

# Competition Dynamics in a Chemical System of Self-replicating Macrocycles

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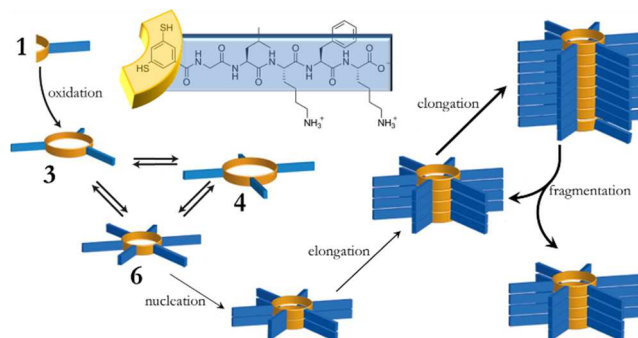
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## Abstract

Central to the origin of life is the question how a chemical system transitioned from interacting molecules to an entity with the capacity for self-replication, diversification and adaptive evolution. Here, we study a chemical system that is comprised of macrocycles that have been shown to spontaneously give rise to self-replicating entities. By combining experimental and theoretical approaches, we strive to understand the evolutionary potential of this system. In particular, we apply eco-evolutionary reasoning to investigate whether and when this system of chemical replicators can diversify. Here, we report first results of a simplified stochastic chemical reaction model that is parameterized on the basis of experimental data. The model considers the competition of two replicators that do not interact directly but need similar building blocks for their growth and reproduction. Interestingly, the replicator that emerges first is being overtaken by the later one. By means of stochastic simulations, we will explore how the competitive ability of a replicator is determined by its chemical characteristics, and under which conditions replicators can coexist. The results will subsequently inform the design of future experiments.

## Introduction

Self-replication is generally considered a defining property of life (Trifonov 2011) and self-replicating molecules are likely to have played a central role in the origin of life. From a molecular point of view self-catalysis results in replication (Orgel 1992). Self-replication can be very basal and take place in the absence of the elaborate machinery in present-day cells (Ashkenasy et al. 2017). Chemical systems showing self-replication are therefore excellent candidates for understanding how the transition from “chemistry” to “biology” proceeded in the early evolution of life. In principle, it should be possible to study chemical evolution by extending principles, tools and techniques from one field to the other (Markovitch et al. 2018). Here we study such a system, which is illustrated in Figure 1 (Carnall et al. 2010; Colomb-Delsuc et al. 2015). In this system, a replicator spontaneously forms from a monomeric building block (**1**) followed by exponential growth of the replicator, according to the following mechanism. Upon oxidation of the monomeric building block smaller macrocycles form. These macrocycles can then interconvert via reversible disulfide exchange reactions leading to larger macrocycles. A particular macrocycle, the hexamer (**6**), was found to self-assemble by stacking onto other hexamers to form nanofibers (Mattia et al. 2017). This self-assembly was found to be catalyzed by fibers



**Figure 1:** Mechanism of self-replication, and the chemical structure of the monomeric building block (peptide-functionalized dimercaptobenzene **1**; peptide sequence is Gly-Leu-Lys-Phe-Lys). Eventually, all the material is contained in hexamer fibers as a result of self-assembly driven replication (see text). Figure adapted from (Frederix et al. 2017).

as new hexamers are adding to fiber ends (Malakoutikhah et al. 2013). Under agitation, such as stirring, these fibers break, thus exposing more fiber ends and leading to exponential growth (Colomb-Delsuc et al. 2015). Firm support for the self-replication capability is obtained by seeding a fresh system with pre-formed fibers which led to an earlier onset of replication (Carnall et al. 2010).

Striking and surprising behavior emerged when two building blocks, of similar design but different peptide sequence, were mixed (Sadownik et al. 2016). Without mixing, each building block gave rise to its own replicator: a hexamer and an octamer, respectively. When mixed, all combinations of hexamer replicators emerged and persisted, suggesting that all these hexamers are replicators. Further seeding experiments suggested that one particular subset of replicators is the ancestor of another subset (Sadownik et al. 2016). In other words, mixing different building blocks leads to a variety of replicators with mutational (or mutational-like) relations between them.

Given these properties, the above chemical system is ideally suited for investigating how Darwinian evolution operates in a system devoid of the elaborate machinery of present-day cells. We are particularly interested in the potential diversification of such a system. To this end, we will apply principles of Adaptive Dynamics (Brännström et al. 2013), a biological toolbox for

studying adaptive evolution and diversification (such as the origin of new species (Weissing et al. 2011)).

