Impact of composition on the dynamics of autocatalytic sets

Alessandro Ravoni
Department of Mathematics and Physics, University of Roma Tre, Via della Vasca Navale 84, 00146 Rome, Italy

Autocatalytic sets are sets of entities that mutually catalyse each other’s production through chemical reactions from a basic food source. Recently, the reflexively autocatalytic and food generated theory has introduced a formal definition of autocatalytic sets which has provided promising results in the context of the origin of life. However, the link between the structrue of autocatalytic sets and the possibility of different long-term behaviours is still unclear. In this work, we study how different interactions among autocatalytic sets affect the emergent dynamics. To this aim, we develop a model in which interactions are presented through composition operations among networks, and the dynamics of the networks is reproduced via stochastic simulations. We find that the dynamical emergence of the autocatalytic sets depends on the adopted composition operations. In particular, operations involving entities that are sources for autocatalytic sets can promote the formation of different autocatalytic subsets, opening the door to various long-term behaviours.

Keywords: Autocatalytic sets; Origin of life; Network Composition; Stochastic Petri nets; Binary polymer model

I. INTRODUCTION

The exact sequence of events that led to the formation of the first living organisms from non-living matter is still a topic under debate (Benner et al., 2012; Bernhardt, 2012; Luisi, 2016; Rasmussen et al., 2004; Szostak, 2017). On the other hand, some particular qualities of early organisms are commonly accepted and are well known. Among these, we can point out the self-replication ability of the early life forms (Higgs and Lehman, 2015; Kauffman, 1993; Luís, 2016; Nghe et al., 2015; Rasmussen et al., 2016). In this scenario, the autocatalytic sets (ASs) are of great interest. Introduced by Kauffman (Kauffman, 1971, 1986, 1993), ASs are sets of entities capable of spontaneous emergence and self-reproduction through catalytic reactions, starting from a finite set of entities assumed to be available in the environment. There are several definitions of ASs in the literature (see, for instance, Jain and Krishna, 1998; Sharov, 1991). Recently, Hordijk et al. (2011) have introduced the notion of reflexively autocatalytic and food generated (RAF) sets, a formal definition of ASs in the framework of chemical reaction systems (CRSs) (see Section II for definitions). Properties of RAF sets have been studied by various authors, and among the most important results (see Hordijk and Steel, 2017, 2018 for more details) note the implementation of a polynomial time algorithm able to identify the presence of a RAF set in a general network of interacting entities (Hordijk and Steel, 2004; Hordijk et al., 2011; Hordijk and Steel, 2012) and the detection of an autocatalytic structure in the metabolic network of Escherichia Coli (Sousa et al., 2015) and ancient anaerobic autotrophs (Xavier et al., 2020).

Moreover, RAF theory has successfully proved that RAF sets have a hierarchical structure, where the largest set of reactions within a CRS satisfying RAF property (the so-called maxRAF) has many subsets that are smaller RAF sets themselves (Hordijk et al., 2012; Hordijk and Steel, 2014, 2018). The latter is a peculiarity that makes RAF sets potentially suitable for experiencing adaptive evolution (a feature that is generally referred to as evolvability); i.e., to collect evolutionary changes beneficial for survival and reproduction in a given environment. Indeed, it has been argued that the autocatalytic subsets present within the structure of the maxRAF could be the elementary units on which natural selection can act (Hordijk and Steel, 2014; Hordijk et al., 2018b; Vasas et al., 2012): the availability of spontaneous reactions would allow the occurrence of mutations and, consequently, the appearance of novel autocatalytic subsets able to replicate themselves with different rates and competing with each other.

However, first results show that the asymptotic dynamics of simple RAF sets eventually reaches the state in which all the reactions of the maxRAF occur catalytically (Hordijk et al., 2018b; Vasas et al., 2012). In this state, all the elementary autocatalytic units coexist without effectively competing with each other, thus leaving no room for adaptive evolution (Hordijk et al., 2018b; Vasas et al., 2010, 2012). The evolvability of RAF sets can be restored by embedding them into compartments and allowing the sharing of resources and the exchange of chemical molecules (Hordijk et al., 2018b; Kauffman, 2011; Serra and Villani, 2019; Vasas et al., 2012). In fact, through numerical simulations, it has been observed that RAF sets enclosed in semipermeable protocells can reach
different asymptotic states \cite{Serra and Villani 2019}, and that spatially separated RAF sets consuming the same food source can give rise to different combinations of competing autocatalytic subsets \cite{Hordijk et al. 2018b}, suggesting that the evolvability of RAF sets is related to the interactions among RAF sets themselves.

In this work we investigate this latter point. In particular, we study the role of various interactions among RAF sets in order to understand how these interactions affect the emergent dynamics. To this aim, we use the stochastic Petri nets (SPNs) formalism \cite{Haas 2006 Molloy 1982} to represent and evolve RAF sets. Furthermore, we introduce some composition operations acting on nets, which correspond to different interactions among RAF sets. In this framework, assuming that the entire maxRAF set always emerges in an isolated RAF set, our goal is to find some composition operations under which the dynamical appearance of the maxRAF is not invariant. This means that the corresponding interaction causes only some of the maxRAF subsets to emerge, allowing the existence of multiple long-term behaviours required for the evolvability of RAF sets.

The paper is organised as follows. In Section II we introduce the definitions of RAF sets and SPNs. In Section III we describe the model we use to evolve nets and we introduce the composition operations. In Section IV we present and analyse the results obtained by simulating the dynamics of various composed RAF sets. Finally, in Section V we discuss and demonstrate the conclusions.

II. BACKGROUND

A. Reflexively autocatalytic and food generated sets

In RAF theory \cite{Hordijk and Steel 2004 Hordijk et al. 2011}, a network of interacting entities is represented by a CRS. Following previous definitions \cite{Hordijk and Steel 2004 Hordijk and Steel 2017 Lohn et al. 1998}, we introduce a CRS as a tuple \((S,R,C,F)\) such that:

- \(S\) is a set of entities;
- \(R\) is a set of reactions, \(\rho \to \pi\), where \(\rho, \pi \in S\) are the reactants and products of a reaction, respectively;
- \(C\) is a catalysis set, that is, a set of pairs \(\{(s,r), s \in S, r \in R\}\) indicating the entity \(s\) as the catalyst of reaction \(r\).

