Graph-theoretic characterizations of monotonicity of chemical networks in reaction coordinates

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Abstract   This paper derives new results for certain classes of chemical reaction networks, linking structural to dynamical properties. In particular, it investigates their monotonicity and convergence under the assumption that the rates of the reactions are monotone functions of the concentrations of their reactants. This is satisfied for, yet not restricted to, the most common choices of the reaction kinetics such as mass action, Michaelis-Menten and Hill kinetics. The key idea is to find an alternative representation under which the resulting system is monotone. As a simple example, the paper shows that a phosphorylation/dephosphorylation process, which is involved in many signaling cascades, has a global stability property. We also provide a global stability result for a more complicated example that describes a regulatory pathway of a prevalent signal transduction module, the MAPK cascade.

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1 Introduction

The study of the qualitative behavior of chemical reaction networks is an area of growing interest, especially in view of the challenges posed by molecular and systems biology. One of the goals, in this respect, is to understand cell function at the level of chemical interactions. This will impact the design of drugs and of therapeutic treatment schedules. In this context, the chemical reaction networks are typically highly dimensional and quite complex, and this calls for the development of systematic tools to handle questions such as: What is the functionality of a specific "pathway" or what is its qualitative behavior? How robust (or insensitive) is the network to parameter changes? Sometimes, such type of questions can be addressed using the theory of monotone systems, which was largely developed in the eighties of the last century by Hirsch (see Smith 1995 for a review).

Monotone systems techniques have been used advantageously for the analysis of several biological systems, arising in ecology and microbiology, see for instance Smith and Waltman (1995) and Angeli and Sontag (2003), and, for obvious historical reasons, to a lesser extent in molecular and systems biology. As a result, a comprehensive theory of chemical reaction networks grounded in the theory of monotone dynamical systems, is still missing.

In general it is not clear when a chemical reaction network gives rise to a monotone system. To the best of our knowledge, this question was only addressed before in Volpert et al. (1994), Banaji (2009) where certain algebraic conditions were provided. In contrast, our goal is to provide conditions in terms of properties of particular graphs which are routinely used by molecular and systems biologists. Once monotonicity has been established in this way, one may at least in principle appeal to monotone dynamical systems theory to derive non-trivial statements concerning the asymptotic behavior of all (or almost all) solutions. However, not all of the available results are sufficient in the context of chemical reaction networks, and some new theory concerning the dynamics of certain monotone systems had to be developed Angeli and Sontag (2008). Also, our convergence results are achieved in conjunction with another important qualitative property that a chemical reaction network may or may not possess, namely that of persistence. Roughly speaking, a network is persistent, if none of the chemicals vanishes asymptotically, when they are all present initially. Fortunately, there is also a graphical theory to verify persistence, see our earlier work Angeli et al. (2007), and we will briefly review it in the Appendix for convenience of the reader.

In summary, the purpose of this paper is twofold:

1. To provide graphical conditions to check monotonicity of a chemical reaction network in the reaction representation.
2. To establish (almost) global convergence to equilibria of chemical reaction networks that fulfill the previous test, provided that a 'persistence' condition is satisfied.

The reaction representation of the network considered here is closely related to a representation used in metabolic engineering Jamshidi and Palsson (2008), see also Othmer (2003).

Two benchmark examples are provided that will illustrate our theory. The first example concerns a model of a single phosphorylation reaction which was also investigated in Angeli and Sontag (2008) where a certain strong monotonicity property had to be verified in an ad-hoc manner. Here, we show that this can be avoided by checking a persistence property instead, using the general methods from Angeli et al. (2007).

The rest of the paper is organized as follows. In Sect. 2 we provide some basic definitions and assumptions that lead to the model of a chemical reaction network. In Sect. 3 we define and briefly review some aspects of monotone systems, and we formulate our first problem, namely determining which reaction networks give rise to monotone systems. Section 4 introduces several graph theoretic concepts related to chemical networks. Our main (almost) global convergence results are presented in Sect. 5, and we show in Sect. 6 that they cover all possible networks that satisfy our main assumptions. Several examples are provided in Sect. 7 and conclusions are drawn in Sect. 8. The notion of persistence, and a persistence result are reviewed in the Appendix.

Some of the results in this paper were initially announced in Angeli et al. (2006) without detailed proofs. We provide the details of these proofs here, and make several extensions. We also present several new examples of biochemical reaction networks studied in the literature to which our theoretical results apply.

2 Basic definitions, terminology and assumptions

The set of vectors in $\mathbb{R}^n$ whose components are non-negative is denoted by $\mathbb{R}^n_{\geq 0}$, sometimes called the *non-negative orthant*, the boundary of $\mathbb{R}^n_{\geq 0}$ is denoted by $\partial \mathbb{R}^{\bar{n}}_{\geq 0}$, and the interior by $\text{int}(\mathbb{R}^n_{\geq 0})$.

A *chemical reaction network* (CRN) is a list of chemical reactions $\mathcal{R}_i$, where the index i takes values in $\mathcal{R} := \{1, 2, \ldots, n_r\}$. Reactions specify how certain certain combinations of chemical species are converted into other combinations of chemical species. The various chemical species are denoted as S_j, where the index j takes values in $\mathcal{S} := \{1, 2, \ldots n_s\}$.

Chemical reactions are written as follows:

$$\mathcal{R}_i : \quad \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \rightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j,$$

or

$$\mathcal{R}_i : \quad \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \leftrightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j$$
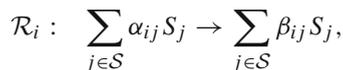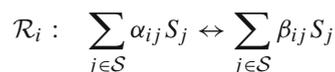
where the α_{ij} and β_{ij} are non-negative integers called the *stoichiometry coefficients*. The first type of reactions are called *irreversible*, while the second are called *reversible*. In the case of a reversible reaction, we call the $\rightarrow (\leftarrow)$ the *forward (back ward)*

reaction. Notice that in both cases the compounds on the left-hand side are usually referred to as the *reactants*, and these on the right-hand side are called the *products* of the reaction. Of course, for reversible reactions this may seem ambiguous, since we are free to decide which species are reactants and which are products. We avoid this ambiguity by agreeing to, once and for always, *fix the reactant side and the product side of every reversible reaction* at the start of our modeling process.

Throughout this paper we

H1 Exclude auto-catalytic reactions,

i.e. reactions (either reversible or not) in which a chemical appears both as a reactant and as a product. More formally, if $\alpha_{ij} > 0$ for some i, j, then $\beta_{ij} = 0$ and vice versa. Notice however, that we are not excluding autocatalysis which occurs in one ore more intermediate steps such as the autocatalysis of S_1 in $S_1 + S_2 \to S_3 \to 2S_1 + S_4$, and therefore **H1** is not as restrictive as it might first appear. The reasons for introducing **H1** will become clear when we discuss the assumptions on the reaction rates in the CRN.

For convenient use later on we arrange the stoichiometry coefficients in a matrix, called the *stoichiometry matrix* Γ, defined as follows:

$$[\Gamma]_{ij} = \beta_{ji} - \alpha_{ji}, \tag{1}$$

for all $i \in \mathcal{R}$ and all $j \in \mathcal{S}$. The stoichiometry matrix will be used later to write the differential equation associated to the CRN. For future reference, we introduce the terminology of the *reaction vector associated to reaction* j, which is defined as the jth column of the stoichiometry matrix.

Next we discuss how the speed of the reactions is affected by the concentrations of the different species. Each chemical reaction takes place continuously in time with its own *reaction rate*. We make the natural and fundamental assumption that

H2 Every reaction rate depends monotonically on the concentrations

   of the species taking part in the reaction.

To make this more precise we first 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 $R(S) := [R_1(S), R_2(S), \ldots R_{n_r}(S)]'$. Then for an irreversible reaction i, the rate at which it takes place is a $\mathcal{C}^1$ function and satisfies the following monotonicity conditions:

$$\frac{\partial R_i(S)}{\partial S_j} \begin{cases} \geq 0 & \text{if } \alpha_{ij} > 0 \\ = 0 & \text{if } \alpha_{ij} = 0. \end{cases} \tag{2}$$

Similarly, if reaction i is reversible, then we assume that

$$R_i(S) = R_{i,f}(S) - R_{i,b}(S), \tag{3}$$

where $R_{i,f}(S)$ satisfies (2), and $R_{i,b}(S)$ satisfies (2) but with α_{ij} replaced by β_{ij}.

Our assumption that the partial derivatives of the reaction rates never change sign, is crucial to the development of our theory. This will become clear in the following sections when we introduce particular graphs associated to a CRN. These graphs can only be defined if the above partial derivatives are sign definite. In other words, if sign definiteness fails, then these graphs cannot be defined and the rest of the theory cannot be developed.

We assume that

H3   If any of the reactants of an irreversible reaction are missing, then the corresponding reaction does not take place. If any of the reactants (products) of a reversible reaction are missing, then the forward (backward) reaction does not take place.

More precisely, if $S_{i_1}, \ldots, S_{i_N}$ are the reactants of the irreversible (reversible) reaction j, then $R_j(S) = 0$ $(R_{j,f}(S) = 0)$ for all S such that $[S_{i_1}, \ldots, S_{i_N}] \in \partial \mathbb{R}_{\geq 0}^N$. And if reaction $S_{i_1}, \ldots, S_{i_M}$ are the products of the reversible reaction j, then $R_{j,b}(S) = 0$ for all S such that $[S_{i_1}, \ldots, S_{i_M}] \in \partial \mathbb{R}_{\geq 0}^M$.

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

$$\dot{S} = \Gamma R(S), \quad S \in \mathbb{R}_{\geq 0}^{n_s}. \tag{4}$$

Pick a reference concentration S_0 (for instance the initial condition to (4)). Note that

$$\mathcal{C}_{S_0} := \mathbb{R}_{\geq 0}^{n_s} \cap (\{S_0\} + \text{Im}(\Gamma))$$

is forward invariant for (4). We call $\mathcal{C}_{S_0}$ the *stoichiometry class* associated to the reference concentration S_0 and we assume that

H4   All stoichiometry classes are compact sets.

Notice that **H4** implies that all solutions of (4) are bounded, and that it is satisfied if there is some vector $d \in (0, +\infty)^{n_s}$ such that $d^T \Gamma = 0$. Indeed, in that case the linear function $d^T S$ remains constant along solutions of (4), and clearly the level sets of this function intersect $\mathbb{R}_{\geq 0}^{n_s}$ in a compact sets.

A basic question, one which is the main focus of this paper, is what happens to solutions in each stoichiometry class.

For technical reasons, related to the use of certain results on monotone dynamical systems, we will sometimes also need a stronger version of (2) and (3):

H5   The inequalities in (2) and (3) hold strictly for all $S \in \text{int}(\mathbb{R}_{\geq 0}^{n_s})$.

From now on, unless explicitly stated otherwise, we assume that

H1, H2, H3, H4 *and* **H5** *hold.*

The most common examples found in the literature that satsify **H2, H3** and **H5** are *mass action kinetics*, *Michaelis-Menten (or Monod) kinetics* and *Hill kinetics*, which in case of an irreversible reaction are given by the following specific functional forms:

$$k_i \prod_{j:\alpha_{ij}>0} S_j^{\alpha_{ij}}, \quad m_i \prod_{j:\alpha_{ij}>0} \frac{S_j}{S_j + a_j}, \quad M_i \prod_{j:\alpha_{ij}>0} \frac{S_j^{n_j}}{S_j^{n_j} + b_j},$$

respectively, where the real parameters k_i, m_i, a_j, M_i, b_j are positive, and the parameters n_j are positive integers. However, our theory also allows that we mix the factors in the above functional forms. For instance, for the reaction $X + 2Y + 3Z \rightarrow P$, the reaction rate

$$K \frac{x^2}{x^2+1} \frac{y}{y+1} z^3,$$

for some $K > 0$, fits our framework. But in general, the reaction rates should not necessarily be products of factors of these three forms: We only require them to satisfy conditions **H2, H3** and **H5**.

