

A Petri net approach to the study of persistence in chemical reaction networks

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Abstract

Persistence is the property, for differential equations in \mathbb{R}^n , that solutions starting in the positive orthant do not approach the boundary of the orthant. For chemical reactions and population models, this translates into the non-extinction property: provided that every species is present at the start of the reaction, no species will tend to be eliminated in the course of the reaction. This paper provides checkable conditions for persistence of chemical species in reaction networks, using concepts and tools from Petri net theory, and verifies these conditions on various systems which arise in the modeling of cell signaling pathways.

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1. Introduction

One of the main goals of molecular systems biology is the understanding of cell behavior and function at the level of chemical interactions, and, in particular, the characterization of qualitative

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features of dynamical behavior (convergence to steady states, periodic orbits, chaos, etc.). A central question, thus, is that of understanding the long-time behavior of solutions. In mathematical terms, and using standard chemical kinetics modeling, this problem may be translated into the study of the set of possible limit points (the ω -limit set) of the solutions of a system of ordinary differential equations.

1.1. Robustness

A distinguishing feature of this study in the context of cell biology, in contrast to more established areas of applied mathematics and engineering, is the very large degree of uncertainty inherent in models of cellular biochemical networks. This uncertainty is due to environmental fluctuations, and variability among different cells of the same type, as well as, from a mathematical analysis perspective, the difficulty of measuring the relevant model parameters (kinetic constants, cooperativity indices, and many others) and thus the challenge to obtain a precise model. Thus, it is imperative to develop tools that are ‘robust’ in the sense of being able to provide useful conclusions based only upon information regarding the *qualitative* features of the network, and not the precise values of parameters or even the forms of reactions. Of course, this goal is often unachievable, since dynamical behavior may be subject to phase transitions (bifurcation phenomena) which are critically dependent on parameter values.

Nevertheless, and surprisingly, research by many, notably by Clarke [10], Horn and Jackson [29,30], Feinberg [18–20], and many others in the context of complex balancing and deficiency theory, and by Hirsch and Smith [41,26] and many others including the present authors [2,17,3,9] in the context of monotone systems, has resulted in the identification of rich classes of chemical network structures for which such robust analysis is indeed possible. In this paper, we present yet another approach to the robust analysis of dynamical properties of biochemical networks, and apply our approach in particular to the analysis of persistence in chemical networks modeled by ordinary differential equations. Our approach to study persistence is based on the formalism and basic concepts of the theory of Petri nets. Using these techniques, our main results provide conditions (some necessary, and some sufficient) to test persistence. We then apply these conditions to obtain fairly tight characterizations in non-trivial examples arising from the current molecular biology literature.

1.2. Persistence

Persistence is the property that, *if every species is present at the start of the reaction, no species will tend to be eliminated in the course of the reaction*. Mathematically, this property can be equivalently expressed as the requirement that the ω -limit set of any trajectory which starts in the interior of the positive orthant (all concentrations positive) does not intersect the boundary of the positive orthant (more precise definitions are given below). Persistence can be interpreted as non-extinction: if the concentration of a species would approach zero in the continuous differential equation model, this means, in practical terms, that it would completely disappear in finite time, since the true system is discrete and stochastic. Thus, one of the most basic questions that one may ask about a chemical reaction network is if persistence holds for that network. Also from a purely mathematical perspective persistence is very important, because it may be used in conjunction with other

techniques in order to guarantee convergence of solutions to equilibria. For example, if a strictly decreasing Lyapunov function exists on the interior of the positive orthant (see e.g. [29,30,18–20,42] for classes of networks where this can be guaranteed), persistence allows such a conclusion.

An obvious example of a non-persistent chemical reaction is a simple irreversible conversion $A \rightarrow B$ of a species A into a species B ; in this example, the chemical A empties out, that is, its time-dependent concentration approaches zero as $t \rightarrow \infty$. This is obvious, but for complex networks determining persistence, or lack thereof, is, in general, an extremely difficult mathematical problem. In fact, the study of persistence is a classical one in the (mathematically) related field of population biology, where species correspond to individuals of different types instead of chemical units; see for example [22,7] and much other foundational work by Waltman. (To be precise, what we call ‘persistence’ coincides with the usage in the above references, and is also sometimes called ‘strong persistence,’ at least when all solutions are bounded, a condition that we will assume in most of our main results, and which is automatically satisfied in most examples. Also, we note that a stronger notion, ‘uniform’ persistence, is used to describe the situation where all solutions are eventually bounded away from the boundary, uniformly on initial conditions, see [8,44].) Most dynamical systems work on persistence imposes conditions ruling out phenomena such as heteroclinic cycles on the boundary of the positive orthant, and requiring that the unstable manifolds of boundary equilibria should intersect the interior, and more generally studying the chain-recurrence structure of attractors, see e.g. [27].

1.3. Petri nets

Basic ideas introduced by Carl Adam Petri in 1962 [38] led to the notion of a *Petri net*, also called a place/transition nets, and they constitute a popular mathematical and graphical modeling tool used for concurrent systems modeling [37,47]. Our modeling of chemical reaction networks using Petri net formalism is not in itself a new idea: there have been many works, at least since [39], which have dealt with biochemical applications of Petri nets, in particular in the context of metabolic pathways, see e.g. [23,28,32,35,36,46]. In this paper, we associate both a Petri net and a system of differential equations to a chemical reaction network. The latter describes the behavior of the concentrations of the chemicals in the network. We intend to draw conclusions about the asymptotic behavior of the solutions of the system of differential equations, based on the graphical and algebraic properties of the associated Petri net. This is very related to open questions which have been raised in recent works by Gilbert and Heiner as well as Silva and Recalde, [24,40], where a similar point of view is taken, of either complementing discrete analysis by means of continuous techniques or integrating the two approaches for a deeper understanding (see [16] for an introduction to continuous Petri nets).

Although we do not use any results from Petri net theory, we employ several concepts (siphons, P-semiflows, etc.), borrowed from that formalism and introduced as needed, in order to formulate new, powerful, and verifiable conditions for persistence and related dynamical properties.

1.4. Application to a common motif in systems biology

In molecular systems biology research, certain ‘motifs’ or subsystems appear repeatedly, and have been the subject of much recent research. One of the most common ones is that in which

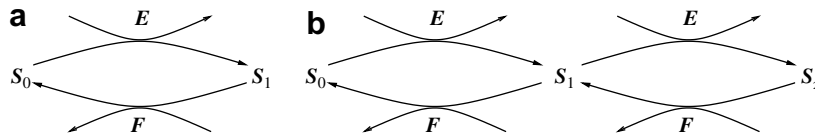


Fig. 1. (a) One-step and (b) two-step transformations.

a substrate S_0 is ultimately converted into a product P , in an ‘activation’ reaction triggered or facilitated by an enzyme E , and, conversely, P is transformed back (or ‘deactivated’) into the original S_0 , helped on by the action of a second enzyme F . This type of reaction is sometimes called a ‘futile cycle’ and it takes place in signaling transduction cascades, bacterial two-component systems, and a plethora of other processes. The transformations of S_0 into P and vice versa can take many forms, depending on how many elementary steps (typically phosphorylations, methylations, or additions of other elementary chemical groups) are involved, and in what order they take place. Fig. 1 shows two examples, (a) one in which a single step takes place changing S_0 into S_1 , and (b) one in which two sequential steps are needed to transform S_0 into S_2 , with an intermediate transformation into a substance S_1 . A chemical reaction model for such a set of transformations incorporates intermediate species, compounds corresponding to the binding of the enzyme and substrate. (In ‘quasi-steady state’ approximations, a singular perturbation approach is used in order to eliminate the intermediates. These approximations are much easier to study, see e.g. [2].) Thus, one model for (a) would be through the following reaction network:



(double arrows indicate reversible reactions) and a model for (b) would be:



where ‘ ES_0 ’ represents the complex consisting of E bound to S_0 and so forth.