We also define a food set \(F \subset S\) such that entities \(s \in F\) are assumed to be available from the environment. We describe a CRS as a bipartite graph such that:

- nodes are of two kinds \(V = S \cup R\);
- edges are of two kinds \(E = E_r \cup C\);
- \(\emptyset\) is a pseudo-entity representing the environment and \(s \in S\) is a food entity if there exists a reaction \(r \in R\) such that \((\emptyset, r) \in E_r\) and \((r, s) \in E_r\).

Note that \(E_r\) is called the spontaneous reactions set. The edges \((s, r) \in E_r\) can be interpreted as source entity \(s\) is consumed by reaction \(r\), while in edges \((r, s) \in E_r\) entity \(s\) is produced by reaction \(r\). \(i_s\) denotes a reaction such that \((\emptyset, i_s), (i_s, s) \in E_r\), implying an input reaction producing a food entity. Moreover, we introduce outflow reactions \(o_s\) such that \((s, o_s), (o_s, \emptyset) \in E_r\). Thus, the CRS is a flow reactor that allows inflow and outflow of entities from and towards the environment.

Let \(R'\) represent a subset of \(R\). The closure \(cl_{R'}(F)\) is defined to be the (unique) minimal subset of \(S\) that contains \(F\) together with all entities that can be produced from \(F\) by repeated applications of reactions in \(R\). Note that \(cl_{R'}(F)\) is well defined and finite \cite{Hordijk and Steel 2004}. Given a CRS \((S,R,C,F)\), a RAF set is a set of reactions \(R' \subseteq R\) (and associated entities) that satisfies the following properties:

- Reflexively autocatalytic: for each reaction \(r \in R'\) there exists at least one entity \(s \in cl_{R'}(F)\) such that \((s,r) \in C\);
- \(F\)-generated: for each reaction \(r \in R'\) and for each entity \(s \in S\) such that \((s,r) \in E_r\), it is \(s \in cl_{R'}(F)\).

Thus, a RAF set is a set of reactions able to catalytically produce all its source entities starting from a suitable food set. It is also possible to define the closure of a set of reactions, introducing the notion of closed RAF sets \cite{Smith et al. 2014}. Given a CRS \((S,R,C,F)\), a subset \(R'\) of \(R\) is said to be a closed RAF set if:

- \(R'\) is a RAF set;
- \(\forall r\) such that all its source entities and at least one catalyst are either part of the set \(F\) or are produced by a reaction from \(R'\), it is \(r \in R'\).

Authors of \cite{Hordijk et al. 2018a} describe a procedure to detect closed RAF sets in a generic CRS. It has been argued that closed RAF sets are associated with the attractors of the dynamics of a CRS and therefore represent the relevant units capable of experiencing adaptive evolution \cite{Hordijk et al. 2018a Smith et al. 2014 Vasas et al. 2012}. FIG. 1 shows an example of a RAF set and its constituent closed RAF sets. We exemplify our point by introducing a set of entities \(S = \{A,B,C,D,E,F,G,H,I,L,M,N,O,P\}\) and a food set \(F = \{A,B,D,F,I,M\}\). In this example, the RAF set is composed of the following reactions (an entity above the arrow of a reaction indicates the catalyst associated
FIG. 1 RAF set example. Entities are represented by letters (dark-green for food-entities, black for non-food entities). The food set is surrounded by a dark-green dashed rectangle. Reactions are displayed by coloured squares. A black arrow emerging from a letter towards a square (from a square pointing at a letter) indicates the corresponding entity is a source (a product) for that reaction. Red dashed arrows indicate catalysis. The maxRAF set consists of three closed RAF sets: \( R^{(1)} = \{r_1, r_3\} \) (green squares), \( R^{(2)} = \{r_1, r_3, r_5, r_8\} \) (magenta and green squares), \( R^{(2)} = \{r_1, r_3, r_2, r_4, r_6, r_7\} \) (blue and green squares).

with that reaction):

\[
\begin{align*}
 r_1 : & \quad A + B \xrightarrow{M} C \\
 r_2 : & \quad A + D \xrightarrow{O} E \\
 r_3 : & \quad C + F \xrightarrow{A} G \\
 r_4 : & \quad B + E \xrightarrow{N} H \\
 r_5 : & \quad C + I \xrightarrow{P} L \\
 r_6 : & \quad C + M \xrightarrow{E} N \\
 r_7 : & \quad D + M \xrightarrow{H} O \\
 r_8 : & \quad F + L \xrightarrow{D} P
\end{align*}
\]

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B. Stochastic Petri nets

A Petri net consists of \cite{Petri2008} (see Petri and Reisig \cite{Petri2008} for further details):

\begin{itemize}
  \item a finite set of place \( P \);
  \item a finite set of transitions \( W \);
  \item functions \( b, e : P \times W \to \mathbb{N} \).
\end{itemize}

Here \( b(p, w) \) and \( e(p, w) \) are the number of edges from place \( p \) to transition \( w \) and from transition \( w \) to place \( p \), respectively. The sets \( b(w) \subset P \) and \( e(w) \subset P \) are the sets of places connected to transition \( w \) by at least one edge. A marking \( X \) of a Petri net is a map \( X : P \to \mathbb{N} \) that assigns a number of tokens to each place. In fact, a marking \( X \) identifies a state of the system in the space of possible configurations of tokens available in each place. With \( x_p \), we indicate the number of tokens of place \( p \) available in marking \( X \). Firing a transition \( w \) consumes \( b(p, w) \) tokens from each of its input places \( p \in b(w) \), and produces \( e(p', w) \) tokens in each of its output places \( p' \in e(w) \). For each marking \( X \), a transition \( w \) is enabled (it may fire) if there are enough tokens in its input places making the consumption possible. This shall occur, if and only if \( X(p) \geq b(p, w), \forall p \in P \). A stochastic Petri net \cite{KAMP2006} is a Petri net for which each transition is equipped with a (possibly marking dependent) rate for the exponentially distributed transition firing times. \( \lambda \) denotes the set of firing rates of a SPN. Note that the evolution of a SPN with exponentially distributed transition rates is isomorphic to continuous-time Markov chain \cite{Molloy1982}.

