Existing Self-Replicators Can Direct the Emergence of New Ones

Yigit Altay, Meniz Altay and Sijbren Otto

Abstract: The study of the interplay between different self-replicating molecules constitutes an important new phase in the synthesis of life and in unravelling the origin of life. Here we show how existing replicators can direct the nature of a newly formed replicator. Starting from the same building block, 6-ring replicators formed when the mixture was exposed to pre-existing 6-membered replicators, while pre-formed 8-membered replicators funnelled the building block into 8-ring replicators. Not only ring size, but also the mode of assembly of the rings into stacks was inherited from the pre-existing replicators. These results show that the nature of self-replicating molecules can be strongly influenced by the interplay between different self-replicators, overriding preferences innate to the structure of the building block.

The process through which chemistry can transition into biology remains shrouded in mystery, yet represents one of the grand challenges in contemporary science. At some stage in the origin of life and in the process of synthesizing life de-novo, the ability to replicate needs to emerge. A good understanding of the requirements for self-replication now exists and several chemical self-replicating systems have been reported. Also the emergence of self-replicators from relatively complex mixtures (in particular dynamic combinatorial libraries, DCLs) has been described by Philp and by us. We previously developed DCLs using building blocks equipped with two thiol groups that can oxidize to form a disulfide macrocycles which continuously exchange building blocks through reversible disulfide exchange reactions. These building blocks were equipped with short peptide chains, containing alternating hydrophilic and hydrophobic amino acids, predisposing them to the formation of β-sheet assemblies. Beyond a critical macrocycle size (which depends on the peptide sequence) macrocycles can assemble into stacks, held together by β-sheets. This assembly process stabilizes the macrocycles that assemble and leads to the autocatalytic formation of more of these assembling macrocycles (i.e. self-replication) through a nucleation-growth mechanism. Exponential replication can be enabled by mechanical agitation that causes growing fibers to fragment, thereby increasing the number of fiber ends from which the fibers grow. Until now, most efforts have focused on systems in which only a single replicator emerges and persists.

And how do existing replicators affect the emergence of new ones? While answers to the former question can be informed by knowledge from contemporary biology, where much is known about how species interact, the latter question has no known counterpart in biology. In current life, all new species derive from existing ones and no new life seems to emerge from scratch. Yet in the early stages of biogenesis replicator emergence is likely to have been much more common.

Herein we describe that existing self-replicators can steer the emergence of new ones, resulting in self-replicators that are different from those that would have emerged in the absence of pre-existing replicators. Thus, replicator composition is not merely dictated by the availability of specific precursors, but becomes dependent on the history of the sample and interactions with pre-existing replicators.

The next phase in the development of such inanimate systems towards life involves the evolution of replicators and, subsequently, replicator communities. In this new phase new questions arise, including: How do replicators interact and what are the consequences of such interactions?

[1] A. Yigit, M. Altay and Prof. Dr. S. Otto, University of Groningen, Centre for Systems Chemistry, Stratingh Institute, Nijenborgh 4, 9747 AG, Groningen, The Netherlands, E-mail: s.otto@rug.nl

Supporting information for this article can be found under:
In the course of our work on the emergence of replicators from DCLs\textsuperscript{6} we prepared building block 1 (Scheme 1a), differing from our previously reported building blocks by featuring a tyrosine residue. We prepared DCLs by oxidizing 1 at 3.8 mM concentration in borate buffer (50 mM, pH 8.2). Depending on the speed of oxidation and the mode of agitation the product distribution differs markedly. When slowly oxidizing a stirred solution by exposing it to oxygen from the air, the cyclic trimer 1\textsubscript{3} is the dominant product (Figure 1a). Repeating this experiment in the absence of agitation produced a mixture of trimer and tetramer macrocycles (Figure 1b). Oxidizing the solution rapidly to 80\% using perborate and subsequently placing it under an inert atmosphere also produced a mixture of 1\textsubscript{3} and 1\textsubscript{4} (Figure 1c). However, when this experiment was repeated but now the sample was exposed to air after being oxidized with perborate, cyclic octamer was formed (Figure 1d). This behavior was found to be qualitatively reproducible (see Figure S1).

For the samples that produced mixtures of 1\textsubscript{3} and 1\textsubscript{4} we did not detect any self-assembled structures by TEM analysis. However, for the experiments of Figure 1a and d, producing mainly 1\textsubscript{3} or 1\textsubscript{4}, respectively, TEM analysis revealed the presence of fibers (Figure S72). Seeding experiments confirmed that 1\textsubscript{3} is able to self-replicate (vide supra). Similar experiments on 1\textsubscript{4} were inconclusive, as the rate of emerge of 1\textsubscript{3} is limited by the rate of oxidation of 1 (cf. Figure 1a).

