A Petri Net Approach to Persistence Analysis in Chemical Reaction Networks

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Summary. A positive dynamical system is said to be persistent if every solution that starts in the interior of the positive orthant does not approach the boundary of this orthant. For chemical reaction networks and other models in biology, persistence represents a non-extinction property: if every species is present at the start of the reaction, then no species will tend to be eliminated in the course of the reaction. This paper provides checkable necessary as well as sufficient conditions for persistence of chemical species in reaction networks, and the applicability of these conditions is illustrated on some examples of relatively high dimension which arise in molecular biology. More specific results are also provided for reactions endowed with mass-action kinetics. Overall, the results exploit concepts and tools from Petri net theory as well as ergodic and recurrence theory.

1 Introduction

Molecular systems biology is a cross-disciplinary and currently very active field of science which aims at the understanding of cell behavior and function at the level of chemical interactions. A central goal of this quest is the characterization of qualitative dynamical features, such as convergence to steady states, periodic orbits, or possible chaotic behavior, and the relationship of this behavior to the structure of the corresponding chemical reaction network. The interest in these questions in the context of systems biology lies on the hope that their understanding might shed new light on the principles underlying the evolution and organization of complex cellular functionalities. Understanding the long-time behavior of solutions is, of course, a classical topic in dynamical systems theory, and is usually formulated in the language of *ω-limit sets*, that is, the study of the set of possible limit points of trajectories of a dynamical system. That is the approach taken in this paper.

Persistency

Persistency is the property that, *if every species is present at the initial time, no species will tend to be eliminated in the course of the reaction.* Mathematically,

we ask 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). Roughly speaking, persistency can be interpreted as non-extinction: if the concentration of a species would approach zero in the continuous differential equation model, for the corresponding stochastic discrete-event model this could be interpreted by thinking that it would completely disappear in finite time due to its discrete nature.

Thus, one of the most basic questions that one may ask about a chemical reaction is if persistency holds for that network. Also from a purely mathematical perspective persistency is very important, because it may be used in conjunction with other tools in order to guarantee convergence of solutions to equilibria and perform other kinds of Input-Output analysis. For example, if a strictly decreasing Lyapunov function exists on the interior of the positive orthant (see e.g. [26, 27, 15, 16, 17, 40] for classes of networks where this can be guaranteed), persistency 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 persistency, 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 (see for example [19, 8] and much other foundational work by Waltman) where species correspond to individuals of different types instead of chemical units; with respect to such studies, chemical networks have one peculiar feature which strongly impacts the invariance property of the boundary and the overall persistence analysis. Lotka-Volterra systems, indeed, are characterized by the property that any extinct species will never make its way back into the ecosystem. As a matter of fact species only interact by influencing the reciprocal death and birth rates but cannot convert into each other, which is instead the typical situation in chemistry.

Petri Nets

Petri nets, also called place/transition nets, were introduced by Carl Adam Petri in 1962 [36], and they constitute a popular mathematical and graphical modeling tool used for concurrent systems modeling [35, 45]. Our modeling of chemical reaction networks using Petri net formalism is a well-established idea: there have been many works, at least since [37],which have dealt with biochemical applications of Petri nets, in particular in the context of metabolic pathways, see e.g. [20, 25, 30, 33, 34], and especially the excellent exposition [44]. However, there does not appear to have been previous work using Petri nets for a nontrivial study of dynamics. In this paper, we provide a new set of tools for the robust analysis of persistence in chemical networks modeled by ordinary differential equations endowed both with arbitrary as well as mass-action kinetics (in the latter case we exploit the knowledge of the convergence speed to zero of

mass-action reaction rates in approaching the orthant boundary in order to relax some of the assumptions needed in the general case).

Our conclusions are robust in the sense that persistence is inferred regardless of the specific values assumed by kinetic constants and comes as a result of both structural (for instance topology of the network) as well as dynamical features of the system (mass-action rates).

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 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. Figure 1 shows two examples, (a) one in which a single step takes place changing S_0 into $P = S_1$, and (b) one in which two sequential steps are needed to transform S_0 into $P = S_2$, 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:

$$
\begin{aligned}
E + S_0 &\leftrightarrow ES_0 \to E + S_1 \\
F + S_1 &\leftrightarrow FS_1 \to F + S_0
\end{aligned}
\tag{1}
$$
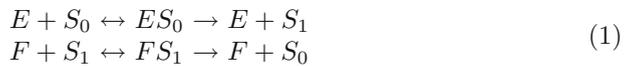
(double arrows indicate reversible reactions) and a model for (b) would be:

$$
\begin{aligned}
E + S_0 &\leftrightarrow ES_0 \to E + S_1 \leftrightarrow ES_1 \to E + S_2 \\
F + S_2 &\leftrightarrow FS_2 \to F + S_1 \leftrightarrow FS_1 \to F + S_0
\end{aligned}
\tag{2}
$$
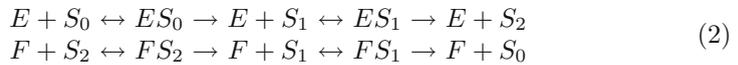
where "ES_0" represents the complex consisting of E bound to S_0 and so forth.
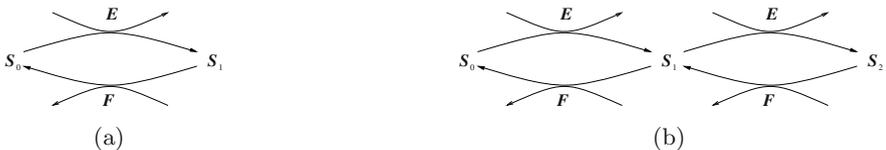
Fig. 1. (a) One-step. (b) Two-step transformations.

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 [28, 31, 43] 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. [32, 13]. We will show how our results can be applied to test persistence of this model, as well as several variants.

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 illustrate, respectively, necessary and sufficient conditions for persistence analysis of broad classes of biochemical networks, regardless of the specific kinetics considered; in Section 6, we show how our results apply to the enzymatic mechanisms described above. Section 7 draws some parallels between liveness analysis for standard and stochastic Petri nets (the so called Commoner's Theorem) and the main result in Section 5. Section 8 further motivates the systematic study of persistence by illustrating a simple toy example for which stochastic and deterministic analysis yield different predictions in terms of qualitative behaviour, while Section 9 presents specific results for attacking such questions in the case of mass-action kinetics. Finally, Section 10 illustrates applicability of the latter analysis results and draws a comparison with the discrete liveness analysis for two simple networks. Conclusions are drawn in Section 11.

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 $\mathcal{R} := \{1, 2, \ldots, n_r\}$. We next define precisely

what one means by reactions, and the differential equation associated to a CRN, using the formalism from chemical networks theory.

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

$$\mathcal{R}_i : \quad \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \rightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j \tag{3}$$
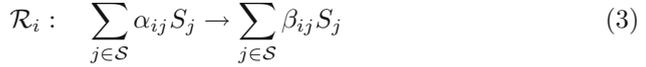
where the α_{ij} and β_{ij} are nonnegative integers called the *stoichiometry coefficients*. The compounds on the left-hand side are usually referred to as the *reactants*, and the ones on the right-hand side are called the *products*, of the reaction. Informally speaking, the forward arrow means that 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:

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

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

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

Each reaction rate R_i is a real-analytic function defined on an open set which contains the non-negative orthant $\mathcal{O}_+ = \mathbb{R}^{n_s}_{\geq 0}$ 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:

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

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 $S_{i_1}, \ldots, S_{i_N}$ are the reactants of reaction j, then we ask that

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

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

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

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

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

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.

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

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

with S evolving in $\mathcal{O}_+$ 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 $i \in \{1, 2, \ldots, n_c\}$. 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:

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

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 $C_i \to C_j$ 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 $C_i \to C_j$ is a reaction of the network). With this notations, we have the following formula, to be used later:

$$\Gamma = \Gamma_c \mathcal{I}. \tag{7}$$

We denote solutions of (6) as follows: $S(t) = \varphi(t, S_0)$, where $S_0 \in \mathcal{O}_+$ 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:

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

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

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. In the present chapter we make an effort to be self-contained with respect, however, for a more in depth introduction see for instance one of the many books devoted to this subject, [36, 45].

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 (usually referred to as "places" in Petri Net literature), V_R is a finite set of nodes (disjoint from V_S), each one corresponding to a reaction (usually named the "transitions" of

the network), 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:

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

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*.

Accordingly, we also talk about *input and output reactions* for a given set $\Sigma \subset \mathcal{S}$ of species. This is defined in the obvious way, viz. by considering the union of all input (and respectively output) reactions over all species belonging to Σ.

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

The last element to fully define the Petri net is the function $W : E \to \mathbb{N}$, 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 2. *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 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. *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 ([44]). (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. Biochemical models are most often non-reversible. For this reason, a far milder notion was introduced [26, 27, 15, 16, 17]: 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 [18]), 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.

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

Definition 4. *A nonempty 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 a* deadlock *if its set of output reactions is all of V_R. A deadlock is* minimal *if it does not contain (strictly) any other deadlocks. A siphon is* minimal *if it does not contain (strictly) any other siphons. Notice that a minimal deadlock need not be a minimal siphon (and viceversa, which is obvious). A pair of distinct deadlocks Σ_1 and Σ_2 is said to be* nested *if either $\Sigma_1 \subset \Sigma_2$ or $\Sigma_2 \subset \Sigma_1$.*

Similarly one defines the notion of *trap*.