Petri net formalism provides a suitable environment for studying the composition of networks, with both a computational and theoretical approach; the latter, in particular, in the context of category theory \cite{Baez2017}. Moreover, this formalism can describe nets with different dynamics \cite{Vazquez2011}. The authors will investigate these aspects in a forthcoming work.

III. THE MODEL

A. Building the net

Given a CRS \((S, R, C, F)\) (or a set of reactions and associated entities that is a RAF set), we build a SPN by adding a place \( p \) for each species \( s \in S \) and a transition \( w \) for each reaction \( r \in R \) such that \( b(w) = \rho(r) \) and \( e(w) = \pi(r) \), where \( \rho(r) \) and \( \pi(r) \) are the set of all sources and targets entities of edges \((s, r), (r, s) \in E_r\), respectively. Note that with this notation we consider both inflowing and outflowing transitions. Moreover, for each catalysis \((s, r) \in C \) we add a transition \( w \) such that \( b(w) = \rho(r) \cup s \) and \( e(w) = \pi(r) \cup s \). Hereafter, we use \( S \) and \( R \) to...
indicate both species and reactions of a CRS and places and transitions of a SPN. The rates $\lambda(r)$ associated with each transition $r$ are marking dependent rates:

$$\lambda(r) = h_r(X)\lambda_r.$$  

Here, $\lambda_r$ is a fixed constant depending on the type of its corresponding reaction in the CRS ($\lambda_r = \{\lambda_s, \lambda_c, \lambda_t, \lambda_0\}$ in which $\lambda_s$ specifies spontaneous, catalysed, inflowing and outflowing reactions, respectively) and $h_r(M)$ is a value proportional to the number of combinations of tokens available in the input places of transition $r$ in the state $X$. Thus, explicitly, we shall have:

$$h_r(X) = \frac{\prod_j b(j, r)}{\sqrt{|b(r)|}-1} \prod_j \left( x_j \right),$$

where the product is among all the input places of transition $r$. We set functions $b$ and $e$ such that the inflowing transitions do not consume tokens of the pseudo-entity $\emptyset$ and produce a fixed value of tokens of the food entities, while the outflowing transitions consume a token of the outflowing entities and do not produce tokens of the pseudo-entity $\emptyset$. Thus, the rate of outflowing transitions is proportional to the amount of tokens of the outflowing entity, while the rate of inflowing transitions is independent of the state of the system. It is noteworthy that the inflowing of food elements still remains a stochastic event. We add inflowing transitions for entities not belonging to the originary food set $F$ (setting the rate of such transition equal to zero), if required for the purpose of composition between nets (see Section III.B). The dynamics of the obtained SPN is described by the stochastic mass action kinetics, that is the classical dynamics used to represent chemical reactions, assuming a well-stirred system (Anderson and Kurtz [2011]).

The CRSs used in this work are generated according to the binary polymer model (BPM) (Kauffman [1986]). The BPM produces a CRS where the entities set $S$ consists of all bit strings up to (and including) a maximum length $N$, and the reaction set $R$ consists of condensation and cleavage reactions. Condensation reaction is a concatenation of two bit strings resulting in a longer string, and cleavage reaction cats a bit string into two smaller ones. The food set is represented by all entities with a length less than or equal to a fixed length $l_f$ (we set $l_f = 2$), and each entity can be a catalyst of each reaction with a certain probability fixed a priori.

We chose to only allow the condensation reactions to occur in the system. Note that the technique we use is also applicable in the case where cleavage reactions are allowed. In fact, none of the operations we introduce in Section III.B is affected by the reversibility of the network reactions. Including cleavage reactions would produce networks, in principle, with different dynamics, since a network with irreversible reactions can have different topology with respect the equivalent reversible network (Feinberg [1995]). The authors are currently investigating this aspect in an upcoming work.

With this limitation, all the spontaneous reactions of our model are binary reactions (i.e., reactions with two reactants, possibly of the same entity type), while all the catalysed reactions are ternary reactions. Even if ternary reactions are rare, they can represent a first approximation of two or more elemental reactions, such as the sequence of reactions of an enzyme catalysis (Gillespie [2007]).

B. Composition

We model interactions between CRSs as composition operations between SPNs. First, note that RAF sets satisfy the following conditions (hereafter, we refer to these as the inclusion conditions) (Horidick and Steel [2004]):

- if $R'_1$ is RAF in $(S_1, R_1, C_1, F_1)$, it is RAF also in $(S_2, R_2, C_2, F_2)$, if conditions $S_1 \subseteq S_2$, $R_1 \subseteq R_2$, $C_1 \subseteq C_2$, $F_1 \subseteq F_2$ are satisfied;
- if $R'_1$ is RAF $(S_1, R_1, C_1, F_1)$ and $R'_2$ is RAF in $(S_2, R_2, C_2, F_2)$, then $R'_1 \cup R'_2$ is RAF in $(S_1 \cup S_2, R_1 \cup R_2, C_1 \cup C_2, F_1 \cup F_2)$.

Thus, if composition does not remove entities from the food set or reactions belonging to a RAF set, it will not have any impact on its RAF property. However, the dynamical behaviour of the composed system can be, generally, different from that of the starting one.

