On the structural monotonicity of chemical reaction networks

David Angeli
Dip. di Sistemi e Informatica, University of Firenze

Patrick De Leenheer
Dep. of Mathematics, University of Florida, Gainesville, FL

Eduardo D. Sontag
Dep. of Mathematics, Rutgers University, Piscataway, NJ

***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 without making assumptions on the structure (e.g., mass-action kinetics) of the dynamical equations involved, and relying only on stoichiometric constraints. The key idea is to find a suitable set of coordinates under which the resulting system is cooperative. As a simple example, the paper shows that a phosphorylation/dephosphorylation process, which is involved in many signaling cascades, has a global stability property.**

I. 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 the understanding of cell functions at the level of chemical interactions. This understanding will have an impact on drug design as well as on therapeutic treatments. While this is a truly formidable task which will likely never be completely accomplished, it is nevertheless apparent that the complexity and high dimensionality of the chemical reaction networks typically found in this context 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 ? A conceptual set of tools in dynamical systems theory, introduced precisely in order to answer questions concerning asymptotic dynamics and their robustness to parameter variations, is based upon the notion of a monotone system. A monotone system is a system whose forward flow preserves some order defined on the state space (precise definitions are given later). Despite the fact that chemical and biological systems (for instance, ecosystem models) were among the most recurrent sources of examples for the rich literature devoted to the subject, a clear connection between chemical reaction networks and 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. In fact, one of the purposes of this paper in to provide easily verifiable conditions. Once monotonicity has been established, one may appeal to the huge body of results in monotone dynamical systems theory to derive non-trivial statements concerning the asymptotic convergence of all (or almost all) solutions to steady state.

The intuitive idea of our construction is as follows. We associate to each chemical reaction network a labeled graph, called the reaction graph, whose vertices are the reactions, and whose edges are labeled either $+$ or $-$. A positive edge is drawn between two reactions if there is any species which is a product in one reaction and a reactant in another; intuitively, the reactions "cooperate" with each other. A negative edge is drawn if there is any species which is a reactant in both reactions (or a product of both reactions); intuitively, the reactions "compete" with each other. Suppose that there are no odd-signed closed paths in the reaction graph. Then, the dynamics on each stoichiometry class can be viewed as a "quotient" dynamics of a monotone system, whose "states" are the reactions, and thus global or almost-global conclusions can be derived by means of the theory of monotone systems. The existence of odd-signed closed paths can be verified, in turn, through a graph-theoretic condition in another graph, the "species-reaction graph," which is canonically associated to each reaction network.

II. BASIC DEFINITIONS

A *chemical reaction network* is just a list of chemical reactions $\mathcal{R}_i$, where the index i takes values in $\mathcal{R} := \{1, 2, \ldots, n_r\}$. 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:

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

where the α_{ij} and β_{ij} are nonnegative integers called the stoichiometry coefficients. Notice that 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. 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 transformations may occur, then, the reaction is reversible and we may sometimes denote it with a double arrow, as in $\leftrightarrow$. Alternatively, we may decide to include its inverse in the chemical reaction network. Throughout this paper we exclude autocatalytic reactions, i.e. reactions (either reversible or not) in which a chemical appears both as a reactant and as a product. More formally, there is no pair $(\alpha_{ij}, \beta_{ij})$ such that $\alpha_{ij}\beta_{ij} > 0$.

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

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

for all $i \in \mathcal{R}$ and all $j \in \mathcal{S}$. This will be later used in order to write down the differential equation associated to the chemical reaction network.

Next we discuss 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 only a function of the concentration of the species taking part to it. This is a natural and fundamental assumption. In order to make this more precise we may define the vector $S = [S_1, S_2, \dots S_{n_s}]'$ of species concentrations and, as a function of it, the vector of reaction rates $R(S) := [R_1(S), R_2(S), \dots R_{n_r}(S)]'$.

In particular, for irreversible reactions, the rate at which R_i 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, for reversible reactions we assume:

$$\frac{\partial R_i(S)}{\partial S_j} = \begin{cases} \geq 0 & \text{if } \alpha_{ij} > 0 \text{ and } \beta_{ij} = 0 \\ \leq 0 & \text{if } \beta_{ij} > 0 \text{ and } \alpha_{ij} = 0 \\ = 0 & \text{if } \alpha_{ij} = 0 \text{ and } \beta_{ij} = 0. \end{cases} \tag{3}$$

This set of assumptions is very natural and amounts to asking that reaction rates increase when reactant concentration is higher, and the same applies to the inverse reaction whenever product concentration is higher (provided that the inverse reaction takes place at all). Notice that we did not assume any specific expression for the reaction rates; in particular we do not need to assume mass-action or any other kind of kinetics. For technical reasons, related to certain results on monotone dynamical systems, we also need the monotonicity property in (2) and (3) to hold strictly for S in the interior of $\mathbb{R}^{n_s}_{\geq 0}$ (this is always the case, for instance, if mass-action kinetics are assumed).