As a concrete example, case (b) may represent a reaction in which the enzyme E reversibly adds a phosphate group to a certain specific amino acid in the protein S_0 , resulting in a single-phosphorylated form S_1 ; in turn, E can then bind to S_1 so as to produce a double-phosphorylated form S_2 , when a second amino acid site is phosphorylated. A different enzyme reverses the process. (Variants in which the individual phosphorylations can occur in different orders are also possible; we discuss several models below.) This is, in fact, one of the mechanisms believed to underlie signaling by MAPK cascades. *Mitogen-activated protein kinase (MAPK) cascades* constitute a motif that is ubiquitous in signal transduction processes [31,33,45] in eukaryotes from yeast to humans, and represents a critical component of pathways involved in cell apoptosis, differentiation, proliferation, and other processes. These pathways involve chains of reactions, activated by extracellular stimuli such as growth factors or hormones, and resulting in gene expression or other cellular responses. In MAPK cascades, several steps as in (b) are arranged in a cascade, with the ‘active’ form S_2 serving as an enzyme for the next stage.

Single-step reactions as in (a) can be shown to have the property that all solutions starting in the interior of the positive orthant globally converge to a unique (subject to stoichiometry constraints) steady state, see [4], and, in fact, can be modeled by monotone systems after elimination of the variables E and F , cf. [1]. The study of (b) is much harder, as multiple equilibria can appear, see e.g. [34,12]. We will show how our results can be applied to test consistency of this model, as well as several variants.

1.5. Organization of paper

The remainder of paper is organized as follows. Section 2 sets up the basic terminology and definitions regarding chemical networks, as well as the notion of persistence, Section 3 shows how to associate a Petri net to a chemical network, Sections 4 and 5 provide, respectively, necessary and sufficient conditions for general chemical networks. In Section 6, we show how our results apply to the enzymatic mechanisms described above. We present some conclusions and directions for future research in Section 8.

2. Chemical networks

A *chemical reaction network* (‘CRN’, for short) is a set of chemical reactions \mathcal{R}_i , where the index i takes values in $\mathcal{R} := \{1, 2, \dots, n_r\}$. We next define precisely what one means by reactions, and the differential equations associated to a CRN, using the formalism from chemical networks theory.

Let us consider a set of chemical species $\mathcal{S} := \{S_j | j \in \{1, 2, \dots, n_s\}\}$ which are the compounds taking part in the reactions. Chemical reactions are denoted as follows:



where the α_{ij} and β_{ij} are non-negative integers called the *stoichiometry coefficients*. The compounds on the left-hand side are usually referred to as the *reactants*, and the ones on the right-hand side are called the *products*, of the reaction. Informally speaking, the forward arrow means that the transformation of reactants into products only happens in the direction of the arrow. If also the converse transformation occurs, then, the reaction is reversible and we need to also list its inverse in the chemical reaction network as a separate reaction.

It is convenient to arrange the stoichiometry coefficients into an $n_s \times n_r$ matrix, called the *stoichiometry matrix* Γ , defined as follows:

$$[\Gamma]_{ji} = \beta_{ij} - \alpha_{ij}, \quad (4)$$

for all $i \in \mathcal{R}$ and all $j \in \mathcal{S}$ (notice the reversal of indices). This will be later used in order to write down the differential equation associated to the chemical reaction network. Notice that we allow Γ to have columns which differ only by their sign; this happens when there are reversible reactions in the network.

We discuss now how the speed of reactions is affected by the concentrations of the different species. Each chemical reaction takes place continuously in time with its own rate which is assumed to be only a function of the concentration of the species taking part in it. In order to make this

more precise, we define the vector $S = [S_1, S_2, \dots, S_{n_s}]'$ of species concentrations and, as a function of it, the vector of reaction rates

$$R(S) := [R_1(S), R_2(S), \dots, R_{n_r}(S)]'.$$

Each reaction rate R_i is a real-analytic function defined on an open set which contains the non-negative orthant $\mathcal{O}_+^{n_s} = \mathbb{R}_{\geq 0}^{n_s}$ of \mathbb{R}^{n_s} , and we assume that each R_i depends only on its respective reactants. (Imposing real-analyticity, that is to say, that the function R_i can be locally expanded into a convergent power series around each point in its domain, is a very mild assumption, verified in basically all applications in chemistry, and it allows stronger statements to be made.) Furthermore, we assume that each R_i satisfies the following monotonicity conditions:

$$\frac{\partial R_i(S)}{\partial S_j} = \begin{cases} \geq 0 & \text{if } \alpha_{ij} > 0, \\ = 0 & \text{if } \alpha_{ij} = 0. \end{cases} \quad (5)$$

We also assume that, whenever the concentration of any of the reactants of a given reaction is 0, then, the corresponding reaction does not take place, meaning that the reaction rate is 0. In other words, if S_{i_1}, \dots, S_{i_N} are the reactants of reaction j , then we ask that

$$R_j(S) = 0 \text{ for all } S \text{ such that } [S_{i_1}, \dots, S_{i_N}] \in \partial \mathcal{O}_+^N,$$

where $\partial \mathcal{O}_+^N = \partial \mathbb{R}_{\geq 0}^N$ is the boundary of \mathcal{O}_+^N in \mathbb{R}^N . Conversely, we assume that reactions take place if reactants are available, that is:

$$R_j(S) > 0 \text{ whenever } S \text{ is such that } [S_{i_1}, \dots, S_{i_N}] \in \text{int}[\mathbb{R}_{\geq 0}^N],$$

where $\text{int}[\mathbb{R}_{\geq 0}^N]$ denotes the interior of the orthant $\mathbb{R}_{\geq 0}^N$.

A special case of reactions is as follows. One says that a chemical reaction network is equipped with *mass-action kinetics* if

$$R_i(S) = k_i \prod_{j=1}^{n_s} S_j^{\alpha_{ij}} \quad \text{for all } i = 1, \dots, n_r.$$

Note that the exponents of each chemical participating in the reaction is the same as the stoichiometric coefficient this chemical has in that reaction. This is a commonly used form for the functions $R_i(s)$ and amounts to asking that the reaction rate of each reaction is proportional to the concentration of each of its participating reactants. The results in this paper do not require this assumption; in a paper in preparation we will specialize and tighten our results when applied to systems with mass-action kinetics.

With the above notations, the chemical reaction network is described by the following system of differential equations:

$$\dot{S} = \Gamma R(S). \quad (6)$$

with S evolving in $\mathcal{O}_+^{n_s}$ and where Γ is the stoichiometry matrix.

There are several additional notions useful when analyzing CRN's. One of them is the notion of a *complex*. We associate to the network (3) a set of complexes, C_i 's, with $i \in \{1, 2, \dots, n_c\}$. Each complex is an integer combination of species, specifically of the species appearing either as products or reactants of the reactions in (3). We introduce the following matrix $\tilde{\Gamma}$ as follows:

$$\tilde{\Gamma} = \begin{bmatrix} \alpha_{11} & \alpha_{21} & \dots & \alpha_{n_r,1} & \beta_{11} & \beta_{21} & \dots & \beta_{n_r,1} \\ \alpha_{12} & \alpha_{22} & \dots & \alpha_{n_r,2} & \beta_{12} & \beta_{22} & \dots & \beta_{n_r,2} \\ \vdots & \vdots & & \vdots & \vdots & \vdots & & \vdots \\ \alpha_{1n_s} & \alpha_{2n_s} & \dots & \alpha_{n_r,n_s} & \beta_{1n_s} & \beta_{2n_s} & \dots & \beta_{n_r,n_s} \end{bmatrix}.$$

Then, a matrix representing the complexes as columns can be obtained by deleting from $\tilde{\Gamma}$ repeated columns, leaving just one instance of each; we denote by $\Gamma_c \in \mathbb{R}^{n_s \times n_c}$ the matrix which is thus constructed. Each of the columns of Γ_c is then associated with a complex of the network. We may now associate to each chemical reaction network, a directed graph (which we call the *C-graph*), whose nodes are the complexes and whose edges are associated to the reactions (3). An edge (C_i, C_j) is in the C-graph if and only if $C_i \rightarrow C_j$ is a reaction of the network. Note that the C-graph need not be connected (the C-graph is connected if for any pair of distinct nodes in the graph there is an undirected path linking the nodes), and lack of connectivity cannot be avoided in the analysis. (This is in contrast with many other graphs in chemical reaction theory, which can be assumed to be connected without loss of generality.) In general, the C-graph will have several connected components (equivalence classes under the equivalence relation ‘is linked by an undirected path to’, defined on the set of nodes of the graph).