In order to study diversification in the chemical system, it is important to construct an experimentally relevant model, the predictions of which can be used to guide new experiments. Here, we sketch such a model.

## Results

### The model

The model was developed in two stages. First, a basic mass-action kinetics model was constructed and the reactions and rate-constants were underpinned via data-fitting to experiments (not shown). This basic model includes all the reactions occurring in the system (Figure 1). Second, this mass-action model was extended to a stochastic one by considering all the different chemical structures that can occur when two building blocks, labeled A and B, are mixed (for example, the cyclic tetramer of composition  $A_2B_2$  has two structural isomers: AABB and ABAB) and their reactions. For hexamers, there are 13 different structural isomers: AAAAAA, AAAAAB, AAAABB, AAABAB, AABAAB, AAABBB, AABABB, ABABAB, AABBBB, ABABBB, ABBABB, ABBBBB and BBBBBB.

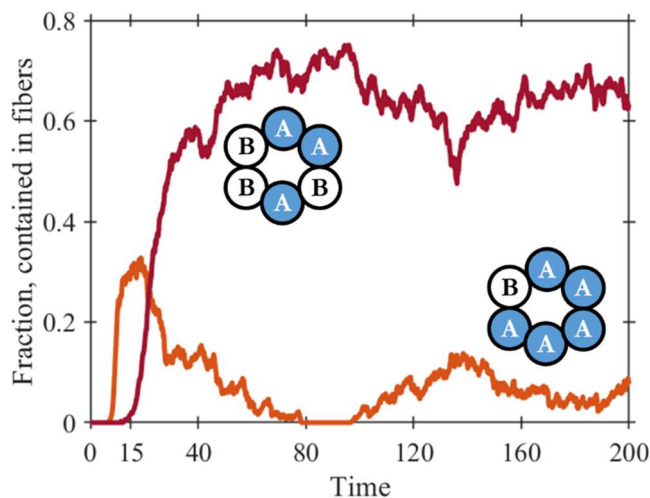
The stochastic model explicitly considers the fiber structure as they occur via hexamer stacking. A hexamer can in principle stack on same hexamer (self-stacking) or on another hexamer (cross-stacking). While presently we do not have a handle on the rates at which hexamers cross-stack, we envision that these rates are largely determined by the interaction energy between two hexamers, which is a function of how well their building blocks can interact when next to each other.

### An example: takeover

In a preliminary analysis, the model was simulated using Gillespie's stochastic algorithm (Gillespie 1976) under a constant-volume reactor condition, with the same volume being flown in and out. The initial conditions and the inflow contained equal amounts of two monomeric building blocks: A and B. For this first case hexamers were only allowed to stack on the same hexamers (i.e. no cross-stacking).

Under these conditions, only two types of hexamer fibers emerged (Figure 2). Interestingly, the hexamer that arises first (isomer AAAAAB) gave way to the second hexamer (isomer AABABB) in what was previously termed as a "takeover" (Markovitch and Lancet 2014).

There are two main factors that differentiate hexamers and their dynamics: 'synthesis' due to oxidations and exchanges, and assembling into fibers which excludes most of replicators stacked in the fiber, except those at its ends, from participating in further reactions. Because the relative self-stacking of the first hexamer is 4 times faster than that of the second hexamer (Figure 2) it is able to self-assemble first. However, in the flow regime it is being continuously diluted and gives way to the second hexamer because the second hexamer is being synthesized more (due to the intrinsic combinatorics; e.g. making  $A_5B_1$  is less likely than  $A_3B_3$ ) and therefore eventually is able to take over.



**Figure 2:** An example of a simulation showing takeover. In this example, a hexamer is only allowed to self-stack on same hexamer (i.e. no cross-stacking). The relative rates of self-stacking are proportional to the number of building-block A in a hexamer ( $n$ ), given by the formula:  $2^{-(6-n)}$ . The simulation considered all possible isomers at size 1.6 (monomer to hexamer) and their reaction. Reactor turnover time is  $3\frac{1}{3}$ .

### Away from randomness

The above result demonstrates how replicators' competition dynamics can be studied. We strive to understand how mixtures of similar and different building blocks would give rise to non-random distribution of replicators and fibers, and what are the limits and potential of evolvability of this chemical system. We are particularly interested in under what circumstances would smaller groups of co-existing replicators be observed and how could large group of replicators give rise to several co-existing replicators subgroups. The emergence of such smaller subgroups could be interpreted as diversification of chemical replicators.

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