Definition 5. *A non-empty set $\mathcal{T} \subset V_S$ is called a* trap *if each output reaction associated to $\mathcal{T}$ is also an input reaction associated to $\mathcal{T}$.*

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

$$L_\Sigma = \{x \in \mathcal{O}_+ \,|\, x_i = 0 \Longleftrightarrow i \in \Sigma\}.$$

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

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

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

$$S_i \overset{k}{\rightarrowtail} S_j$$

(where $k \in \mathcal{R}$). With this notation, the notion of siphon can be rephrased as follows: $Z \subset \mathcal{S}$ is a siphon for a chemical reaction network if for every $S \in Z$ and $k \in \mathcal{R}$ such that $\tilde{S}_k := \{T \in \mathcal{S} : T \overset{k}{\rightarrowtail} S\} \neq \emptyset$, 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 $S_0 \in \text{int}(\mathcal{O}_+)$ be any initial condition. By conservativity, solutions satisfy $cS(t) \equiv cS_0$, and hence remain bounded, and therefore $\omega(S_0)$ is a nonempty compact set. Moreover, by persistence, $\omega(S_0) \cap \partial\mathcal{O}_+ = \emptyset$, 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:

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

(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 $T_n \to +\infty$ so that there is a finite limit:

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

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

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 a real number p, let $\operatorname{sign} p := 1, 0, -1$ if $p > 0$, $p = 0$, or $p < 0$ respectively, and, similarly for any real vector $x = (x_1, \ldots, x_n)$, let $\operatorname{sign} x := (\operatorname{sign} x_1, \ldots, \operatorname{sign} x_n)'$. When x belongs to the closed positive orthant $\mathbb{R}^n_+$, $\operatorname{sign} x \in \{0, 1\}^n$.

Lemma 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, $\operatorname{sign} \bar{x}(t)$ 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 $Z = \{r + 1, \ldots, n\}$, with $0 \leq r \leq n$, and we write equations in the following block form:

$$\dot{y} = g(y, z)$$
$$\dot{z} = h(y, z)$$

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 $i \in \{1, \ldots, r\}$. Since the vector field is real-analytic, each coordinate function $\bar{y}_i$ is real-analytic (see e.g. [41], 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 $t \in G$, $\bar{y}(t) \in \operatorname{int} \mathbb{R}^r_+$, 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 $i \in \{1, \ldots, r\}$ 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 $i \in \{1, \ldots, r\}$ such that $y_i = 0$, $g_i(y, z) \geq 0$. Thus, the required property holds (case $z = 0$). In particular, $\operatorname{int} \mathbb{R}_+^r$ is also forward invariant (see e.g. [2], Lemma III.6). By construction, $\bar{y}$ is a solution of $\dot{y} = g(y, 0)$, $\bar{y}(t) \in \operatorname{int} \mathbb{R}_+^r$ for each $t \in G$, Since G is dense and $\operatorname{int} \mathbb{R}_+^r$ is forward invariant, it follows that $\bar{y}(t) \in \operatorname{int} \mathbb{R}_+^r$ for all $t \in J$. Therefore,

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

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

We then have an immediate corollary:

Lemma 2. *Suppose that $\Omega \subset \mathcal{O}_+$ 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), $\varphi(t, S_0) \in \mathcal{O}_+$ for all t (negative as well as positive). Therefore, it also belongs to L_Z, since its sign is constant by Lemma 1.

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

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

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 3. *Let $Z \subset \mathcal{S}$ 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 $l \in \mathcal{S}$ 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 $\tilde{S}_k = \{ l \in \mathcal{S} : S_l \xrightarrow{k} S_i \}$ 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.

The above Lemmas are instrumental to proving the following Proposition:

Proposition 1. *Let $\xi \in \mathcal{O}^+$ be such that $\omega(\xi) \cap L_Z \neq \emptyset$ for some $Z \subset \mathcal{S}$. Then Z is a siphon.*

Proof. Let Ω be the closed and invariant set $\omega(\xi)$. Thus, by Lemma 2, the non-empty set $L_Z \cap \Omega$ is also invariant. Notice that

$$\mathrm{cl}[L_Z] \; = \; \bigcup_{W \supseteq Z} L_W \,.$$

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

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

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

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

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 3 we may conclude Z is a siphon.

Although at this point Proposition 1 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. This explains why a siphon is sometimes also called a "locking set" in the Petri net literature. A precise statement of the foregoing remarks is as follows.

Proposition 2. *Let $Z \subset \mathcal{S}$ be non-empty. Then Z is a siphon if and only if $cl(L_Z)$ is forward invariant for (6).*

Proof.
Sufficiency: Pick $\xi \in L_Z \neq \emptyset$. Then forward invariance of $\mathrm{cl}(L_Z)$ implies that $\Gamma R(\xi) \in TC_\xi(\mathrm{cl}(L_Z)) = TC_\xi(L_Z)$, where the last equality holds since $\xi \in L_Z$. It follows from Lemma 3 that Z is a siphon.

Necessity: Pick $\xi \in \mathrm{cl}(L_Z)$. This implies that $\xi_i = 0$ for all $i \in Z \cup Z'$, where $Z' \subset \mathcal{S}$ 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),

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

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 2.

Proof of Theorem 2

Let $\xi \in \text{int}(\mathcal{O}_+)$ 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 $\mathcal{O}_+$ is empty.

Indeed, suppose that the intersection is nonemty. Then, Ω would intersect L_Z, for some $\emptyset \neq Z \subset \mathcal{S}$. In particular, by Proposition 1, 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 $cS(t_n, \xi) \to 0$ at least along a suitable sequence $t_n \to +\infty$. However, $cS(t, \xi) = c\xi > 0$ for all $t \geq 0$, thus giving a contradiction. □

6   Applications

We now apply our results to obtain persistence results for variants of the reaction (b) shown in Figure 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 "↔" in order to avoid having to rewrite them twice. The Petri net associated to (2) is shown if 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 $E + S_1 \to ES_1$ 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:

$E + ES_0 + ES_1 = \text{const}_1$,
$F + FS_2 + FS_1 = \text{const}_2$, and
$S_0 + S_1 + S_2 + ES_0 + ES_1 + FS_2 + FS_1 = \text{const}_3$,
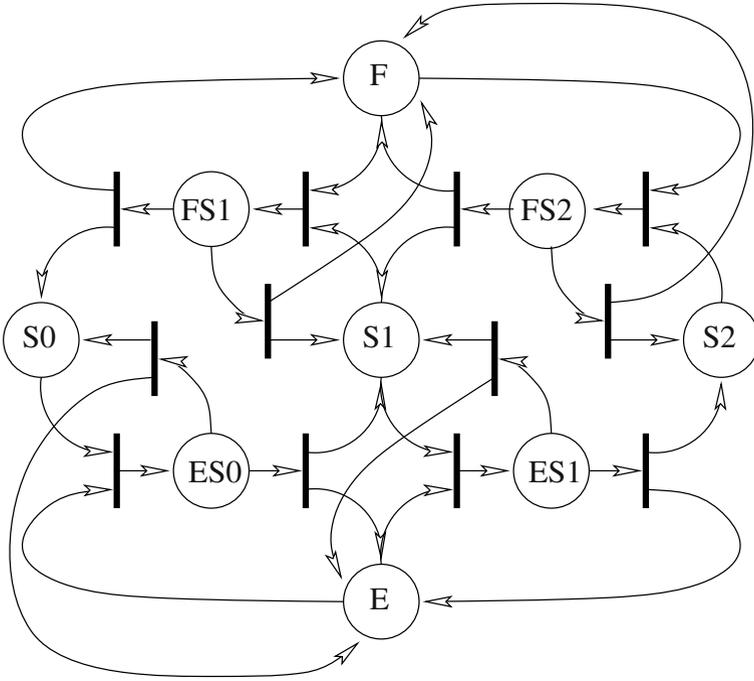
Fig. 2. Petri net associated to reactions (2)

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 $E^\star$ in order to emphasize its role as a kinase for the subsequent stage):

$$
\begin{aligned}
E + S_0 &\leftrightarrow ES_0 \rightarrow E + S_1 \leftrightarrow ES_1 \rightarrow E + E^\star \\
F + E^\star &\leftrightarrow FS_2 \rightarrow F + S_1 \leftrightarrow FS_1 \rightarrow F + S_0 \\
E^\star + S_0^\star &\leftrightarrow ES_0^\star \rightarrow E^\star + S_1^\star \leftrightarrow ES_1^\star \rightarrow E^\star + S_2^\star \\
F^\star + S_2^\star &\leftrightarrow FS_2^\star \rightarrow F^\star + S_1^\star \leftrightarrow FS_1^\star \rightarrow F^\star + S_0^\star.
\end{aligned}
\tag{12}
$$
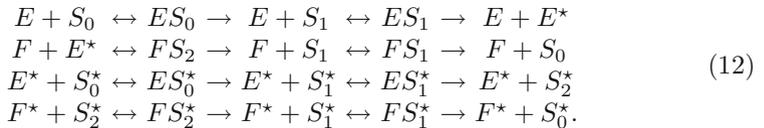
The overall reaction is shown in Fig. 3. Note – using the labeling of species and reaction as in the previous example – that $\Gamma v = 0$ with $v = [v_1'\, v_1'\, v_1'\, v_1']'$ and
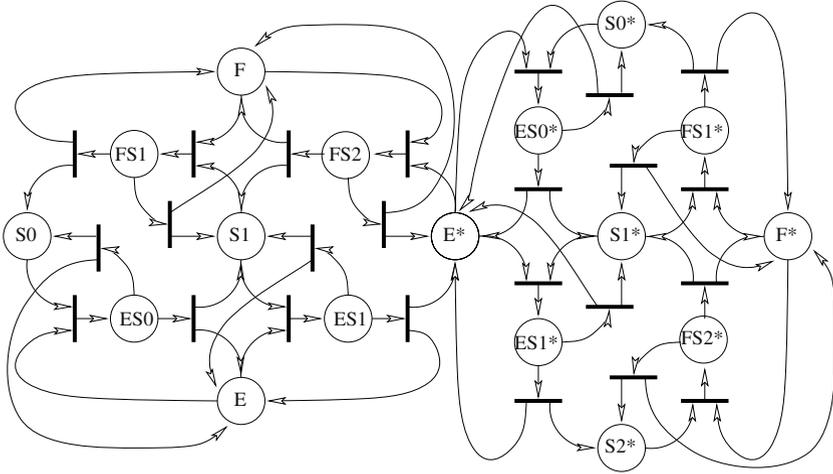
Fig. 3. Petri net associated to reactions (12)

$v_1 = [2\,1\,1\,2\,1\,1]'$, and hence the network is consistent. There are five minimal siphons for this network, namely:

$$\{E, ES_0, ES_1\},$$
$$\{F, FS_2, FS_1\},$$
$$\{F^\star, FS_2^\star, FS_1^\star\},$$
$$\{S_0^\star, S_1^\star, S_2^\star, ES_0^\star, ES_1^\star, FS_2^\star, FS_1^\star\},$$

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:

$$E + ES_0 + ES_1 = \mathrm{const}_1,$$
$$F + FS_2 + FS_1 = \mathrm{const}_2,$$
$$F^\star + FS_2^\star + FS_1^\star = \mathrm{const}_3,$$
$$S_0^\star + S_1^\star + S_2^\star + ES_0^\star + ES_1^\star + FS_2^\star + FS_1^\star = \mathrm{const}_4,$$

and

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

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.