Let \mathcal{I} be the incidence matrix of the C-graph, namely the matrix whose columns are in one-to-one correspondence with the edges (reactions) of the graph and whose rows are in one-to-one correspondence with the nodes (complexes). Each column contains a -1 in the i th entry and a $+1$ in the j th entry (and zeroes in all remaining entries) whenever (C_i, C_j) is an edge of the C-graph (equivalently, when $C_i \rightarrow C_j$ is a reaction of the network). With this notations, we have the following formula, to be used later:

$$\Gamma = \Gamma_c \mathcal{I}. \quad (7)$$

We denote solutions of (6) as follows: $S(t) = \varphi(t, S_0)$, where $S_0 \in \mathcal{O}_+^{n_s}$ is the initial concentration of chemical species. As usual in the study of the qualitative behavior of dynamical systems, we will make use of ω -limit sets, which capture the long-term behavior of a system and are defined as follows:

$$\omega(S_0) := \{S \in \mathcal{O}_+^{n_s} : \varphi(t_n, S_0) \rightarrow S \text{ for some } t_n \rightarrow +\infty\} \quad (8)$$

(implicitly, when talking about $\omega(S_0)$, we assume that $\varphi(t, S_0)$ is defined for all $t \geq 0$ for the initial condition S_0). We will be interested in asking whether or not a chemical reaction network admits solutions in which one or more of the chemical compounds become arbitrarily small. The following definition, borrowed from the ecology literature, captures this intuitive idea.

Definition 2.1. A chemical reaction network (6) is *persistent* if $\omega(S_0) \cap \partial \mathcal{O}_+^{n_s} = \emptyset$ for each $S_0 \in \text{int}(\mathcal{O}_+^{n_s})$.

We will derive conditions for persistence of general chemical reaction networks. Our conditions will be formulated in the language of Petri nets; these are discrete-event systems equipped with an algebraic structure that reflects the list of chemical reactions present in the network being studied, and are defined as follows.

3. Petri nets

We associate to a CRN a bipartite directed graph (i.e., a directed graph with two types of nodes) with weighted edges, called the *species-reaction Petri net*, or SR-net for short. Mathematically, this is a quadruple

$$(V_S, V_R, E, W),$$

where V_S is a finite set of nodes each one associated to a species, V_R is a finite set of nodes (disjoint from V_S), each one corresponding to a reaction, and E is a set of edges as described below. (We often write S or V_S interchangeably, or R instead of V_R , by identifying species or reactions with their respective indices; the context should make the meaning clear.) The set of all nodes is also denoted by $V \doteq V_R \cup V_S$.

The edge set $E \subset V \times V$ is defined as follows. Whenever a certain reaction R_i belongs to the CRN:

$$\sum_{j \in S} \alpha_{ij} S_j \rightarrow \sum_{j \in S} \beta_{ij} S_j, \quad (9)$$

we draw an edge from $S_j \in V_S$ to $R_i \in V_R$ for all S_j 's such that $\alpha_{ij} > 0$. That is, $(S_j, R_i) \in E$ iff $\alpha_{ij} > 0$, and we say in this case that R_i is an *output reaction* for S_j . Similarly, we draw an edge from $R_i \in V_R$ to every $S_j \in V_S$ such that $\beta_{ij} > 0$. That is, $(R_i, S_j) \in E$ whenever $\beta_{ij} > 0$, and we say in this case that R_i is an *input reaction* for S_j .

Notice that edges only connect species to reactions and vice versa, but never connect two species or two reactions.

The notion of an SR-net is very closely related to that of an SR-graph in [14,15]. The only difference is that an SR-net is a directed graph, while an SR-graph is not, and that reversible reactions in an SR-net are represented by two distinct reaction nodes, while only one reaction node appears in the SR-graph for a reversible reaction.

The last element to fully define the Petri net is the function $W : E \rightarrow \mathbb{N}$, which associates to each edge a positive integer according to the rule:

$$W(S_j, R_i) = \alpha_{ij} \quad \text{and} \quad W(R_i, S_j) = \beta_{ij}.$$

Several other definitions which are commonly used in the Petri net literature will be of interest in the following. We say that a row or column vector v is non-negative, and we denote it by $v \succeq 0$ if it is so entry-wise. We write $v \succ 0$ if $v \succeq 0$ and $v \neq 0$. A stronger notion is instead $v \gg 0$, which indicates $v_i > 0$ for all i .

Definition 3.1. A *P-semiflow* is any row vector $c \succ 0$ such that $c\Gamma = 0$. Its *support* is the set of indices $\{i \in V_S : c_i > 0\}$. A Petri net is said to be *conservative* if there exists a P-semiflow $c \gg 0$.

Notice that without loss of generality a P-semiflow has integer components since the entries of Γ are integers. Notice also that P-semiflows for the system (6) correspond to non-negative linear first integrals, that is, linear functions $S \mapsto cS$ such that $(d/dt)cS(t) \equiv 0$ along all solutions of (6) (assuming that the span of the image of $R(S)$ is \mathbb{R}^{n_r}). In particular, a Petri net is conservative if and only if there is a positive linear conserved quantity for the system. (Petri net theory views Petri nets as ‘token-passing’ systems, and, in that context, P-semiflows, also called *place-invariants*, amount to conservation relations for the ‘place markings’ of the network, that show how many tokens there are in each ‘place,’ the nodes associated to species in SR-nets. We do not make use of this interpretation in this paper.)

Definition 3.2. A *T-semiflow* is any column vector $v \succ 0$ such that $\Gamma v = 0$. A Petri net is said to be *consistent* if there exists a T-semiflow $v \gg 0$.

The notion of T-semiflow corresponds to the existence of a collection of positive reaction rates which do not produce any variation in the concentrations of the species. In other words, v can be viewed as a set of *fluxes* that is in equilibrium [46]. (In Petri net theory, the terminology is ‘T-invariant,’ and the fluxes are flows of tokens.)

A chemical reaction network is said to be *reversible* if each chemical reaction has an inverse reaction which is also part of the network. (This is different from the meaning reversibility has in the Petri net literature.) Biochemical models are most often non-reversible. For this reason, a far milder notion was introduced [29,30,18–20]: A chemical reaction network is said to be *weakly reversible* if each connected component of the C-graph is strongly connected (meaning that there is a directed path between any pair of nodes in each connected component). In algebraic terms, weak reversibility amounts to existence of $v \gg 0$ such that $\mathcal{I}v = 0$ (see Corollary 4.2 of [21]), so that in particular, using (7), also $\Gamma v = \Gamma_c \mathcal{I}v = 0$. Hence a chemical reaction network that is weakly reversible has a consistent associated Petri net (the converse is in general not true, see for instance Example 1).

A few more definitions are needed in order to state our main results.

Definition 3.3. A non-empty set $\Sigma \subset V_S$ is called a *siphon* if each input reaction associated to Σ is also an output reaction associated to Σ . A siphon is *minimal* if it does not contain (strictly) any other siphons.

For later use we associate a particular set to a siphon Σ as follows:

$$L_\Sigma = \{x \in \mathcal{O}_+^{n_s} | x_i = 0 \iff i \in \Sigma\}.$$

The set L_Σ is therefore characterized as the set of concentration vectors whose entries are zero if (and only if) the corresponding chemical species are in the siphon Σ .

