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# Intermediates, Catalysts, Persistence, and Boundary Steady States

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## Abstract

For dynamical systems arising from chemical reaction networks, persistence is the property that each species concentration remains positively bounded away from zero, as long as species concentrations were all positive in the beginning. We describe two graphical procedures for simplifying reaction networks without breaking known necessary or sufficient conditions for persistence, by iteratively removing so-called intermediates and catalysts from the network. The procedures are easy to apply and, in many cases, lead to highly simplified network structures, such as monomolecular networks. For specific classes of reaction networks, we show that these conditions are equivalent to one another and, thus, necessary and sufficient for persistence. Furthermore, they can also be characterized by easily checkable strong connectivity properties of the underlying graph. In particular, this is the case for (conservative) monomolecular networks, as well as cascades of a large class of post-translational modification systems (of which the MAPK cascade and the  $n$ -site futile cycle are prominent examples). Since the aforementioned sufficient conditions for persistence preclude the existence of boundary steady states, our method also provides a graphical tool to check for that.

**Keywords:** Reaction Network Theory · Model Reduction · Persistence · Boundary Steady States · Intermediates · Catalysts · Post-Translational Modification

**MSC Codes:** 34C99 80A30 92C42

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## 1 Introduction

Since the seminal works of Horn, Jackson and Feinberg in the 70's ([8, 13, 14], and references therein), chemical reaction network theory (CRNT) has provided a fruitful framework to study the dynamical systems describing how the concentrations of the involved chemical species evolve over time. Of great interest has been the long-term behavior of these systems, for example, whether they may exhibit oscillatory behavior [9], local asymptotic stability [1, 2, 9, 22], or persistence [3, 5, 7, 11].

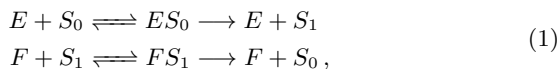
The mathematical concept of persistence models the property that every species concentration remains above a certain threshold, as long as there were positive amounts of each species in the beginning. Besides its intrinsic relevance to the applied sciences, most notably in population biology [21], the concept of persistence has also drawn attention in the context of CRNT on account of its connection with the global attractor conjecture [12].

It can be difficult to determine if the solutions to a system of ordinary differential equations are persistent case by case. A recent contribution was given by Angeli, De Leenheer and Sontag [3], who provided two checkable conditions, one sufficient, and the other one necessary, for the persistence of conservative reaction networks. Their criteria work under fairly general assumptions on the reaction kinetics. But perhaps unsurprisingly, reaction networks become more difficult to analyze the larger they are, often times exponentially so [6]. Thus, criteria for persistence in terms of a simplified "skeleton" of the given network are desirable. More importantly, simplified versions retaining the properties of interest of the original network may also give insight into the underlying biological mechanism, suggesting what might be the leading causes of the presence (or absence) of said properties. For example, for the class of post-translational modification (PTM) systems of Thomson and Gunawardena [23], persistence can be characterized in terms of strong connectedness of the underlying substrate network, as we shall see below.

That is the motivation for our model simplification approach to study persistence. Model reduction has been yet another active line of research in CRNT [10]. In this work we describe a process through which one may

simplify a reaction network by iteratively removing “intermediates” [10], and/or “catalysts.” Intuitively speaking, an intermediate is an indivisible, transient species appearing in the middle of a chain of reactions. Catalysts, on the other hand, are reactants which remain unchanged in every reaction, except possibly for interactions exclusively with other catalysts. Our main contribution in this paper is to show that the removal of intermediates and/or catalysts does not break the conditions for persistence given in [3].

As shown by various examples throughout this work, all taken from the systems biology literature, reaction networks naturally exhibit many intermediate complexes and catalysts. So, their removal will often reduce dramatically the size of the network, facilitating its inspection for persistence. To illustrate this, consider a simple one-site phosphorylation process, which can be modeled by the reaction network



where  $S_0, S_1$  represent, respectively, the dephosphorylated and phosphorylated forms of a substrate,  $E$  acts as a kinase,  $F$  acts as a phosphatase, and  $ES_0$  and  $FS_1$  are intermediate steps in the phosphorylation/dephosphorylation mechanism. Using our results, one may show that necessary or sufficient conditions for persistence for (1) are a consequence of the same necessary or sufficient conditions for its much simpler underlying substrate model,



For monomolecular models such as (2), the necessary or sufficient conditions for persistence are actually equivalent, and, furthermore, characterized by the strong connectedness of each connected component. In fact, (1) will turn out to be a special case of PTM system. As mentioned earlier, we will see how persistence for systems like those can also be characterized by necessary and sufficient conditions in terms of strong connectedness of the connected components of their underlying substrate networks.

We emphasize that iteratively removing intermediates and catalysts—and, if eventually obtaining a monomolecular network, then checking it for strong connectedness of its connected components—is essentially a graphical procedure.