6.3   Example 3

An alternative mechanism for dual phosphorilation in MAPK cascades, considered in [32], differs from the previous ones in that it becomes relevant in what order the two phosphorylations occur. (These take place at two different sites,
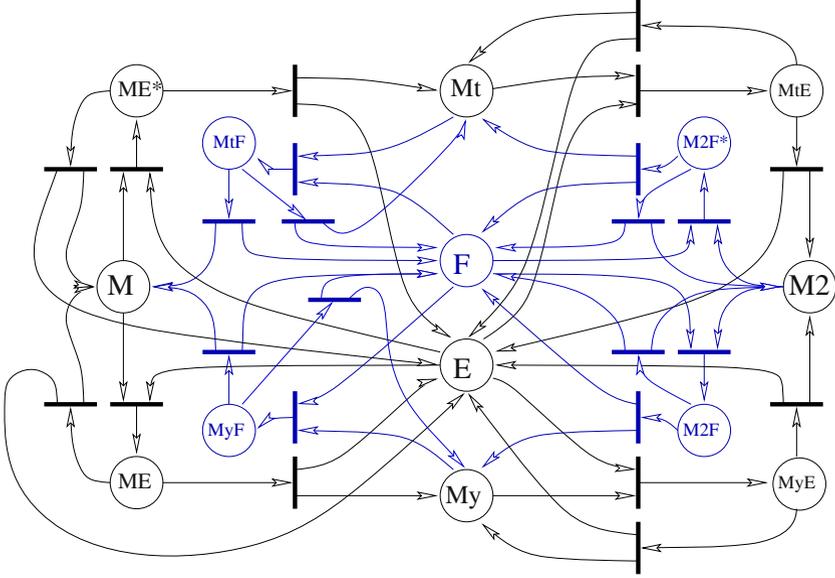
Fig. 4. Petri net associated to the network (13)

a threonine and a tyrosine residue). The corresponding network can be modeled as follows:

$$
\begin{aligned}
M + E &\leftrightarrow ME \rightarrow M_y + E \leftrightarrow M_y E \rightarrow M_2 + E \\
M + E &\leftrightarrow ME^\star \rightarrow M_t + E \leftrightarrow M_t E \rightarrow M_2 + E \\
M_2 + F &\leftrightarrow M_2 F \rightarrow M_y + F \leftrightarrow M_y F \rightarrow M + F \\
M_2 + F &\leftrightarrow M_2 F^\star \rightarrow M_t + F \leftrightarrow M_t F \rightarrow M + F.
\end{aligned}
\tag{13}
$$
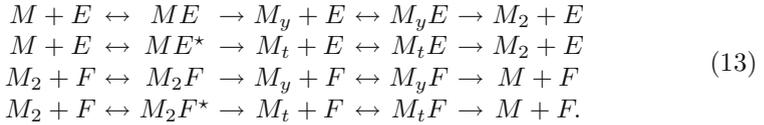
See Fig. 4 for the corresponding Petri net. This network is consistent. Indeed, $\Gamma v = 0$ for the same v as in the previous example. Moreover it admits three siphons of minimal support:

$\{E, ME, ME^\star, M_y E, M_t E\},$
$\{F, M_y F, M_t F, M_2 F, M_2 F^\star\},$
and
$\{M, ME, ME^\star, M_y, M_t, M_y E, M_t E, M_2, M_2 F, M_2 F^\star, M_t F, M_y F\}.$

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.

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 [36], 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-one correspondence with the places of the network, i.e. $M = [m_1, m_2, \ldots, m_{n_s}]' \subset \mathbb{N}^{n_s}$, and the m_is, $i = 1 \ldots n_s$, stand for the number of "tokens" associated to the places $S_1 \ldots S_{n_p}$. 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 tokes 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 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), whose study is classical not only in Petri Net theory but also in the chemical kinetics literature. In the latter, the equation governing the probability evolution is in fact the so-called *Chemical Master Equation*, which is often simulated by using a method known as "Gillespie's algorithm".

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 is indeed 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 [45] for a precise definition).

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, [22] and [12]):

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 looses 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   Networks with Mass-Action Kinetics: A Toy Example

The results presented so far are independent of the type of kinetics assumed for the chemical reaction network. A special case, which is of particular interest in many applications, is that of systems with *mass-action* kinetics, as already mentioned in Section 2. For systems with mass-action kinetics, we will next derive sufficient conditions for persistence that exploit the additional structure in order to relax some of the structural assumptions on the chemical reaction network under consideration. As shown in the proof of Theorem 2, whenever the omega-limit set of an interior solution of a chemical reaction network intersects the boundary of $\mathcal{O}_+$, the zero components of any intersection point correspond to some siphon. There are two ways to rule this out situation. One way is to check whether a siphon contains the support of a P-semiflow, as done in Theorem 2. In this case, we say that the siphon is *structurally non-emptiable*; otherwise, we say that the siphon is *critical*.

The conditions we are seeking will apply to chemical reaction networks whose siphons are allowed to be critical (actually they need not even contain traps).

To further motivate our results, we first of all discuss a toy example which can be easily analyzed both in a deterministic and a stochastic set-up and will illustrate the usefulness of a systematic approach to the problem.

Consider the following simple chemical reaction network:

$$2A + B \rightarrow A + 2B \qquad B \rightarrow A \qquad (14)$$
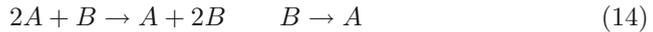
which we assume endowed with mass-action kinetics. The associated Petri Net is shown in Fig. 5 and it has the following properties:

1. it is conservative, with P-semiflow $[1, 1]$
2. it is consistent, with T-semiflow $[1, 1]'$
3. it admits a unique non-trivial siphon: $\{B\}$, which is also critical
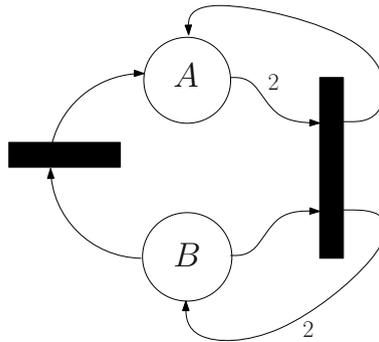
Fig. 5. A persistent chemical network whose associated Petri Net is not alive

The net effect of the first reaction is to transform one molecule of species A into one molecule of species B, and, clearly, the second reaction produces the reverse transformation. Hence, given any positive initial number of molecules for A and B, say n in total, we build the corresponding finite dimensional Markov chain (see [29] for basic definitions), which in this case has the following graphical structure:

$$[1, n-1] \leftrightarrow [2, n-2] \leftrightarrow \ldots \leftrightarrow [n-2, 2] \leftrightarrow [n-1, 1] \rightarrow [n, 0],$$

where a pair $[n_a, n_b]$ denotes the number of A and B molecules respectively. Notice that the above graph has a unique absorbing component, corresponding to the node $[n, 0]$. Such a state, when reached, basically shuts off the chemical reaction network, since the reactions do not allow the production of a molecule of A if there are no B molecules. Note that the node $[0, n]$ is never reached from another state, since consumption of an A-molecule requires that at least 2 molecules of A be available beforehand. This is why we do not include it in the diagram.

It turns out that, in the case of Petri Nets, the topology for the associated reachability graph shown in Fig. 6 is not infrequent: namely, there exist one or more absorbing components for the Markov Chain and a central strongly-connected transient component; moreover, as the number of tokens increases,
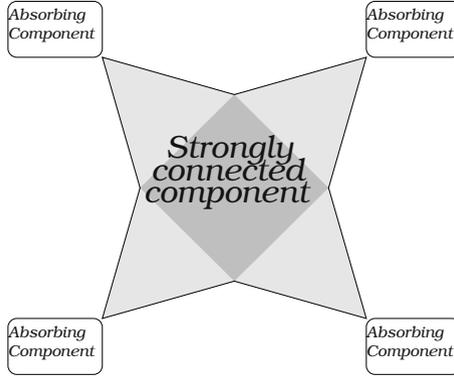
Fig. 6. Reachability graph of a Petri Net with critical siphons

the average-time that it takes to reach the absorbing components (the *time to absorption*) from the central region rapidly grows to infinity. The absorbing components of the graph correspond to the situation in which one or more critical siphons are emptied, while the central region corresponds to situations in which markings are oscillating, yet without reaching simultaneously a zero marking for all places within any given critical siphon.