It is also useful to introduce a binary relation ‘reacts to’, which we denote by \succrightarrow , and we define as follows: $S_i \succrightarrow S_j$ whenever there exists a chemical reaction \mathcal{R}_k , so that

$$\sum_{l \in S} \alpha_{kl} S_l \rightarrow \sum_{l \in S} \beta_{kl} S_l$$

with $\alpha_{ki} > 0$, $\beta_{kj} > 0$. If the reaction number is important, we also write

$$S_i \xrightarrow{k} S_j$$

(where $k \in \mathcal{R}$). With this notation, the notion of siphon can be rephrased as follows: $Z \subset S$ is a siphon for a chemical reaction network if for every $S \in Z$ and $k \in \mathcal{R}$ such that $\tilde{S}_k := \{T \in S : T \xrightarrow{k} S\} \neq \emptyset$, it holds $\tilde{S}_k \cap Z \neq \emptyset$.

4. Necessary conditions

Our first result will relate persistence of a chemical reaction network to consistency of the associated Petri net.

Theorem 1. *Let (6) be the equation describing the time-evolution of a conservative and persistent chemical reaction network. Then, the associated Petri net is consistent.*

Proof. Let $S_0 \in \text{int}(\mathcal{O}_+^{n_s})$ be any initial condition. By conservativity, solutions satisfy $cS(t) \equiv cS_0$, and hence remain bounded, and therefore $\omega(S_0)$ is a non-empty compact set. Moreover, by persistence, $\omega(S_0) \cap \partial\mathcal{O}_+^{n_s} = \emptyset$, so that $R(\tilde{S}_0) \gg 0$, for all $\tilde{S}_0 \in \omega(S_0)$. In particular, by compactness of $\omega(S_0)$ and continuity of R , there exists a positive vector $v \gg 0$, so that

$$R(\tilde{S}_0) \succeq v \text{ for all } \tilde{S}_0 \in \omega(S_0).$$

Take any $\tilde{S}_0 \in \omega(S_0)$. By invariance of $\omega(S_0)$, we have $R(\varphi(t, \tilde{S}_0)) \succeq v$ for all $t \in \mathbb{R}$. Consequently, taking asymptotic time averages, we obtain:

$$0 = \lim_{T \rightarrow +\infty} \frac{\varphi(T, \tilde{S}_0) - \tilde{S}_0}{T} = \lim_{T \rightarrow +\infty} \frac{1}{T} \int_0^T \Gamma R(\varphi(t, \tilde{S}_0)) dt \quad (10)$$

(the left-hand limit is zero because $\varphi(T, \tilde{S}_0)$ is bounded). However,

$$\frac{1}{T} \int_0^T R(\varphi(t, \tilde{S}_0)) dt \succeq v$$

for all $T > 0$. Therefore, taking any subsequence $T_n \rightarrow +\infty$ so that there is a finite limit:

$$\lim_{n \rightarrow +\infty} \frac{1}{T_n} \int_0^{T_n} R(\varphi(t, \tilde{S}_0)) dt = \bar{v} \succeq v.$$

We obtain, by virtue of (10), that $\Gamma \bar{v} = 0$. This completes the proof of consistency, since $\bar{v} \gg 0$. \square

5. Sufficient conditions

In this present Section, we derive sufficient conditions for insuring persistence of a chemical reaction network on the basis of Petri net properties.

Theorem 2. *Consider a chemical reaction network satisfying the following assumptions:*

1. *its associated Petri net is conservative;*
2. *each siphon contains the support of a P-semiflow.*

Then, the network is persistent.

We first prove a number of technical results. The following general fact about differential equations will be useful.

For each real number p , let $\text{sign } p = 1, 0, -1$ if $p > 0$, $p = 0$, or $p < 0$ respectively, and for each vector $x = (x_1, \dots, x_n)$, let $\text{sign } x = (\text{sign } x_1, \dots, \text{sign } x_n)'$. When x belongs to the closed positive orthant \mathbb{R}_+^n , $\text{sign } x \in \{0, 1\}^n$.

Lemma 5.1. *Let f be a real-analytic vector field defined on some open neighborhood of \mathbb{R}_+^n , and suppose that \mathbb{R}_+^n is forward invariant for the flow of f . Consider any solution $\bar{x}(t)$ of $\dot{x} = f(x)$, evolving in \mathbb{R}_+^n and defined on some open interval J . Then, $\text{sign}\bar{x}(t)$ is constant on J .*

Proof. Pick such a solution, and define

$$Z := \{i | \bar{x}_i(t) = 0 \text{ for all } t \in J\}.$$

Relabeling variables if necessary, we assume without loss of generality that $Z = \{r+1, \dots, n\}$, with $0 \geq r \geq n$, and we write equations in the following block form:

$$\begin{aligned}\dot{y} &= g(y, z), \\ \dot{z} &= h(y, z),\end{aligned}$$

where $x' = (y', z')'$ and $y(t) \in \mathbb{R}^r$, $z(t) \in \mathbb{R}^{n-r}$. (The extreme cases $r = 0$ and $r = n$ correspond to $x = z$ and $x = y$ respectively.) In particular, we write $\bar{x}' = (\bar{y}', \bar{z}')'$ for the trajectory of interest. By construction, $\bar{z} \equiv 0$, and the sets

$$B_i := \{t | \bar{y}_i(t) = 0\}$$

are proper subsets of J , for each $i \in \{1, \dots, r\}$. Since the vector field is real-analytic, each coordinate function \bar{y}_i is real-analytic (see e.g. [43], Proposition C.3.12), so, by the principle of analytic continuation, each B_i is a discrete set. It follows that

$$G := J \setminus \bigcup_{i=1}^r B_i.$$

is an (open) dense set, and for each $t \in G$, $\bar{y}(t) \in \text{inter}\mathbb{R}_+^r$, the interior of the positive orthant.

We now consider the following system on \mathbb{R}^r :

$$\dot{y} = g(y, 0).$$

This is again a real-analytic system, and \mathbb{R}_+^r is forward invariant. To prove this last assertion, note that forward invariance of the closed positive orthant is equivalent to the following property:

for any $y \in \mathbb{R}_+^r$ and any $i \in \{1, \dots, r\}$ such that $y_i = 0$, $g_i(y, 0) \geq 0$.

Since \mathbb{R}_+^n is forward invariant for the original system, we know, by the same property applied to that system, that for any $(y, z) \in \mathbb{R}_+^n$ and any $i \in \{1, \dots, r\}$ such that $y_i = 0$, $g_i(y, z) \geq 0$. Thus, the required property holds (case $z = 0$). In particular, $\text{inter}\mathbb{R}_+^r$ is also forward invariant (see e.g. [2], Lemma III.6). By construction, \bar{y} is a solution of $\dot{y} = g(y, 0)$, $\bar{y}(t) \in \text{inter}\mathbb{R}_+^r$ for each $t \in G$. Since G is dense and $\text{inter}\mathbb{R}_+^r$ is forward invariant, it follows that $\bar{y}(t) \in \text{inter}\mathbb{R}_+^r$ for all $t \in J$. Therefore,

$$\text{sign}\bar{x}(t) = (1_r, 0_{n-r})' \text{ for all } t \in J,$$

where 1_r is a vector of r 1's and 0_{n-r} is a vector of $n - r$ 0's. \square

We then have an immediate corollary:

Lemma 5.2. *Suppose that $\Omega \subset \mathcal{O}_+^n$ is a closed set, invariant for (6). Suppose that $\Omega \cap L_Z$ is non-empty, for some $Z \subset \mathcal{S}$. Then, $\Omega \cap L_Z$ is also invariant with respect to (6).*

Proof. Pick any $S_0 \in \Omega \cap L_Z$. By invariance of Ω , the solution $\varphi(t, S_0)$ belongs to Ω for all t in its open domain of definition J , so, in particular (this is the key fact), $\varphi(t, S_0) \in \mathcal{O}_+^{n_s}$ for all t (negative as well as positive). Therefore, it also belongs to L_Z , since its sign is constant by Lemma 5.1. \square

In what follows, we will make use of the Bouligand tangent cone $TC_\xi(K)$ of a set $K \subset \mathcal{O}_+^{n_s}$ at a point $\xi \in \mathcal{O}_+^{n_s}$, defined as follows:

$$TC_\xi(K) = \left\{ v \in \mathbb{R}^n : \exists k_n \in K, k_n \rightarrow \xi \text{ and } \lambda_n \searrow 0 : \frac{1}{\lambda_n} (k_n - \xi) \rightarrow v \right\}.$$