Let $(S_1, R_1, b_1, e_1, \lambda_1)$ and $(S_2, R_2, b_2, e_2, \lambda_2)$ be two SPNs and let $I, O$ be subsets of their inflowing and outflowing transitions sets. Let $\sim$ be the equivalence relation such that $s \sim s'$ if $i_s \in I$ and $o_{s'} \in O$ for some choice of $I, O$. $S_{\sim}$ denotes the set of places identified by relation $\sim$. We define the following composition operations:

**CO1:**

$$\begin{align*}
(S_1, S_2) \rightarrow S_s &= S_1 \cup S_2;
(R_1, R_2) \rightarrow R_s &= (R_1 \cup R_2 \cup R_{II}) \setminus (O_1 \cup I_2);
(b_1, b_2) \rightarrow b_s &= b_1 \cup b_2 \cup b_I;
(e_1, e_2) \rightarrow e_s &= e_1 \cup e_2 \cup e_I;
(\lambda_1, \lambda_2) \rightarrow \lambda_s &= \lambda_1 \cup \lambda_2 \cup \lambda_I;
R_I := \{ r \mid b(r) = s, e(r) = s', \forall s \in S_1, s' \in S_2 \text{ such that } s \sim s' \};
\lambda_I := \{ \lambda(r) \mid \lambda(r) = h_r(X)\lambda_f, \forall r \in R_I \}.
\end{align*}$$

**COII:**

$$\begin{align*}
(S_1, S_2) \rightarrow S_s &= S_1 \cup S_2;
(R_1, R_2) \rightarrow R_s &= (R_1 \cup R_2 \cup R_{II}) \setminus (O_1 \cup I_2);
(b_1, b_2) \rightarrow b_s &= b_1 \cup b_2 \cup b_{II};
(e_1, e_2) \rightarrow e_s &= e_1 \cup e_2 \cup e_{II};
(\lambda_1, \lambda_2) \rightarrow \lambda_s &= \lambda_1 \cup \lambda_2 \cup \lambda_{II};
R_{II} := \{ r \mid b(r) = b(r'), e(r) = b(r''), \forall r'' \in R_2 \text{ such that } b(r'') \in S_{\sim} \text{ and } b(r') \sim b(r'') \}.
\end{align*}$$
\[ \lambda_{II} := \{ \lambda(r) \mid \lambda(r) = h_r(X)\lambda_f, \forall r \in R_{II}\}. \]

\[
\text{CO}_{III}: \\
(S_1, S_2) \to S_* = \{S_1 \cup S_2\}/\sim; \\
(R_1, R_2) \to R_* = R_1 \cup R_2; \\
(b_1, b_2) \to b_* = b_1 \cup b_2; \\
(e_1, e_2) \to e_* = e_1 \cup e_2; \\
(\lambda_1, \lambda_2) \to \lambda_* = \lambda_1 \cup \lambda_2.
\]

Here \( \lambda_f \) is a constant value and \((S_*, R_*, b_*, e_*, \lambda_*)\) is the composed SPN.

To summarise, all the composition operations we define relate a set of places \( S \) that are input for outflowing transitions of a SPN, together with a set of places \( S' \) that are input for inflowing transitions of another SPN. The formal addition of inflowing transitions for places not belonging to the food set enlarges the possible composition operations between SPNs.

Given a set \( S_* \), operation \( \text{CO}_I \) adds a transition from \( S \) to \( S' \) for each pair of places in \( S_* \), while operation \( \text{CO}_{II} \) adds a transition from \( S \) to \( S' \) for each combination of places that appears as input of a transition in the inflowing net. Each combination corresponds to the definition of complex \(^1\) in the framework of chemical reaction networks (Feinberg, 1995). In fact, given a set of chemical species, a complex is defined as a member of the vector space generated by the species that provides the inputs (or the outputs) of a reaction (Anderson and Kurtz, 2011; Feinberg, 1995).

Both operation \( \text{CO}_I \) and \( \text{CO}_{II} \) introduce a flux of entities from one net to another. The composite network can therefore be seen as the union of two separate networks that evolve in parallel, communicating only with (asymmetrical) exchange of chemical species. This could be, for instance, the case of two spatially separated protocols, one of which can release molecules towards the other. According to this interpretation, the flowing rate \( \lambda_f \) is a parameter that encompasses the characteristics of the flow process (for example, cell permeability). Operation \( \text{CO}_{II} \), instead, merges each pair of places in \( S_* \), allowing transitions of the two original SPNs to operate on the glued set of places. Composing nets via operation \( \text{CO}_{II} \) actually produces a new single network. In this case, one can think of composite net as the result of mutations that enlarge a network (for instance, net 1), introducing new possible reactions and, consequently, new chemical species (corresponding to net 2).

It is worth to underline that, if there exists a \( s \in S_* \) such that \( s \in F \), operations \( \text{CO}_I \) and \( \text{CO}_{II} \) can actually modify the RAF property of net 2. In particular, if transitions \( r \in R_{I,II} \) are assumed to be spontaneous transitions (\( \lambda_f < \lambda_c \)), net 2 could not be catalytically produced starting from the food set \( F \). In this case, the composed net contains a RAF set \( R' \) such that \( R_1 \subseteq R' \subseteq (R_1 \cup R_2) \), with \( R_1 = R' \) if \( F \subseteq S_* \). Instead, if transitions \( r \in R_{I,II} \) are assumed to be (auto) catalysed transitions (\( \lambda_f \geq \lambda_c \)), the whole composed net shall be a RAF set.

### IV. RESULTS AND DISCUSSION

In this Section, we present the results regarding the impact of composition on the dynamics of RAF sets as follows: we first introduce the characteristics of simulations and the quantities taken into account. In Section [IV.A] we present results for the non interacting nets, in order to have a reference model for the interacting cases, presented in Section [IV.B]

Starting from different instances of the BPM with \( N < 8 \), we use the RAF algorithm introduced in (Hordijk et al., 2011) in order to detect and select three different RAF sets, each of which contains more than one closed RAF set. Note that, even if different RAF sets have the same species set \( S \), the set of reactions \( E \) will be different; i.e., various RAF sets have different chemistry. The RAF sets identified through this procedure constitute the collection on which we will carry out the study. Even if such a small collection cannot be taken as a solid statistical basis, it is still sufficient for providing us with interesting information. We duplicate each RAF set and let it interacts with its copy by switching to representation of RAF set as an SPN and using one of the composition operations introduced above. We simulated the dynamics of the system using the standard Gillespie algorithm (Gillespie, 1976, 1977), setting the volume of the system at \( V = 1 \) (arbitrary units). For each simulation, we perform 100 independent runs of \( 10^6 \) time steps. One of the necessary conditions for a RAF set is the ability to produce itself starting from the elements of the food set. Indeed, the initial state of the SPN is set such that:

\[
\begin{align*}
x_s(t=0) &= x_0 \text{ if } s \in F; \\
x_s(t=0) &= 0 \text{ otherwise.}
\end{align*}
\]

Here \( x_0 \) is an arbitrary constant. The values of \( x_0, \lambda_c, \lambda_i \) and \( \lambda_s \) are set such that the number of tokens of food places at \( t \to \infty \) is equal to \( 10^5 \), for an SPN with inflow, outflow and all (and only) binary transitions having, as input, food places only (and firing rate \( \lambda_c \)). The value of \( \lambda_s \) is fixed at \( \lambda_s = \lambda_c/10 \), while \( \lambda_f \) varies such that \( \lambda_f \in [\lambda_c 10^{-1}, \lambda_c 10^0] \). It is noteworthy that the values of these parameters are not taken from “in vivo” data.

