathbf{T_1}$ is a catalyst for its own formation and an inhibitor for the formation of $\mathbf{T_2}$. In the presence of added template $\mathbf{T_2}$, $\mathbf{T_1}$ will be amplified through the cross-catalytic action of $\mathbf{T_2}$, and the more $\mathbf{T_1}$ is produced, the more dominant autocatalytic cycle 1 will become and the more the formation of $\mathbf{T_2}$ will be inhibited. In the presence of both templates,

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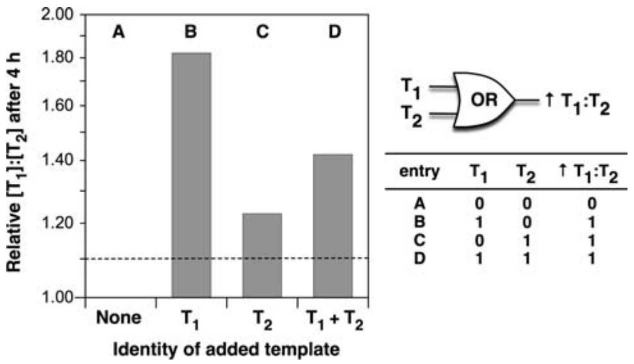
(7) Maximum rates (velocities) of reaction were calculated by determining the largest value of the first derivative of the polynomial function describing the concentration time profile for each reaction. For bimolecular reactions, this metric is equivalent to the initial rate. For autocatalytic reactions, this represents the point of inflection of the sigmoidal curve.

$\mathbf{T_1}$ will be amplified and $\mathbf{T_2}$ suppressed because $\mathbf{T_1}$ is a catalyst for its own formation and an inhibitor for the formation of $\mathbf{T_2}$. This behavior represents the logical OR operation: the presence of either template or both templates together results in the enhanced formation of $\mathbf{T_1}$ ($\mathbf{T_1}/\mathbf{T_2} > 1$).

With this expectation in mind, we conducted a series of four experiments, in each of which the starting concentrations of **A**−**C** were all 25 mM and 20 mol % template $\mathbf{T_1}$ and/or $\mathbf{T_2}$ was added as appropriate at the start of the reactions. All reactions were carried out in $CDCl_3$ at 10 °C, and the reaction mixtures were assayed[8] after 4 and 8 h by 400 MHz ^1H NMR spectroscopy (see the Supporting Information for details). The results are summarized in Figure 4.



entry	T_1	T_2	↑ T_1:T_2
A	0	0	0
B	1	0	1
C	0	1	1
D	1	1	1

Figure 4. Ratio of [$\mathbf{T_1}$]/[$\mathbf{T_2}$] is higher when the identity of the input template is $\mathbf{T_1}$ OR $\mathbf{T_2}$.

The results of these experiments confirm our predictions. Taking experiment A (Figure 4) as the baseline, the formation of $\mathbf{T_1}$ is enhanced significantly, by 1.8 times, when only $\mathbf{T_1}$ is added (experiment B, Figure 4). Clearly, in this case, autocatalytic cycle 1 is dominant. When only $\mathbf{T_2}$ is added (experiment C, Figure 4), $\mathbf{T_1}$ is once again amplified at the expense of $\mathbf{T_2}$. This outcome can be rationalized readily by realizing that $\mathbf{T_2}$ is, in fact, also a catalyst for the formation of $\mathbf{T_1}$. Therefore, cross-catalytic cycle 1 (Figure 1b) will generate significant quantities of $\mathbf{T_1}$. The production of $\mathbf{T_1}$ will, in turn, allow autocatalytic cycle 1 (Figure 1b) to become operative and, in addition, impose an inhibitory effect on the operation of cross-catalytic cycle 2 (Figure 1b). The net effect is amplification of $\mathbf{T_1}$ at the expense of $\mathbf{T_2}$. When both templates are added together (experiment D, Figure 4), $\mathbf{T_1}$ is once again amplified strongly at the expense of $\mathbf{T_2}$. In this case, autocatalytic cycle 1 and cross-catalytic cycle 1 are the dominant pathways in the system.

This series of experiments confirms our expectation that this small network of simple synthetic replicating systems is capable of acting in concert to perform the logical OR operation based on the input template. Template $\mathbf{T_1}$ is enhanced when the template input is $\mathbf{T_1}$ or $\mathbf{T_2}$ or both. Although the amplification effects reported here are not particularly large, we believe that this can be remedied by a careful computationally assisted replicator design. However, it is striking that two templates whose molecular weights are around 400 and which differ by a single CH_2 unit can exhibit the behavior observed here. The results presented here bode well for the development of more complex recognition-mediated reaction networks[9] that rely on multiple recognition events, such as a combination of minimal and reciprocal[10] replicators. Such systems can potentially generate and express more complex programmed responses to chemical inputs through template-directed processes. These strategies are currently under development in our laboratory.

Acknowledgment. The financial support of the Engineering and Physical Sciences Research Council and the Biotechnology and Biological Sciences Research Council is gratefully acknowledged.

Supporting Information Available: Experimental procedures and characterization for compunds **A**−**C**, $\mathbf{T_1}$, and $\mathbf{T_2}$ and procedures for conducting and assaying kinetic experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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[8] Analysis of complex systems by NMR spectroscopy relies on there being sufficient dispersion of the resonances utilized in the assay. ^1H NMR spectroscopy is not ideal for this purpose because ^1H chemical shifts span a relatively small range. In the system presented here, dispersion is not an issue. However, in cases where signal dispersion does become a problem, ^{19}F NMR spectroscopy provides a suitable alternative (see ref 9a for an example).

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