Notice also that if we would have allowed auto-catalytic reactions, then in the case of mass action kinetics, the partial derivative of the reaction rate with respect to a species which appears on both reactant and product side of the reaction, would change sign. As we pointed out earlier, it is crucial in the development of our theory that these partial derivatives never change sign, explaining the introduction of **H1**.

3 Problem formulation

To state the problem of interest it is useful to review some definitions from the theory of monotone dynamical systems Smith (1995). We consider autonomous nonlinear systems of the form $\dot{x} = f(x)$, where $f : X \rightarrow \mathbb{R}^n$ is a locally Lipschitz vector field, and x takes values in a closed set $X \subset \mathbb{R}^n$. We assume that a partial order $\succeq$ is defined on X, viz. a binary relation satisfying the following axioms:

- Reflexivity: $x \succeq x$ for all $x \in X$
- Transitivity: $x_1 \succeq x_2$ and $x_2 \succeq x_3$ $\Rightarrow$ $x_1 \succeq x_3$, for all $x_1, x_2, x_3 \in X$
- Antisymmetry: $x_1 \succeq x_2$ and $x_2 \succeq x_1$ $\Rightarrow$ $x_1 = x_2$ for all x_1, x_2.

We also assume that this partial order is closed, i.e. that if $x_n \rightarrow x$ and $y_n \rightarrow y$ as $n \rightarrow \infty$ and $x_n \succeq y_n$ for all n, then also $x \succeq y$.

Typically such closed partial order relations will be defined by first introducing a closed pointed convex cone $K \subset \mathbb{R}^n$ of "positive vectors" and calling $x_1 \succeq x_2$ iff $x_1 - x_2 \in K$. The geometric properties of such cones are easily translated into the axioms above. We say that a system is *monotone* if for all $x_1 \succeq x_2$ and all $t \geq 0$ we have $x(t, x_1) \succeq x(t, x_2)$, where $x(t, x_i)$ denotes the solution at time t with initial condition x_i (Notice that we implicitly assumed forward completeness of the system, viz. global existence of solutions in the future). If the partial order is the one induced by the positive orthant (viz. $K = \mathbb{R}^n_{\geq 0}$), then we say that the system is *cooperative*. We will also need stronger monotonicity notions, based on the following definitions: $x_1 \succ x_2$ iff $x_1 \succeq x_2$ and $x_1 \neq x_2$, and if the partial order is generated by cone a with non-empty interior, we have an even stronger notion of $x_1 \gg x_2$ iff $x_1 - x_2 \in \text{int}(K)$. We say that a system is *strongly monotone* if: $x_1 \succ x_2$ implies $x(t, x_1) \gg x(t, x_2)$ for all $t > 0$.

Testing monotonicity of a system with respect to the partial order induced by an orthant cone is particularly simple for $\mathcal{C}^1$ vector fields $f(x)$, $x \in X$, see Smith (1995).

The property is in fact equivalent to the matrix $\Sigma Df(x)\Sigma$ having non-negative off-diagonal entries for all $x \in X$, where $Df(x)$ denotes the Jacobian and Σ is some suitably chosen diagonal matrix with -1 and 1 entries along the diagonal (Σ canonically identifies the orthant). Alternatively, we may check monotonicity with respect to an orthant induced order graphically as follows. We start by inspecting the signs of the off-diagonal entries of the Jacobian matrix $Df(x)$, and require that these entries never change sign (i.e. either an entry is always positive or zero in X, or it is negative or zero in X). Now we can define the crucial ingredient, an undirected, signed graph which we call the *J-graph* for lack of better terminology, as follows. The J-graph has n nodes (where n is the dimension of x), and we draw an undirected positive (negative) edge from node i to node j if $[Df(x)]_{ji} \geq (\leq)0$ in X and $[Df(x^*)]_{ji} > (<)0$ for at least one $x^* \in X$. If $[Df(x)]_{ji} = 0$ for all x in X, then we don't draw an edge from node i to node j. Notice that al least in principle, since the J-graph is an undirected graph, there could either be one edge, two edges of the same sign, or two edges of different sign between two given nodes. To verify if the system $\dot{x} = f(x)$ is monotone with respect to an order induced by some orthant cone, it will suffice to check if the associated J-graph (note that assuming the existence of the graph is already a nontrivial assumption) has a particular property which will be reviewed in the next section, after we have introduced some more graph theory terminology.

Since the monotonicity property of a system has important implications for its asymptotic behavior, some of which will be summarized later, we are interested in providing sufficient conditions that guarantee that system (4) is monotone with respect to an orthant cone, after a suitably coordinate transformation that depends only on the stoichiometry matrix Γ and not on the particular functional form of the reaction rate vector $R(S)$ in (4). In this way monotonicity can be inferred just by looking at the list of chemical reactions involved in the network, without having to write down equations and reaction rates explicitly. In Volpert et al. (1994) a similar problem was considered, but treated in a purely algebraic fashion. In Banaji (2009) instead, conditions are determined in order to characterize the set of cones and associated partial orders which make a certain reaction monotone. Importantly, it is established that monotonicity of a network with respect to a given partial order independent of reaction kinetics (with some minimal monotonicity assumptions) is equivalent to asking that each individual reaction be monotone with respect to that same order.

It is also worth pointing out that our change of coordinates turns out to be the same for all possible choices of $R(S)$. A weaker but more general approach, for which systematic tools are still not available, is to allow the change of variables to depend on the parameters of $R(S)$. This is interesting, for instance, if mass-action kinetics are adopted, because in that case the reaction vector $R(S)$ is parameterized by a finite number of parameters, namely the positive rate constants. The results of this paper extend those of Angeli et al. (2004) which were limited to reactions with certain tree topology.

4 Graph theoretical preliminaries

Most assumptions in our main results are of a graph theoretical nature, and are stated in terms of several graphs associated to a CRN which we introduce below.

4.1 The species-reaction graph

First we define an undirected bipartite $\{+, -\}$-labeled graph, i.e. a graph having two types of nodes and two types of edges, called the *species-reaction graph* of a CRN, or *SR-graph* for short. Mathematically, such a graph is specified by a quadruple

$$(V_S, V_R, E_+, E_-),$$

where V_S is a finite set of nodes, each one associated to a species, V_R is a finite set of nodes (disjoint from V_S), each one corresponding to a reaction (either irreversible or reversible; in the latter case, the forward and backward reactions are taken into account only once in the graph), while E_+ and E_- are the sets of positive and negative edges, technically subsets of $V_S \times V_R$. Whenever a certain reaction R_i of the form:

$$\sum_{j \in \mathcal{S}} \alpha_{ij} S_j \to \sum_{j \in \mathcal{S}} \beta_{ij} S_j, \quad \text{or} \quad \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \leftrightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j, \tag{5}$$

belongs to the network, we draw a positive edge between $S_j \in V_S$ and $R_i \in V_R$ for all S_j's such that $\alpha_{ij} > 0$ (recall that, as mentioned in Sect. 2, at the start of the modeling process, we declare for every reversible reaction, one side of the reaction to be the reactant side, and the other side to be the product side); formally, we say that $(S_j, R_i) \in E_+$ iff $\alpha_{ij} > 0$. Intuitively, we draw a positive edge between $S_j \in V_S$ and $R_i \in V_R$ if S_j is a reactant, and hence contributes to, the reaction R_j. Similarly, we draw a negative edge between R_i and every $S_j \in V_S$ such that $\beta_{ij} > 0$. Formally, this means that $(S_j, R_i) \in E_-$ whenever $\beta_{ij} > 0$.

It is interesting to note that the notion of the species-reaction graph, and close analogs, were also considered in Feinberg (1991), Reddy et al. (1993), Zevedei-Oancea and Schuster (2003), Craciun and Feinberg (2006), yet in different contexts. Here we will use species-reaction graphs with a new purpose, namely to identify a class of monotone reaction networks.

For future reference, we define a *leaf* of the SR-graph as a node N in $V_S \cup V_R$ for which there is precisely one edge (N, M) (or (M, N)) in $E_- \cup E_+$, for some M in $V_S \cup V_R$.

4.2 The reaction graph and the species graph

Next, we introduce the *reaction graph* (or R-graph for short) which can be derived from the SR-graph. The reaction graph is usually simpler than the SR-graph, since it only has nodes corresponding to reactions, yet it also carries meaningful sign information on its edges. Formally, the reaction graph is defined as a triple $(V_R, \tilde{E}_+, \tilde{E}_-)$, where V_R is again a finite set of reactions, and where $\tilde{E}_-$ and $\tilde{E}_+$ are the positive and negative undirected edges of the graph, defined as follows. We let $\{R_i, R_j\} \in \tilde{E}_-$ $(i \neq j)$ whenever there exists $S_k \in V_S$ so that (S_k, R_i) and (S_k, R_j) both belong either to E_- or E_+. Symmetrically, we let $\{R_i, R_j\} \in \tilde{E}_+$ $(i \neq j)$ whenever there exists $S_k \in V_S$ so that (S_k, R_i) and (S_k, R_j) both belong to $E_- \cup E_+$ but have opposite signs.

In other words, a signed edge is drawn between R_i and R_j whenever there exists a path of length two in the SR-graph, between the two reactions, and the corresponding sign σ is computed as the *opposite* of the product of the signs of the edges included in the path. At this stage the procedure does not mean much, but we will show in later Sections that it is tied to the sign pattern of the Jacobian obtained by writing the network in a suitable set of coordinates. Notice that more than one path (of length 2) can exist in the SR-graph between two given reactions. Accordingly, up to two edges (of opposite signs) might exist between any pair of reactions in the reaction graph.

A similar procedure can be adopted to define the *species graph* (S-graph for short) associated to a CRN. This is again a triple $(V_S, \hat{E}_+, \hat{E}_-)$, defined according to the following set of rules. We let $\{S_i, S_j\} \in \hat{E}_-$ $(i \neq j)$ whenever there exists $R_k \in V_R$ so that (S_i, R_k) and (S_j, R_k) both belong either to E_- or E_+. Symmetrically, we let $\{S_i, S_j\} \in \hat{E}_+$ $(i \neq j)$ whenever there exists $R_k \in V_R$ so that (S_i, R_k) and (S_j, R_k) both belong to $E_- \cup E_+$ but have opposite signs. In other words, a signed edge is drawn between S_i and S_j whenever there exists a path of length two in the SR-graph, between the two species, and the corresponding sign σ is computed as the *opposite* of the product of the signs of the edges included in the path. Of course more than one path (of length 2) can exist in the SR-graph between two given species. Accordingly, up to two edges (of opposite signs) might exist between any pair of species in the S-graph.

4.3 The positive loop property

For an arbitrary J-, SR-, R- or S-graph, a *simple loop* is a path connecting nodes via edges, whose first and last node coincide and with the property that no nodes or edges are repeated twice, with the exception of the first and last node. The *length* of a simple loop is defined as its total number of edges.

Definition 4.1 We say that a J-, SR-, R- or S-graph has the *positive loop property* if any simple loop includes an even number of negative edges.