Asymptotic analysis of Stochastic Petri Nets with an associated reachability graph which has the topology of Fig. 6 may lead to results which are in sharp contrast, to say the least, with what is experienced in practice for any sufficiently large initial number of tokens in the network. In fact, it may be argued that, although in theory the only stationary steady-states are indeed reached when at least one of the critical siphons gets emptied, such evolutions are so unlikely to happen in any finite time (at the scale of what is meaningful to consider for the application at hand), that, though possible in principle, they are however violating some "vague" entropic principle which one expects at the core of chemical kinetics.

Let us analyze our example (14) in further detail. To make our model suitable for computations, we associate to it a homogeneous continuous time Markov chain, assigning to each reaction a positive rate, denoted as k_1 and k_2 for reactions 1 and 2 respectively. If we adopt mass-action kinetics, the matrix corresponding to the associated chemical master equation for a total number of n molecules of A and B is given by:

$$M = \begin{bmatrix} 0 & k_2 & 0 & 0 & \ldots & 0 \\ 0 & \star & 2k_2 & 0 & \ldots & 0 \\ 0 & (n-1)^2k_1 & \star & 3k_2 & & \vdots \\ 0 & 0 & 2(n-2)^2k_1 & \star & \ddots & \vdots \\ 0 & \ldots & 0 & \ddots & \ddots & nk_2 \\ 0 & \ldots & 0 & 0 & (n-1)k_1 & \star \end{bmatrix} \tag{15}$$

where $\star$ is chosen so that the matrix M has each row summing to 0. Hence, the vector $p(t) = [p_{[n,0]}(t), \ldots, p_{[1,n-1]}(t)]'$, evolves according to the following equation:

$$\dot{p}(t) = Mp(t)$$

where $p_{[n_a,n_b]}(t)$ denotes the probability of having n_a molecules of A and n_b molecules of B at time t. One can easily compute the average absorption time for any initial number of molecules of species B. Performing the computation using a symbolic computational package, there results (for k_1 and k_2 equal to 1) the exponential growth rate plotted in Fig. 7. Even with as few as 30 molecules, the average time it takes to have all the Bs transformed into As is so large that no real life experiment nor simulation will ever meet such conditions.

In other words, while a Petri Net graphical analysis leads one to conclude that extinction is theoretically possible, this is an event with vanishingly small probability. On the other hand, as it will be shown next, sometimes such chemical reaction networks can still be proved to be persistent when modeled by means of differential equations for concentrations. Thus, the ODE model (in which no species ever vanishes) provides a more accurate description of the true asymptotic behavior of the physical system in question. Of course, in general it is not clear which modeling framework should be used under what circumstances. Our aim is merely to point out certain discrepancies that may arise between the two kinds of models, in order to further motivate an in depth study of persistence on the basis of ODE techniques.
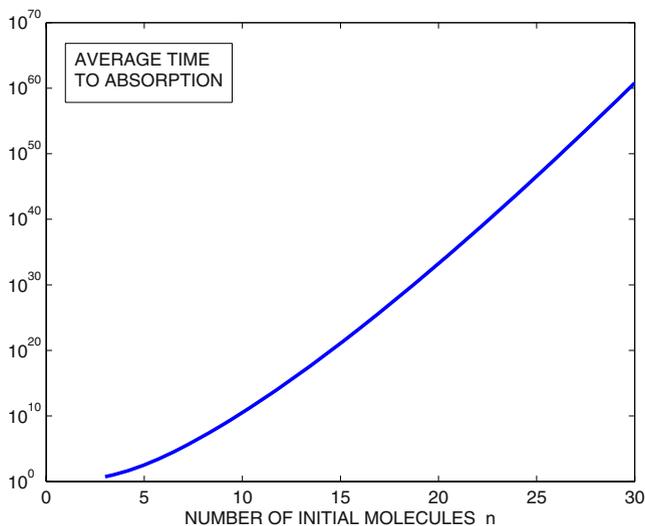
Fig. 7. Exponential increase of average time to absorption

So, let us now perform a simple deterministic analysis of the model. The equations associated to the chemical reaction network are:

$$\dot{a} = -k_1 a^2 b + k_2 b \qquad \dot{b} = -k_2 b + k_1 a^2 b \tag{16}$$

Exploiting the conservation law $a(t) + b(t) = M_{tot}$ we can bring down dimension by 1 and study the simpler system:

$$\dot{a} = (k_2 - k_1 a^2)(M_{tot} - a)$$

with a belonging to $[0, M_{tot}]$. Equilibria of the above equation are located at: $a = M_{tot}$, $a = \pm\sqrt{k_2/k_1}$. Two different scenarios arise, namely:

1. if $\sqrt{k_2/k_1} \geq M_{tot}$ only one equilibrium exists in $[0, M_{tot}]$ and all solutions converge to it; this is a boundary equilibrium and therefore persistence does not hold in this case.
2. if $\sqrt{k_2/k_1} < M_{tot}$, two equilibria exist in $[0, M_{tot}]$ and all solution starting in $[0, M_{tot})$ converge to the interior equilibrium $\sqrt{k_2/k_1}$. In this case persistence holds.

For example, in the above example, we had $k_1 = k_1 = 1$ and $M_{tot} = 30$, so the second case holds.

9   A Notion of Dynamic Non-emptiability

A low-dimensional system such as the example in Section 8 may be easily analyzed by direct computation or phase-plane analysis techniques. However, for higher dimensional examples, it is desirable to have systematic tools that can predict persistence in Petri Nets with critical siphons. We will show next that one can still rule out solutions approaching the set L_Z, for certain kinds of critical siphons Z, by exploiting the additional information that comes from having imposed mass-action kinetics. To this end, we associate to each siphon a hierarchy between its output reactions, as follows.

Let $\Sigma \subset \mathcal{S}$ be a siphon. We say that $R_i \preccurlyeq_\Sigma R_j$ if $\alpha_{ik} \geq \alpha_{jk}$ for all $k \in \Sigma$ and at least one of the inequalities is strict for some $k \in \Sigma$. The meaning of this order relationship becomes clearer thanks to the following Lemma, whose proof is a direct consequence of the definition of mass-action kinetics.

Lemma 4. *Let $\Sigma \subset V_S$ be a siphon and $R_i \preccurlyeq_\Sigma R_j$. Let us consider a network (6) endowed with mass action kinetics. Then, for each $\varepsilon > 0$, and each compact subset $K \subset L_\Sigma$, there exists an open neighborhood U_K of K such that, for all $S \in U_K$, it holds $R_i(S) \leq \varepsilon R_j(S)$.*

Accordingly, for each siphon Σ and each $\varepsilon > 0$, we may define the cone of feasible reaction rates when approaching the boundary region L_Σ, as follows:

$$\mathcal{F}_\varepsilon(\Sigma) := \{v \succeq 0 : v_i \leq \varepsilon v_j, \, \forall i, j \in \mathcal{R} : R_i \preccurlyeq_\Sigma R_j\}. \tag{17}$$

The following Lemma is a well-known fact in Petri Net theory and we recall it here for the sake of completeness.

Lemma 5. *Consider a conservative and consistent chemical reaction network and let Σ be an arbitrary subset of V_S. Then either 1. or 2. holds:*

1. *there exists $c \succ 0$ such that $c\,\Gamma = 0$ and $c_k = 0$ for all $k \notin \Sigma$.*
2. *there exists $v \gg 0$ such that $[\Gamma v]_k < 0$ for all $k \in \Sigma$.*

Proof. Without loss of generality assume that Σ comprises the first h species of V_S. Accordingly, we may partition Γ as follows:

$$\Gamma = \begin{bmatrix} \Gamma_\Sigma \\ \Gamma_{\bar\Sigma} \end{bmatrix}.$$

Consider the Petri Net associated to Γ_Σ. One of the following conditions holds:

1. the net admits a place which is structurally bounded,
2. the net does not admit structurally bounded places.

By Theorem 15, page 333 of [38], the two conditions are respectively equivalent to:

1. there exists some $c \succ 0$ so that $c\,\Gamma_\Sigma \preceq 0$; in particular then, there exists $\tilde c := [c, 0] \succ 0$ such that $\tilde c\,\Gamma \preceq 0$; moreover, by consistency of the original net, this is equivalent to $\tilde c\,\Gamma = 0$.
2. for each $p \in \Sigma$, there is a $v_p \succeq 0$ such that $\Gamma_\Sigma v_p \succeq e_p$ (p-th canonical basis vector); this, in turn, implies that $[\Gamma \sum_p v_p]_k > 0$ for all $k \in \Sigma$. By consistency, we can find some $w \gg \sum_p v_p$ be such that $\Gamma w = 0$. We pick $v := w - \sum_p v_p \gg 0$. Clearly, $\Gamma v = -\Gamma \sum_p v_p$, which then gives, as desired, that $[\Gamma v]_k < 0$ for all $k \in \Sigma$.

This completes the proof of the Lemma.

In particular, applying the previous Lemma to a siphon Σ, condition 1. is equivalent in our terminology to saying that the siphon is structurally non-emptiable, while condition 2. is therefore a characterization of criticality for a siphon. Notice that condition 2. is equivalent to asking that the following cone has non-empty interior:

$$\mathcal{C}(\Sigma) = \{v \succeq 0 : [\Gamma v]_k \le 0, \, \forall\, k \in \Sigma\}$$

Hence, for a critical siphon Σ, we may find suitable positive reaction rates, which overall produce a decrease in the concentration of all of its species. On the other hand, when solutions approach the boundary we know that a certain hierarchy may hold between the output reaction rates of the siphon, due to the mass-action kinetics.

