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Effector-Triggered Self-Replication in Coupled Subsystems

Dávid Komáromy, Meniz Tezcan, Gaël Schaeffer, Ivana Marić, and Sijbren Otto*

Abstract: In living systems processes like genome duplication and cell division are carefully synchronized through subsystem coupling. If we are to create life *de novo*, similar control over essential processes such as self-replication need to be developed. Here we report that coupling two dynamic combinatorial subsystems, featuring two separate building blocks, enables effector-mediated control over self-replication. The subsystem based on the first building block shows only self-replication, whereas that based on the second one is solely responsive toward a specific external effector molecule. Mixing the subsystems arrests replication until the effector molecule is added, resulting in the formation of a host–effector complex and the liberation of the building block that subsequently engages in self-replication. The onset, rate and extent of self-replication is controlled by the amount of effector present.

Minimal living systems are generally believed to feature three main characteristics: replication, compartmentalization and metabolism.^[1] Efforts towards the construction of fully synthetic living systems have focused mostly on isolated subsystems capable of self-replication^[2] or compartment formation.^[3] The functional integration of these subsystems^[4] represents an arduous challenge and new concepts for achieving subsystem coupling are badly needed. In this context, systems of replicators for which the onset and extent of replication can be triggered and controlled by effector molecules are highly relevant research targets. However, triggered self-replication has received only little attention and currently triggers are limited to light^[5] and stabilization of the replicator by binding.^[6]

We envisaged that triggered replication systems may also be achieved using a conceptually different modular approach, by coupling two subsystems: one devoted to replication and another to effector recognition. The coupling of these two chemical subsystems may be realized through dynamic processes (i.e. chemical equilibria) which can interfere with each other via common components; that is, dynamic molecular networks or dynamic combinatorial libraries.^[7] In such networks building blocks, appended with functional groups capable of reversible covalent bond formation, react with each other to form a diverse collection of oligomeric compounds, a dynamic combinatorial library (DCL). The members of such libraries constantly exchange building

blocks via the formation and breakage of the reversible covalent bonds, that is, the distribution of the members is governed by a large number of interconnected equilibria. Consequently, perturbing one equilibrium in one part of the network in principle affects the distribution of all DCL members, including those that are only indirectly connected to the perturbed dynamic process. Coupling of thermodynamically controlled subsystems in the form of such signal-cascading networks has advanced experimental^[8] and theoretical^[9] underpinnings.

Individually, many dynamic combinatorial systems that feature effector recognition through host–guest chemistry have been reported.^[10] In such systems guest addition leads to the selective amplification of those library members that bind the guest. More recently, also dynamic combinatorial systems showing self-replication have been developed.^[11] In such systems binding of specific library members to copies of themselves leads to their proliferation in an often autocatalytic manner. However, no functional integration of guest recognition and replication subsystems has yet been reported.

We now show how an effector molecule can trigger self-replication in a DCL which is composed of two building blocks: the subsystem constructed from the first serves as a platform for self-replication, whereas the second building block is responsible solely for effector recognition.

Recently we reported that in disulfide-based^[12] dynamic combinatorial libraries of dithiol building block **1** (Scheme 1) stirring induces the emergence of a self-replicator (see the Supporting Information (SI), Figure S66) in the form of the cyclic hexamer (**1₆**), self-assembling first into nanoribbons, then transforming into nanoplatelets.^[13] We set out to couple

Scheme 1. Dynamic combinatorial chemistry of a system made from building blocks **1** and **2** and guest **3**.

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this self-replicating system to a host–guest system also established previously^[14] in which spermine guest (**3**) is able to amplify the formation of a cyclic tetramer host (**2₄**) in a DCL made from building block **2**. We reasoned that, upon oxidation of a mixture of thiols **1** and **2**, a DCL of various cyclic disulfide oligomers should be formed in which assembly nucleation, and thereby replicator emergence, is hampered by the low concentration of the replicator in the mixture. Upon addition of effector **3**, the cyclic tetramer **2₄** should be amplified, leading to the depletion of building block **2** from the DCL. This, in turn, allows the library to re-equilibrate, leaving behind **1**-only oligomers, besides the **2₄3** complex. Nucleation of the **1₆** replicator should now be more likely and the continued stirring of this re-equilibrated library would lead to the emergence of self-replicator **1₆** through a growth-breakage mechanism.

Indeed, stirring building block **1** and **2** ($[1] = [2] = 2.0$ mM) in aqueous borate buffer (50 mM, pH 8.2) gave a DCL composed mainly of mixed trimers and tetramers, alongside larger macrocycles, but essentially devoid of replicators (Figure 1 A). However, addition of 0.5 equivalent of **3** (relative to **1**) and subsequent stirring for 1 day induced a dramatic change in the library composition which was now dominated by self-replicator **1₆** and spermine binder **2₄** (Figure 1 C). Interestingly, when the same experiment was repeated, but the mixture was not agitated following the addition of effector **3** the most abundant **1**-containing species were **1₃** and **1₄**, which are also the main products in a non-agitated DCL

prepared from **1** only (Figure 1 B).^[13] These results show that upon addition of **3**, the host–guest complex **2₄3** is formed first, accompanied by the buildup of a range of **1**-only oligomers. It also shows that in the coupled subsystems the emergence of the replicator is promoted by mechanical agitation, as is the case for the separate replication subsystem.