An important result, see e.g. Smith (1995), Smith and Hirsch (2003), is the following:

Lemma 4.2 *Let $\dot{x} = f(x)$ be defined on X, where f is $\mathcal{C}^1$, and assume that the J-graph exists. Then the system is monotone with respect to some orthant cone iff the J-graph has the positive loop property.*

We now turn to the question of establishing the positive loop property of the R- or S-graph directly in terms of properties of the SR-graph. In the following Section, this property, together with Lemma 4.2, will then lead to the monotonicity of an important dynamical system -defined later- that is related to each CRN.

Bearing in mind that simple loops in the SR-graph always have an even number of edges, we make the following definitions:

Definition 4.3 Let L be a simple loop in the SR-graph. We say that L is an *e-loop* if letting λ be half of its length and σ the product of the signs of all of its edges, there holds that $(-1)^\lambda = \sigma$. Otherwise, we say that L is an *o-loop*.

The concepts of e-loops and o-loops have also been used in different contexts, namely in the analysis of multi-stability for chemical reaction networks with mass-action kinetics (Craciun and Feinberg 2006). We need to develop some properties of e-loops and start by their characterization.

Lemma 4.4 (*e-loops characterization*) *The following facts are equivalent for a given simple loop L in the SR-graph*:

1. *L is an e-loop.*
2. *L contains an even number of segments $R_x S_y R_z$ with (S_y, R_x) and (S_y, R_z) being of the same sign.*
3. *L contains an even number of segments $S_x R_y S_z$ with (S_x, R_y) and (S_z, R_y) being of the same sign.*

Proof We prove the statement for loops starting at an S node; the proof for loops starting at an R node is similar. Let $E_1, E_2, \ldots E_n$ be the ordered sequence of edges comprised in the loop L. Let $\sigma(E_i)$ be equal to $+1$ if $E_i \in E_+$ and -1 if $E_i \in E_-$. Since n is an even number we can define $\lambda = n/2$. We have,

$$\sigma = \prod_{i=1}^{n} \sigma(E_i) = \prod_{k=1}^{\lambda} \sigma(E_{2k-1})\sigma(E_{2k}) = (-1)^{\lambda - n_p}$$

where n_p denotes the number of times E_{2k-1} and E_{2k} have the same sign Hence σ equals $(-1)^{\lambda}$ iff n_p is even. This completes the proof of the Lemma.   □

We have the following result that shows equivalence of the R-graph and S-graph having the positive loop property to certain properties of the SR-graph.

Proposition 4.5 *The R-graph* (*respectively the S-Graph*) *have the positive loop property if and only if the following two conditions are met*:

1. *all simple loops in the SR-graph are e-loops*;
2. *in the SR-graph, each node in V_S (respectively V_R) is linked to at most two nodes in V_R (V_S).*

Proof We show first the sufficiency part for the case of an R-graph (the proof for S-graph is entirely analogous). Let G denote the SR-graph and G_R the reaction graph. We need to show that each of the simple loops of G_R contains an even number of negative edges. If L is a simple loop of length two for G_R, then we can lift this loop to a -not necessarily unique- loop $\tilde{L}$ in G by following any length-2 path joining consecutive reactions in L. By **H1** the loop L must necessarily contain two distinct species nodes (if not, the single species would be a reactant and product of both reactions). Thus L is of the form $R_1 - S_1 - R_2 - S_2 - R_1$ with $R_1 \neq R_2$ and $S_1 \neq S_2$. Now by condition 1, L is an e-loop, and hence by Lemma 4.4, the segments $R_1 - S_1 - R_2$ and $R_2 - S_2 - R_1$ are such that their edge pairs either have different sign (within each segment), or all four edge pairs have the same sign. In the former case L has no negative edges, while in the former L has two negative edges. In either case, L has contains an even number of edges.

If L is a simple loop in G_R of length 3 or higher, we again lift the loop in G_R to a -not necessarily unique- loop $\tilde{L}$ in G by following any length-2 path joining consecutive reactions in L. We claim that the lifted loop $\tilde{L}$ is simple. Indeed, no reaction node can be repeated twice, for otherwise L would not be simple; and this in turn implies that no species node can be repeated twice for otherwise it would be connected to at least 3 reactions, thereby violating condition 2. By condition 1, $\tilde{L}$ is an e-loop, and hence, by virtue of Lemma 4.4, it contains an even number of segments $R_x S_y R_z$ with edges (S_y, R_x) and (S_y, R_z) of the same sign. Since these segments correspond to negative edges in G_R, we have that L contains an even number of negative edges, completing the sufficiency part of the proof.

To prove the converse, we assume that either condition 1 or condition 2 is violated.

If condition 1 is violated, then there exist simple o-loops in the SR-graph G. By Lemma 4.4, such o-loops have an odd number of segments $R_x S_y R_z$ with (S_y, R_x) and (S_y, R_z) of the same sign. The corresponding simple loop in G_R therefore has an odd number of negative edges, and thus G_R does not have the positive loop property.

If condition 2 is violated, then there is a species node S_i in G which is linked to more than two reactions; let us fix three of these reactions and without loss of generality label them as R_1, R_2 and R_3. Consider the following loop in the G_R graph:

$$L = \{R_1, R_2\}, \{R_2, R_3\}, \{R_3, R_1\}.$$

Lift this loop to the following (non-simple) loop in G: $\tilde{L} = R_1 - S_i - R_2 - S_i - R_3 - S_i - R_1$, where for simplicity we only indicated the sequence of nodes met along the loop rather than its edges. By the definition of the sign of an edge in G_R on the basis of the corresponding signs in G (see the definition of R-graphs in the previous subsection), it follows that the sign of L can be computed as $(-1)^3 \cdot \text{sign}(\tilde{L})$. Notice however, that each edge is repeated twice in $\tilde{L}$. Therefore, $\text{sign}(\tilde{L}) = +1$ and as a consequence $\text{sign}(L) = -1$. Hence, L contains an odd number of negative edges, and thus G_R does not have the positive loop property. □

4.4 The directed species-reaction graph

In the subsequent Sections, we will not only require that a certain system is monotone, but that it is in fact strongly monotone. This stronger notion can be verified by checking if its Jacobian matrix is irreducible, which in turn can be derived -as we shall see later- from a graph associated to a CRN, which we define here and call the *directed species-reaction graph* (or directed SR-graph for short). The directed SR-graph is a bipartite graph, $(V_S, V_{R_i}, V_{R_r}, E)$, in which V_S is again a finite set of nodes, each one associated to a chemical species, V_{R_i} is a finite set of nodes, each one associated to an irreversible reaction and V_{R_r} is a finite set of nodes, each one associated to a reversible reaction. In particular, $V_{R_i} \cap V_{R_r} = \emptyset$ and we may define $V_R = V_{R_i} \cup V_{R_r}$ and $V = V_R \cup V_S$. The set $E \subset V \times V$ is a set of edges, which are defined according to the following set of rules:

Fig. 1 **a** SR-graph and
b directed SR-graph of (6)
(*solid lines* refer to positive,
dashed lines to negative edges)

(a)   (b)
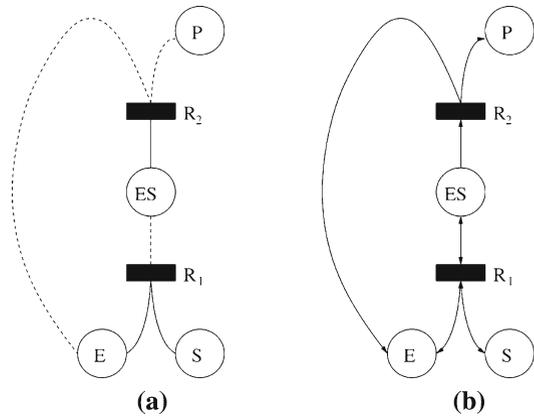
1. an edge from $S_j \in V_S$ to $R_k \in V_{R_r}$ exists iff S_j is a species involved in R_k;
2. an edge from $S_j \in V_S$ to $R_k \in V_{R_i}$ exists iff S_j is a reactant species of R_k;
3. an edge from $R_k \in V_R$ to $S_j \in V_S$ exists iff S_j is a species involved in R_k.

We say that the directed species-reaction graph is *R-strongly connected* if for every
pair of distinct nodes R_a and R_b in V_R, there is a directed path of pairwise distinct edges
in E such that the first edge originates from R_a and the last edge ends in R_b. Notice that
if every species in a CRN is both a reactant of at least one reaction, and a product of at
least one reaction (these reactions must be different by **H1**), then strong connectedness
and R-strong connectedness are equivalent for a directed species-reaction graph.

4.5 Illustrative example

In order for the reader to familiarize himself with the various graphs introduced in this
Section we consider the following well-known enzymatic reaction network:

$$E + S \leftrightarrow ES \rightarrow E + P, \tag{6}$$

where E is an enzyme and S a substrate which upon reacting give rise to the inter-
mediate complex ES. The complex then splits into the product P and the original
enzyme. We depict the associated SR-, R-, S-, and directed SR-graph in Figs. 1 and 2.

Notice from the SR-graph that it has two leaves, namely the species nodes S and
P, and that it has a single simple loop $R_1 - ES - R_2 - E - R_1$ and that it is an e loop.
Thus, it follows from the structure of the SR-graph by Proposition 4.4 that the R-graph
has the positive loop property, a fact which can also be easily seen by direct inspection
of the R-graph. It is also clear that the directed SR-graph is R-strongly connected.

5 Analysis in reaction coordinates

For a given CRN, we now introduce the so-called *system in reaction coordinates*.
Choosing an arbitrary representative S_0 of a given stoichiometry class $\mathcal{C}_{S_0}$, the system

Fig. 2 **a** R-graph and **b** S-graph of (6)

(a)   **(b)**
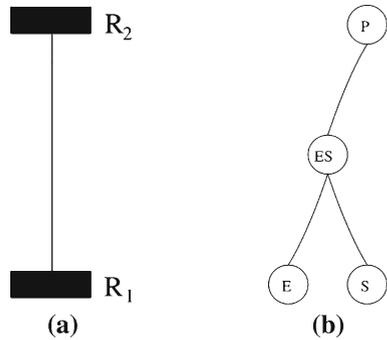
in reaction coordinates is defined as follows:

$$\dot{x}(t) = R(S_0 + \Gamma x(t)), \quad x \in X_0 := \{x \in \mathbb{R}^{n_r} \mid S_0 + \Gamma x \geq 0\}, \qquad (7)$$

where x_i ($i \in \mathcal{R}$) is called the *extent of the i-th reaction* Othmer (2003). System (7) is closely related to representations of CRN's frequently used in metabolic engineering. There, systems whose state is a 'flux vector' are considered, see for instance Jamshidi and Palsson (2008).

In fact, the linearization of (7) at some steady state, is such a system.

The following result shows that, at least in principle, the dynamics of system (4) can be understood by studying the dynamics of system (7).

Proposition 5.1 *Let $x(t)$ be a solution of* (7). *Then*

$$S(t) = S_0 + \Gamma x(t) \qquad (8)$$

is a solution of (4) *in* $\mathcal{C}_{S_0}$. *Conversely, let $S(t)$ be a solution of* (4) *in* $\mathcal{C}_{S_0}$. *Then there is a* (*not necessarily unique*) *solution $x(t)$ of* (7) *such that* (8) *holds.*

Proof The first assertion is immediate. To prove the second, since $S(0) \in \mathcal{C}_{S_0}$, we can find some (not necessarily unique) x_0 such that $S(0) = S_0 + \Gamma x_0$. Then define

$$x(t) = x_0 + \int_0^t R(S(\tau)) d\tau.$$

Clearly, (8) holds for $t = 0$. Moreover, as $d/dt(S_0 + \Gamma x(t)) = \Gamma \dot{x}(t) = \Gamma R(S(t)) = d/dt(S(t))$, for all t. But since solutions of (4) are unique, it follows that (8) must hold for all t. It is then also immediate that $x(t)$ is a solution of (7). $\square$

In the following subsections we will thoroughly analyze system (7).