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\(^1\) Note that complexes play a major role in the framework of chemical reaction networks theory. For instance, the deficiency theorems (Feinberg, 1995) are able to predict whether the dynamics of a large class of networks will have a stationary distribution, starting from the topology of the reaction graph having complexes as nodes.
but they have phenomenological motivations. Thus, although we can reasonably generalise the characteristics of the dynamics, quantities such as the species’ production rate or the time evolution of the concentrations may differ from those in other similar stochastic simulations [Hordijk and Steel 2012, Hordijk et al. 2018b].

We focus our attention on the effective appearance of a maxRAF set during the evolution of the system. In particular, we introduce the following quantities:

1) \( M_s(t) = \sum_s \frac{1}{1 + \tau_i}, \forall s \notin F; \)

2) \( \tau_i = \min\{t \mid n(r) \geq i, \forall r \in R\}. \)

Here \( n(r) \) is the number of executions of transition \( r \), and \( R \) is the maxRAF set. Note that the natural condition \( x_s(t) \geq 0 \) implies that each term contributing to the computation of \( M_s(t) \) can possess, at most, the value one. Both \( M_s(t) \) and \( \tau_i \) are calculated for each single net that forms the composed net. Let \( m_M = \overline{M_s(t)} \) be the mean value of \( M_s(t) \) for \( t \to \infty \). If all the non-food entities of a maxRAF set are efficiently produced, then \( M_s(t) \to 0 \) for \( t \to \infty \) and \( m_M = 0 \). However, the definition of a RAF set does not ensure that all the entities associated with such a set are present in large amount during the evolution of the system. For instance, a non-food entity that is a source for a transition of a RAF set could be continuously consumed by that transition as soon as it is produced, resulting in a fluctuating evolution of its number of tokens. For this reason, we consider a less restrictive condition than \( m_M = 0 \) for the emergence of a maxRAF set. In particular, for large \( t \), we require a strictly positive concentration for all non-food entities and a high concentration for most of them. This implies that the relation \( \frac{1}{1 + \tau_i} < 1 \) holds for all the terms contributing to the calculation of \( M_s \), and that the relation \( \frac{1}{1 + \tau_i} \approx 0 \) holds for most of them. Thus, we introduce the following condition:

\[
m_M < 1. \tag{4}
\]

We assume that, if condition (4) holds, the entire maxRAF set emerges.

Note, moreover, that different growing rates among entities of a RAF set correspond to different effective firing rates of the transitions. Therefore, even if a maxRAF R set appears, the time \( \tau \) necessary to perform all the transition of \( R \) can exhibit different slopes during the evolution of the net, based on the various effective rates of subsets of \( R \). We use the slope \( m_r \) of the straight line \( y_r(i) = m_r i + q_r \) that approximates \( \tau_i \) for \( i \to \infty \) in order to compare the efficiency of the (total) self-production of different RAF sets. We summarise the results obtained from the simulated composition operations and the different RAF sets of our collection through a scatter plot showing the values \((m_M, m_r)\) averaged over independent runs (FIG. 7).

We start investigating the non interacting nets by simulating the evolution of three isolated RAF sets that constitute the collection, in order to obtain the dynamics that will be the benchmark for the evolution of the composite nets. FIG. 2 shows the time evolution of the number of tokens of non-food entities for the isolated RAF set represented in FIG. 1. Hereafter, we refer to this simulation as “reference”, since it will be used as a benchmark for the other simulations.

It is evident that, after a transient time of approximately 0.06 time units, all the entities associated with the RAF set grow in number, with the exception of entity \( C \): \( C \) is the only entity in the set to be a source for more than one transition (FIG. 1). The green line in FIG. 3 shows the corresponding trend of \( M_s(t) \). As expected, after the same transient time of \( \approx 0.06 \) time units, \( M_s(t) \) decreases down to values close to zero, the entire maxRAF set appears and condition (4) is satisfied (FIG. 7).

We find that the asymptotic dynamics of simple isolated RAF sets is not affected by changing the initial state. This is obvious from FIG. 5 and FIG. 4 by comparing the trends of \( M_s(t) \) and \( \tau_i \) obtained for the same RAF set for various initial conditions. In particular, we perform different simulations by setting \( x_s(t = 0) \) equal to a random number less than \( 10^4 \) for all non-food entities of the maxRAF set, and by setting \( x_s(t = 0) = 10^4 \) for only those entities associated with a particular closed RAF set. For all the RAF sets in the collection, the resulting values of \( m_M \) and \( m_r \) are in agreement with those corresponding to the initial conditions described by Eq.
These results suggest once again that simple RAF sets have an effective advantage in self-reproduction over non-RAF set. Also, the structure of RAF sets alone is not sufficient to guarantee the presence and the dynamical selectability of different long-term behaviours. Using the dynamics of isolated RAF sets as reference, we can now move on to the dynamics of composed RAF sets.

B. Composite nets

In order to compose nets, we choose five different sets $S_i$: the set of places belonging to the food set, the set of places non belonging to the food set, the set of places corresponding to the molecules of length $l = l_f + 1$ and $l \leq l_f + 1$ of the BPM and the set of places that are not input places for spontaneous transitions (not selected for operation $CO_{II}$). We compose copies of the RAF sets according to the composition operations $CO_1$, $CO_{II}$ and $CO_{III}$. The initial states of the nets are set according to Eq. \ref{eq:initial_conditions}. Moreover, for operation $CO_{III}$, simulations are performed in which the transitions belonging to net 2 cannot proceed for a certain time interval of $10^4$ time steps. Hereafter, we refer to this particular configuration as the “delayed configuration”.