This motivates the following definition which is a key notion needed in the formulation of our main result:

Definition 6. *We say that a critical siphon Σ is* dynamically non-emptiable *if there is some $\varepsilon > 0$ such that the following condition holds:*

$$\mathcal{C}(\Sigma) \cap \mathcal{F}_\varepsilon(\Sigma) = \{0\}.$$

Intuitively speaking, this definition excludes the possibility of having trajectories which monotonically decrease to the set L_Σ for a given dynamically non-emptiable siphon. Its technical meaning will be clearer in the following developments.

We are now ready to state our main result:

Theorem 4. *Consider a conservative CRN as in (6), endowed with mass-action kinetics. Associate to it a Petri Net and assume that*

1. *All of its critical siphons are dynamically non-emptiable.*
2. *There are no nested distinct critical deadlocks.*

Then, the chemical reaction network is persistent.

We start with a result which clarifies the role of dynamic non-emptiability.

Lemma 6. *Consider a chemical reaction network having a dynamically non-emptiable siphon $\Sigma \subset V_S$. Let S_0 be arbitrary in $\mathcal{O}^+ \setminus L_\Sigma$. Then, provided $\omega(S_0)$ is compact, we have that $\omega(S_0) \nsubseteq L_\Sigma$.*

Proof. Assume by contradiction that $\omega(S_0) \subset L_\Sigma$, and pick an increasing sequence $t_n \to +\infty$ such that $S_i(t_n) \geq S_i(t_{n+1})$ for all $i \in \Sigma$ and all $n \in \mathbb{N}$. Let $\varepsilon > 0$ be sufficiently small, as required by the definition of dynamic non-emptiability for Σ. By Lemma 4 (applied with $K = \omega(S_0)$) there exists $T > 0$, so that for all $t \geq T$, it holds:

$$R(S(t)) \in \mathcal{F}_\varepsilon(\Sigma) \tag{18}$$

Taking averages of $\dot{S}(t)$ on intersample intervals of such a sequence yields:

$$\frac{1}{t_{n+1} - t_n} \int_{t_n}^{t_{n+1}} \Gamma R(S(\tau)) \, d\tau = \frac{S(t_{n+1}) - S(t_n)}{t_{n+1} - t_n}.$$

Hence, factoring out Γ from the integral above yields

$$\frac{1}{t_{n+1} - t_n} \int_{t_n}^{t_{n+1}} R(S(\tau)) \, d\tau \ \in \ \mathcal{C}(\Sigma). \tag{19}$$

Now, since $\mathcal{F}_\varepsilon(\Sigma)$ is a closed, convex cone, and exploiting (18), we also have that for all sufficiently large n's, $\frac{1}{t_{n+1}-t_n} \int_{t_n}^{t_{n+1}} R(S(\tau)) \, d\tau \in \mathcal{F}_\varepsilon(\Sigma)$. By dynamic non-emptiability of Σ then $\frac{1}{t_{n+1}-t_n} \int_{t_n}^{t_{n+1}} R(S(\tau)) \, d\tau = 0$ for all sufficiently large n's; this implies $S(t)$ is an equilibrium for all sufficiently large t's, and therefore, by uniqueness of solutions, S_0 is also an equilibrium. Hence $\{S_0\} = \omega(S_0) \subset L_\Sigma$ which is clearly a contradiction.

The following Lemma is crucial to the proof of Theorem 4.

Lemma 7. *Let $C \subsetneq \omega(S_0)$ be a non-empty closed, invariant set such that there are no other closed invariant sets nearby (i.e., in $[\omega(S_0) \cap U_C] \setminus C$ for some open neighborhood $U_C \supset C$). Then, there exists $\tilde{S}_0 \in [\omega(S_0) \cap U_C] \setminus C$ such that $\omega(\tilde{S}_0) \subset C$.*

Proof. Consider the set $N := [\omega(S_0) \cap V_C] \setminus C$, where V_C is an open neighborhood of C such that $\mathrm{cl}[V_C] \subset U_C$. We claim that N is non-empty. If N were empty, then $\omega(S_0) = [\omega(S_0) \cap V_C] \cup [\omega(S_0) \setminus C]$ would be the union of two non-empty open sets $[\omega(S_0) \cap V_C]$ and $[\omega(S_0) \setminus C]$. Note that their intersection would be N, hence empty, by assumption. This would imply that $\omega(S_0)$ is not connected, a contradiction to connectedness of omega limit sets.

We wish to show that there is some $\tilde{S}_0 \in N$ such that $\omega(\tilde{S}_0) \subset C$. Assume by contradiction that this is not the case, i.e. that $\omega(\tilde{S}_0) \not\subset C$ for all $\tilde{S}_0 \in N$.

Fact 1: All solutions starting in N leave $\mathrm{cl}[V_C]$ in forward time.
If not, then there would be some $p \in N$ whose forward orbit is contained in $\mathrm{cl}[V_C]$. But then the definition of omega limit sets implies that $\omega(p) \subset \mathrm{cl}[V_C]$ ($\subset U_C$) as well. In addition, $p \in \omega(S_0)$ implies that $\omega(p) \subset \omega(S_0)$ (by invariance and closedness of omega limit sets), and thus we have that $\omega(p) \subset \omega(S_0) \cap U_C$. On the other hand, our assumption implies that $\omega(p) \not\subset C$, and therefore the set $\Delta = \omega(p) \setminus C$ is not empty. Moreover, we claim that Δ is invariant. To see that Δ is forward invariant, we argue by contradiction. If not, then there must be some forward solution starting in Δ which must enter C in some finite forward time (since every forward solution starting in Δ certainly remains in $\omega(p)$ by invariance of omega limit sets). But this contradicts backward invariance of C. A similar argument shows that Δ is backward invariant. In conclusion, the set Δ is non-empty, invariant, and contained in $[\omega(S_0) \cap U_C] \setminus C$. This contradicts the hypothesis that there are no invariant sets in $[\omega(S_0) \cap U_C] \setminus C$.

Now we partition N into two subsets: a subset N_1 consistinf of those states whose solutions also leave $\mathrm{cl}[V_C]$ in backward time and a subset N_2 consisting of those states whose backwards solution do not leave $\mathrm{cl}[V_C]$:

$$N_1 := \{\tilde{S}_0 \in N : \exists t < 0 : S(t, \tilde{S}_0) \notin \mathrm{cl}[V_C]\}$$

$$N_2 := \{\tilde{S}_0 \in N : \forall t \leq 0, S(t, \tilde{S}_0) \in \mathrm{cl}[V_C]\}.$$

Fact 2: $N_1 \cap \tilde{U}_C = \emptyset$, for some sufficiently small neighborhood $\tilde{U}_C \supset C$.
If this were not the case, then there would be a sequence of points $S_n \in N_1$ so that $S_n \to S_c$ for some $S_c \in C$. Then we could define $\tilde{S}_n := \varphi(-\tau_n, S_n)$ where $\tau_n > 0$ is the first time τ for which $\varphi(-\tau, S_n)$ belongs to ∂V_C. Let $\tilde{S} := \lim_{n \to +\infty} \tilde{S}_n$ (which without loss of generality always exists after possibly passing to a subsequence). Clearly, $\tilde{S} \in \partial V_C$. We claim that $\omega(\tilde{S}) \subset C$, thus giving rise to a contradiction. The claim can be shown in 3 steps.

1. First we prove that $\tau_n \to +\infty$. If not, then there exists a bounded subsequence admitting a finite limit; without loss of generality, let us relabel this subsequence as τ_n. Let $0 \leq \bar{\tau} = \lim_{n \to +\infty} \tau_n$. By continuity:

$$\varphi(\bar{\tau}, \tilde{S}) = \lim_{n \to +\infty} \varphi(\bar{\tau}, \tilde{S}_n) = \lim_{n \to +\infty} \varphi(\bar{\tau} - \tau_n, S_n) = \varphi(0, S_c) = S_c \in C.$$

This, however, violates invariance of C.

2. Next, we show that $\varphi(t, \tilde{S}) \in W_C$ for all $t \geq 0$, for some open W_C with $\mathrm{cl}[V_C] \subset W_C$ and $\mathrm{cl}[W_C] \subset U_C$. If not, then there would exist a finite $\bar{t} > 0$ so that $\varphi(\bar{t}, \tilde{S}) \notin \mathrm{cl}[V_C]$. But since $\varphi(\bar{t}, \tilde{S}) = \lim_{n \to \infty} \varphi(\bar{t}, \tilde{S}_n)$, it follows that for all sufficiently large n's, $\varphi(\bar{t}, \tilde{S}_n) \notin \mathrm{cl}[V_C]$. This violates unboundedness of the sequence $\{\tau_n\}$, because by definition of τ_n, there holds that $\varphi(t, \tilde{S}_n) \in \mathrm{cl}[V_C]$ for all $t \in [0, \tau_n]$.

3. Since $\omega(\tilde{S}) \subset \mathrm{cl}[W_C] \subset [\omega(S_0) \cap U_C]$, we are left to conclude that $\omega(\tilde{S}) \subset C$. Indeed, if this were not the case, then it can be proved (using the same arguments used to prove invariance of Δ in the proof of *Fact 1*) that $\omega(\tilde{S}) \backslash C$ is a non-empty invariant set contained in $[\omega(S_0) \cap U_C] \backslash C$. But this contradicts that there are no invariant sets in $[\omega(S_0) \cap U_C] \backslash C$.

Hence we are only left to deal with the smaller set $\tilde{U}_C \cap \omega(S_0)$ where only solutions of type N_2 exist. Notice that, for all $p \in N_2$ we have $\alpha(p) \subseteq C$ (once more, this can be proved by contradiction, by showing that $\alpha(p) \backslash C$ is non-empty and invariant using similar arguments from the proof of invariance of Δ in the proof of *Fact 1*; this in turns yields a contradiction to the fact that there are no invariant sets in $[\omega(S_0) \cap U_C] \backslash C$). On the other hand, by *Fact 1*, the solutions starting in N_2 must leave $\mathrm{cl}[V_C]$ in forward time.