5.1 Strong monotonicity

We start by establishing the following result.

Theorem 1 *Pick any $S_0 \in \mathbb{R}^{n_s}$. Then system* (7) *is monotone with respect to an order induced by some orthant cone iff the R-graph has the positive loop property.*

Proof By Lemma 4.2, system (7) is monotone with respect to the order induced by some orthant cone iff the J-graph associated to system (7) exists and has the positive loop property. Therefore, it suffices to show that the latter holds iff the R-graph has the positive loop property.

Assume first that the R-graph has the positive loop property. We start by showing that in this case, the J-graph of (7) exists. The Jacobian matrix of (7) reads as follows:

$$DR \cdot \Gamma.$$

Let us see if the sign of the edge joining x_l to x_m with $l \neq m$ can be determined unambiguously. First note that matrix multiplication yields that the (m, l)-th entry of the Jacobian matrix is:

$$\sum_{j \in \mathcal{S}} [DR]_{mj} [\Gamma]_{jl}.$$

Notice that by **H2** (and in particular by using (2) and (3)), together with (1), there holds that

$$[DR]_{mj} \simeq -[\Gamma]_{jm},$$

where we used the notation that $a \simeq b$ iff $ab \geq 0$. Consequently, it follows that the sign of each term in the above sum is determined as follows:

$$[DR]_{mj} [\Gamma]_{jl} \simeq -[\Gamma]_{jm} [\Gamma]_{jl}.$$

We claim that the sign of every term is the same. If not, then there would be $j_1 \neq j_2$ such that $[\Gamma]_{j_1 m} [\Gamma]_{j_1 l}$ and $[\Gamma]_{j_2 m} [\Gamma]_{j_2 l}$ have different sign. But then the SR-graph would contain a simple o-loop $R_l - S_{j_1} - R_m - S_{j_2} - R_l$. This in turn would imply by Proposition 4.5, that the R-graph does not have the positive loop property, a contradiction. This establishes our claim. Consequently, none of the entries of the Jacobian matrix change sign in X_0, and this in turn implies that the J-graph does indeed exist.

Next we show that the J-graph has the positive loop property. Notice that the above formula that determines the sign of the (m, l)th entry of the Jacobian matrix (and equivalently, of the sign of the edge connecting the lth and mth node of the J-graph), shows that this sign is the same as the sign of the edge connecting reaction node R_l to reaction node R_m in the R-graph. Thus, the J-graph of system (7) must have the positive loop property, because the the R-graph has this property.

To conclude the proof, we need to show that if the J-graph exists, and has the positive loop property, then the R-graph has the positive loop property. This follows again from the fact that the sign of the edge connecting the R_l node to the R_m node in the R-graph, is the same as the sign of the edge connecting the lth node to the mth node in the J-graph. □

Remark 5.2 Obviously, monotonicity of system (7) could be established in the traditional way Smith (1995), Smith and Hirsch (2003) using the J-graph as in Lemma 4.2. But for a given CRN it is not clear what the J-graph looks like without a (often lengthy) calculation of the Jacobian matrix of the vector field of system (7). The R-graph on the other hand is easily derived from the CRN, and hence the monotonicity test described in Theorem 1 is much more natural and user-friendly in this context. In fact, this feature is one of the main reasons for introducing the R-graph in this work.

For technical reasons, it is also useful to provide sufficient conditions ensuring that the Jacobian matrix of system (7) is irreducible. Indeed, for orthant-monotone systems with irreducible Jacobian throughout their state-space, the flow is strongly monotone, and this typically allows to draw stronger conclusions regarding the possible qualitative asymptotic behaviors of the system, see for instance Angeli and Sontag (2008) and Hirsch (1988).

We claim that irreducibility for the Jacobian matrix associated to system (7) can be established by inspection of the directed SR-graph.

Proposition 5.3 *Assume that the R-graph has the positive loop property. Then the Jacobian matrix of system (7) is irreducible in $int(X_0) = \{x \in \mathbb{R}_{\geq 0}^{n_r} \mid x_0 + \Gamma x \gg 0\}$ iff the directed SR-graph is R-strongly connected.*

Proof Before starting the proof, we point out that the restriction of irreducibility of the Jacobian matrix of system (7) to a proper subset of its state space, stems from the fact that **H5** only holds on $int(\mathbb{R}_{\geq 0}^{n_s})$, but not necessarily on $\mathbb{R}_{\geq 0}^{n_s}$.

We start by showing that the (j, i)th entry of the Jacobian matrix of (7) is nonzero iff there is a path of length 2 in the directed SR-graph connecting R_i to R_j.

Denoting the vector field of (7) by $f(x)$, we use the chain rule to compute the (j, i)th entry of the Jacobian matrix of (7), yielding

$$Df_{ji} = \sum_k [DR]_{jk} \Gamma_{ki}.$$

Since the R-graph has the positive loop property, it follows from the proof of Theorem 1 that no two terms in this sum have opposite sign. Therefore, the above sum is different from zero iff it contains at least one nonzero term. By **H5** this will happen iff in the directed SR-graph, there exists a path of length 2, connecting R_i to R_j.

By definition, the Jacobian matrix of (7) is irreducible if given any pair of distinct indices (j, i), there are pairwise distinct indices $k_1, \ldots, k_l$ with $k_1 = i$ and $k_l = j$ such that

$$Df_{k_l k_{l-1}} \ldots Df_{k_2 k_1} \neq 0.$$

This happens if each factor in the product is nonzero, which, by the above argument is equivalent to asking that in the directed SR-graph, there is a path of length 2, connecting the reactions R_{k_r} to $R_{k_{r+1}}$ for all r. This in turn is equivalent to requiring that the directed SR-graph is R-strongly connected. Thus we have established that the Jacobian matrix of (7) is irreducible iff the directed SR-graph is R-strongly connected. □

Combining Theorem 1 and Proposition 5.3 we find that

Corollary 1 *If the R-graph has the positive loop property, and if the directed SR-graph is R-strongly connected, then system* (7) *is monotone on X_0 with respect to the order induced by some orthant cone, and also strongly monotone on $int(X_0)$ with respect to that same order.*

5.2 Convergence

The change to the system in reaction variables introduced so far is not particularly useful if we cannot establish a link between the dynamics of the original chemical reaction network and those of the system in reaction coordinates, even if we assume that the latter system is strongly monotone. This may at first seem a bit surprising, given the simple relationship between solutions of systems (4) and (7) established in Proposition 5.1. But there, we ignored that a priori there is no guarantee that solutions of (7) are bounded (notice that the state space of system (7) is not compact, unlike the state space $\mathcal{C}_{S_0}$ of system (4)). In fact, this issue constitutes the main technical difficulty that needs to be surmounted in order for us to obtain some meaningful convergence results for system (4).

Our subsequent analysis aims at establishing convergence for (4), using the strong monotonicity of system (7), for instance, under the assumptions of Corollary 1. We will distinguish two cases and each case will be treated with different techniques. In particular, letting K be the orthant cone that induces the partial order preserved by (7), we assume that either one of the following holds:

$$\mathrm{Ker}[\Gamma] \cap \mathrm{int}(K) \neq \emptyset \,, \tag{9}$$

$$\mathrm{Ker}[\Gamma] \cap K = \{0\}. \tag{10}$$

Interestingly, under the conditions of our main result in Theorem 2 below, we will show in the next Section that the intermediate case, in which the Kernel of Γ intersects K in non-trivial points of ∂K, but not in points of $\mathrm{int}(K)$, never occurs.

In case (9) holds, we will use a recently obtained theoretical result Angeli and Sontag (2008), that adds to the tools available for monotone systems. It is a global convergence result which exploits strong monotonicity and translation invariance in order to build a suitable Lyapunov function for the system. In general, we say that a semiflow $\Phi : \mathbb{R}_{\geq 0} \times X \to X$ is *translation invariant* with respect to a set V, if $x \in X$ implies that $x + v \in X$, and that $\Phi(t, x+v) = \Phi(t, x) + v$ for all $v \in V$ and $t \in \mathbb{R}_{\geq 0}$. Notice that the flow induced by the solutions of system (7) is translation-invariant with respect to $\mathrm{Ker}[\Gamma]$.

In case (10) holds, we will exploit Hirsch's generic convergence Theorem Hirsch (1988) instead; not for the system (7) in reaction coordinates, which may have unbounded solutions, but for a suitable reduced system which is also strongly monotone.

Theorem 2 below requires that the CRN's of interest are persistent, a topic worthy of the development of a theory in its own right, see our previous work in Angeli et al.

(2007). For convenience we have included an Appendix reviewing the main definitions and result.

Theorem 2 *Pick any* $S_0 \in int(\mathbb{R}^{n_s})$.

Assume that system (4) *is persistent.*

Assume also that system (7) *is monotone on* X_0 *with respect to the partial order induced by some orthant* K, *and strongly monotone on* $int(X_0)$ *with respect to that same order.*

If (9) *holds, then all solutions of* (4) *in* $int(\mathbb{R}^{n_s}_{\geq 0})$ *converge to an equilibrium, and moreover, this equilibrium is unique within each stoichiometry class.*

If (10) *holds, then almost all solutions of* (4) *in* $int(\mathbb{R}^{n_s}_{\geq 0})$ *converge to the set of equilibria, the measure of the set of possibly non-converging initial conditions being zero.*

Proof We pick $S_0 \in int(\mathbb{R}^{n_s})$ and consider its corresponding stoichiometry class $\mathcal{C}_{S_0}$.

We first establish that for every solution $x(t)$ of system (7) in $int(X_0)$, there exist $\delta > 0$ and $T > 0$ such that

$$d(x(t), \partial X_0) \geq \delta, \quad \text{for all } t \geq T, \tag{11}$$

where $d(y, \partial X_0)$ denotes the Euclidean distance from y to the boundary of X_0, ∂X_0. If this were not the case, then there would be a solution $x(t)$ in $int(X_0)$ and an increasing sequence $t_k \to +\infty$ such that

$$\lim_{t_k \to +\infty} d(x(t_k), \partial X_0) = 0. \tag{12}$$

Since $\{S(t_k)\}$ is a sequence in the compact set $\mathcal{C}_{S_0}$, we may pass to a converging subsequence with limit S^*. Then (12), (8) in Proposition 5.1 and the definition of X_0, imply that $S^* \in \partial(\mathbb{R}^{n_s}_{\geq 0})$. And since $S^* \in \omega(S(0))$, it follows that $\omega(S(0)) \cap \partial(\mathbb{R}^{n_s}_{\geq 0}) \neq \emptyset$, contradicting the assumption that system (4) is persistent.

Assume that (9) holds. In this case, it can be shown Angeli and Sontag (2008) that Ker[Γ] is 1-dimensional, and thus Ker[Γ] := span(v) for some unit vector v belonging to $int(K)$. Then all the assumptions of the Main Result in Angeli and Sontag (2008) hold for system (7), except for the fact that here, system (7) is strongly monotone in $int(X_0)$, but not necessarily in X_0. However, the same proof given there in fact shows that the conclusion of the Main Result in Angeli and Sontag (2008) remains valid, if for every solution $x(t)$ of (7) in $int(X_0)$, there is some $\delta > 0$ and some $T > 0$ such that (11) holds. But earlier, we have already established that this is indeed the case.