Bouligand cones provide a simple criterion to check forward invariance of closed sets (see e.g. [5]): a closed set K is forward invariant for (6) if and only if $\Gamma R(\xi) \in TC_\xi(K)$ for all $\xi \in K$. However, below we consider a condition involving tangent cones to the sets L_Z , which are not closed. Note that, for all index sets Z and all points ξ in L_Z ,

$$TC_\xi(L_Z) = \{v \in \mathbb{R}^n : v_i = 0 \ \forall i \in Z\}.$$

Lemma 5.3. *Let $Z \subset S$ be non-empty and $\xi \in L_Z$ be such that $\Gamma R(\xi) \in TC_\xi(L_Z)$. Then Z is a siphon.*

Proof. By assumption $\Gamma R(\xi) \in TC_\xi(L_Z)$ for some $\xi \in L_Z$. This implies that $[\Gamma R(\xi)]_i = 0$ for all $i \in Z$. Since $\xi_i = 0$ for all $i \in Z$, all reactions in which S_i is involved as a reactant are shut off at ξ ; hence, the only possibility for $[\Gamma R(\xi)]_i = 0$ is that all reactions in which S_i is involved as a product are also shut-off. Hence, for all $k \in \mathcal{R}$, and all $l \in S$ so that $S_l \xrightarrow{k} S_i$, we necessarily have that $R_k(\xi) = 0$.

Hence, for all $k \in \mathcal{R}$ so that $\tilde{S}_k = \{l \in S : S_l \xrightarrow{k} S_i\}$ is non-empty, there must exist an $l \in \tilde{S}_k$ so that $\xi_l = 0$. But then necessarily, $l \in Z$, showing that Z is indeed a siphon. \square

The above Lemmas are instrumental to prove the following Proposition:

Proposition 5.4. *Let $\xi \in \mathcal{O}_+^{n_s}$ be such that $\omega(\xi) \cap L_Z \neq \emptyset$ for some $Z \subset S$. Then Z is a siphon.*

Proof. Let Ω be the closed and invariant set $\omega(\xi)$. Thus, by Lemma 5.2, the non-empty set $L_Z \cap \Omega$ is also invariant. Notice that

$$\text{cl}[L_Z] = \bigcup_{W \supseteq Z} L_W.$$

Moreover, $L_W \cap \Omega$ is invariant for all $W \subset S$ such that $L_W \cap \Omega$ is non-empty. Hence,

$$\text{cl}[L_Z] \cap \Omega = \bigcup_{W \supseteq Z} [L_W \cap \Omega]$$

is also invariant. By the characterization of invariance for closed sets in terms of Bouligand tangent cones, we know that, for any $\eta \in \text{cl}[L_Z] \cap \Omega$ we have

$$\Gamma R(\eta) \in TC_\eta(\Omega \cap \text{cl}(L_Z)) \subset TC_\eta(\text{cl}(L_Z)).$$

In particular, for $\eta \in L_Z \cap \Omega$ (which by assumption exists), $\Gamma R(\eta) \in TC_\eta(L_Z)$ so that, by virtue of [Lemma 5.3](#) we may conclude Z is a siphon. \square

Although at this point [Proposition 5.4](#) would be enough to prove [Theorem 2](#), it is useful to clarify the meaning of the concept of a ‘siphon’ here. It hints at the fact, made precise in the Proposition below, that removing all the species of a siphon from the network (or equivalently setting their initial concentrations equal to 0) will prevent those species from being present at all future times. Hence, those species literally ‘lock’ a part of the network and shut off all the reactions that are therein involved. In particular, once emptied a siphon will never be full again. A precise statement of the foregoing remarks is as follows.

Proposition 5.5. *Let $Z \subset S$ be non-empty. Then Z is a siphon if and only if $\text{cl}(L_Z)$ is forward invariant for (6).*

Proof. *Sufficiency:* Pick $\xi \in L_Z \neq \emptyset$. Then forward invariance of $\text{cl}(L_Z)$ implies that $\Gamma R(\xi) \in TC_\xi(\text{cl}(L_Z)) = TC_\xi(L_Z)$, where the last equality holds since $\xi \in L_Z$. It follows from [Lemma 5.3](#) that Z is a siphon.

Necessity: Pick $\xi \in \text{cl}(L_Z)$. This implies that $\xi_i = 0$ for all $i \in Z \cup Z'$, where $Z' \subset S$ could be empty. By the characterization of forward invariance of closed sets in terms of tangent Bouligand cones, it suffices to show that $[\Gamma R(\xi)]_i = 0$ for all $i \in Z$, and that $[\Gamma R(\xi)]_i \geq 0$ for all $i \in Z'$ whenever $Z' \neq \emptyset$. Now by (6),

$$[\Gamma R(\xi)]_i = \sum_k \beta_{ki} R_k(\xi) - \sum_l \alpha_{li} R_l(\xi) = \sum_k \beta_{ki} R_k(\xi) - 0 \geq 0, \quad (11)$$

which already proves the result for $i \in Z'$. Notice that the second sum is zero because if $\alpha_{li} > 0$, then species i is a reactant of reaction l , which implies that $R_l(\xi) = 0$ since $\xi_i = 0$. So we assume henceforth that $i \in Z$. We claim that the sum on the right side of (11) is zero. This is obvious if the sum is void. If it is non-void, then each term which is such that $\beta_{ki} > 0$ must be zero. Indeed, for each such term we have that $R_k(\xi) = 0$ because Z is a siphon. This concludes the proof of [Proposition 5.4](#). \square

Proof of [Theorem 2](#)

Let $\xi \in \text{int}(\mathcal{O}_+^{n_s})$ be arbitrary and let Ω denote the corresponding ω -limit set $\Omega = \omega(\xi)$. We claim that the intersection of Ω and the boundary of $\mathcal{O}_+^{n_s}$ is empty.

Indeed, suppose that the intersection is non-empty. Then, Ω would intersect L_Z , for some $\emptyset \neq Z \subset S$. In particular, by [Proposition 5.4](#), Z would be a siphon. Then, by our second assumption, there exists a non-negative first integral cS , whose support is included in Z , so that necessarily $cS(t_n, \xi) \rightarrow 0$ at least along a suitable sequence $t_n \rightarrow +\infty$. However, $cS(t, \xi) = c\xi > 0$ for all $t \geq 0$, thus giving a contradiction. \square

6. Applications

We now apply our results to obtain persistence results for variants of the reaction (b) shown in Fig. 1 as well as for cascades of such reactions.

6.1. Example 1

We first study reaction (2). Note that reversible reactions were denoted by a ‘ \leftrightarrow ’ in order to avoid having to rewrite them twice. The Petri net associated to (2) is shown in Fig. 2. The network comprises nine distinct species, labeled $S_0, S_1, S_2, E, F, ES_0, ES_1, FS_2, FS_1$. It can be verified that the Petri net in Fig. 2 is indeed consistent (so it satisfies the necessary condition). To see this, order the species and reactions by the obvious order obtained when reading (2) from left to right and from top to bottom (e.g., S_1 is the fourth species and the reaction $E + S_1 \rightarrow ES_1$ is the fourth reaction). The construction of the matrix Γ is now clear, and it can be verified that $\Gamma v = 0$ with $v = [2 \ 1 \ 1 \ 2 \ 1 \ 1 \ 2 \ 1 \ 1 \ 2 \ 1 \ 1]'$. The network itself, however, is not weakly reversible, since neither of the two connected components of (2) is strongly connected. Computations show that there are three minimal siphons:

$$\{E, ES_0, ES_1\},$$

$$\{F, FS_1, FS_2\},$$

and

$$\{S_0, S_1, S_2, ES_0, ES_1, FS_2, FS_1\}.$$

Each one of them contains the support of a P-semiflow; in fact there are three independent conservation laws:

