

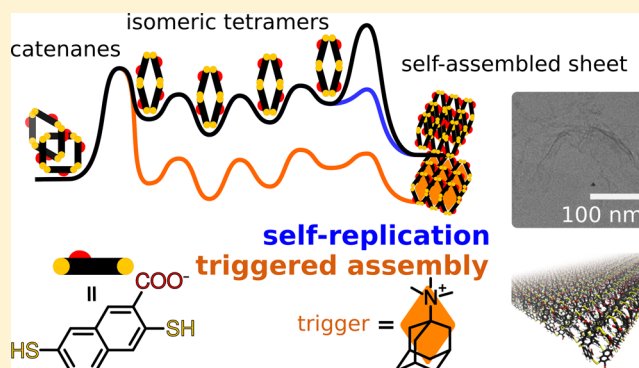
# Template-Triggered Emergence of a Self-Replicator from a Dynamic Combinatorial Library

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**S** Supporting Information

**ABSTRACT:** Self-assembly of a specific member of a dynamic combinatorial library (DCL) may lead to self-replication of this molecule. However, if the concentration of the potential replicator in the DCL fails to exceed its critical aggregation concentration (CAC), then self-replication will not occur. We now show how addition of a template can raise the concentration of a library member–template complex beyond its CAC, leading to the onset of self-replication. Once in existence, the replicator aggregates promote further replication also in the absence of the template that induced the initial emergence of the replicator.



## INTRODUCTION

Synthetic self-replicating chemical systems<sup>1,2</sup> have received considerable attention since the first example was shown by von Kiedrowski almost three decades ago.<sup>2a</sup> They are instrumental in addressing two of the grand challenges in science: the origin of life and creating life de novo.<sup>3</sup> Self-replication has recently found its place also in materials science, where it gives rise to self-synthesizing materials if the replicators undergo a self-assembly process, in which the assembly drives the replication.<sup>4</sup> In such cases, the information contained in the structure of the replicator directs the reaction network toward the formation of the very molecules that self-assemble. This process has been developed in the context of dynamic combinatorial chemistry. In a dynamic combinatorial library (DCL)<sup>5</sup> building blocks react with each other reversibly to yield multiple library members that are at equilibrium. Self-assembly of one of these library members will shift this equilibrium in favor of the assembling molecule,<sup>6</sup> resulting in a material that is therefore not only self-assembling but also self-synthesizing.

While self-synthesis can, in principle, create materials starting from the inactive subcomponent, there is one significant obstacle: the formation of the replicator (nucleation) is normally spontaneous and thus gives little room to make the process controllable. However, in Nature materials do not emerge spontaneously; their nucleation is usually triggered by separate entities or processes. Microtubule self-assembly is a representative example. The constituting proteins ( $\alpha$ - and  $\beta$ -tubulin) assemble into microtubules spontaneously, but the nucleation barrier is high. To trigger the formation of microtubules, cells use the  $\gamma$ -tubulin ring complex, which templates the assembly.<sup>7</sup> Control over assembly and replication by a molecular signal has been elusive in synthetic replicating systems.<sup>8</sup>