Thus, we exposed freshly prepared DCLs made from building block 1 to a series of different replicators: 2\textsubscript{2}, 3\textsubscript{3}, 4\textsubscript{4}, 5\textsubscript{5}, 6\textsubscript{6}, and 6\textsubscript{7} (10 mol\%) which we prepared following previously described procedures.\textsuperscript{6} Remarkably, all DCLs to which hexamer replicators (2\textsubscript{2}, 3\textsubscript{3}, 4\textsubscript{4}, and 5\textsubscript{5}) were added exhibited product distributions dominated by hexamer 1\textsubscript{6} (Figure 2a-d). Furthermore, all libraries seeded with octamer replicators (5\textsubscript{5} and 6\textsubscript{6}) formed 1\textsubscript{8} dominated libraries (Figure 2e-f). In some cases also mixed macrocycles were observed (1\textsubscript{2}\textsubscript{2} or 1\textsubscript{5}\textsubscript{5} in the presence of 2\textsubscript{2} and 1\textsubscript{6} in the presence of 5\textsubscript{5}). Thus, pre-existing replicators control the ring size of the new replicators made from building block 1, in some cases overriding the inherent preference of 1 to produce octamer replicator.

The results above show that the behavior of DCLs made from 1 is unusually sensitive to small changes in the experimental conditions and that the different products that are formed are separated by relatively high activation energy barriers. The relatively high plasticity of this system (even some hexamer can be formed transiently – cf. Figure 1a) makes it an ideal candidate to probe the extent to which replicator emergence can be directed by introducing other replicators.
In order to confirm that $1_8$ and $1_8$ are self-replicators, seeding experiments were performed using the seeds obtained from the libraries corresponding to Figure 2a and 2f. When a freshly prepared library of peptide 1 is seeded with $10\text{ mol}\%$ seed of $1_8$ or $1_8$, we observed rapid growth of the corresponding macrocycles (Figure 3), relative to the non-seeded control (blue lines), confirming that $1_8$ and $1_8$ are indeed able to self-replicate.

![Figure 4](image)

**Figure 4.** CD spectra of samples dominated by (a) $1_8$ and (b) $1_8$ and (c) normalized maximum thioflavin T fluorescence emission intensity (at 492 nm) of non-seeded or seeded (10 mol $\%$) DCLs made from peptide 1 (3.8 mM in 50 mM borate buffer pH 8.2): i, non-agitated; ii, stirred at 1200 rpm and kept under a nitrogen atmosphere; iii, stirred at 1200 rpm in the presence of air; iv, seeded with $4_a$; v, seeded with $5_a$; vi, seeded with $3_a$; vii, seeded with $2_a$; viii, oxidized to 80% using perborate and stirred at 1200 rpm; ix, seeded with $5_a$; x, seeded with $6_a$.

The structures of the newly formed $1_8$ and $1_8$ replicators were characterized by circular dichroism (CD) spectroscopy, thioflavin T fluorescence assays, TEM and IR spectroscopy. The CD spectra of the samples dominated by $1_8$ showed a positive helicity at around 190 nm and a negative helicity at 210 nm (Figure 4a). These bands appear at wavelengths that are somewhat smaller than those typical for B-sheets. According to Pribic et al., such shifted signals may arise in tyrosine containing peptides due to $\pi-\pi^*$ transitions that complicate the far UV region of the CD spectrum and are still in agreement with a B-sheet structure. The three libraries that produced $1_8$ replicators showed three rather different signatures in their CD spectra (Figure 4b) which we tentatively assigned to parallel B-sheet (for the sample in which $1_8$ emerged autonomously), anti-parallel B-sheet (when the formation of $1_8$ was triggered by $5_a$) and mixed (parallel and anti-parallel) B-sheet structures (when the formation of $1_8$ was triggered by $6_a$). Thioflavin T assays showed an at least 40 times increase in emission for the $1_8$ containing sample and an at least 20 times increase for the $1_8$ containing samples, compared to samples in the absence of replicator, which supports amyloid type B-sheet structures for all samples.

Negative staining transmission electron microscopy (TEM) revealed bundles of fibers having a right-handed helicity for all samples dominated by $1_8$ (Figure 5a-d and Figure S73). For the samples of $1_8$ that showed parallel B-sheets we observed single fibers with a width of $\approx 4.5$ nm. In contrast, for samples of $1_8$ that showed anti-parallel B-sheets we observed a high degree of lateral association of the fibers. In the sample of $1_8$ that showed mixed B-sheets we observed laterally associated fibers along with single fibers. Thus, it appears that the lateral association of the fibers occurs through anti-parallel B-sheet formation.