In order to prove that self-replication is indeed a consequence of subsystem coupling and only happens when both stimuli (addition of **3** and stirring) are present, control experiments were performed. First, in the absence of **3**, mechanical agitation of a DCL made from equimolar amounts of **1** and **2** showed only trace amounts of the replicator, even upon 4 months incubation (see SI Figure S63). Interestingly, when **1** was applied in excess, stirring led to a DCL featuring mixed macrocycles, as well as self-replicator **1₆** (see SI Figure S61). Second, in the absence of mechanical agitation, addition of **3** resulted in a DCL featuring **2₄** as the main **2**-containing species, whereas the relative amount of other oligomers featuring **2** decreased substantially (Figure 1 B). Furthermore, the presence of **3** did not have any impact on the behavior of the DCL made from **1**: mechanical agitation resulted in the formation of **1₆**, whereas in the absence of stirring, trimers, tetramers and larger macrocycles formed (see SI Figure S60).

In order to provide further mechanistic insight into the triggered replication process, the time evolution of the stirred system following addition of **3** was monitored (Figure 2). First, **2₄** appeared almost instantaneously after the addition of **3**, showing that its formation was the first event in the reaction cascade. The concentration of **2₄** reached its equilibrium value at a time scale (< 12 hours; see also SI Figure S65) that is one order of magnitude smaller than timescale of the replication of **1₆** (140 hours). Thus, the addition of **3** is indeed a trigger. Second, simultaneously with the appearance of **2₄**, the relative concentrations of **1₃** and **1₄** rapidly increased, followed by a slow decrease that coincided with the formation of the self-replicator **1₆**. This result shows that the formation of **1₆**

Figure 1. UPLC traces of a DCL composed of equimolar amounts of **1** and **2** ($[1] = [2] = 2.0$ mM) in aqueous borate buffer (50 mM, pH 8.2) A) after 2 days of stirring and B) subsequent addition of 0.50 equivalents (1.0 mM) of **3** and 1 day in the absence of agitation. C) After 2 days of stirring, followed by addition of 0.5 equivalents (1.0 mM) of **3** and 1 day of stirring.

Figure 2. Time evolution of a DCL composed of equimolar amounts of **1** and **2** ($[1] = [2] = 2.0$ mM) in aqueous borate buffer (50 mM, pH 8.2) after the addition of **3** (0.9 equivalents) at $t = 0$ h.

proceeded via intermediates **1**₃ and **1**₄. Third, the increase in the relative amount of **1**₆ showed a lag phase, indicative of a slow initial nucleation event, followed by a rapid increase, most likely arising from an autocatalytic breakage-elongation mechanism as described before.^[11b]

We then explored whether the trigger allows also quantitative control over the onset, rate and extent of replication. Thus, we prepared libraries with the same initial composition as described before ($[1] = [2] = 2.0$ mM), added different amounts of effector **3** (0.3, 0.6 and 0.9 mM) and monitored the time evolution of the system with UPLC. This set of experiments was carried out in three repeats and the overall trends were reproducible (see SI Figure S71). One of the three datasets is shown in Figure 3, revealing that the lag

Figure 3. Example of the time evolution of a DCL composed of equimolar amounts of **1** and **2** (2.0 mM each) in aqueous borate buffer (50 mM, pH 8.2), upon addition of increasing amounts (0.3, 0.6 and 0.9 mM) of **3**. Two repeats of this experiment are shown in the SI, Figure S71.

phase, the replication rate and the extent of replication all depend strongly on the amount of effector present. These findings can be interpreted as follows: The higher the effector concentration, the larger the amount of **2** sequestered as **2**₄-effector complex, and consequently, the larger the amount of **1**-only containing macrocycles accessible for replication, leading to a higher final replicator concentration. Furthermore, an increasing concentration of **1**-only containing macrocycles increases the chance of nucleation of assemblies of **1**₆, hence the shortening of the lag phase prior to replication. Finally, the higher rate of replication at higher effector concentrations reflects the higher achievable concentration of the autocatalytic replicator. Another factor that contributes to the higher replication rate induced by **3** is the fact that fiber growth is faster when the composition of the trimer and tetramer macrocycles is biased towards those rich in **1**. This is corroborated by the observation that, when the distribution of the two building blocks over the trimer and tetramer rings is 1:1, no significant replicator growth occurs in the course of 30 hours even upon seeding with as much as 60% pre-formed replicator (see SI Figure S70).

Next, we verified whether the process of effector-induced triggering of self-replication interfered with the supramolecular organization of the replicator. Transmission electron microscopy (see SI Figure S68A and B) and atomic force microscopy (Figure S68C and D) show nanoribbons with dimensions that are very similar for the isolated replication subsystem as compared to the case when the subsystems are coupled. Analyses on solutions containing only the host-effector complex **2**₄**3** did not reveal any nanoscale objects detectable by TEM; and the mixing of **2**₄**3** and pre-formed nanoribbons of **1**₆ revealed nanoribbons similar to those of **1**₆ by itself (see SI Figure S69). Thus, subsystem coupling does not appear to affect the molecular and supramolecular characteristics of the replication process, confirming the modular character of the system and the compatibility between the subsystems.