Denoting the projection $\pi_v(x) := x - (v'x)v$ of x on the linear space $v^\perp$, it now follows from this modification of the Main Result in Angeli and Sontag (2008) that $\pi_v(x(t)) \to \bar{x}$ for some $\bar{x} \in v^\perp$, and that this value is uniquely defined and independent from initial conditions. Therefore, in original coordinates, $S(t) = S_0 + \Gamma x(t) = S_0 + \Gamma \pi_v(x(t)) \to S_0 + \Gamma \bar{x}$, as $t \to +\infty$. Thus, $S_0 + \Gamma \bar{x}$ is the unique globally attractive equilibrium contained in the stoichiometry class of S_0.

Now we assume that (10) holds. Consider (7) and let's perform a linear coordinate transformation $x = Tz$, where the last m columns of the matrix T are m basis vectors

of Ker[Γ] (the first $n_r - m$ columns don't really matter as long as T is invertible):

$$\dot{z} = T^{-1}R(S_0 + \Gamma T z), \quad z \in Z_0 := \{z \in \mathbb{R}^{n_r} | S_0 + \Gamma T z \geq 0\},$$

or, in blocks of coordinates:

$$\dot{z}_1 = \tilde{R}_1 \left(S_0 + \Gamma \begin{pmatrix} z_1 \\ 0 \end{pmatrix} \right) \tag{13}$$

$$\dot{z}_2 = \tilde{R}_2 \left(S_0 + \Gamma \begin{pmatrix} z_1 \\ 0 \end{pmatrix} \right) \tag{14}$$

on Z_0, where $\tilde{R}_1$ and $\tilde{R}_2$ are suitably defined smooth vector fields. Since (7) is monotone (strongly monotone) on X_0 (int(X_0)) with respect to the partial order induced by the cone K, it follows that (13)–(14) is monotone (strongly monotone) on Z_0 (int(Z_0)) with respect to the partial order induced by $\tilde{K} := T^{-1}K$ which is also a closed convex pointed cone in $\mathbb{R}^{n_r}$ with non-empty interior.

Now apply Lemma 8.1 with $L = \text{Ker}[\Gamma]$, which yields the cone $K' = \pi(\tilde{K})$

We claim that system (13), defined on

$$\Delta_1 := \pi(Z_0) = \{z_1 \in \mathbb{R}^{n_r - m} \mid S_0 + \Gamma T(z_1 \ 0)' \geq 0\},$$

is monotone with respect to the order induced by the cone K'. (Note also that Δ_1 is compact since it is the preimage of the compact set $\mathcal{C}_{S_0}$ under the isomorphism of $\mathbb{R}^{n_r - m}$ which maps z_1 to $S_0 + \Gamma T(z_1 \ 0)'$).

To prove this, let $p, q \in \pi(Z_0)$ be such that $q - p \in K'$. We need to show that $q(t) - p(t) \in K'$ for all $t \geq 0$. By monotonicity of (13)–(14) on Z_0, we have that:

$$q - p \in K' \Rightarrow \begin{pmatrix} q - p \\ y \end{pmatrix} \in \tilde{K} \quad \text{for some } y \in \mathbb{R}^m \Rightarrow$$

$$\begin{pmatrix} q \\ y \end{pmatrix} - \begin{pmatrix} p \\ 0 \end{pmatrix} \in \tilde{K} \Rightarrow \begin{pmatrix} q(t) \\ y(t) \end{pmatrix} - \begin{pmatrix} p(t) \\ r(t) \end{pmatrix} \in \tilde{K} \quad \text{for all } t \geq 0 \Rightarrow$$

$$q(t) - p(t) \in K' \quad \text{for all } t \geq 0,$$

where $(q(t) \ y(t))'$ and $(p(t) \ r(t))'$ are solutions of (13)–(14) starting at $t = 0$ in $(q \ y)'$ and $(p \ 0)'$ respectively.

Moreover, we claim that (13) is strongly monotone on int(Δ_1) (which is clearly non-empty since π is an open mapping and Z_0 has non-empty interior). To prove this, assume that $p, q \in \text{int}(\Delta_1)$ with $q - p \in K' \backslash \{0\}$. We need to show that $q(t) - p(t) \in \text{int}(K')$ for all $t > 0$. By strong monotonicity of (13)–(14) on int(Z_0), we have that:

$$q - p \in K' \backslash \{0\} \Rightarrow \begin{pmatrix} q \\ y \end{pmatrix} - \begin{pmatrix} p \\ 0 \end{pmatrix} \in \tilde{K} \backslash \{0\} \quad \text{for some } y \in \mathbb{R}^m \Rightarrow$$

$$\begin{pmatrix} q(t) \\ y(t) \end{pmatrix} - \begin{pmatrix} p(t) \\ r(t) \end{pmatrix} \in \text{int}(\tilde{K}) \quad \text{for all } t > 0 \Rightarrow$$

$$q(t) - p(t) \in \text{int}(K') \quad \text{for all } t > 0,$$

All the assumptions required for the application of the generic convergence Theorem Hirsch (1988) to the strongly monotone system (13) on $\text{int}(\Delta_1)$ are now satisfied, except that we need to check that $\omega(z_1) \subset \text{int}(\Delta_1)$ for all $z_1 \in \text{int}(\Delta_1)$ (We already know by compactness of Δ_1, that the omega limit sets of solutions in Δ_1 are nonempty, compact sets in Δ_1).

If this were not the case, then there would be some $z_{1,0} \in \text{int}(\Delta_1)$ such that there is some $p \in \omega(z_{1,0})$ with $p \in \partial\Delta_1$. Consider a solution $z(t) = (z_1(t)\ z_2(t))$ of system (13)–(14) starting in $(z_{1,0}\ z_{2,0})$ for some $z_{2,0}$ at $t = 0$. Then there is an increasing sequence $t_k \to \infty$ such that $z_1(t_k) \to p$. This implies that

$$\lim_{t_k \to \infty} d(z(t_k), \partial Z_0) = 0,$$

and hence via $x(t) = \Gamma z(t)$ that

$$\lim_{t_k \to \infty} d(x(t_k), \partial X_0) = 0,$$

contradicting (11).

Hence, we conclude that for almost all $z_1(0) \in \text{int}(\Delta_1)$, $z_1(t)$ converges to the set of equilibria in $\text{int}(\Delta_1)$. But by Proposition 5.1, and since $z = Tx$, every solution $S(t)$ of (4) in $\mathcal{C}_{S_0}$ satisfies

$$S(t) = S_0 + \Gamma x(t) = S_0 + \Gamma T \begin{pmatrix} z_1(t) \\ 0 \end{pmatrix},$$

for some solution $z(t)$ of (13), and thus almost all solutions of (4) in $\mathcal{C}_{S_0} \cap \text{int}(\mathbb{R}^{n_s})$ converge to the set of equilibria. $\qquad\square$

6 Have all cases been covered?

In principle it is possible that

$$\{0\} \neq \text{Ker}[\Gamma] \cap K \subset \partial K, \tag{15}$$

a case we are seemingly neglecting when proving Theorem 2. However, we will prove in this Section that if the R-graph has the positive loop property and if the directed SR-graph is R-strongly connected (notice that these are precisely the conditions from Corollary 1 that imply that (7) is strongly monotone in $\text{int}(X_0)$, which is the main hypothesis in Theorem 2), and if K is the corresponding orthant cone from Theorem 1, then (15) cannot hold.

In, other words, we have the following dichotomy.

Lemma 6.1 *Suppose that the R-graph has the positive loop property and that the directed SR-graph is R-strongly connected, and let K be the orthant cone from Theorem 1. Then either one of* (9) *or* (10) *must hold.*

Before proving Lemma 6.1, we make the following definition:

Definition 6.2 Let

$$L = R_1, S_1, R_2, S_2, \ldots, S_{m-1}, R_m, S_m, R_1,$$

be a simple loop in the SR-graph [The assumption that the species and reactions in the loop are ordered like this means no loss of generality since we can always relabel them.] We say that L is *unitary* if

$$(-1)^m \prod_{j=1}^{m} \frac{\Gamma_{jj}}{\Gamma_{j(j+1)}} = 1,$$

where $m + 1$ means 1.

Proof We will assume without loss of generality that $K = \mathbb{R}^{n_r}_{\geq 0}$. Indeed, this can always be achieved by performing a linear diagonal coordinate transformation $z = \Sigma x$ for system (7), where the diagonal entries of Σ are either $+1$ or -1. Since we assume that the R-graph has the positive loop property, it follows from Proposition 4.5 that every chemical species participates in at most 2 reactions. This implies in particular that every row of Γ contains at most two non-zero entries.

Since we assume that the directed SR-graph is R-strongly connected, both the SR- and R-graph alike must be connected (that is, for each pair of nodes in these graphs, there exists a path of undirected edges in the graph that connects the two nodes). We now distinguish two cases.

Case A. There are species which take part in just one reaction.

Such species are leaves of the SR-graph. Let $v \in \mathbb{R}^n_{\geq 0}$ be in $\mathrm{Ker}[\Gamma]$. We will show that necessarily $v = 0$. Let species S_i be such a species taking part in just one reaction $\mathcal{R}_j$. Then necessarily $v_j = 0$. Now consider the set of all species $S_{i'}$ which participate either as a reactant or as a product in reaction $\mathcal{R}_j$ (if this set is empty, the proof is done). Then either $S_{i'}$ participates in a second reaction $\mathcal{R}_{j(i')}$, in which case $v_{j(i')} = 0$ (indeed, in this case the i'th row in Γ has exactly two nonzero entries, one in the ith column, the second in the $j(i')$th column; in order for the dot product of the i'th row and the vector v to be zero, there must then necessarily hold that $v_{j(i')} = 0$), or not, in which case $S_{i'}$ is also a leaf of the SR-graph. Since the SR-graph is connected, this argument can be applied recursively by propagating through the SR-graph, and it leads to the conclusion that $v = 0$.

Case B. Every species takes part in exactly two reactions.

Without loss of generality we assume that the two non-zero entries in each row of Γ have opposite signs: Indeed, if this were not the case for, say row i, then there would exist $j_1 \neq j_2$ so that $\Gamma_{ij_1}\Gamma_{ij_2} > 0$. But then $v \in \mathrm{Ker}[\Gamma]$ would imply that $v_{j_1} = v_{j_2} = 0$, and one could argue as in Case A to show that necessarily $v = 0$.

There are two sub-cases to consider:

Case B1: All simple loops in the SR-graph are unitary.

We will show that in this case there always exists a positive vector v such that $\Gamma v = 0$, and we will construct this vector. It is useful to interpret the components of this vector v as positive weights assigned to the reaction nodes in the SR-graph. These weights will be assigned according to a simple rule outlined below by propagation through the connected SR-graph.

The process starts by choosing an arbitrary species node, say S_1, and considering the two distinct reactions in which it participates. Suppose these reactions correspond to the nodes R_j and R_k in the SR-graph. Label node R_j by an arbitrary positive number $v_j > 0$, and assign the weight v_k of node R_k as follows:

$$v_k = -\frac{\Gamma_{1j}}{\Gamma_{1k}} v_j.$$

Note that v_k is positive since Γ_{1j} and Γ_{1k} have opposite signs, as pointed out above. Notice also that every vector v^* whose jth and kth components equal v_j and v_k respectively, is such that the dot product of the first row of Γ and v^* is zero, regardless of the values of the other components of v^*. In other words, any weight assignments which we will make later to reaction nodes other than R_j and R_k, have no effect on the zero value of this dot product.