$$E + ES_0 + ES_1 = \text{const}_1,$$

$$F + FS_2 + FS_1 = \text{const}_2, \text{ and}$$

$$S_0 + S_1 + S_2 + ES_0 + ES_1 + FS_2 + FS_1 = \text{const}_3,$$

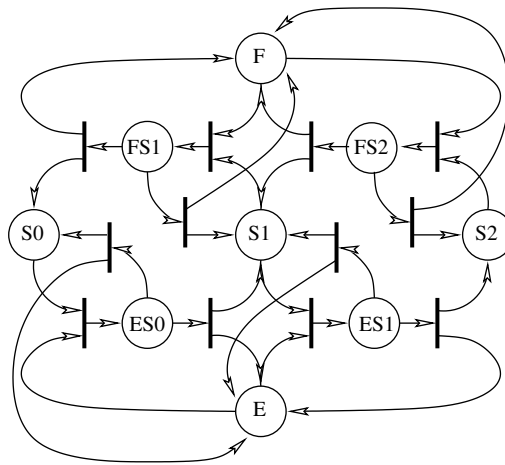
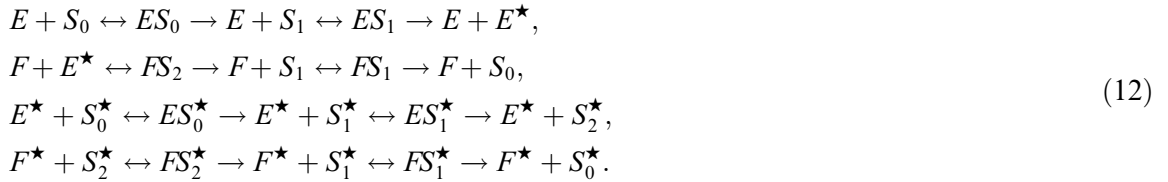


Fig. 2. Petri net associated to reactions (2).

whose supports coincide with the three mentioned siphons. Since the sum of these three conservation laws is also a conservation law, the network is conservative. Therefore, application of [Theorem 2](#) guarantees that the network is indeed persistent.

6.2. Example 2

As remarked earlier, examples as the above one are often parts of cascades in which the product (in MAPK cascades, a doubly-phosphorilated species) S_2 in turn acts as an enzyme for the following stage. One model with two stages is as follows (writing S_2 as E^* in order to emphasize its role as a kinase for the subsequent stage):



The overall reaction is shown in [Fig. 3](#). Note – using the labeling of species and reaction as in the previous example – that $\Gamma v = 0$ with $v = [v_1' \ v_1' \ v_1' \ v_1']'$ and $v_1 = [2 \ 1 \ 1 \ 2 \ 1 \ 1]'$, and hence the network is consistent. There are five minimal siphons for this network, namely:

$$\begin{aligned}
 &\{E, ES_0, ES_1\}, \\
 &\{F, FS_2, FS_1\}, \\
 &\{F^*, FS_2^*, FS_1^*\}, \\
 &\{S_0^*, S_1^*, S_2^*, ES_0^*, ES_1^*, FS_2^*, FS_1^*\},
 \end{aligned}$$

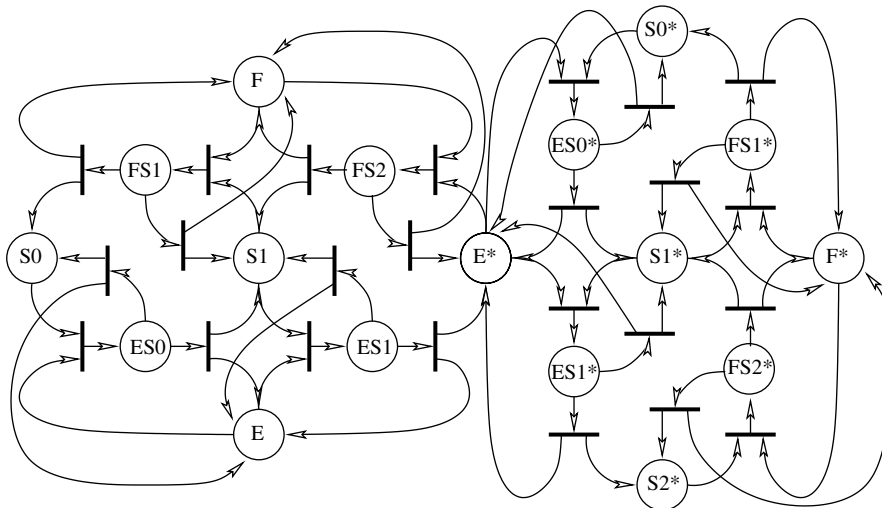


Fig. 3. Petri net associated to reactions (12).

and

$$\{S_0, S_1, E^\star, ES_0, ES_1, FS_2, FS_1, ES_0^\star, ES_1^\star\}.$$

Each one of them is the support of a P-semiflow, and there are five conservation laws:

$$\begin{aligned} E + ES_0 + ES_1 &= \text{const}_1, \\ F + FS_2 + FS_1 &= \text{const}_2, \\ F^\star + FS_2^\star + FS_1^\star &= \text{const}_3, \\ S_0^\star + S_1^\star + S_2^\star + ES_0^\star + ES_1^\star + FS_2^\star + FS_1^\star &= \text{const}_4, \end{aligned}$$

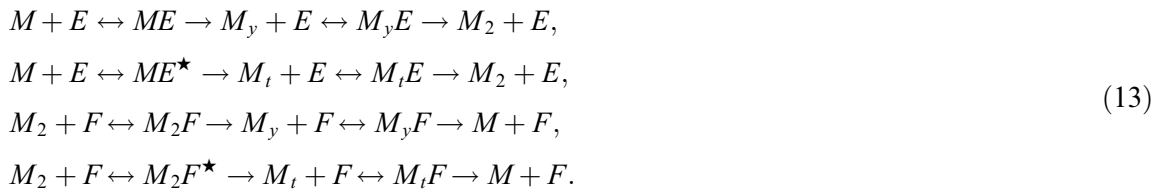
and

$$S_0 + S_1 + E^\star + ES_0 + ES_1 + FS_2 + FS_1 + ES_0^\star + ES_1^\star = \text{const}_5.$$

As in the previous example, the network is conservative since the sum of these conservation laws is also a conservation law. Therefore the overall network is persistent, by virtue of [Theorem 2](#). It is worth pointing out that the number of minimal siphons of a network may grow even exponentially with the size of the network. For large scale networks, it becomes therefore crucial to obtain algorithms for the determination of all minimal siphons in order to automatically check the assumptions of [Theorem 2](#). The paper [\[13\]](#) presents one such algorithm, together with some numerical and analytical results dealing with problem complexity.

6.3. Example 3

An alternative mechanism for dual phosphorylation in MAPK cascades, considered in [\[34\]](#), differs from the previous ones in that it becomes important at which sites the two phosphorylations occur. (These take place at two different sites, a threonine and a tyrosine residue). The corresponding network can be modeled as follows:



See [Fig. 4](#) for the corresponding Petri net. This network is consistent. Indeed, $\Gamma v = 0$ for the same v as in the previous example. Moreover it admits three siphons of minimal support:

$$\begin{aligned} \{E, ME, ME^\star, M_yE, M_tE\}, \\ \{F, M_yF, M_tF, M_2F, M_2F^\star\}, \end{aligned}$$

and

$$\{M, ME, ME^\star, M_y, M_t, M_yE, M_tE, M_2, M_2F, M_2F^\star, M_tF, M_yF\}.$$

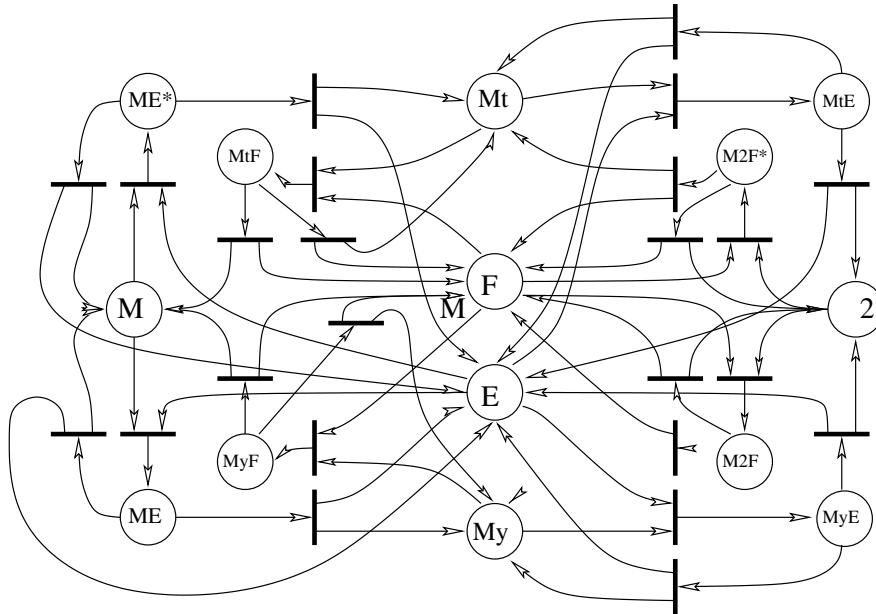


Fig. 4. Petri net associated to the network (13).