We show next that this situation contradicts chain transitivity of $\omega(S_0)$ (for a proof that this set must be chain transitive, see for instance Lemma 2.1' in [21]), and this will complete the proof of Lemma 7. Let $\varepsilon > 0$ be sufficiently small, and $\tilde{V}_C$ an open neighborhood of C so that $x + z \in \tilde{U}_C$ for all $x \in \tilde{V}_C$ and all z with $|z| \leq \varepsilon$. First notice that by *Fact 1*, for all $S_i \in N_2$, there is some $t_{S_i} > 0$ such that $S(t_{S_i}, S_i) \notin \mathrm{cl}[V_C]$, hence also $S(t_{S_i}, S_i) \notin N_2$. Then by backward invariance of N_2 we obtain the stronger conclusion that $S(t, S_i) \notin N_2$ for all $t \geq t_{S_i}$.

Denote for each $S_i \in N_2$ the infimum of such t_{S_i}'s by t_i (the so-called first crossing time). We claim that $\sup\{t_i | S_i \in [\mathrm{cl}[V_c] \backslash \tilde{V}_C] \cap N_2\} < +\infty$. The proof is based on a standard compactness argument. To see this, fix $S_i \in N_2$, pick some t_{S_i} as above, and consider an open neighborhood V_i of $S(t_{S_i}, S_i)$ which is contained in the complement of $\mathrm{cl}[V_C]$. Then by continuity of the flow, the sets $U_{S_i} := \varphi^{-1}(t_{S_i}, V_i)$ are open neighborhoods of S_i, and are such that for all $x \in U_i \cap N_2$, t_{S_i} is certainly an upper bound of the first crossing-time of the solution starting in x, and in particular -by backward invariance of N_2- there holds that $S(t, x) \notin N_2$ for all $x \in U_i \cap N_2$ and all $t \geq t_{S_i}$. Now since N_2 is compact, and the collection of open sets $\{U_{S_i} | S_i \in N_2\}$ is an open cover of N_2, we can extract a finite subcover $\{U_{S_1}, \ldots, U_{S_N}\}$. Let $\tau = \max_{i=1,\ldots N}\{t_{S_i}\}$. Then it follows that $S(t, S_i) \notin N_2$ for all $t \geq \tau$ and all $S_i \in [\mathrm{cl}[V_C] \backslash \tilde{V}_C] \cap N_2$, which proves our claim.

Let $S_1, \ldots, S_N$ be an arbitrary (ε, τ)-chain relative to the flow $\varphi(t, S_0)$ restricted to $\omega(S_0)$ and with $S_1 \in \omega(S_0) \backslash V_C$; hence, there exist $t_1, t_2 \ldots t_{N-1} \geq \tau$ so that $|\varphi(t_j, S_j) - S_{j+1}| \leq \varepsilon$ for all $j \in \{1, 2, \ldots N - 1\}$. We claim that $S_j \notin \tilde{V}_C$ for all $j \in \{1 \ldots N\}$. We prove the result by induction. Assume $S_j \notin \tilde{V}_C$ (which is obviously true for $j = 1$); following the flow t_j seconds ahead gives $\varphi(t_j, S_j) \notin N_2$. Indeed, if $S_j \in [\mathrm{cl}[V_C] \backslash \tilde{V}_C] \cap N_2$, this follows from the fact that $t_j \geq \tau$, while

if $S_j \notin [\mathrm{cl}[V_C] \setminus \tilde{V}_C] \cap N_2$ (and thus in particular $S_j \notin N_2$), this follows from backward invariance of N_2. Hence, $\varphi(t_j, S_j) \notin \tilde{U}_C$ (since $\omega(S_0) \cap \tilde{U}_C \subset N_2$); therefore, $S_{j+1} \notin \tilde{V}_C$ by our choice of ε. This shows that indeed $\omega(S_0)$ is not chain transitive, since it is not possible to reach C starting outside $\tilde{V}_C$ by means of (ε, τ)-chains (provided that ε and τ are chosen as specified).

Lemma 8. *Assume that all the critical siphons of (6) are dynamically non-emptiable, and let Z be a critical siphon. Suppose that $S_0 \in \mathcal{O}_+$ is such that $\omega(S_0) \cap \mathrm{cl}[L_Z] \doteq \Omega$ is non-empty. Assume further that $\omega(S_0) \cap \mathrm{cl}[L_Z]$ is separated from $\omega(S_0) \cap \mathrm{cl}[L_\Sigma]$ for all deadlocks Σ for which it is not the case that $Z \subseteq \Sigma$. Then, there exists an open neighborhood U of Ω such that $[\omega(S_0) \cap U] \setminus \Omega$ does not contain closed invariant sets.*

Remark. Notice that in the above separation condition, we may assume without loss of generality that the deadlock Σ is critical. Indeed, if it were not critical, and hence structurally non-emptiable, then the arguments in the proof of Theorem 2 show that $\omega(S_0) \cap \mathrm{cl}[L_\Sigma] = \emptyset$.

Proof. The lemma is trivial if $\omega(S_0) \subset \mathrm{cl}[L_Z]$. Hence, we are only left to deal with the case in which this inclusion does not hold. We recall that $\mathrm{cl}[L_Z] = \bigcup_{\Sigma \supseteq Z} L_\Sigma$, so that

$$\omega(S_0) \cap \mathrm{cl}[L_Z] = \left\{ \bigcup_{\Sigma \text{ is a siphon: } \Sigma \supseteq Z} L_\Sigma \cap \omega(S_0) \right\} \doteq \Omega,$$

where the restriction to siphons Σ in the union above, follows from Proposition 1. Assume, by contradiction, that every neighborhood U of Ω contains a closed minimal invariant set $C \subset \omega(S_0) \setminus \mathrm{cl}[L_Z]$ (every closed invariant set contains a minimal invariant subset, henceforth minimality of C can be assumed without loss of generality). Hence, using the superscript c to denote the complement with respect to $\mathcal{O}^+$:

$$C \subset U \cap \omega(S_0) \cap \left[\bigcup_{\Sigma \text{ is a siphon: } \Sigma \supseteq Z} L_\Sigma \right]^c$$

Now, since

$$\mathcal{O}^+ = \bigcup_{\text{all } \Sigma, \text{ including } \emptyset} L_\Sigma,$$

it follows that

$$C \subset U \cap \omega(S_0) \cap \bigcup_{\text{all } \Sigma, \text{ including } \emptyset: \Sigma \not\supseteq Z} L_\Sigma$$

$$= U \cap \bigcup_{\text{all siphons } \Sigma, \text{ including } \emptyset: \Sigma \not\supseteq Z} L_\Sigma \cap \omega(S_0), \quad (20)$$

where we used Proposition 1 in the last equality. Pick U sufficiently small, so that $\omega(S_0) \cap \mathrm{cl}[L_\Sigma] \cap U = \emptyset$ for all deadlocks Σ so that $\Sigma \not\supseteq Z$. As a consequence,

we may without loss of generality restrict the union in equation (20) to critical siphons which are not deadlocks. We claim that $R(S) \succ 0$ for all $S \in C$. Suppose the claim is false, Then there is some $S^* \in C \subset L_\Sigma$ with $R(S^*) = 0$ for some critical siphon Σ not being a deadlock. Then for all $i \in \mathcal{R}$, there is some $j \in \Sigma$ (and thus in particular $S_j = 0$), such that $\alpha_{ij} > 0$. This implies that the set of output reactions associated to the siphon Σ consists of all the reactions of the network, and hence Σ is a deadlock. We have a contradiction.

Consider next any $\hat{S} \in C$. By boundedness of solutions, time-averages of reaction rates are also bounded and in particular

$$\lim_{n \to +\infty} \frac{1}{T_n} \int_0^{T_n} R(S(t, \hat{S})) \, dt = v \succeq 0 \tag{21}$$

along some subsequence $T_n \to +\infty$ and for some vector v, possibly depending upon $\hat{S}$. Moreover,

$$0 = \lim_{T_n \to +\infty} \frac{S(T_n, \hat{S}) - \hat{S}}{T_n} = \lim_{n \to +\infty} \frac{1}{T_n} \int_0^{T_n} \Gamma R(S(t, \hat{S})) \, dt = \Gamma v,$$

implying that $v \in \text{Ker}[\Gamma] \subset \mathcal{C}(W)$ (actually for all W).

Next, it is a well known fact in Ergodic Theory, that minimal flows (in our case the flow restricted to C) admit a unique invariant ergodic probability measure. Let $m(\cdot)$ be such a measure; by the Ergodic Theorem (see [7]) for m-almost all $\hat{S} \in C$ it holds:

$$\lim_{T \to +\infty} \frac{1}{T} \int_0^T R(S(t, \hat{S})) \, dt = \int_C R(S) \, dm \tag{22}$$

Hence, (21) and (22) imply that $v = \int_C R(S) \, dm$, and then the above considerations imply that $v \succ 0$. Moreover, compactness of C, the definition (21) of v, and $\mathcal{F}_\varepsilon(\Sigma)$ being a closed convex cone, imply by virtue of Lemma 4, that $v \in \mathcal{F}_\varepsilon(\Sigma)$. So we have found a non-trivial v in $\mathcal{F}_\varepsilon(\Sigma) \cap \mathcal{C}(\Sigma)$, a contradiction to dynamic non-emptiability of the siphon Σ.