By connectedness of the SR-graph, we can find a second species, say S_2 (this means no loss of generality because we can always relabel the species) which participates in R_k and in a third reaction R_l, which could possibly be R_j. Assume first that R_l is not R_j (the case were R_l equals R_j is treated below in more generality), so that the three reaction nodes encountered so far are distinct. Then we label node R_l by the weight v_l, given by the rule:

$$v_l = -\frac{\Gamma_{2k}}{\Gamma_{2l}} v_k.$$

As before, v_l is positive, and any vector v^* whose k and lth components equal v_k and v_l respectively, is such that the dot product of the second row of Γ and v^* is zero. Using the same rule, it is clear how to continue this process and label all reaction nodes in the SR graph, at least as long as only unlabeled reaction nodes are encountered. If an already labeled reaction node is encountered, the weight we wish to assign to it according to the above rule, could in principle be different from the weight it already carries. We show next that this cannot happen because of our assumption that all simple loops are unitary. Let

$$L = R_1, S_1, R_2, S_2, \ldots, S_{m-1}, R_m, S_m, R_1$$

be a simple loop in the SR-graph for which the assignment of $v_1, v_2, \ldots, v_m$ is unambiguous, but where moving from R_m through S_m to R_1 yields a different weight for

reaction node R_1 (which already carries label v_1). More precisely, we have the following inequality:

$$v_1 \neq v_1^{\text{new}},$$

where

$$v_1^{\text{new}} = \prod_{j=1}^{m} \frac{-\Gamma_{jj}}{\Gamma_{j(j+1)}} v_1 = v_1 (-1)^m \prod_{j=1}^{m} \frac{\Gamma_{jj}}{\Gamma_{j(j+1)}},$$

by the rule described above. Since $v_1 > 0$, this implies that

$$1 \neq (-1)^m \prod_{j=1}^{m} \frac{\Gamma_{jj}}{\Gamma_{j(j+1)}},$$

which violates the assumption that L is unitary.

Case B2: There is a simple loop L in the SR-graph which is not unitary.

We will show that in this case, whenever $v \in K = \mathbb{R}_{\geq 0}^{n_r}$ satisfies $\Gamma v = 0$, then necessarily $v = 0$. First, after a possible relabeling, we let

$$L = R_1, S_1, R_2, S_2, \ldots, S_{m-1}, R_m, S_m, R_1$$

be a simple loop in the SR-graph which is not unitary. We claim that necessarily $v_1 = v_2 = \cdots = v_m = 0$. Suppose not, then $v_1 > 0$, and as we argued in Case B1, we have that:

$$v_1 = \prod_{j=1}^{m} \frac{-\Gamma_{jj}}{\Gamma_{j(j+1)}} v_1.$$

Since $v_1 > 0$, it follows that

$$\prod_{j=1}^{m} \frac{-\Gamma_{jj}}{\Gamma_{j(j+1)}} = 1,$$

contradicting the fact that L is not unitary.

We have thus shown in particular that whenever $\Gamma v = 0$, there must be at least one $v_j = 0$. From here on, we can argue as in the proof of Case A (the only difference is that the possibility of encountering leaves in the SR graph as in Case A, does not occur here).

Summarizing, we have shown that the intersection of $\text{Ker}[\Gamma]$ and K is either the zero vector, or contains a vector in $\text{int}(K)$.   □

7 Examples

The application of Theorem 2 to the following two examples requires that we establish that the CRN's are persistent. To avoid diverting attention from the main purpose of this Section, which is to establish global or almost global convergence, we will here only state, but not prove, that the respective CRN's are indeed persistent. In the Appendix we will review the required material from Angeli et al. (2007) to establish persistence for an arbitrary CRN, and we will only illustrate it for Example 2 (and not for Example 1), which treats the most complex CRN encountered in this Section.

We also point out that although both examples are usually modeled under the assumption of mass action kinetics for all involved reactions, this requirement can be relaxed as long as the reactions rates satisfy **H2, H3** and **H5**. We illustrate how non-mass action reaction rates arise as a result of quasi steady state approximations in the first example.

7.1 Example 1: single phosphorylation

In molecular systems biology, certain "motifs" or subsystems appear repeatedly, and have been the subject of much recent research. One of the most common is that in which a substrate S_1 is ultimately converted into a product S_2, in an "activation" reaction triggered or facilitated by an enzyme E, and, conversely, S_2 is transformed back (or "deactivated") into the original S_1, 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_1 into S_2 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. A chemical reaction model for such a set of transformations incorporates intermediate species, called complexes, corresponding to the binding of the enzyme and substrate. The simplest such CRN is as follows:

$$\begin{aligned} S_1 + E &\leftrightarrow ES_1 \rightarrow S_2 + E \\ S_2 + F &\leftrightarrow FS_2 \rightarrow S_1 + F, \end{aligned} \tag{16}$$
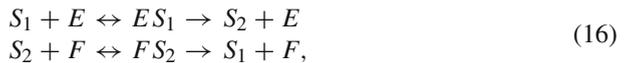
Ordering the species as $S = (S_1, \ E, \ ES_1, \ S_2, \ F, \ FS_2)'$, the stoichiometry matrix is

$$\Gamma = \begin{pmatrix} -1 & 0 & 0 & 1 \\ -1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & -1 & 1 \\ 0 & 0 & 1 & -1 \end{pmatrix}$$

and we note that $1^T \Gamma = 0$ which implies that **H2** holds. The SR-graph of this network is shown in Fig. 3. We point out that the above reaction is not *weakly reversible* (using the language of Feinberg (1979)). This is because the following directed graph of

Fig. 3 SR-graph associated to (16): *dashed edges are negative* (−), while *solid edges are positive* (+)
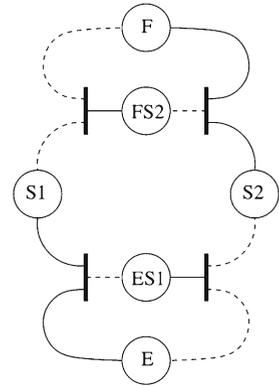
complexes associated to the network:

$$C_1 \leftrightarrow C_2 \rightarrow C_3 \\ C_4 \leftrightarrow C_5 \rightarrow C_6 , \tag{17}$$

is such that neither of its connected components $\{C_1, C_2, C_3\}$ and $\{C_4, C_5, C_6\}$ are strongly connected (indeed, there is no path from C_3 to C_1 or C_2 for instance; similarly, there is no path from C_6 to C_4 or C_5). The lack of weak reversibility implies that even if we would restrict to mass action kinetics, the zero-deficiency theorem Feinberg (1979) is not applicable to study the dynamics of this system. (The zero deficiency theorem implies local stability of unique steady states within each stoichiometry class, provided all reaction rates are mass action and the network satisfies additional conditions, one of which being weak reversibility).

However, notice that all simple loops in the SR-graph are e-loops and that each species node is linked to at most two reaction nodes. Then by Proposition 4.5, the R-graph (not displayed; it has 4 reaction nodes connected in a simple loop through positive edges) has the positive loop property. It is easily verified that the directed SR-graph (not displayed, but easily obtained from modifying the SR-graph by adding arrows to the edges based on the CRN (16)) is R-strongly connected. Thus, by Corollary 1 the associated reaction coordinate system (7) is monotone on X_0 with respect to an orthant cone, and strongly monotone on int(X_0). It turns out that the orthant cone is $\mathbb{R}^4_{\geq 0}$, and thus (7) is cooperative. (As a side remark notice that the S-graph does not have the positive loop property, showing that analysis in species coordinates does not allow to derive similar conclusions. On the other hand, eliminating E and F would allow proving monotonicity of a reduced system, but this approach does not help directly in establishing global convergence properties.) Moreover, the kernel of the stoichiometry matrix ker[Γ] is given by span$[1, 1, 1, 1]'$, and thus (9) holds. It was shown in Angeli et al. (2007) that (16) is persistent, see also the Appendix. It follows from Theorem 2 that all solutions of (4) corresponding to initial conditions in int($\mathbb{R}^6_{\geq 0}$), converge to a unique equilibrium in (the interior of) each stoichiometry class.

We remark that this example was also investigated in Angeli and Sontag (2008). The same convergence result was established there, but the proof relied on an ad-hoc

and complicated verification of strong monotonicity of system (7) on certain parts of X_0. This is avoided here, by instead establishing the persistence property of system (16), which is based on generally applicable methods from our work in Angeli et al. (2007).

Non-mass action kinetics It is standard practice in the analysis of CRN's with mass action kinetics to make the quasi steady state approximation (QSSA) assumption Keener and Sneyd (1998), typically when certain reactions are known to be fast compared to others (for instance, dimerization reactions of proteins are much faster than the binding of RNA polymerase to DNA, which is the first step in transcription). In general, this leads to simplified CRN's with non-mass action kinetics which we illustrate next for the network (16), assuming that the complex FS_2 is at quasi steady state. It is well-known Keener and Sneyd (1998) that the CRN then simplifies to (after first using a conservation law to eliminate one of the system variables, and then making the QSSA assumption):

$$S_1 + E \leftrightarrow ES_1 \rightarrow S_2 + E \tag{18}$$
$$S_2 \rightarrow S_1 \tag{19}$$
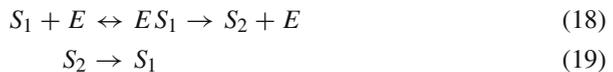
where the reaction rates of the reactions in (18) still obey the mass action kinetics law, while the reaction rate of the reaction in (19) follows the Michaelis-Menten law:

$$R_3(S_2) = k \frac{S_2}{a + S_2},$$

for suitable positive constants k and a that can be expressed in terms of the rate constants of the original CRN. Note that **H1**, **H2**, **H3** and **H5** hold. Here, the stoichiometry matrix is:

$$\Gamma = \begin{pmatrix} -1 & 0 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix}$$

and thus $1^T \Gamma = 0$ which implies that **H4** holds. We depict the SR-graph of CRN (18)–(19) in Fig. 4 and note that all simple loops are e-loops, and each species node is linked to at most two reaction nodes. Thus the R-graph (not depicted) has the positive loop property by Proposition 4.5. Moreover, it is easily established that the directed SR-graph is R-strongly connected. By Corollary 1 the associated reaction coordinate system (7) is monotone on X_0 with respect to the orthant cone $\mathbb{R}^3_{\geq 0}$, and strongly monotone on $\text{int}(X_0)$. Also, $\ker[\Gamma] = \text{span}[1, 1, 1, 1]'$, and thus (9) holds. Finally, it can be shown that (18)–(19) is persistent. It follows from Theorem 2 that all solutions of (4) corresponding to initial conditions in $\text{int}(\mathbb{R}^4_{\geq 0})$, converge to a unique equilibrium in (the interior of) each stoichiometry class. We also note that the zero-deficiency theorem Feinberg (1979) is not applicable here because the reaction rate of the reaction in (19) is not of mass-action type.

Fig. 4 SR-graph associated to (18)–(19): *dashed edges* are negative (−), while *solid edges* are positive (+)
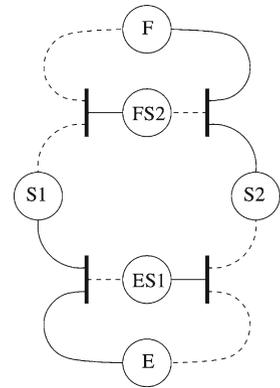
7.2 Example 2: An EGF pathway model

Consider Fig. 5 which depicts a model of (part of) the EGF pathway, a particular example of a signal transduction pathway. This pathway is triggered by the binding of extracellular growth factors on the cell's receptors, and ultimately affects transcription (by activating transcription factors for instance) and translation (by activating ribosomal proteins for instance, thereby altering translation of mRNA into proteins).