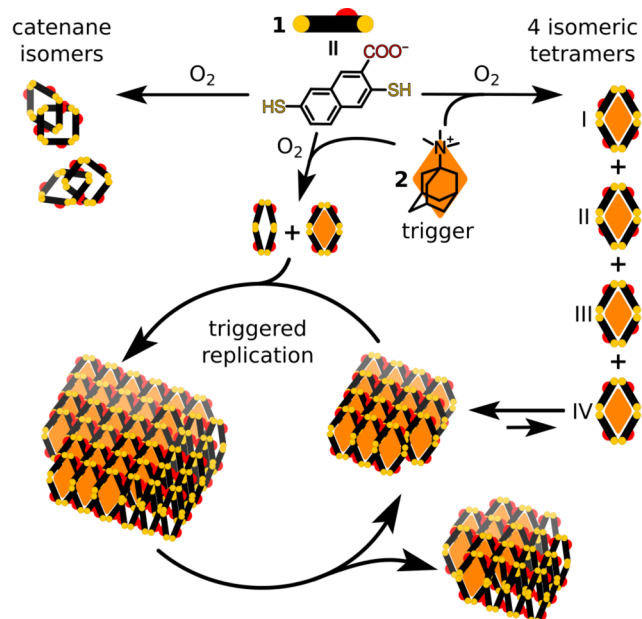
## RESULTS AND DISCUSSION

We now report the first example of a small dynamic combinatorial system in which the onset of replication is triggered by a molecular signal. In the absence of the signal the equilibrium concentration of the replicator remains below its critical aggregation concentration (CAC), so self-assembly and concomitant self-replication do not occur. However, following the Le Chatelier–Brown principle, introduction of a template molecule that can bind to a library member can cause the concentration of the latter to increase.<sup>9–11</sup> In our system introducing a template raised the concentration of the replicator–template complex above the CAC, triggering self-replication. Once replication had started, it could also take place in the absence of the template that was responsible for its initiation. These results illustrate the power of the dynamic combinatorial approach in systems chemistry,<sup>3b,12</sup> revealing how the interplay between supramolecular interactions and reversible covalent chemistry can lead to emergent behavior.

The template triggered self-replication was discovered in our investigations of the DCL made from dithiol building block **1** (Scheme 1), in which we encountered unexpected behavior that we only recently have been able to interpret. Oxidizing building block **1** in the presence of oxygen from the air resulted in a small DCL dominated by a series of isomeric catenanes held together by reversible disulfide linkages.<sup>13</sup> Disulfide exchange in these systems is mediated by attack of residual thiolate anion on disulfide bonds.<sup>14</sup> As reported previously, upon the addition of cationic adamantane guests **2** the catenanes open up to form a series of four isomeric tetramers,

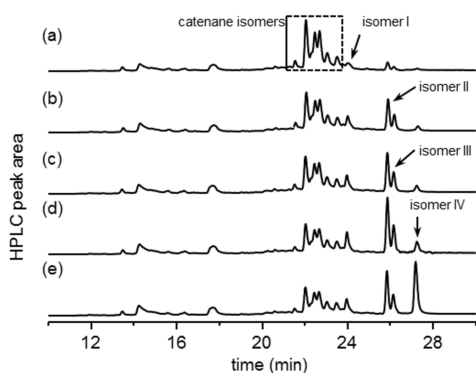
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Scheme 1. Systems Chemistry of Building Block 1<sup>a</sup>

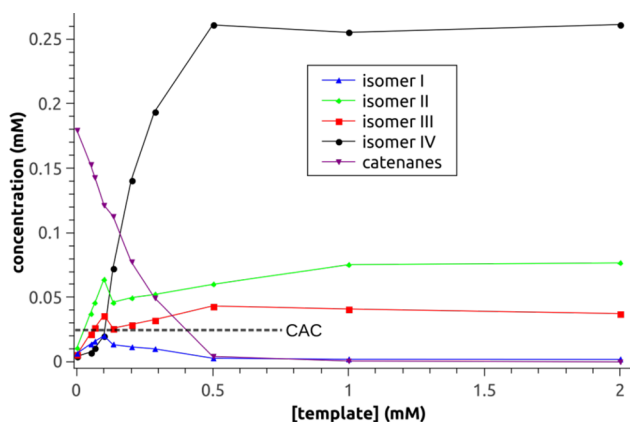
<sup>a</sup>In the absence of a template the library is mostly composed of catenanes. In the presence of the trigger template the library yields a mixture of four tetramers (nomenclature in the order of their elution). The complex of tetramer IV and the trigger can self-assemble into sheet-like structures above its CAC and initiate the replication process.

which differ in the position of the carboxylate groups.<sup>13a</sup> The four tetramers (for the assignment, see ref 13b) are all amplified upon adding the guest. However, when studying the library composition as a function of the amount of template 2 that was added, we observed a puzzling effect: increasing the template concentration from 0.10 to 0.13 mM led to a sudden dramatic increase of the concentration of the least abundant of the four tetramer isomers (Figure 1).



**Figure 1.** HPLC analysis of the DCL made from 2.0 mM building block 1 in aqueous borate buffer (50 mM, pH 8.2) (a) without any template and with template 2 at a concentration of (b) 0.050, (c) 0.067, (d) 0.10, and (e) 0.13 mM.