We also assume that, whenever any of the reactants of a given irreversible reaction is 0, then, the corresponding reaction does not take place, viz. the reaction rate is 0. So, if $S_{i_1}, \dots, S_{i_N}$ are the reactants of reaction j, $R_j(S) = 0$ for all S such that $[S_{i_1}, \dots, S_{i_N}] \in \partial\mathbb{R}^N_{\geq 0}$. Similarly, for reversible reactions, $R_j(S) \leq 0$ for all S such that $[S_{i_1}, \dots, S_{i_N}] \in \partial\mathbb{R}^N_{\geq 0}$; symmetrically, if $S_{i_1}, \dots, S_{i_N}$ are the products of reaction j, $R_j(S) \geq 0$ for all S such that $[S_{i_1}, \dots, S_{i_N}] \in \partial\mathbb{R}^N_{\geq 0}$.

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

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

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

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

is forward invariant for (4). We call $\mathcal{C}_{S_0}$ the stoichiometry class associated to the reference concentration S_0 and assume that all stoichiometry classes are compact sets. This implies in particular that all solutions of (4) are bounded. A basic question, one which is the main focus of this paper, is what happens to solutions in each stoichiometry class.

III. PROBLEM FORMULATION

In order to state precisely the problem of interest it is useful to recall a few definitions concerning the theory of monotone dynamical systems. We consider autonomous nonlinear systems of the form $\dot{x} = f(x)$, where $f : \mathbb{R}^n \to \mathbb{R}^n$ 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 $\succeq$ be 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.

Typically such partial orders will be defined by first introducing a closed pointed cone $K \subset \mathbb{R}^n$ of “positive vectors” which is the closure of its interior, and calling $x_1 \succeq x_2$ iff $x_1 - x_2 \in K$. Geometric properties of the cone 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*. Stronger monotonicity notions are also of interest and are obtained by defining strict orders as follows: $x_1 \succ x_2$ iff $x_1 \succeq x_2$ and $x_1 \neq x_2$, or the even stronger notion $x_1 \gg x_2$ iff $x_1 - x_2 \in \mathrm{int}(K)$. 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 is particularly simple for $\mathcal{C}^1$ vector fields, $\dot{x} = f(x)$. 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 that associating to $Df(x)$ a directed graph with signed edges (corresponding to the signs of $Df(x)$), each undirected loop of the graph contains an even number of negative edges. Due to the simplicity and physical appeal of this property, as well as the important implications in terms of asymptotic dynamics which are later summarized, we are interested in providing sufficient conditions for (4) to be cooperative in suitably chosen coordinates. To the best of our knowledge this problem was first posed in the monograph [9], and some algebraic conditions for its solutions proposed. If this change of coordinates and/or dimensionality reduction exists, then we say that the chemical reaction network is **structurally monotone**. The word "structurally" emphasizes that monotonicity only depends upon the qualitative information provided by 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. It is worth pointing out that the change of coordinates we are after is 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 upon the parameters of $R(S)$. This is interesting, for instance, if mass-action kinetics are adopted and all the possible choices of $R(S)$ are then finitely parametrized by a certain number of positive kinetic constants. The results discussed extend those of [3] which were limited to reactions with certain tree topology.

IV. GRAPH THEORETICAL PRELIMINARIES

It is useful for the subsequent developments to explicitly illustrate the graphical representations of a CRN that will be used throughout the rest of the paper. As a matter of fact, most assumptions in the results to follow can be easily understood in terms of graph theoretic properties.