We find that composition operations do not have any impact on the emergence of the maxRAF sets for any choice of $S_i$ that does not include food entities (FIG. \ref{fig:behavior_maxRAF}). However, a primordial form of biological interactions can be established; namely, facilitation and cheating. In particular, in nets composed through operations $CO_1$ and $CO_{III}$, inflowing of external entities can facilitate the appearance and the sustenance of a RAF set, improving its production efficiency. At the same time, withdrawing entities produced by a RAF set can counteract its production. These aspects are well highlighted by various trends of the generated $m_s$ due to different conditions.

On the other hand, as expected, composition operations involving the food set have a major role on the emergence of the maxRAF set. FIG. \ref{fig:behavior_maxRAF_composition} and FIG. \ref{fig:behavior_maxRAF_composition_2} show the behaviour of $M_s(t)$ and $\tau_i$ associated with any RAF sets that are composed by operations $CO_1$, $CO_{II}$ and $CO_{III}$ for $S_{II} = F$. Similar trends are obtained from the composition of the other RAF sets in our collection. Results show that operations $CO_1$ and $CO_{III}$ prevent the entire maxRAF set in at least one of the two copies from emerging. In particular, we find that, if the rate $\lambda_f$ of transitions allowing the flux of food elements from net 1

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Total production of non-food entities by isolated nets. $M_s(t)$ obtained in a simulation run of the isolated RAF set shown in FIG. \ref{fig:behavior_maxRAF} for different initial conditions: solid green line (reference simulation): $x_s(t=0) = 100$ if $s \in F$, $x_s(t=0) = 0$ otherwise; dotted magenta line: $x_s(t=0) = 100$ if $s \in F$, $x_s(t=0) = rand(0,10^4)$ otherwise; dashed blue line: $x_s(t=0) = 100$ if $s \in F$, $x_s(t=0) = 10^4$ if $s \in \{E,H,N,O\}$; $x_s(t=0) = 0$ otherwise. After a transient time of $\tau = 0.006$ (arbitrary units) $M_s(t)$ always takes values close to zero, indicating that the maxRAF set has emerged for all the different tested configurations. Dash-dotted red line: net that failed to be a RAF set, obtained by switching off the catalysis $(D,8)$ in the RAF set shown in FIG. \ref{fig:behavior_maxRAF}. In this case, $M_s(t)$ is always greater than 1, indicating that at least one non-food entity is not produced by transitions of the system. (FIG. \ref{fig:behavior_maxRAF_composition}). Conversely, the dynamics emerging in a net that failed to be a RAF set is significantly different (FIG. \ref{fig:behavior_maxRAF_composition} and FIG. \ref{fig:behavior_maxRAF_composition_2} red lines).
}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Cycles of transitions completed by isolated nets. $\tau_i$ obtained for the isolated RAF set shown in FIG. \ref{fig:behavior_maxRAF} with different initial conditions: solid green line (reference simulation): $x_s(t=0) = 100$ if $s \in F$, $x_s(t=0) = 0$ otherwise; dotted magenta line: $x_s(t=0) = 100$ if $s \in F$, $x_s(t=0) = rand(0,10^4)$ otherwise; dashed blue line: $x_s(t=0) = 100$ if $s \in F$, $x_s(t=0) = 10^4$ if $s \in \{E,H,N,O\}$; $x_s(t=0) = 0$ otherwise. The similar slope of $\tau_i$ for $t \to \infty$ associated with different tested configurations indicates that, after a transient time, the efficiency of performing all the transitions of the RAF set does not depend on the initial conditions. Red circle: the net that failed to be a RAF set, obtained by switching off the catalysis $(D,8)$ in the RAF set shown in FIG. \ref{fig:behavior_maxRAF}. In this case, the net is not able to perform all its transitions.}
\end{figure}
to net 2 is low enough ($\lambda_f < 10^6 \lambda_c$), operation $CO_f$ prevents the emergence of the maxRAF set in net 2, while net 1 exhibits the same dynamics of the isolated net, as can be concluded from FIG. 7. On the other hand, for the same composition operation, if the rate $\lambda_f$ is sufficiently high ($\lambda_f \geq 10^6 \lambda_c$), the flow of food elements is such that the maxRAF set of net 1 does not contain available resources to perform all its transitions, while net 2 evolves as if it is isolated and able to drawn food directly from the environment (FIG. 7). For operation $CO_{I\!I\!I}$, we have achieved significantly different results. In this case, we find different threshold values $\lambda_f = (\lambda_{f1}, \lambda_{f2})$ (depending on the specific RAF set of the collection) such that the emergence of the maxRAF set in net 2 is prevented for $\lambda_f \leq \lambda_{f1}$, while the opposite situation is obtained for $\lambda_f \geq \lambda_{f2}$. Moreover, for intermediate values $\lambda_{f1} < \lambda_f < \lambda_{f2}$, no maxRAF set emerges but only some of the closed RAF sets (FIG. 5 and FIG. 6). Therefore, for this range of values, the flux of entities is such that both nets 1 and 2 have enough food elements to fire transitions and perform (complementary) subsets of the maxRAF set, namely, the closed RAF sets. Furthermore, the stochastic nature of the flow process allows the emergence of different closed RAF sets in each run, hence showing the possible selectability of asymptotic dynamics for composite nets.

The observed dynamics lead to some important considerations: first, it is clear that the actual availability of resources is a crucial element in the dynamical realisation of a RAF set, and the rate at which food elements enter the net is as relevant as the definition of the food set itself, as expected. Moreover, the differences emerging due to the impact of the $CO_f$ and $CO_{I\!I\!I}$ operations suggest that the complexes play an important role in the dynamics of the RAF sets, even if they are defined starting from the single entities. We will investigate these points in a following work. Finally, as previously observed in (Hordijk et al., 2018b), biological interactions different from competition among RAF sets are plausible.