Each of them is also the support of a conservation law, respectively for M, E and F molecules. The sum of these conservation laws, is also a conservation law and therefore the network is conservative. Thus the [Theorem 2](#) again applies and the network is persistent.

6.4. Example 4

We give next an example of Reaction Network which cannot be analyzed by means of our results; this is a chemical reaction network for which siphons and P-semiflow do not coincide:



Notice that there is only one conservation law for the network, namely $A + B + 3C + 3D$; there are, however, 2 non-trivial siphons $\{A, C, D\}$ and $\{B, C, D\}$, none of which contains the support of the unique P-semiflow. Hence, [Theorem 2](#) cannot be applied to network (14); on the other hand, the associated Petri net is consistent and numerical evidence shows that the network is indeed persistent when simulated with reaction rates expressed according to mass-action kinetics. Specific criteria which exploit this additional structure of the system are currently under investigation. This trivial example shows that indeed even very simple examples can violate the assumptions of our main result; it is therefore remarkable that fairly complex examples taken from the biochemical literature can indeed be treated by means of such analytical tools.

7. Discrete vs. continuous persistence results

As a matter of fact, and this was actually the main motivation for the introduction of Petri Nets in [38], each Petri net (as defined in Section 3) comes with an associated discrete event system, which governs the evolution of a vector M , usually called the *marking* of the net. The entries of M are non-negative integers, in one-to-one correspondence with the places of the network, i.e. $M = [m_1, m_2, \dots, m_{n_s}]' \subset \mathbb{N}^{n_s}$, and the m_i s, $i = 1, \dots, n_s$, stand for the number of ‘tokens’ associated to the places S_1, \dots, S_{n_p} . In our context, each token may be thought of as a molecule of the corresponding species. Once a certain initial condition $M_0 \subset \mathbb{N}^{n_s}$ has been specified for a given net, we have what is usually called a marked Petri net. In order to define dynamical behavior, one considers the following *firing rules* for transitions R :

1. A transition R can fire whenever each input place of R is marked with a number of tokens greater or equal than the weight associated to the edge joining such a place to R (in our context a reaction can occur, at a given time instant, only provided that each reagent has a number of molecules greater or equal than the corresponding stoichiometry coefficient); we call such transitions enabled.
2. When a transition R fires, the marking M of the network is updated by subtracting, for each input place, a number of tokens equal to the weight associated to the corresponding edge, while for each output place a number of tokens equal to the weight of the corresponding edge is added.

Together with a rule that specifies the timing of the firings, this specifies a dynamical system describing the evolution of vectors $M \in \mathbb{N}^{n_s}$. There are several ways to specify timings. One may use a deterministic rule in which a specification is made at each time instant of which transition fires (among those enabled). Another possibility is to consider a stochastic model, in which firing events are generated by a random processes with exponentially decaying probability distributions, with a specified rate λ . The timing of the next firing of a particular reaction R might depend on R as well as the state vector M . In this way, an execution of the Petri net is nothing but a realization of a stochastic process (which is Markovian in an appropriate space).

The main results in Sections 4 and 5 are independent of the type of kinetics assumed for the chemical reaction network (for instance mass-action kinetics or Michaelis–Menten kinetics are both valid options at this level of abstraction). This also explains, to a great extent, the similarity between our theorems and their discrete counterparts which arise in the context of liveness’s studies for Petri Nets and Stochastic Petri Nets (liveness can be seen indeed as the discrete analog of persistence for ODEs, even though its definition is usually given in terms of firing of transitions rather than asymptotic averages of markings, see [47] for a precise definition).

It is well known that the following necessary condition for liveness holds:

Liveness of a Conservative PN \Rightarrow Consistence of the PN.

Notice the similarity of the above implication with the statement of Theorem 1. Also in its discrete stochastic counterpart, the result can be thought of as a consequence of ergodicity of the associated Markov chain.

The discrete counterparts of Theorem 2 are more subtle. In particular, we focus our attention on the so called *Siphon-Trap Property* which is a sufficient condition for liveness of conservative

Petri Nets, and actually a complete characterization of liveness if the net is a ‘Free Choice Petri Net’ (this is known as Commoner’s Theorem, [25] and [11]):

Theorem 3. *Consider a conservative Petri net satisfying the following assumption:*

each (minimal) siphon contains a non-empty trap.

Then, the PN is alive.

Notice the similarity between the assumptions and conclusions in Theorem 2 and in Theorem 3. There are some subtle differences, however. Traps for Petri nets enjoy the following invariance property: if a trap is non-empty at time zero (meaning that at least one of its places has tokens), then the trap is non-empty at all future times. In contrast, in a continuous set-up (when tokens are not integer quantities but may take any real value), satisfaction of the siphon-trap property does not prevent (in general) concentrations of species from decaying to zero asymptotically. This is why we needed a strengthened assumption 2, and asked that each siphon contains the support of a P-semiflow (which is always, trivially, also a trap). In other words, in a continuous set-up the notion of a trap loses much of its appeal, since one may conceive situations in which molecules are pumped into the trap at a rate which is lower than the rate at which they are extracted from it, so that, in the limit, the trap can be emptied out even though it was initially full. A similar situation never occurs in a discrete set-up since, whenever a reaction occurs, at least one molecule will be left inside the trap.

8. Conclusions

In ecology, persistence is the property of an ecosystem to asymptotically preserve non-zero populations of all the species which are present at the initial time. In the present paper we obtain both necessary and sufficient conditions for persistence in chemical reaction networks under a general monotonicity assumption for the reaction rates. The conditions are stated in terms of graphical and algebraic properties of Petri nets which are associated to the chemical reaction network. In a subsequent paper we will present tighter results for networks in which all reaction rates are of mass action type. The result presented here may also serve as a preliminary step towards the construction of a systematic input/output theory for chemical reaction networks, by allowing systems with inflows and outflows.

References

- [1] D. Angeli, P. De Leenheer, E.D. Sontag, On the structural monotonicity of chemical reaction networks, in: Proc. IEEE Conf. Decision and Control, San Diego, Dec. 2006, IEEE Publications, 2006, paper WeA01.2.
- [2] D. Angeli, E.D. Sontag, Monotone control systems, IEEE Trans. Autom. Control 48 (2003) 1684.
- [3] D. Angeli, J.E. Ferrell Jr., E.D. Sontag, Detection of multi-stability, bifurcations, and hysteresis in a large class of biological positive-feedback systems, Proc. Natl. Acad. Sci. USA 101 (2004) 1822.
- [4] D. Angeli, E.D. Sontag, Translation-invariant monotone systems, and a global convergence result for enzymatic futile cycles Nonlinear Anal. Series B: Real World Appl., in press, (Summarized version in A note on monotone systems with positive translation invariance, in: Proc. 14th IEEE Mediterranean Conference on Control and Automation, June 28–30, 2006, Ancona, Italy. Available from: <<http://www.diiga.univpm.it/MED06>>).