We associate to a CRN a "bipartite undirected $\{+,-\}$-labeled graph,", i.e. a graph having two types of nodes and two types of edges, called the *species-reaction graph* of a chemical reaction network, 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 belongs to the network:

$$\sum_{j \in \mathcal{S}} \alpha_{ij} S_j \quad \leftrightarrows \quad \sum_{j \in \mathcal{S}} \beta_{ij} S_j \,, \qquad (5)$$

we draw a positive edge between $S_j \in V_S$ and $R_i \in V_R$ for all S_js such that $\alpha_{ij} > 0$; 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 $\beta_{ij} > 0$. Notice that in (5) we decided on purpose to leave the direction of the chemical reaction unspecified. In fact, the results to follow, will not depend on the specific orientation that one may choose for each of the reactions in the network and only rely on the fact that all of the products (and similarly all of the reactants) of a given reaction are linked to it through edges of the same kind (either positive or negative) while, at the same time, edges linking reactants and products of a same reaction should have opposite signs. This kind of representation is entirely analogous to a Petri Net, at least from a formal point of view.

Next, we illustrate a procedure for deriving from the SR-graph a *reaction graph* (R-graph for short), which only involves reactions, yet carries meaningful sign information on edges. 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.

Definition We say that a graph (and in particular the R-graph) is ***sign consistent*** if any cycle includes an even number of negative edges.

Alternatively, sign-consistence may be more easily checked by assigning to each node a $+$ or $-$ label and verifying that every edge has a sign equal to the product of the signs of the nodes it is attached to. It is well known that a $\mathcal{C}^1$ dynamical system with sign-definite Jacobian is monotone with respect to the partial order induced by some orthant if and only if considering the sign of the Jacobian as the incidence matrix of a graph with signed edges, the corresponding graph is sign-consistent. This is how the property will be used in the following Sections. For the time being we are interested in purely graph theoretical results which will help us establishing whether sign-consistency holds for the reaction graph by checking conditions expressed in terms of the SR-graph. A similar procedure can be adopted to define the *species graph* (S-graph for short) associated to the network. 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. We define sign-consistent S-graphs in a manner analogous as done for R-graphs.

In order to state the main results for this section, we need the following definitions:

Definition Let L be a simple loop in the SR-graph viz. a path whose first and last node coincide and with the property that no node and no edge is repeated twice. We say that L is an **e-loop** if letting λ be half of its length (viz. the number of reactions included in the loop) and σ the product of the signs of all of its edges, it holds that $(-1)^\lambda = \sigma$. Otherwise, we say that L is an **o-loop**.

This definition is not new, and it plays a major role also in the analysis of multistability for chemical reaction networks with mass-action kinetics using "Advanced Deficiency Theory" ([5]). Its meaning will be clearer thanks to the following Lemma:

Lemma (e-loops characterization) The following facts are equivalent for a given 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. Let $E_1, E_2, \dots E_n$ be the ordered sequence of edges comprised in the loop. Let $\sigma(E_i)$ be equal to $+1$ if $E_i \in E_+$ and -1 if $E_i \in E_-$. Clearly n is an even number and we may let $\lambda = n/2$. We have obviously:

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

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

The following Proposition will be used in the subsequent developments:

Proposition 1 The R-graph (respectively the S-Graph) is **sign-consistent** if and only if the following two conditions are met:

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

Proof of Proposition 1. We show first the sufficient 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 only need to show that each of its simple loops contains an even number of negative edges. This is trivially true for loops of length 2, by virtue of the e-loop condition. Let L be a simple loop in G_R of length 3 or higher; we may lift the loop in G_R to a loop $\tilde{L}$ in G by following any length-2 path joining consecutive reactions in L (the lifted loop might not be unique) Moreover, by assumption 2, the lifted loop $\tilde{L}$ will be simple (in fact no reaction can be repeated twice, otherwise this would violate L being simple, and this in turn yields no species can be repeated twice for otherwise it would be connected to at least 3 reactions; hence no edge is repeated twice). As a consequence of 1., $\tilde{L}$ is an e-loop and hence, by virtue of the Lemma on e-loops, 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 corresponds to negative edges in G_R, we have that L contains exactly an even number of negative edges; this completes the sufficient part of the proof.

Conversely, assume that either condition 1. or 2. is violated. In particular, if condition 1. is violated, this means that there exist simple o-loops in the SR-graph G. Projecting them down in G_R, yields a simple loop in G_R with an odd number of negative edges, and therefore violates sign-consistence of G_R. If condition 2. is violated instead, there exists an element S_i which is linked to more than two reactions; let without loss of generality identify three of them as R_1, R_2 and R_3. Consider the 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 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 rule used to compute signs of an edge in G_R on the basis of the corresponding signs in G, it follows that the sign of L can be computed according to $(-1)^3 \cdot \text{sign}(\tilde{L})$. 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 necessarily contains an odd number of negative edges, which violates sign consistence.