It is also intriguing that an effective competition can emerge if two nets share the same food, as in case of composition operation $CO_{I\!I\!I}$ and $S_\infty$. In particular, we observed that in the delayed configuration, the presence of the maxRAF of net 1 prevents the emergence of the maxRAF of net 2. In fact, once the maxRAF of net 1 has had enough time to appear, the number of tokens of its associated entities increases. Since the effective rate of a transition is proportional to the number of tokens of its sources, entities of net 1 have a higher chance of reacting with respect to their counterparts belonging to the delayed net 2. Most of the food elements are therefore consumed by transitions of net 1. Once activated,
only some of the transitions of net 2 are able to be performed efficiently, leading to the emergence of only some of the closed RAF sets which constitute the maxRAF set of net 2. Different runs show that the emerging closed RAF sets can vary due to the stochastic nature of the system evolution, thus guaranteeing the selectability of the different long-term behaviours.

This result is in contrast with the previous ones where it has been observed that isolated RAF sets are not able to experiment different asymptotic dynamics. In fact, composing two RAF sets using operation $CO_{III}$ produces a composite net in which all transitions form a (larger) RAF set (see the inclusion conditions, Section III.B), and the effect of the delay can be seen as a selection of a particular initial state. However, the same composite net is not able to produce competition if the delay is not introduced. Also, an effective competition between closed RAF sets has not been observed in an isolated net with initial conditions containing RAF sets already emerged at time $t = 0$ (FIG. 7, FIG. 3, and FIG. 4).

We suggest that key elements for this form of competition are both the structure of the composite RAF set and the particular choice of initial conditions. In fact, in nets composed by operation $CO_{III}$ and $S_\infty = F$, each transition that consumes at least one food entity as a source or a catalyst, always has at least one competitor represented by its copy. By contrast, the hierarchical structure of RAF sets does not guarantee such level of competition between different close RAF sets. Moreover, the delay brings the system into a state that promotes the formation of some subsets of the RAF set. The system can hardly reach this state only through random fluctuations. The results presented in this paper show that these two conditions allow RAF sets to have different accessible asymptotic dynamics.

V. CONCLUSIONS

In this work we study the impact of composition operations on the dynamics of simple RAF sets. This allows us to test whether the interactions among RAF sets permit different long-term dynamical behaviour of the RAF sets themselves, that is a necessary condition for evolvability. To this aim, we generate various RAF sets starting from different instances of the BPM and represent RAF sets as SPNs. Moreover, we introduce composition operations that, acting on SPNs, correspond to interactions among RAF sets. We find that, if the composition operations do not involve the food entities of a RAF set, the dynamics of the system always reaches a state in which all the composed RAF sets appear. However, how fast the RAF sets emerge and their efficiency in self-reproduction depends on the exchange of entities, showing that the composition operations can give rise to interactions with ecological features.

On the other hand, composition operations involving food entities hinders the appearance of, at least, one of the two original RAF sets, giving rise to possible different long-term behaviours. In particular, if the food entities can be exchanged between one RAF set and another, as the case of composition operations $CO_{I}$ and $CO_{II}$, the emergence of the maxRAF set of the starting nets depends on the rate of the exchange transitions (the flow). For sufficiently low rates, only the RAF set capable of acquiring food directly from the environment is able to form. Conversely, for sufficiently high rates the elements of the food are exchanged in such portions to allow only the receiving RAF set to appear. Furthermore, if complexes of a reaction are involved in the exchange and not individual entities (as in the case of the composition operation $CO_{III}$), we find an intermediate interval of flow rate values within which the exchange of food elements between the nets allows the emergence of just some of the closed RAF sets that constitute the starting maxRAF sets. For these intermediate flow rates, therefore, the necessary evolvability conditions are met. Finally, we find that only some closed RAF sets within a maxRAF set can appear if the maxRAF set shares the food entities with its copy that has already fully emerged. The latter is a relevant result, since sharing the same food set by two RAF sets produces a maxRAF set that is the union of the two starting RAF sets. The dynamics observed in
this case shows that different long-term behaviours are possible for a single RAF set, at least as long as the system is in a particular initial state and the subsets of the RAF set compete with each other for each reaction that needs food elements.

In previous works it was theorised that separate RAF networks could compete for shared resources [Hordijk and Steel 2014, Kauffman 2011, Vasas et al. 2012], and a competitive dynamics was observed in spatially separate RAF sets [Hordijk et al. 2018b]. Results presented in this paper confirm the possible evolvability of a system of RAF sets separated into compartments. We also noticed that isolated (composite) RAF sets can experience different asymptotic dynamics. Furthermore, recent results show the presence of RAF sets in real biological systems (Sousa et al. 2015, Xavier et al. 2020), confirming their biological interest. However, since all the simple (tested) isolated RAF sets experience the emergence of the entire maxRAF set, the definition of the RAF sets does not seem to be sufficient for implying dynamics with multiple selectable long-term behaviours.

In order to further clarify this last point, we will explore a larger ensemble of RAF sets in a forthcoming paper. Moreover, in the future it might be interesting to study the composition of RAF sets enclosed in protocols, investigating how the coupling between internal networks and boundaries affects the global dynamics of the system.

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REFERENCES

Anderson, D. F., Kurtz, T. G., 2011. Continuous time Markov chain models for chemical reaction networks. In Design and analysis of biomolecular circuits (pp. 3-42). Springer, New York, NY.

Baez, J. C., Pollard, B. S., 2017. A compositional framework for reaction networks. Reviews in Mathematical Physics, 29(09), 1750028.

Benner, S. A., Kim, H. J., Yang, Z., 2012. Setting the stage: the history, chemistry, and geobiology behind RNA. Cold Spring Harbor perspectives in biology, 4(1), a003541.

Bernhardt, H. S., 2012. The RNA world hypothesis: the worst theory of the early evolution of life (except for all the others). Biology direct, 7(1), 23.

Feinberg, M., 1995. The existence and uniqueness of steady states for a class of chemical reaction networks. Archive for Rational Mechanics and Analysis, 132(4), 311-370.