- [5] J.-P. Aubin, A. Cellina, *Differential Inclusions: Set-Valued Maps and Viability Theory*, Springer, 1984.
- [7] G. Butler, P. Waltman, Persistence in dynamical systems, *J. Diff. Equ.* 63 (1986) 255.
- [8] G. Butler, H.I. Freedman, P. Waltman, Uniformly persistent systems, *Proc. Am. Math. Soc.* 96 (1986) 425.
- [9] M. Chaves, E.D. Sontag, R.J. Dinerstein, Steady-states of receptor–ligand dynamics: a theoretical framework, *J. Theor. Biol.* 227 (2004) 413.
- [10] B.L. Clarke, Stability of complex reaction networks, *Adv. Chem. Phys.* 43 (1980) 1.
- [11] F. Commoner, Deadlocks in Petri nets, Tech. Report, Applied Data Research Inc. Wakefield, Massachusetts, 1972.
- [12] C. Conradi, J. Saez-Rodriguez, E.-D. Gilles, J. Raisch, Using chemical reaction network theory to discard a kinetic mechanism hypothesis: in: *Proc. FOSBE 2005 (Foundations of Systems Biology in Engineering)*, Santa Barbara, Aug. 2005, pp. 325–328.
- [13] R. Cordone, L. Ferrarini, L. Piroddi, Enumeration algorithms for minimal siphons in Petri Nets based on place constraints in: *IEEE Trans. Syst., Man, Cybern.-A: Syst. Hum.* 35 (2005) 844.
- [14] G. Craciun, M. Feinberg, Multiple equilibria in complex chemical reaction networks: II. The Species-Reaction Graph, *SIAM J. Appl. Math.* 66 (2006) 1321.
- [15] G. Craciun, Y. Tang, M. Feinberg, Understanding bistability in complex enzyme-driven reaction networks, *Proc. Natl. Acad. Sci.* 103 (2006) 8697.
- [16] R. David, H. Alla *Discrete, Continuous, and Hybrid Petri Nets*, Springer, Berlin, 2005.
- [17] P. De Leenheer, D. Angeli, E.D. Sontag, Monotone chemical reaction networks, *J. Math. Chem.* 41 (2007) 295.
- [18] M. Feinberg, F.J.M. Horn, Dynamics of open chemical systems and algebraic structure of underlying reaction network, *Chem. Eng. Sci.* 29 (1974) 775.
- [19] M. Feinberg, Chemical reaction network structure and the stability of complex isothermal reactors – I. The deficiency zero and deficiency one theorems, *Review Article* 25, *Chem. Eng. Sci.* 42 (1987) 2229–2268.
- [20] M. Feinberg, The existence and uniqueness of steady states for a class of chemical reaction networks, *Arch. Ration. Mech. Anal.* 132 (1995) 311.
- [21] M. Feinberg, *Lectures on chemical reaction networks* Lectures at the Mathematics Research Center, University of Wisconsin, 1979. Available from: <http://www.che.eng.ohio-state.edu/~feinberg/LecturesOnReactionNetworks/>.
- [22] T.C. Gard, Persistence in food webs with general interactions, *Math. Biosci.* 51 (1980) 165.
- [23] H. Genrich, R. Küffner, K. Voss, Executable Petri net models for the analysis of metabolic pathways, *Int. J. Software Tools Technol. Transfer (STTT)* 3 (2001) 394.
- [24] D. Gilbert, M. Heiner, From Petri Nets to differential equations – an integrative approach for biochemical network analysis, in: *Proc. of 27th ICAPTN 2006*, Springer, 2006.
- [25] M.H.T. Hack, *Analysis of production schemata by Petri-Nets*, Master Thesis, MIT, 1972.
- [26] M. Hirsch, H.L. Smith, Monotone dynamical systems, in: A. Canada, P. Drabek, A. Fonda (Eds.), *Handbook of Differential Equations, Ordinary Differential Equations*, second vol., Elsevier, 2005.
- [27] J. Hofbauer, J.W.-H. So, Uniform persistence and repellers for maps, *Proc. Am. Math. Soc.* 107 (1989) 1137.
- [28] R. Hofestädt, A Petri net application to model metabolic processes, *Syst. Anal. Mod. Simul.* 16 (1994) 113.
- [29] F.J.M. Horn, R. Jackson, General mass action kinetics, *Arch. Rational Mech. Anal.* 49 (1972) 81.
- [30] F.J.M. Horn, The dynamics of open reaction systems, in: *Mathematical Aspects of Chemical and Biochemical Problems and Quantum Chemistry (Proc. SIAM-AMS Sympos. Appl. Math., New York, 1974)*, pp. 125–137. SIAM-AMS Proceedings, vol. VIII, Amer. Math. Soc., Providence, 1974.
- [31] C.-Y.F. Huang, J.E. Ferrell, Ultrasensitivity in the mitogen-activated protein kinase cascade, *Proc. Natl. Acad. Sci. USA* 93 (1996) 10078.
- [32] R. Küffner, R. Zimmer, T. Lengauer, Pathway analysis in metabolic databases via differential metabolic display (DMD), *Bioinformatics* 16 (2000) 825.
- [33] A.R. Asthagiri, D.A. Lauffenburger, A computational study of feedback effects on signal dynamics in a mitogen-activated protein kinase (MAPK) pathway model, *Biotechnol. Prog.* 17 (2001) 227.
- [34] N.I. Markevich, J.B. Hoek, B.N. Kholodenko, Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades, *J. Cell Biol.* 164 (3) (2004) 353.
- [35] J.S. Oliveira, C.G. Bailey, J.B. Jones-Oliveira, D.A. Dixon, D.W. Gull, M.L.A. Chandler, A computational model for the identification of biochemical pathways in the Krebs cycle, *J. Comput. Biol.* 10 (2003) 57.

- [36] M. Peleg, I. Yeh, R. Altman, Modeling biological processes using workflow and Petri net models, *Bioinformatics* 18 (2002) 825.
- [37] J.L. Peterson, *Petri Net Theory and the Modeling of Systems*, Prentice Hall, Lebanon, Indiana, 1981.
- [38] C.A. Petri, *Kommunikation mit Automaten*, Ph.D. Thesis, University of Bonn, 1962.
- [39] V.N. Reddy, M.L. Mavrouniotis, M.N. Liebman, Petri net representations in metabolic pathways, *Proc. Int. Conf. Intell. Syst. Mol. Biol.* 1 (1993) 328–336.
- [40] M. Silva, L. Recalde, Continuization of timed Petri nets: from performance evaluation to observation and control, in: *Proc. 26th ICAPTN 2005*, Springer, 2005, pp. 26–47.
- [41] H.L. Smith, *Monotone dynamical systems: An introduction to the theory of competitive and cooperative systems*, *Mathematical Surveys and Monographs*, vol. 41, AMS, Providence, RI, 1995.
- [42] E.D. Sontag, Structure and stability of certain chemical networks and applications to the kinetic proofreading model of T-cell receptor signal transduction, *IEEE Trans. Autom. Control* 46 (2001) 1028, Errata in *IEEE Trans. Autom. Control* 47(2002): 705..
- [43] E.D. Sontag, *Mathematical Control Theory: Deterministic Finite Dimensional Systems*, second ed., Springer, New York, 1998.
- [44] H.R. Thieme, Uniform persistence and permanence for non-autonomous semiflows in population biology, *Math. Biosci.* 166 (2000) 173.
- [45] C. Widmann, G. Spencer, M.B. Jarpe, G.L. Johnson, Mitogen-activated protein kinase: Conservation of a three-kinase module from yeast to human, *Physiol. Rev.* 79 (1999) 143.
- [46] I. Zevedei-Oancea, S. Schuster, Topological analysis of metabolic networks based on Petri net theory: in: *Silico Biol.* 3 (2003), paper 0029.
- [47] M. Zhou, *Modeling, Simulation, and Control of Flexible Manufacturing Systems: A Petri Net Approach*, World Scientific Publishing, Hong Kong, 1999.