Figure 2 shows the dependence of the concentration of the four isomers on the concentration of template 2 that was present (see Supporting Information (SI), Figures S15 and S16 for similar experiments performed at different building block concentrations). At low concentration of the template, all tetramers show an approximately linear dependence on the concentration of 2. DCLFit analysis<sup>11d,g,15</sup> (Supporting



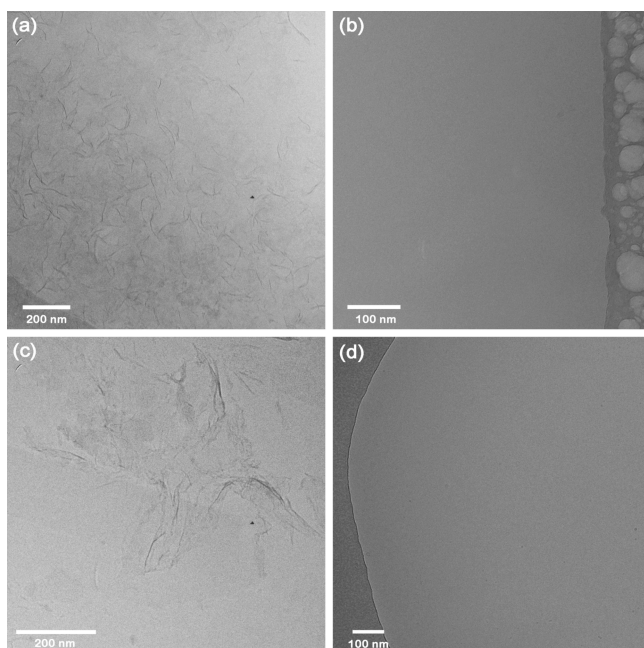
**Figure 2.** Concentration of the library members as a function of the concentration of template 2 in DCLs made from 2.0 mM building block 1 in aqueous borate buffer (50 mM, pH 8.2).

Information, page S14) indicates that the equilibrium constant for binding between the template and isomer IV is approximately three times smaller than the corresponding binding constant for isomer II. Nevertheless, the template binds strongly to all tetramers (we have previously shown that the average binding constants of the template to the mixture of tetramers exceeds  $10^7 \text{ M}^{-1}$ ),<sup>13a</sup> hence the concentration of tetramers that have not bound any template is small. After exceeding a critical concentration, tetramer IV—the weakest binder—dominates the library at the expense of its isomers. Such behavior is indicative of aggregate formation, which occurs once the complex of isomer IV and the template exceeds a CAC. This hypothesis was verified by analyzing the sample of Figure 1d (below the CAC of the isomer IV–template complex) and Figure 1e (above its CAC) by cryo-TEM. Only the sample above the CAC showed the presence of aggregates in the form of sheets with a thickness of approximately 2–3 nm (see Figure 3a,b, respectively).

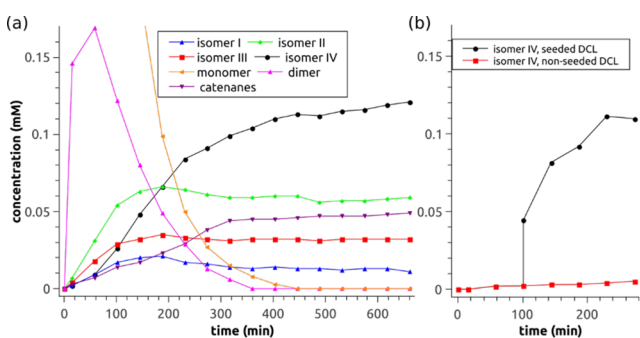
We decided to investigate the early stages of DCL evolution. The growth of isomer IV in a library templated with 10 mol % 2 initially lags behind the more stable isomers II and III, but after exceeding the CAC, it accelerates causing isomer IV to eventually dominate the library (Figure 4a). In contrast to the other library members, the sigmoidal growth of isomer IV suggests an autocatalytic self-assembly process. Tetramers within the self-assembled sheets presumably have lower energies than the free library members or the tetramers at the edges of the assembly. Thus, the sheets can grow at the expense of the species in the solution and close the self-replication cycle upon sheet fracture. We have already observed similar behavior in fiber-based replicators.<sup>6h–k</sup>

Interestingly, after 11 h the total concentration of the tetramers in the library is 50% higher than the total template concentration, suggesting that tetramer IV was to some extent amplified not only because of the stabilization by the template but also because the tetramer IV–template complex triggered the aggregation of the empty tetramer IV.

To check if isomer IV alone can form aggregates, we isolated it by preparative HPLC and dissolved it in borate buffer (50 mM, pH 8.2) at two different concentrations; one below (0.020 mM) and one above (0.040 mM) the CAC of the isomer IV–template complex. Cryo-TEM analysis did not show any aggregates for the former sample (Figure 3d), while the latter showed nanosheets with a thickness of about 2 nm (Figure 3c)



**Figure 3.** Cryo-TEM images of (a) a DCL made from building block 1 (2.0 mM) and template 2 (0.13 mM, above the CAC); (b) a DCL made from building block 1 (2.0 mM) and template 2 (0.10 mM, below the CAC); (c) a solution of isomer IV (0.040 mM, above its CAC); (d) a solution of isomer IV (0.020 mM, below its CAC). All samples were prepared in borate buffer (50 mM, pH 8.2). Full-size images are shown in Figures S11–S14.