Gillespie, D. T., 1976. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. Journal of computational physics, 22(4), 403-434.

Gillespie, D. T., 1977. Exact stochastic simulation of coupled chemical reactions. The journal of physical chemistry, 81(25), 2340-2361.

Gillespie, D. T., 2007. Stochastic simulation of chemical kinetics. Annu. Rev. Phys. Chem., 58, 35-55.

Haas, P. J., 2006. Stochastic petri nets: Modelling, stability, simulation. Springer Science and Business Media.

Higgs, P. G., Lehman, N., 2015. The RNA World: molecular cooperation at the origins of life. Nature Reviews Genetics, 16(1), 7-17.

Hordijk, W., Steel, M., 2004. Detecting autocatalytic, self-sustaining sets in chemical reaction systems. Journal of theoretical biology, 227(4), 451-461.

Hordijk, W., Kauffman, S. A., Steel, M., 2011. Required levels of catalysis for emergence of autocatalytic sets in models of chemical reaction systems. International journal of molecular sciences, 12(5), 3085-3101.

Hordijk, W., Steel, M., 2012. Autocatalytic sets extended: Dynamics, inhibition, and a generalization. Journal of Systems Chemistry, 3(1), 5.

Hordijk, W., Steel, M., Kauffman, S. A., 2012. The structure of autocatalytic sets: Evolvability, enablement, and emergence. Acta biotheoretica, 60(4), 379-392.

Hordijk, W., Steel, W., 2014. Conditions for evolvability of autocatalytic sets: A formal example and analysis. Origins of Life and Evolution of Biospheres, 44(2), 111-124.

Hordijk, W., Steel, M., 2017. Chasing the tail: The emergence of autocatalytic networks. Biosystems, 152, 1-10.

Hordijk, W., Steel, M., Dittrich, P., 2018. Autocatalytic sets and chemical organizations: Modeling self-sustaining reaction networks at the origin of life. New Journal of Physics, 20(1), 015011.

Hordijk, W., Naylor, J., Krasnogor, N., Fellermann, H., 2018. Population dynamics of autocatalytic sets in a compartmentalized spatial world. Life, 8(3), 33.

Hordijk, W., Steel, M., 2018. Autocatalytic networks at the basis of life’s origin and organization. Life, 8(4), 62.

Jain, S., Krishna, S., 1998. Autocatalytic sets and the growth of complexity in an evolutionary model. Physical Review Letters, 81(25), 5684.

Kauffman, S. A., 1971. Cellular homeostasis, epigenesis and replication in randomly aggregated macromolecular systems. J. Cybernetics 1, 71-96.

Kauffman, S. A., 1986. Autocatalytic sets of proteins. Journal of theoretical biology, 119(1), 1-24.

Kauffman, S. A., 1993. The origins of order: Self-organization and selection in evolution. Oxford University Press, USA.

Kauffman, S. A., 2011. Approaches to the origin of life on earth. Life, 1(1), 34-48.

Lohn, J. D., Colombano, S. P., Scargle, J., Stassinopoulos, D., Haith, G. L., 1998. Evolving catalytic reaction sets using genetic algorithms. Proceedings of the 1998 IEEE International Conference on Evolutionary Computation, Anchorage, AK,USA. pp. 487-492.

Lu, P. L., 2016. The emergence of life: from chemical origins to synthetic biology. Cambridge University Press.

Molloy, M., 1982. Performance analysis using stochastic Petri nets. IEEE Transactions on computers, (9), 913-917.

Mossel, E., Steel, M., 2005. Random biochemical networks: The probability of self-sustaining autocatalysis. Journal of theoretical biology, 233(3), 327-336.

Nghe, P., Hordijk, W., Kauffman, S. A., Walker, S. I.,
Schmidt, F. J., Kemble, H., Yeates, J. A. M., Lehman, N., 2015. Prebiotic network evolution: six key parameters. Molecular BioSystems, 11(12), 3206-3217.

Petri, C. A., Reisig, W., 2008. Petri net. Scholarpedia, 3(4), 6477.

Rasmussen, S., Chen, L., Deamer, D., Krakauer, D. C., Packard, N. H., Stadler, P. F., Bedau, M. A., 2004. Transitions from nonliving to living matter. Science, 303(5660), 963-965.

Rasmussen, S., Constantinescu, A., Svaneborg, C., 2016. Generating minimal living systems from non-living materials and increasing their evolutionary abilities. Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1701), 20150440.

Serra, R., Villani, M., 2019. Sustainable growth and synchronization in protocell models. Life, 9(3), 68.

Sharov, A. A., 1991. Self-reproducing systems: structure, niche relations and evolution. BioSystems, 25(4), 237-249.

Smith, J. I., Steel, M., Hordijk, W., 2014. Autocatalytic sets in a partitioned biochemical network. Journal of Systems Chemistry, 5(1), 2.

Sousa, F. L., Hordijk, W., Steel, M., Martin, W. F., 2015. Autocatalytic sets in E. coli metabolism. Journal of Systems Chemistry, 6(1), 4.

Szostak, J. W., 2017. The narrow road to the deep past: in search of the chemistry of the origin of life. Angew. Chem. Int. Ed. Engl. 56, 11 037 - 11 043.

Vasas, V., Szathmry, E., Santos, M., 2010. Lack of evolvability in self-sustaining autocatalytic networks constraints metabolism-first scenarios for the origin of life. Proceedings of the National Academy of Sciences, 107(4), 1470-1475.

Vasas, V., Fernando, C., Santos, M., Kauffman, S. A., Szathmry, E., 2012. Evolution before genes. Biology direct, 7(1), 1.

Vazquez, C. R., Silva, M., 2011. Stochastic continuous Petri nets: An approximation of Markovian net models. IEEE Transactions on Systems, Man, and Cybernetics-Part A: Systems and Humans, 42(3), 641-653.

Xavier, J. C., Hordijk, W., Kauffman, S. A., Steel, M., Martin, W. F., 2020. Autocatalytic chemical networks at the origin of metabolism. Proceedings of the Royal Society B, 287(1922), 20192377.