![Figure 5](image)

**Figure 5.** Transmission electron microscopy images of DCLs made from 1 seeded with (a) $2_c$; (b) $3_c$; (c) $4_c$; (d) $5_c$; (e) $5_a$ and (f) $6_a$.

We further characterized the different assemblies of $1_8$ and $1_8$ by IR spectroscopy (Figure 6). The frequency of the C=O bands are in the range expected for B-sheet assemblies. Only for the sample of $1_8$ where we suspect the formation of antiparallel B-sheets and which exhibited extensive lateral association of the fibers, we observed an additional band at 1615 cm$^{-1}$, which is associated with the phenyl ring of tyrosine. The fact that this band is only observed for the $1_8$ sample that shows extensive laterally associated fibers suggests that in this sample those phenols are in an environment that differs from the one in a non-associated fiber, which would be in agreement with the postulated occurrence of parallel and antiparallel B-sheet assemblies.
COMMUNICATION

Taken together these results suggest that replicator 1 can exhibit different modes of assembly: one in which its fibers show lateral association through anti-parallel β-sheet formation and one in which such interactions are absent. Remarkably, this mode of assembly is dictated by the replicator that triggered its emergence. Thus, not only the information regarding ring size is transferred, but also information regarding the mode of assembly of these rings. Seeding experiments showed that both forms of information are to some extent heritable: seeding a DCL made from 1 with a sample of 1a that showed laterally associated fibers induced the formation of more 1a that also showed fiber bundles, while seeding a similar DCL with a sample of 1 that showed no fiber bundling induced more 1a that existed as non-associated fibers (See SI Figure S74).

![Figure 6. ATR-IR spectra of different replicators obtained from DCLs made from peptide 1 (3.8 mM in 50 mM borate buffer pH 8.2): vii, seeded with 2a; viii, oxidized to 80% using perobarate and stirred at 1200 rpm in the presence of air; ix, seeded with 5a. Assignment: TFA at 1675 cm⁻¹; amide I band (C=O) at 1615 cm⁻¹; amide II band (C-N) at 1537 cm⁻¹; C-C ring stretching at 1515 cm⁻¹.](image)

Finally, we investigated the influence of the sample history on replicator composition. We prepared DCLs having the same building block compositions as those shown in Figures 2a-f, but now the building blocks were mixed prior to oxidation. All of the DCLs produced 1a and 1a along with mixed trimers and tetramers (See SI Figure S45-68), but no significant amount of any replicator. These data show that the history of the sample is an essential factor determining not only the nature, but also the presence or absence of replicators.

In summary, our results show that both the molecular structure (ring size) and the mode of assembly (parallel or antiparallel β-sheets and degree of fiber bundling) of newly emerging replicators can be controlled by pre-existing replicators. The interactions between replicators can override the preference for a particular structure and ring size innate to the structure of the building blocks of the replicator. While involving similar (but not identical) replicators to the ones reported in a previous study, our current system shows behavior that is exactly opposite to that reported previously. In the previous work we showed how one specific pre-existing replicator can help the formation of one other specific replicator, that does not readily form by itself. This behavior was only observed for these two specific replicators and the structure of the newly formed replicator appeared to be variable. Our current results show that replicators, rather than having an apparently predetermined structure, can also be highly plastic and respond to the presence of any of a range of pre-existing replicators by adopting the ring size of the specific replicator it was exposed to. As a consequence the composition and nature of the system of replicators reflects sample history and inter-replicator interactions, which are both key prerequisites for Darwinian evolution.

Acknowledgements

We are grateful for support from the ERC, NWO, COST Action CM1304 and the Ministry of Education, Culture and Science (Gravitation program 024.001.035).

Keywords: self-replication • dynamic combinatorial chemistry • de-novo life • origin of life • systems chemistry


Compounds are quantified in terms of the percentage of the total peak area in the chromatogram. This total peak area is comparable for mixtures with very different macrocycle compositions, indicating that the molar absorptivity of the building block unit is independent of the macrocycle in which it resides. Thus, peak areas correlate directly with macrocycle concentrations (expressed in units of building block).


We survey how existing replicators can affect the emergence of new ones in a dynamic combinatorial library made from a tyrosine containing building block. Seeding with pre-existing replicators of different sizes (hexamer or octamer) directs the ring size of the tyrosine-containing replicator.

Yigit Altay, Meniz Altay, Sijbren Otto*