V. ANALYSIS IN REACTION COORDINATES

A possible approach to investigate monotonicity of the flow is fulfilled consists in using "reaction" coordinates, instead of traditional species coordinates. In particular, choosing an arbitrary representative S_0 of a given stoichiometry class, the system in these coordinates may be expressed as follows:

$$\dot{x}(t) = R(S_0 + \Gamma x(t)), \;\; x \in \{x \in \mathbb{R}^{n_r} \,|\, S_0 + \Gamma x \geq 0\}, \tag{6}$$

where x_i ($i \in \mathcal{R}$) denotes the extent of the i-th reaction. Notice that, solutions of (4) are obtained from solutions of (6) just by letting $S(t) = S_0 + \Gamma x(t)$. The main results for this Section are established below:

Theorem 2. The system in (6) is orthant-cooperative if and only if the R-graph is sign-consistent.

Proof. In order to prove the result it is enough to show that the sign rule adopted in the definition of edges of the R-graph, is coincident with the sign pattern obtained computing the Jacobian in (6). The Jacobian matrix of (6) reads as follows $\frac{\partial \dot{x}}{\partial x} = DR \cdot \Gamma$. This is an $n_r \times n_r$ matrix, and each x_i can be associated to a chemical reaction. For all $l \neq m \in \mathcal{R}$ we have $\frac{\partial \dot{x}_m}{\partial x_l} = 0$ if and only if the two chemical reactions do not have species in common. Computation of the sign associated to the edge joining R_l to R_m can be performed according to:

$$[DR \cdot \Gamma]_{lm} = \sum_{j \in \mathcal{S}} [DR]_{lj} [\Gamma]_{jm}.$$

Since $[DR]_{lj} \simeq -[\Gamma]_{jl}$, the sign associated to an edge can be evaluated according to:

$$[DR \cdot \Gamma]_{lm} \simeq - \sum_{j \in \mathcal{S}} [\Gamma]_{jl} [\Gamma]_{jm}.$$

Notice that this is precisely the formula used order to define the sign of edges in the R-graph. Hence, monotonicity holds if and only if an even number of inhibitions are met along any cycle of the Jacobian; accordingly, this is true if and only if the R-graph is sign-consistent.

As pointed out earlier, the change of 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 monotone dynamical system obtained in the new coordinates. The main technical difficulty in this respect appears to be the lack of compactness of the state-space of (6). In particular, even if every solution of (4) is by construction confined to a compact set, namely the stoichiometry class of the considered initial condition, the corresponding solution in reaction coordinates need not be bounded.

Our subsequent analysis aims at establishing convergence, based on monotonicity of (6). Two possibilities appear of interest and will be treated with different techniques; in particular, letting K, denote the orthant associated to the partial order preserved by (6),

- $\text{Ker}[\Gamma] \cap \text{int}(K) \neq \emptyset$
- $\text{Ker}[\Gamma] \cap K = \{0\}$.

The intermediate case, in which the Kernel of Γ only intersects with the boundary of K appears to be more challenging, but seems to represent a rather degenerate case, not particularly frequent in applications. Case 1, will be treated according to a recently obtained result [1], whose formulation was precisely motivated by problems arising in the context of chemical kinetics. It is a global convergence result which exploits strong monotonicity and translation invariance in order to build a suitable Lyapunov function for the system. Case 2 on the other hand will be treated by exploiting Hirsch's generic convergence Theorem [7]; not for the system in reaction coordinates as such, which need not have bounded solutions, but for a suitable quotient system.

Theorem 3. Let the system in (6) be strongly monotone with respect to the partial order induced by some orthant K. Then, if Case 1. holds, all solutions of (4) converge to an equilibrium, moreover this equilibrium is unique within each stoichiometry class. If Case 2. holds, then almost all solutions of (4) converge to equilibria, except possibly those corresponding to a zero-measure set of initial conditions.

Proof. Assume that Case 1 holds. In this case it can be shown [1] that $\text{Ker}(\Gamma)$ is 1-dimensional, and thus $\text{Ker}[\Gamma] := \text{span}(v)$ for some unit vector v belonging to $\text{int}(K)$. Then all the assumptions of the Main Result in [1] hold for system (6). Denote the projection $\pi_v(x) := x - (v'x)v$ of x on the linear space $v^{\perp}$. By the Main Result in [1] we conclude 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 $t \to +\infty$, which is therefore the unique globally attractive equilibrium contained in the stoichiometry class of S_0.