Lemma 9. *Consider a chemical reaction network without nested, distinct critical deadlocks. Let Δ_1 and Δ_2 be a critical siphon and deadlock respectively, such that it is not the case that $\Delta_1 \subseteq \Delta_2$. Then, for any $S_0 \in \text{int}[\mathcal{O}^+]$, we have $\omega(S_0) \cap cl[L_{\Delta_1}] \cap cl[L_{\Delta_2}] = \emptyset$.*

Proof. Arguing by contradiction, we would have

$$\emptyset \neq \omega(S_0) \cap cl[L_{\Delta_1}] \cap cl[L_{\Delta_2}] = \omega(S_0) \cap cl[L_{\Delta_1 \cup \Delta_2}].$$

As usual, $cl[L_{\Delta_1 \cup \Delta_2}] = \bigcup_{W \supseteq \Delta_1 \cup \Delta_2} L_W$ so that there exists $W \supseteq \Delta_1 \cup \Delta_2$ with $L_W \cap \omega(S_0) \neq \emptyset$. By Proposition 1, W is a critical siphon and therefore, since it contains the deadlock Δ_2, it is also a critical deadlock. Moreover, $W \supsetneq \Delta_2$, but this violates the assumption that critical deadlocks are not nested.

We are now ready to prove an improved version of Lemma 6.

Lemma 10. *Consider a chemical reaction network having a dynamically non-emptiable siphon $\Sigma \subset V_S$ and assume that the network is free of nested critical deadlocks. Let S_0 be arbitrary in $\mathcal{O}^+ \setminus L_\Sigma$. Then, provided $\omega(S_0)$ is compact, we have that $\omega(S_0) \nsubseteq cl[L_\Sigma]$.*

Proof. The proof is carried out by considering two separate cases:

1. $\omega(S_0) \cap L_W = \emptyset$ for all $W \supsetneq \Sigma$; Since $cl[L_\Sigma] = \bigcup_{W \supseteq \Sigma} L_W$, the result follows by Lemma 6, considering that $\omega(S_0) \cap cl[L_\Sigma] = \omega(S_0) \cap L_\Sigma$.

2. Assume that $\exists W \supsetneq \Sigma$ such that $\omega(S_0) \cap L_W \neq \emptyset$ and let W be maximal with this property, so that indeed $\omega(S_0) \cap L_W = \omega(S_0) \cap cl[L_W]$. Clearly, W is a critical siphon (by Proposition 1). Pick any critical deadlock Z (if one exists) so that $\omega(S_0) \cap cl[L_Z] \neq \emptyset$ and it is not the case that $W \subseteq Z$. By Lemma 9, $\omega(S_0) \cap cl[L_Z]$ and $\omega(S_0) \cap cl[L_W]$ are separated, as requested by Lemma 8. Hence, there exists an open neighborhood U_W of $\omega(S_0) \cap cl[L_W]$ so that $\omega(S_0) \cap U_W \setminus cl[L_W]$ does not contain closed invariant sets. Finally, by Lemma 7, there exists $\tilde{S}_0 \in \omega(S_0) \cap U_W \setminus cl[L_W]$ so that $\omega(\tilde{S}_0) \subset cl[L_W] \cap \omega(S_0) = L_W \cap \omega(S_0) \subset L_W$. This however contradicts Lemma 6.

Proof of Theorem 4. The proof will be carried out by contradiction. Assume that the reaction network (6) be not persistent. Then, there exists S_0 in $\operatorname{int}(\mathcal{O}_+)$, so that $\omega(S_0) \cap \partial \mathcal{O}^+ \neq \emptyset$. Let $\mathcal{E} = \{\Sigma \subset V_S : \omega(S_0) \cap L_\Sigma \neq \emptyset\}$; clearly $\mathcal{E}$ is non-empty, and by Proposition 1, its elements are necessarily critical siphons. Pick any pair $\Delta_1, \Delta_2 \in \mathcal{E}$ ($\Delta_1 \neq \Delta_2$) of which Δ_1 is maximal in $\mathcal{E}$ with respect to set inclusion and Δ_2 is a deadlock (if there is not such a pair the next conclusion trivially holds). Of course $\Delta_1 \nsubseteq \Delta_2$ (by maximality of Δ_1) and, as a consequence, by Lemma 9 separation of $\omega(S_0) \cap cl[L_{\Delta_1}]$ and $\omega(S_0) \cap cl[L_{\Delta_2}]$ holds. Let Δ be a maximal element of $\mathcal{E}$, with respect to set inclusion. Two possible cases can be ruled out:

1. $\omega(S_0) \subset cl[L_\Delta]$; this can be ruled out by virtue of Lemma 10 and exploiting dynamical non-emptiability of Δ.

2. $\omega(S_0) \nsubseteq cl[L_\Delta]$; by Lemma 2, $\omega(S_0) \cap L_\Delta$ is invariant. Similarly, for all W such that $\omega(S_0) \cap L_W$ is non-empty, there holds that $\omega(S_0) \cap L_W$ is invariant, and hence $\omega(S_0) \cap cl[L_\Delta]$ is invariant as well since $cl[L_\Delta] = \bigcup_{W \supseteq \Delta} L_W$.

   In this case we may apply Lemma 8 to the siphon Δ (which, as we just proved, satisfies the isolation condition) so that we conclude existence a neighborhood U of $\omega(S_0) \cap cl[L_\Delta] \doteq \Omega$ so that $[U \cap \omega(S_0)] \setminus cl[L_\Delta]$ does not contain closed invariant sets. Application of Lemma 7, then, shows existence of $\tilde{S}_0$ in $\omega(S_0) \setminus \Omega$ such that $\omega(\tilde{S}_0) \subseteq \Omega$. By virtue of Lemma 10, however, this violates dynamical non-emptiability of Δ.

This completes the proof of the Theorem.

10   Examples and Discussion

We illustrate applicability of Theorem 4 through some examples which, despite their apparent simplicity, cannot be treated by the results in Section 5.

Consider the Petri Net displayed in Fig. 8, whose associated CRN is given below:

$$2A + B \rightarrow C \rightarrow A + 2B \rightarrow D \rightarrow 2A + B.$$
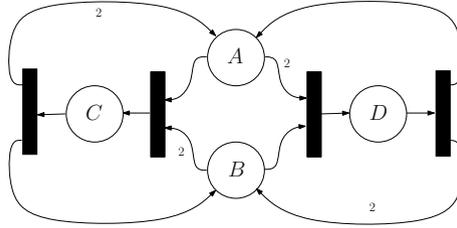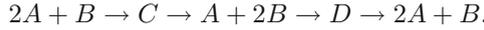
Fig. 8. A live and persistent network with critical siphons

We have that:

$$\Gamma = \begin{bmatrix} -2 & 1 & -1 & 2 \\ -1 & 2 & -2 & 1 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix}.$$

It is easy to verify that the CRN is weakly reversible (and hence its associated Petri Net is consistent, with T-semiflow $[1, 1, 1, 1]'$). Moreover, there is a unique conservation law, $A + B + 3C + 3D$ (associated to the P-semiflow $[1, 1, 3, 3]$), and two non-trivial siphons (in fact, both are deadlocks): $\Sigma_a = \{A, C, D\}$ and $\Sigma_b = \{B, C, D\}$, none of them containing the support of a first integral (both of them are therefore critical). It clearly holds that $R_1 \preccurlyeq_{\Sigma_a} R_3$ and $R_3 \preccurlyeq_{\Sigma_b} R_1$. Notice that both siphons are dynamically non-emptiable. To see this for the critical siphon Σ_a (similar arguments can be used to show it for Σ_b), notice that

$$\mathcal{C}(\Sigma_a) = \{v \succeq 0 \,:\, -2v_1 + v_2 - v_3 + 2v_4 \leq 0, \; v_1 - v_2 \leq 0, \; v_3 - v_4 \leq 0\}.$$

This implies in particular that

$$v \in \mathcal{C}(\Sigma_a) \;\; \Rightarrow \;\; v_3 \leq v_1.$$

Dynamic non-emptiability of Σ_a requires that there is some $\epsilon > 0$ such that the cone $\mathcal{C}$ and the cone

$$\{v \succeq 0 \,|\, v_1 \leq \epsilon v_3\},$$

only intersect in 0. This happens when we choose an ϵ in $(0, 1)$.

Obviously the network does not exhibit nested critical deadlocks since $\Sigma_a \nsubseteq \Sigma_b$ and $\Sigma_b \nsubseteq \Sigma_a$, and therefore Theorem 4 is applicable. We conclude that the network is persistent.

It is worth pointing out that the associated Petri Net does satisfy the assumption of Commoner's theorem; indeed the *traps* of network are the sets $\{A, C, D\}$ and $\{B, C, D\}$ and coincide with the siphons, so that the network is live. In this
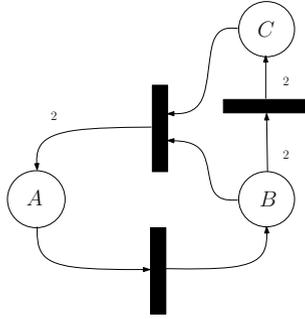
Fig. 9. Non live Petri Net giving rise to persistent CRN

case we expect the stochastic analysis and the deterministic one to give results which are in good agreement with each other.

Consider now the following simple reaction network:

$$A \to B, \quad B + C \to 2A, \quad 2B \to 2C$$
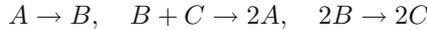
The stoichiometry matrix Γ is given by:

$$\Gamma = \begin{bmatrix} -1 & 2 & 0 \\ 1 & -1 & -2 \\ 0 & -1 & 2 \end{bmatrix}.$$

As before, we associate to it a Petri Net, whose graph is represented in Fig. 9, and compute its invariants. The network is conservative with a unique P-semiflow $[1, 1, 1]$ and consistent with T-semiflow $[4, 2, 1]'$. It exhibits one non-trivial siphon : $\Sigma = \{A, B\}$, which is critical since there cannot contain a support of a P-semiflow. It is worth pointing out that the network does not satisfy the siphon-trap property, in fact there are no non-trivial traps. Indeed, starting with initial marking $[2, 2, 2]$ it is possible to first empty out the A place, by triggering reaction 1 twice, then place B by triggering reaction 2 twice. Once the siphon is emptied, it will be such for all future times and indeed no reaction can take place henceforth. This situation is called a *deadlock* in Petri Net terminology and indeed shows that the net is not live.