A particular part of this pathway is the so-called MAPK (mitogen activated protein kinase) cascade, see the cascade of the kinases RAF, MEK and MAPK in Fig. 5. Each kinase induces the phosphorylation of the next level, and the last kinase, MAPK, can phosphorylate proteins that interfere with transcription and translation directly or indirectly. Here, MEK stands for MAPK/ERK Kinase and ERK for the Extracellular signal Regulated Kinase. Historically, MAPK was called ERK, being the first kinase to be isolated in this pathway.

In Example 1 above, and also in previous work we investigated the properties of particular models for such cascades Angeli and Sontag (2008), Angeli et al. (2004). Here we will focus on the role played by RKIP, a Raf-1 kinase inhibitor protein, using the model described in Cho et al. (2003) and briefly reviewed in the next section. This protein, by binding to Raf-1, inhibits the phosphorylation of MEK by Raf-1.

7.2.1 RKIP network

Consider the following CRN, which we will call the *RKIP network* in the sequel:

$$\text{Raf-1} + \text{RKIP} \leftrightarrow \text{Raf-1/RKIP} \tag{20}$$

$$\text{Raf-1/RKIP} + \text{ERK-PP} \leftrightarrow \text{Raf-1/RKIP/ERK-PP} \tag{21}$$

$$\text{Raf-1/RKIP/ERK-PP} \rightarrow \text{Raf-1} + \text{ERK} + \text{RKIP-P} \tag{22}$$

$$\text{MEK-PP} + \text{ERK} \leftrightarrow \text{MEK-PP/ERK} \tag{23}$$

$$\text{MEK-PP/ERK} \rightarrow \text{MEK-PP} + \text{ERK-PP} \tag{24}$$
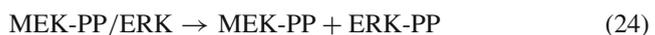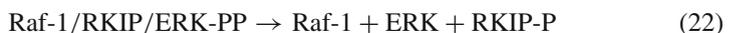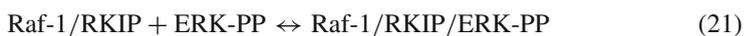
Fig. 5 Model of an EGF pathway, from http://en.wikipedia.org/wiki/MAPK/ERK_pathway

$$\text{RKIP-P} + \text{RP} \leftrightarrow \text{RKIP-P/RP} \qquad (25)$$
$$\text{RKIP-P/RP} \rightarrow \text{RKIP} + \text{RP} \qquad (26)$$
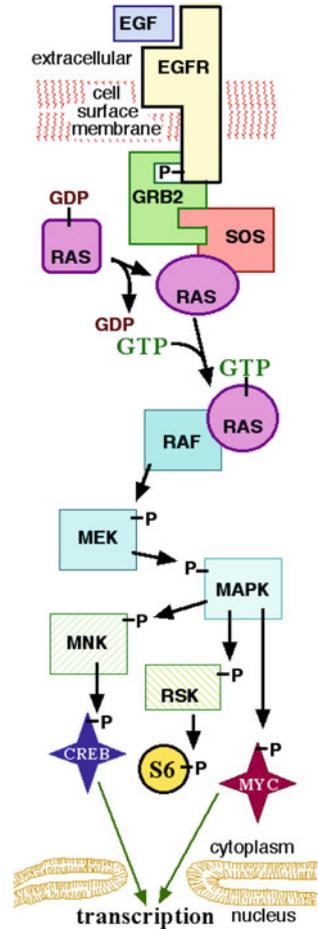
This CRN is illustrated graphically in Fig. 6. The protein RKIP inhibits phosphorylation of MEK by binding to Raf-1, thereby forming the complex Raf-1/RKIP.

Free Raf-1 phosphorylates MEK into its activated form MEK-PP. This activated form binds to ERK to form the MEK-PP/ERK complex which gives rise to the activated ERK-PP, and the original MEK-PP.

The complex Raf-1/RKIP and ERK-PP yield the Raf-1/RKIP/ERK-PP complex. This complex breaks up into the phosphorylated RKIP-P, Raf-1 and dephosphorylated ERK (dephosphorylation occurs via two unmodeled phosphatases). Finally, the phosphatase RP, dephosphorylates RKIP-P into RKIP via the intermediate complex RKIP-P/RP.

When we order the biochemical species in (20)–(26) from left to right and from top to bottom (for instance, RAF-1 is x_1 and RKIP-P is x_7), and we order the reactions
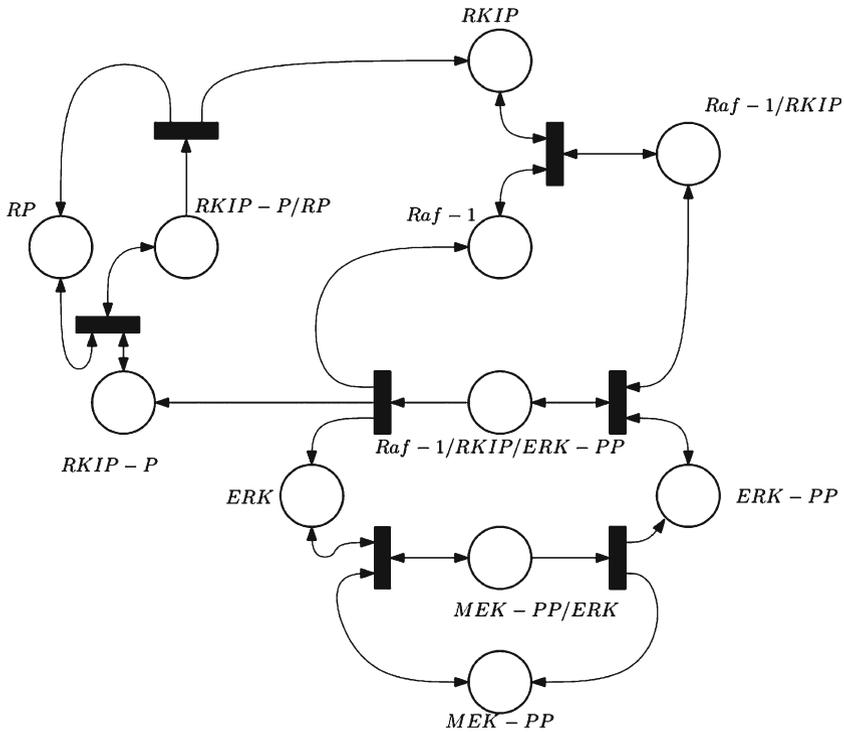
Fig. 6 The RKIP network

similarly, then it is straightforward to write the stoichiometry matrix Γ associated to this CRN.

$$\begin{pmatrix} -1 & 0 & 1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{pmatrix}$$

In Fig. 7 we depict the SR-graph of the RKIP network. Notice that all simple loops in the SR-graph are e-loops and each species node is connected to at most two reaction nodes. This implies that the R graph (not depicted) has the positive loop property by Proposition 4.1. It is easily verified that the directed SR-graph is R-strongly connected, see Fig. 8. Thus, by Corollary 1 the associated reaction coordinate system (7) is monotone on X_0 with respect to an orthant cone, and strongly monotone on int(X_0).

Fig. 7 SR-graph of the RKIP network. *Dashed edges* are negative $(-)$, while *solid edges* are positive $(+)$

It turns out that the orthant cone is $\mathbb{R}^7_{\geq 0}$, and thus (7) is cooperative. Moreover, the following vector belongs to both $\ker[\Gamma]$ and to $\text{int}(\mathbb{R}^7_{\geq 0})$:

$$\left(1, \ 1, \ 1, \ 1, \ 1, \ 1, \ 1\right)'$$

This implies that (9) holds. In the Appendix we show that (16) is persistent using the results from Angeli et al. (2007). It follows from Theorem 2 that all solutions of (4) corresponding to initial conditions in $\text{int}(\mathbb{R}^{11}_{\geq 0})$, converge to a unique equilibrium in (the interior of) each stoichiometry class.

8 Conclusions

We have presented a new method, entirely based on graphical conditions, for analyzing the dynamical behavior of chemical reaction networks. A key idea of our approach is to switch from a system description in the usual species coordinates -where the state vector contains the traditional species concentrations of the various chemicals participating in the network- to a new set of coordinates which we call the reaction coordinates. The main reason for doing this is that quite surprisingly, in many examples, including very large ones taken from the current biochemical literature, the

Fig. 8 Directed SR-graph associated to the RKIP network. Note that three additional edges are now bidirectional, compared to Fig. 6

system description in reaction coordinates turns out to be strongly monotone. Consequently, one can prove convergence or generic convergence for this system, using the recent result in Angeli and Sontag (2008) in case of convergence, and Hirsch's classical results Hirsch (1988) in case of generic convergence. This, coupled with our earlier work on persistence Angeli et al. (2007) allows us to draw similar conclusions for the actual system of interest, which is the traditional species coordinates system.

The theory is illustrated on a benchmark model in the area of enzyme kinetics, and on two more recent and more complex reaction networks which arise in many signal transduction pathways, the futile cycle and the RKIP inhibited ERK pathway from Cho et al. (2003).

Related work by Feinberg (1979) identifies classes of chemical reaction networks for which each positive stoichiometry class has a unique positive equilibrium point which is locally stable. Those results require that all reaction rates are of mass action type. In contrast, we relax this constraint on the reaction rates and moreover, we obtain global stability results.

Other related but more recent work in Craciun and Feinberg (2006) relies on the same graphical concepts -in particular the species-reaction graph- presented here, yet it has a very different scope. While we present global stability results, that work is

concerned with the problem of identifying which chemical reaction networks may have multiple equilibria in their stoichiometry classes.

Our current research efforts are focused on the extension of the presented theory to chemical reaction networks with time-varying kinetics and with inflows and outflows of chemical species.

Appendix

For convenience of the reader, we provide some auxiliary results in this Appendix. We start with the following.

Lemma 8.1 *Let $\tilde{K}$ be a closed and pointed convex cone in $\mathbb{R}^{n_r}$ with non-empty interior, and let L be a subspace in $\mathbb{R}^{n_r}$. Assume that*

$$\tilde{K} \cap L = \{0\}. \tag{27}$$

If L is m-dimensional, we can pick a basis for $\mathbb{R}^{n_r}$ such that the last m basis vectors belong to L and thus $L = \{\mathbf{0}\} \times \mathbb{R}^m$ in this basis. With respect to this basis, we define the projection $\pi : \mathbb{R}^{n_r} = \mathbb{R}^{n_r - m} \times \mathbb{R}^m \to \mathbb{R}^{n_r - m}$ such that $\pi(x, y) = x \in \mathbb{R}^{n_r - m}$. Then

$$K' := \pi(\tilde{K}), \tag{28}$$

is a closed and pointed convex cone in $\mathbb{R}^{n_r - m}$, with non-empty interior.