**Figure 4.** (a) Change in the product distribution with time of a DCL made from 1.5 mM building block 1 in the presence of 0.15 mM template 2, showing the sigmoidal growth of isomer IV. (b) Change in the concentration of isomer IV with time in a DCL made from 2.0 mM building block 1 without seeding (open circles) and upon seeding with 10 mol % (with respect to  $[I]_{\text{total}}$ ) of isomer IV at  $t = 100$  min (filled circles), demonstrating the autocatalytic nature of the formation of isomer IV.

similar to those observed for the library in the presence of template 2 (Figure 3a). Apparently, the CACs of the isomer IV–template complex and that of isomer IV alone are similar. Furthermore, isomer IV does not necessarily require the presence of template 2 to self-assemble. In order to prove that the self-assembled isomer IV sheets can indeed replicate without the help of the template, we performed a seeding experiment. We prepared two identical DCLs from building block 1 (2.0 mM) and monitored their composition with time. To one of the samples we added 10 mol % of tetramer IV (with respect to the building block) after 100 min, resulting in a concentration of IV that is above the CAC. The results of this experiment are shown in Figure 4b and reveal that isomer IV

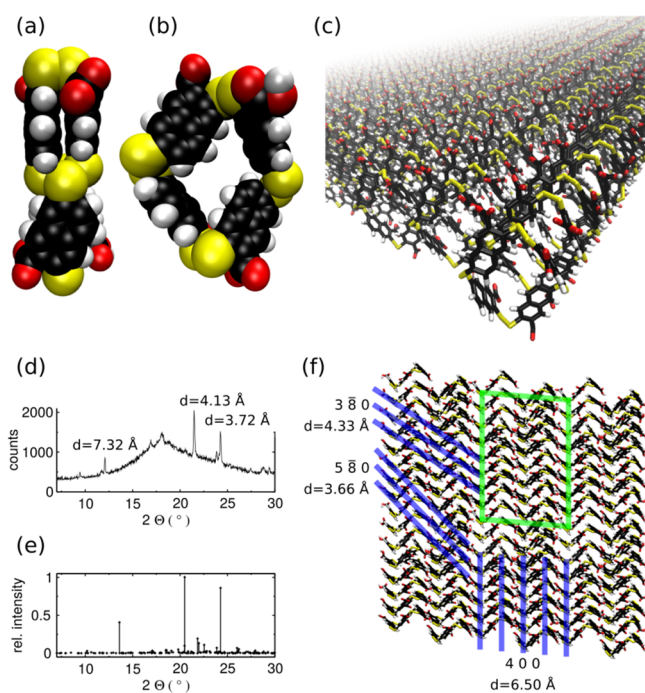
promotes its own formation (and does not require template 2 once the concentration of isomer IV exceeds its CAC). The kinetics of formation of isomers I–III did not change significantly upon addition of isomer IV (see Figure S7).

These results raised the question whether replication of isomer IV is triggered by aggregation of the isomer IV–template complex or whether these two processes are independent. To answer this question we performed a seeding experiment with 2-fold excess of the template over tetramer IV in the seed. The results were similar to the seeding experiment without the template in the seed (apart from amplification of the other tetramers, presumably due to the excess of the template; see Figure S8), proving that the tetramer IV–template complex can trigger replication of the tetramer IV alone.

Note that the DCL in Figure 4a produced more of the tetramers than there is template available, while the total tetramer concentration in the DLCs in Figure 2 and Figure S16, that had more time to equilibrate, did not significantly exceed the template concentration. This observation suggests that the replicating tetramer IV, not bound to the template, is a kinetic product and thermodynamically unstable relative to the catenanes. This hypothesis was corroborated by treating the library seeded by tetramer IV with a substoichiometric amount of reducing agent (dithiothreitol) under nitrogen. Such procedure provides constant amount of thiolates able to quickly catalyze disulfide exchange and leads to thermodynamic equilibrium, where the total amount of tetramers was similar to the template concentration (see Figure S10).