However, further analysis of siphon Σ shows that indeed it is a dynamically non-emptiable siphon. In fact,

$$\mathcal{C}(\Sigma) = \{v \geq 0 : -v_1 + 2v_2 \leq 0 \text{ and } v_1 - v_2 - v_3 \leq 0\}.$$

In combination with the constraint $v_3 \leq \epsilon v_2$ which follows taking into account $R_3 \preceq_\Sigma R_2$ we get

$$2v_2 \leq v_1 \leq v_2 + v_3 \leq (1 + \epsilon)v_2$$

so that indeed for $\epsilon \in (0, 1)$ we obtain $v_2 = 0$ and consequently v_1 and $v_3 = 0$ as well. We can thus apply Theorem 4 and conclude persistence of the chemical reaction network for all values of the kinetic constants.

11   Conclusions

Persistence is the property that species (for instance in chemical reactions or in ecology) will remain asymptotically non-zero provided that they were present at the initial time. This paper provided necessary as well as sufficient conditions for the analysis of persistence in chemical reaction networks.

The results in the first part of the paper were based only upon structural and topological features of the network. Such results are "robust" with respect to uncertainty in model parameters such as kinetic constants and cooperativity indices, and they are in the same spirit as the work of Clarke [11], Horn and Jackson [26, 27], Feinberg [15, 16, 17], and many others in the context of complex balancing and deficiency theory, as well as the work of Hirsch and Smith [39, 23] and many others (including the present authors [2, 14, 3, 10]) in the context of monotone systems.

On the other hand, the knowledge of the functional dependency of reaction rates upon coefficients of the stoichiometry matrix, as in mass action kinetics, allows one to obtain tighter sufficient conditions for robust persistence of chemical reaction networks, again on the basis of topological information and regardless of the kinetics parameters involved of which only positivity is assumed. The second part of the paper takes advantage of such information. In particular, the conditions given here allow one to isolate certain classes of networks for which stochastic and deterministic analysis provide results which are qualitatively very different; in particular, Theorem 4 may sometimes be useful when one needs to decide that a certain chemical reaction network which is not "live" when considered as a stochastic discrete system, turns out to be persistent in a deterministic context, even regardless of parameter values. Our result may also serve as preliminary steps towards the construction of a systematic Input/Output theory for chemical reaction networks, by allowing systems with inflows and outflows.

Acknowledgments

References

1. D. Angeli, P. De Leenheer, E.D. Sontag, "On the structural monotonicity of chemical reaction networks," Proc. IEEE Conf. Decision and Control, San Diego, Dec. 2006, IEEE Publications, (2006), to appear.
2. D. Angeli, E.D. Sontag, "Monotone control systems," *IEEE Trans. Autom. Control* **48** (2003), pages 1684–1698.
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," *Proceedings of the National Academy of Sciences USA* **101** (2004), pages 1822–1827.

4. D. Angeli, E.D. Sontag, "A global convergence result for strongly monotone systems with positive translation invariance," *Nonlinear Analysis Series B: Real World Applications*, to appear.

5. J-P. Aubin, A. Cellina, *Differential Inclusions: Set-Valued Maps and Viability Theory*, Springer-Verlag, 1984.

6. N.P. Bhatia, G.P. Szegö, *Stability Theory of Dynamical Systems*, Springer-Verlag, Berlin, 1970.

7. L.A. Bunimovich, S.G. Dani, R.L. Dobrushin, et al., *Dynamical Systems, Ergodic Theory and Applications*, Springer-Verlag, 2000.

8. G. Butler, P. Waltman, "Persistence in dynamical systems," *J. Differential Equations* **63** (1986), pages 255-263.

9. G. Butler, H.I. Freedman, P. Waltman, "Uniformly persistent systems," *Proc. Am. Math. Soc.* **96** (1986), pages 425-430.

10. M. Chaves, E.D. Sontag, R.J. Dinerstein, "Steady-states of receptor-ligand dynamics: A theoretical framework," *J. Theoretical Biology* **227** (2004), pages 413–428.

11. B.L. Clarke, "Stability of complex reaction networks," *Adv. Chem. Phys.* **43** (1980), pages 1-216.

12. F. Commoner, "Deadlocks in Petri Nets," *Tech. Report, Applied Data Research Inc. Wakefield, Massachussetts* (1972),

13. 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.* pages 325-328.

14. P. De Leenheer, D. Angeli, E.D. Sontag, "Monotone chemical reaction networks," *J. Mathematical Chemistry* (2006), to appear.

15. M. Feinberg, F.J.M. Horn, "Dynamics of open chemical systems and algebraic structure of underlying reaction network," *Chemical Engineering Science* **29** (1974), pages 775-787.

16. M. Feinberg, "Chemical reaction network structure and the stabiliy of complex isothermal reactors - I. The deficiency zero and deficiency one theorems," Review Article 25, *Chemical Engr. Sci.* **42**(1987), pp. 2229-2268.

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

18. M. Feinberg, "Lectures on chemical reaction networks," *Lectures at the Mathematics Research Center, University of Wisconsin*, 1979.
    `http://www.che.eng.ohio-state.edu/feinberg/`

19. T.C. Gard, "Persistence in food webs with general interactions," *Math. Biosci.* **51** (1980), pages 165–174.

20. H. Genrich, R. Küffner, K. Voss, "Executable Petri net models for the analysis of metabolic pathways," *Int. J. on Software Tools for Technology Transfer (STTT)* **3** (2001), pages 394-404.

21. M.W. Hirsch, H.L. Smith, X. Zhao, "Chain transitivity, attractivity, and strong repellors for semidynamical systems," *Journal of Dynamics and Differential Equations* **13** (2001), pages 107-131.

22. M.H.T. Hack, "Analysis of production schemata by Petri-Nets," *Master Thesis, MIT* (1972),

23. M. Hirsch, H.L. Smith, in *Handbook of Differential Equations, Ordinary Differential Equations (second volume)* (A. Canada, P. Drabek, and A. Fonda, eds.), Elsevier, 2005.

24. J. Hofbauer, J.W.-H. So, "Uniform persistence and repellors for maps," *Proceedings of the American Mathematical Society* **107** (1989), pages 1137-1142.

25. R. Hofestädt, "A Petri net application to model metabolic processes," *Syst. Anal. Mod. Simul.* **16** (1994), pages 113-122.

26. F.J.M. Horn, R. Jackson, "General mass action kinetics," *Arch. Rational Mech. Anal.* **49**(1972), pp. 81-116.

27. 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.

28. C.-Y.F. Huang, Ferrell, J.E., "Ultrasensitivity in the mitogen-activated protein kinase cascade," *Proc. Natl. Acad. Sci. USA* **93** (1996), pages 10078–10083.

29. J. G. Kemeny, A.W. Knapp, J.L. Snell and J.G. Kemeny, *Denumerable Markov Chains*, Graduate Texts in Mathematics, Springer-Verlag, 1976.

30. R. Küffner, R. Zimmer, T. Lengauer, "Pathway analysis in metabolic databases via differential metabolic display (DMD)," *Bioinformatics* **16** (2000), pages 825-836.

31. A.R. Asthagiri and 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), pages 227–239.

32. N.I. Markevich, J.B. Hoek, B.N. Kholodenko, "Signaling switches and bistability arising from multisite phosphorilation in protein kinase cascades" *Journal of Cell Biology*, Vol. 164, N.3, pp. 353-359, 2004

33. J.S. Oliveira, C.G. Bailey, J.B. Jones-Oliveira, Dixon, D.A., Gull, D.W., Chandler, M.L.A., "A computational model for the identification of biochemical pathways in the Krebs cycle," *J. Comput. Biol.* **10** (2003), pages 57-82.

34. M. Peleg, M., I. Yeh, R. Altman, "Modeling biological processes using workflow and Petri net models," *Bioinformatics* **18** (2002), pages 825-837.

35. J.L. Peterson, *Petri Net Theory and the Modeling of Systems* Prentice Hall, Lebanon, Indiana 1981.

36. C.A. Petri, *Kommunikation mit Automaten* Ph.D. Thesis, University of Bonn, 1962.

37. V.N. Reddy, M.L. Mavrovouniotis, M.N. Liebman, "Petri net representations in metabolic pathways.," *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **1** (1993), pages 328-336.

38. G. Rozenberg, W. Reisig, *Lectures on Petri Nets Basic Models: Basic Models, Lecture Notes in Computer Science 1491, Springer-Verlag, 1998.*

39. 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).

40. 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), pages 1028–1047. (Errata in *IEEE Trans. Autom. Control* **47**(2002): 705.)

41. E.D. Sontag, *Mathematical Control Theory: Deterministic Finite Dimensional Systems, Second Edition* Springer, New York 1998.

42. H.R. Thieme, "Uniform persistence and permanence for non-autonomous semiflows in population biology," *Math. Biosci.* **166** (2000), pages 173-201.

43. C. Widmann, G. Spencer, M.B. Jarpe, G.L. Johnson, G.L., "Mitogen-activated protein kinase: Conservation of a three-kinase module from yeast to human," *Physiol. Rev.* **79** (1999), pages 143–180.

44. I. Zevedei-Oancea, S. Schuster, "Topological analysis of metabolic networks based on Petri net theory," *In Silico Biol.* **3** (2003), paper 0029.

45. M. Zhou, *Modeling, Simulation, and Control of Flexible Manufacturing Systems: A Petri Net Approach* World Scientific Publishing, Hong Kong, 1999.