Proof 1.   (K' is a convex cone) This is immediate from the fact that the image of a convex cone under the linear mapping π is a convex cone as well.
2.   (K' is closed) Let $\{x_n\}$ be a sequence in K' with $x_n \to x$. We want to prove that $x \in K'$. Pick a sequence $\{y_n\}$ in $\mathbb{R}^m$ such that $(x_n, y_n) \in \tilde{K}$. There are two cases to consider.
     Case (i): The sequence $\{y_n\}$ is bounded. In this case, we can pick a converging subsequence and assume wlog that $y_n \to y$ for some y. Then $(x_n, y_n) \to (x, y) \in \tilde{K}$, because $\tilde{K}$ is closed, and hence $x \in K'$.
     Case (ii): The sequence y_n is unbounded. We will show that this cannot happen. In this case, pick a subsequence and wlog assume $|y_n| \to +\infty$. Let $u_n := x_n/|y_n|$. Since the sequence x_n is bounded (because it is convergent), it follows that $u_n \to 0$. Let $v_n = y_n/|y_n|$. These are unit vectors, so pick a subsequence and wlog assume $v_n \to v$, for some unit (and hence nonzero) vector v. So we have that $(u_n, v_n) \to (0, v)$ with $v \neq 0$. Now, $(u_n, v_n) = (1/|y_n|)(x_n, y_n)$ is in $\tilde{K}$ for all n, since $\tilde{K}$ is a cone. It follows that $(0, v) \in \tilde{K}$ because $\tilde{K}$ is closed. On the other hand, $(0, v) \in L$. From (27), it follows that $v = 0$, a contradiction.
3.   (K' is pointed) If not, then there is $x \neq 0$ such that $x \in K' \cap (-K')$. This implies the existence of y_1 and y_2 such that $(x, y_1) \in \tilde{K}$ and $(-x, y_2) \in \tilde{K}$.

Moreover, $y_2 \neq -y_1$ because $\tilde{K}$ is pointed. Since $\tilde{K}$ is a convex cone, it follows that $(x, y_1) + (-x, y_2) = (0, y_1 + y_2) \in \tilde{K}$, where $y_1 + y_2 \neq 0$. We also have that $(0, y_1 + y_2) \in L$, yielding a contradiction because of (27).

4. (K' has non-empty interior) This is immediate from the fact that π is an open map: If U is a non-empty open set in $\tilde{K}$, then its image $\pi(U)$ is a non-empty open set in K', hence K' has non-empty interior. □

Let us now turn to the concept of persistence of a CRN, and review how it can be established using certain graphical tools. We provide only the essential background and statement of the main persistence result, and readers who wish to consult the proofs are referred to Angeli et al. (2007). The set-up in that paper is slightly different than here because reversible reactions are treated as two separate (irreversible) reactions, the forward and the backward reaction. As a consequence, the stoichiometry matrix is extended to a matrix with additional columns, one for each reversible reaction. This is done by inserting a new column right after the column vector that represents the reaction vector associated to every reversible reaction. For a given reversible reaction, the column vector that is inserted is equal to the opposite of the reaction vector of the given reaction. The resulting matrix is called the *extended stoichiometry matrix* and it is denoted by Γ_e. To illustrate this procedure, recall the stoichiometry matrix Γ of Example 2. Then the corresponding Γ_e is given by:

$$
\begin{pmatrix}
-1 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1
\end{pmatrix}
$$

The 2nd, 4th, 7th and 10th columns in Γ_e are the respective opposite of the preceding columns, which are precisely the reaction vectors of one of the four reversible reactions in the CRN. The reaction rate vector $R(S)$ is also extended to the *extended reaction rate vector* $R_e(S)$ to reflect the replacement of each reaction rate of a reversible reaction by two reaction rates. For a reversible reaction with rate $R_i(S) = R_{i,f}(S) - R_{i,b}(S)$ (recall (3)), we replace the ith coordinate $R_i(S)$ of the reaction rate vector $R(S)$ by the pair $R_{i,f}(S), R_{i,b}(S)$.

It is easy to see that with this new notation, the differential equation (4) for the species concentrations, can also be written as follows:

$$
\dot{S} = \Gamma_e R_e(S), \quad S \in \mathbb{R}^{n_s}_{\geq 0}, \tag{29}
$$

Denoting the omega limit set of the solution of (29) (i.e. (4)) starting from S_0 at $t = 0$ by $\omega(S_0)$, we have:
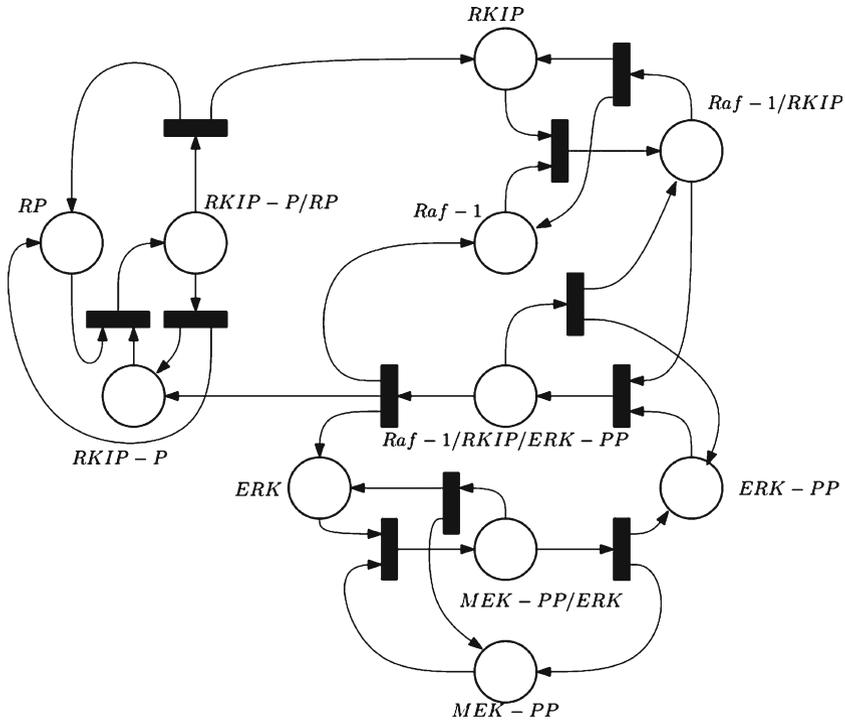
Fig. 9 SR-net of the RKIP network

Definition 8.2 System (29), or equivalently, system (7), and the associated CRN are all said to be persistent if $\omega(S_0) \cap \partial \mathbb{R}^{n_s}_{\geq 0} = \emptyset$, for all $S_0 \in \text{int}(\mathbb{R}^{n_s}_{\geq 0})$.

We now associate to a CRN a bipartite directed graph, called the *species-reaction Petri Net*, or SR-net for short. Mathematically, this is a triple

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

where as before, V_S is a set of species nodes, and V_R the set of reaction nodes, and E is a set of edges as described below. First we denote the set of all nodes by $V := V_R \cup V_S$. Then the edge set $E \subset V \times V$ is defined as follows. If a certain reaction R_i (necessarily irreversible in this context) belongs to the CRN, then there is 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. The set of *input* (*or output*) *reactions* of a nonempty set $\Sigma \subset V_S$ is simply defined as the union of the set of input (output) reactions of every element of Σ.

Definition 8.3 A P-semiflow is any row vector $c \succ 0$ such that $c \, \Gamma_e = 0$. Its support is the set of indices $\{i \in V_S : c_i > 0\}$.
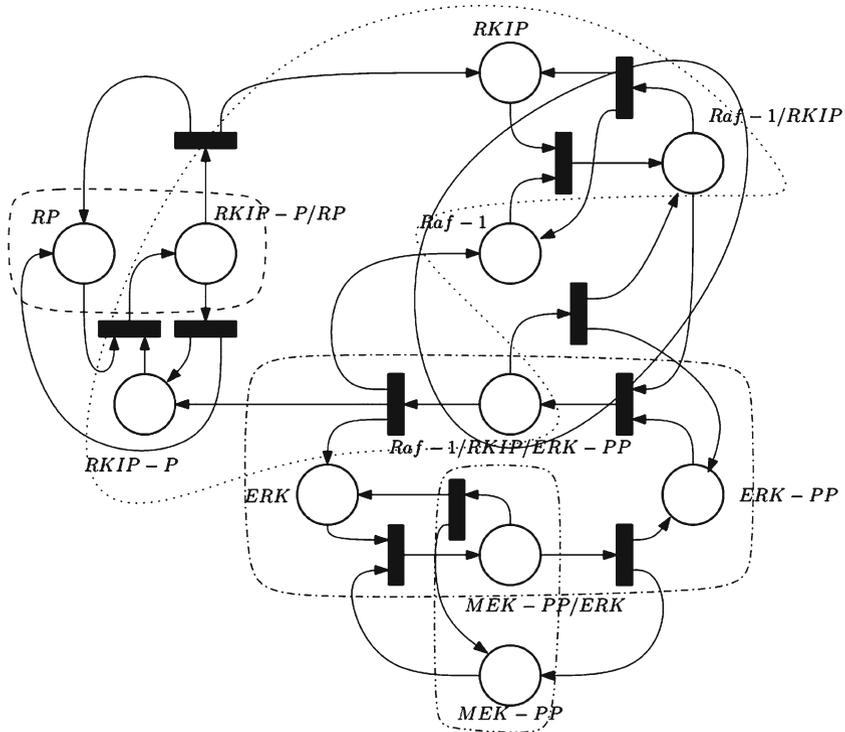
Fig. 10 The five minimal siphons of the RKIP network

Definition 8.4 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 minimal if it does not contain (strictly) any other siphon.

The main persistence result from Angeli et al. (2007) is as follows.

Theorem 3 *Consider a CRN satisfying the following assumptions:*[1]

1. *There is a positive P-semiflow.*
2. *Every minimal siphon of the CRN contains the support of a P-semiflow.*

Then the CRN is persistent.

To illustrate these various concepts, we consider Example 2 and will show that the CRN considered there is persistent. First, we depict the associated SR-net in Fig. 9, and we notice in particular that every reversible reaction in Fig. 6 has now been replaced

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[1] The persistence result in Angeli et al. (2007) requires that all reaction rates are real-analytic functions, but it can be shown that this can be relaxed to demanding that they are only $\mathcal{C}^1$, the assumption made in this paper.

by two reactions. As illustrated in Fig. 10, the RKIP network has five minimal siphons:

$$\mathcal{S}_1 = \{\text{Raf-1, Raf-1/RKIP, Raf-1/RKIP/ERK-PP}\},$$
$$\mathcal{S}_2 = \{\text{ERK-PP, Raf-1/RKIP/ERK-PP, ERK, MEK-PP/ERK}\},$$
$$\mathcal{S}_3 = \{\text{RKIP, Raf-1/RKIP,Raf-1/RKIP/ERK-PP, RKIP-P, RKIP-P/RP}\},$$
$$\mathcal{S}_4 = \{\text{RP, RKIP-P/RP}\}$$
$$\mathcal{S}_5 = \{\text{MEK-PP, MEK-PP/ERK}\}.$$

It is easily verified that the following vectors are P-semiflows:

$$c_1 = \left(1,\, 0,\, 1,\, 0,\, 1,\, 0,\, 0,\, 0,\, 0,\, 0,\, 0\right),$$
$$c_2 = \left(0,\, 0,\, 0,\, 1,\, 1,\, 1,\, 0,\, 0,\, 1,\, 0,\, 0\right),$$
$$c_3 = \left(0,\, 1,\, 1,\, 0,\, 1,\, 0,\, 1,\, 0,\, 0,\, 0,\, 1\right),$$
$$c_4 = \left(0,\, 0,\, 0,\, 0,\, 0,\, 0,\, 0,\, 0,\, 0,\, 1,\, 1\right),$$
$$c_5 = \left(0,\, 0,\, 0,\, 0,\, 0,\, 0,\, 0,\, 1,\, 1,\, 0,\, 0\right).$$

Moreover, for every i, the support of c_i coincides with (the support of) siphon $\mathcal{S}_i$. Notice also that the sum of all vectors c_i is a positive vector, and a P-semiflow. Thus, both assumptions in Theorem 3 are satisfied, and hence the RKIP network is persistent.

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