We then investigated the structure of the aggregates made by isomer IV with molecular dynamics (MD) simulations using the General Amber Force Field<sup>16</sup> with either Amber<sup>17</sup> or GROMACS<sup>18</sup> packages. Similarly to our previous results with isomer III,<sup>13b</sup> isolated tetramer IV assumes a folded conformation (Figure 5a). We hypothesized that hydrophobicity of the naphthalene units would promote self-assembly of the tetramers into sheet-like aggregates. However, our simulations showed that isomer IV in its folded conformation does not form any persistent assemblies. On the other hand, the partially opened tetramer (as shown in Figure 5b) was capable of forming stable sheets characterized by periodic arrangement of isomer IV in two dimensions. The rhomboidal structure of assembled tetramer IV is much more similar to the complex of tetramer IV with template 2<sup>13a</sup> than the nonassembling folded structure, which might explain the initiation of the replication of the empty tetramer by its complex with template 2. We noticed that the powder XRD pattern calculated from the assemblies shown in Figure 5c,f is very similar to the experimentally determined powder XRD data (compare Figure 5d,e), corroborating our model. The three major reflections in the diffractogram (labeled in Figure 5f) allowed us to calculate the unit cell parameters, which were very similar to the parameters obtained from MD simulations (relative difference approximately 10%; difference in area per molecule <1%, for details see SI page S15 and Figure S21). When the MD cell was restrained to match the experimental parameters, the simulated diffraction pattern was matching the experimental one (Figure S22).

The comparison between the structures of free and assembled isomer IV (Figure 5a,b) shows why such sheet-like assemblies are formed. Although the free tetramer minimizes its hydrophobic solvent accessible surface area by folding, it suffers an enthalpic penalty due to conformational strain. The dihedral



**Figure 5.** (a) A representative structure of tetramer IV in solution from MD simulations. (b) A representative structure of tetramer IV from the MD simulation of the self-assembled sheet shown in panel (c). (d) Powder X-ray diffractogram of a lyophilized sheet sample of tetramer IV compared with the simulated diffractogram of the MD structure (e). (f) Assignment of the three major reflections using the simulated sheet structure. The cell used in the simulations (labeled with a green box) consists of 16 tetramer IV molecules.

angles of the two outer disulfide bonds are close to  $0^\circ$ , similarly to other hydrophobic disulfides studied previously.<sup>11g,13b</sup> In contrast, the conformer present in the assemblies has unstrained disulfide bonds because it does not have to fold to decrease hydrophobic hydration. Owing to its bola-amphiphile-like structure, the hydrophilic carboxylates are exposed to the aqueous phase, whereas the hydrophobic aromatic parts are efficiently screened from water by the neighboring tetramers. The thickness of the sheet (2.1 nm) corresponds well with that determined from the cryo-TEM images (2–3 nm).

## CONCLUSIONS

In conclusion, we have shown how one of four isomeric members of a DCL (isomer IV) can be selectively amplified as a result of autocatalytic self-assembly. We attribute the fact that only tetramer IV shows autocatalytic self-assembly to the unique bola-amphiphile-like structure of this isomer, resulting from the pairwise positioning of two carboxylate groups on opposite sides of the hydrophobic core of the molecule. While the isolated isomer IV will self-assemble spontaneously, it will not do so without assistance in the DCL made from only building block 1. In the DCL, self-assembly requires the help of a template molecule (2) to raise the concentration of the isomer IV–template complex above its CAC. In the absence of this template, isomer IV is not produced in large enough concentrations because other library members (in particular the catenanes) provide a lower-energy alternative, siphoning off the building block. While the template is crucial for allowing its complex with tetramer IV to form the aggregate, once those initial self-assembled sheets have been formed, the assemblies

can be propagated without template. This is the first example of the emergence of a self-replicator in a DCL that is dependent on the (one-off) help of a template molecule. This work demonstrates how combinations of molecular recognition phenomena can conspire to lead to the emergence of self-replicating systems and further underlines the potential of the dynamic combinatorial approach for the development and control of self-replicating and self-synthesizing systems<sup>4,6</sup> in particular and for systems chemistry<sup>3b,12</sup> in general.

## ASSOCIATED CONTENT

### Supporting Information

Kinetics of the growth of all isomers upon seeding with isomer IV; seeding experiment in the presence of template 2; seeding experiment with a mixture of isomer IV and template 2, equilibration experiments, relative binding constants estimated with DCLFit, methods and data for analytical and preparative HPLC, LC-MS, cryo-TEM, and XRD analysis, and procedures for MD. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b04380.

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### Notes

The authors declare no competing financial interest.

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