In Case 2, we consider the quotient flow $\varphi(t, [x(0)]) := [x(t)]$, which is obtained by considering the equivalence relation

$$x_1 \sim x_2 \text{ iff } \Gamma(x_1 - x_2) = 0.$$

This dynamical system has a state-space which can be canonically identified with the stoichiometry class of S_0 (it is therefore a compact space), and it is strongly monotone with respect to the quotient orders defined by:

$$[x_1] \succeq (\gg)[x_2] \text{ iff } \forall\, z_1 \in [x_1],\; \exists\, z_2 \in [x_2] : z_1 \succeq (\gg) z_2 \tag{7}$$

Transitivity and reflexivity of the partial order defined in (7) are easy to prove. We show next that also anti-symmetry holds. In fact, by the transversality between K and $\text{Ker}[\Gamma]$ we have: $[x_1] \succeq [x_2]$ and $[x_2] \succeq [x_1]$ implies $x_1 - x_2 + \gamma_0^I \in K$ and $x_2 - x_1 + \gamma_0^{II} \in K$ for some $\gamma_0^I, \gamma_0^{II}$ belonging to $\text{Ker}[\Gamma]$. Hence, taking sums and exploiting convexity of K we obtain $\gamma_0^I + \gamma_0^I I \in K$ and therefore $\gamma_0^I + \gamma_0^{II} = 0$. Hence, $x_1 - x_2 + \gamma_0^I \in K \cap -K = \{0\}$. This indeed shows that $[x_1] = [x_2]$ as desired. Then, by Hirsch's generic convergence Theorem [7] we conclude that for almost all initial conditions in the stoichiometry class of S_0, solutions converge to a single equilibrium (not necessarily unique).

Example: 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,

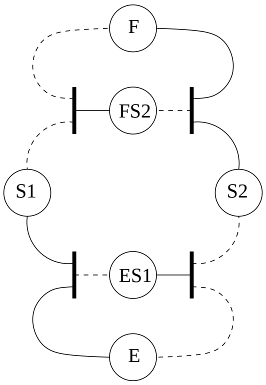

Fig. 1. SR-graph associated to a simple enzymatic reaction: positive (solid) and negative (dashed) edges

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, compounds corresponding to the binding of the enzyme and substrate. The simplest such reaction is modeled by the following reaction network:

$$\begin{array}{ccccc} S1+E & \leftrightarrow & ES1 & \rightarrow & S2+E \\ S2+F & \leftrightarrow & FS2 & \rightarrow & S1+F \end{array}, \tag{8}$$

The SR-graph of this network is shown in Fig. 1. This can be associated to a 6-dimensional system of differential equations (adopting species coordinates). We point out that the above reaction is not *weakly reversible* (using the language of [6]). This is because the following graph of complexes associated to the network:

$$\begin{array}{ccccc} C_1 & \leftrightarrow & C_2 & \rightarrow & C_3 \\ C_4 & \leftrightarrow & C_5 & \rightarrow & C_6 \end{array}, \tag{9}$$

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 [6] 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 loops are e-loops and by virtue of Theorem 2 the system is therefore orthant-cooperative. (As a side remark notice that the S-graph is not sign-consistent, 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, there exists a non-trivial kernel of the stoichiometry matrix $\Gamma = \text{span}[1,1,1,1]'$. With the orientation of the reactions chosen in Figure 1, the system turns out to be cooperative in reaction coordinates, so that $[1,1,1,1]$ belongs to the interior of the positive orthant. Strong monotonicity can be proved by checking irreducibility of the Jacobian matrix in reaction coordinates (by Kamke's condition [8]) (at least on the interior of our state space, namely $X := \{x \in \mathbb{R}^4 : S_0 + \Gamma x \in \text{int}(\mathbb{R}^6_{\geq 0})\}$. It can be proved [2] that ω-limit sets for initial conditions in X are again contained in X. Hence, case 1. holds and this implies global convergence to a unique equilibrium, in each stoichiometry class.

VI. CONCLUSIONS

We have presented a new method, based on graph-topological conditions, for analyzing monotonicity of chemical reaction networks with respect to suitable coordinates. It is sometimes convenient to adopt reaction coordinates rather than the usual concentration of species as a state for our system. In this way, monotonicity with respect to an orthant is easily checked and convergence analysis can be easily carried out by using Hirsch's "generic convergence theorem" or a recently proved result [1] on convergence for systems which are shift-invariant with respect to a positive translation vector. The theory is illustrated through a non-trivial example arising in chemical kinetics.

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