We further tested subsystem compatibility and whether our approach of triggered replication can be extended to structurally related replication systems. Thus, we prepared DCLs by mixing building blocks **2** + **4** and **2** + **5**. Building blocks **4** and **5** contain one ethylene oxide unit less and more than **1**, respectively. Oxidation of compound **4** by itself produces the self-replicating cyclic tetramer **4**₄. In contrast, compound **5** by itself forms trimers and tetramers, together with a range of large macrocycles while no replicator emerges.^[13]

In the DCL composed of building blocks **2** and **4**, addition of **3** triggered the exclusive formation of replicator **4**₄ (Figure 4B). Analogously, upon mixing building blocks **2** and **5**, addition of **3** resulted in the formation of receptor **2**₄ and the formation of a large diversity of macrocycles (up to 12mers), composed almost solely of **5** (Figure 4D). These results demonstrate the compatibility of the effector binding subsystem with the subsystems based on **4** and **5** and the transferability of the concept of triggered replication to the tetramer-based replicator made from building block **4**.

As effector specificity is an important characteristic of biological signaling systems, we also investigated whether the

Figure 4. UPLC traces of stirred DCLs composed of equimolar amounts of **4** (A, B) or **5** (C, D) and **2** ($[4] = [5] = [2] = 2.0$ mM) in aqueous borate buffer (50 mM, pH 8.2) before (A, C) and 1 day after (B, D) the addition of 0.50 equivalents (1.0 mM) of **3**.

ability to trigger self-replication was specific regarding spermine (**3**) as an effector. We found that among four different, structurally related amines, those that feature the structural moiety required for binding to receptor **2**₄^[14] (two secondary amine groups separated by a chain of four aliphatic carbon atoms) are effective triggers, whereas the ones lacking this motif do not induce guest complexation and self-replication (see SI Figure S67).

Finally, we show that the system can be switched between the self-sorted state featuring replicator **1**₆ and the “mixed” state mostly devoid of replicator (Figure 5 and SI Figure S72).

Figure 5. Alternating additions of portions of **3** and **2** to an initially equimolar mixture of **1** and **2** ($[1]=[2]=2.0$ mM) in aqueous borate buffer (50 mM, pH 8.2), resulting in switching between “mixed” (low mol% of **1**₆) and self-sorted (high mol% of **1**₆) states of the system.

These studies were motivated by the observation that, upon addition of one equivalent of **2** to a sample containing only **1**₆, this replicator rapidly disintegrates and a DCL dominated by mixed macrocycles is formed (see SI Figure S64). We performed a similar experiment, but now starting from an equimolar mixture of **1** and **2** (2.0 mM), which gave rise to a mixed DCL, featuring numerous species (Figure 1A). Addition of 0.25 equivalents of **3** resulted in the formation of a self-sorted DCL, consisting nearly exclusively of **1**₆ and **2**₄ (Figure 1C). When 1 equivalent of **2** was added to a self-sorted DCL (containing **1**:**2**:**3** in a 2:2:0.5 molar ratio), the composition of the resulting library was similar to the initial one, featuring only trace amounts of **1**₆. However, ca. 1 equivalent of **2**₄ was still present in the mixture, reflecting the fact that the binding between **2**₄ and **3** is strong. The resulting mixture again showed self-sorting upon addition of 0.5 equivalents of **3** and the resulting self-sorted DCL could again be switched back to the mixed state. Only two switching cycles could be accomplished. Upon the third addition of **3**, self-sorting was incomplete and substantial amounts of mixed species were still present. These results demonstrate that the system can be alternated between replicator-rich and replicator-poor states depending on the ratio between effector and building blocks, but can be operated only in a certain stoichiometric regime.

In conclusion, we have demonstrated effector-triggered self-replication by coupling a host–guest subsystem with

a self-replication subsystem via dynamic combinatorial disulfide chemistry. Mixing the two building block that correspond to the two subsystems results in a diverse library, essentially devoid of replicator. Addition of an effector molecule results in binding between an oligomer of the non-replicating building block and the effector molecule. As a result, the library is depleted of the non-replicating building block, making self-replication possible. The quantitative characteristics of the self-replication process (length of the lag phase, rate and maximal extent of replication) depend on the amount of effector present. Triggering replication by subsystem coupling has an important advantage over previously reported triggered replication systems^[5,6] by being modular: the replication system can in principle evolve independently of the triggering system, since, in contrast to earlier reports, the triggering system does not directly involve the replicating molecules. This method paves the way for better temporal (and possibly also spatial) control over self-replication in dynamic systems. Furthermore, the control over replication by a single effector provides a handle through which the replication system could potentially be integrated with further subsystems, with the prospect of creating supersystems with more intricate emergent properties, such as negative feedback and oscillatory behavior.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: dynamic combinatorial chemistry · host–guest chemistry · molecular networks · self-replication · systems chemistry

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