This paper is organized as follows. In Section 2, we review the basic formalism of reaction networks. We present the conditions for persistence in [3] in the form we shall use in this work, and discuss their relationship with boundary steady states. A few trivial but notable examples we shall refer to several times throughout the work are given, and persistence is characterized for monomolecular networks in terms of strong connectedness of its connected components. In Section 3, we define the concepts of intermediates and catalysts. We describe the networks obtained from their removal, and state our main results (Theorems 30 and 31), concerning how these operations do not break the aforementioned conditions for persistence. Some biologically relevant examples are presented in Section 4, the most important of which being cascades of a class of post-translational modification systems. In Section 5 we return to our main result, giving the details of the proof.

## 2 Reaction Networks

In what follows we denote the set of nonnegative real (respectively, integer) numbers by  $\mathbb{R}_{\geq 0}$  (respectively,  $\mathbb{Z}_{\geq 0}$ ), and denote the set of strictly positive real (respectively, integer) numbers by  $\mathbb{R}_{> 0}$  (respectively,  $\mathbb{Z}_{> 0}$ ). Given  $x \in \mathbb{R}^n$ , for some  $n \in \mathbb{Z}_{> 0}$ , we write  $x \geq 0$  to mean that  $x \in \mathbb{R}_{\geq 0}^n$ , that is, each coordinate of  $x$  is nonnegative. We write  $x > 0$  to mean that  $x \geq 0$ , and at least one coordinate of  $x$  is positive, and write  $x \gg 0$  to mean that  $x \in \mathbb{R}_{> 0}^n$ , in other words, each coordinate of  $x$  is strictly positive. For any finite set  $X$ , the notation  $|X|$  represents the number of elements of  $X$ . Given  $n \in \mathbb{Z}_{> 0}$ , we write  $[n] := \{1, \dots, n\}$ . By convention  $[0] := \emptyset$ .

### 2.1 Basic Formalism

In this work we take the approach of defining reaction networks from their reaction graphs. Thus, a *reaction network* is an ordered triple  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  in which  $\mathcal{S}$  is a finite, possibly empty set,  $\mathcal{C}$  is a finite subset of  $\mathbb{R}_{\geq 0}^n$ , where  $n := |\mathcal{S}|$ , and  $(\mathcal{C}, \mathcal{R})$  is a digraph with no self-loops. The set  $\mathcal{S}$  is called the *species* set of the reaction network. Its elements are tacitly assumed to be ordered a priori in some fixed way, say,

$$\mathcal{S} = \{S_1, \dots, S_n\}.$$

We identify the elements  $(\alpha_1, \dots, \alpha_n)$  of  $\mathcal{C}$ , called the *complexes* of the reaction network, with the formal linear combinations of species

$$\alpha_1 S_1 + \dots + \alpha_n S_n.$$

The digraph  $(\mathcal{C}, \mathcal{R})$  is called the *reaction graph* of  $G$ , and its edges are referred to as the *reactions* of the network. We further assume that each complex takes part in at least one reaction, and that each species is part of at least one complex. Formally, this means that each vertex of  $(\mathcal{C}, \mathcal{R})$  has indegree or outdegree at least one, and that for each  $i \in [n]$ , there exists  $(\alpha_1, \dots, \alpha_n) \in \mathcal{C}$  such that  $\alpha_i > 0$ . It follows that  $\mathcal{S} = \emptyset$  if, and only if  $\mathcal{C} = \emptyset$  or  $\mathcal{R} = \emptyset$ . This is referred to as the *empty reaction network*.

The reactions are also tacitly assumed to be ordered in some way fixed a priori, say,

$$\mathcal{R} = \{R_1, \dots, R_m\},$$

where  $m := |\mathcal{R}|$ . We typically express the reaction

$$R_j = ((\alpha_{1j}, \dots, \alpha_{nj}), (\alpha'_{1j}, \dots, \alpha'_{nj}))$$

as

$$R_j: \sum_{i=1}^n \alpha_{ij} S_i \longrightarrow \sum_{i=1}^n \alpha'_{ij} S_i, \quad j = 1, \dots, m.$$

The complex on the lefthand side is referred to as the *reactant* of the reaction, while the complex on the righthand side is referred to as its *product*. The species  $S_i$  such that  $\alpha_{ij} > 0$  are, accordingly, called the *reactants* of  $R_j$ , while the species  $S_i$  for which  $\alpha'_{ij} > 0$  are called the *products* of the reaction. With the above notation, we may also define the  $n \times m$  matrix  $N$ ,

$$N_{ij} := \alpha'_{ij} - \alpha_{ij}, \quad i = 1, \dots, n, \quad j = 1, \dots, m,$$

known as the *stoichiometric matrix* of the network. The column-space of  $N$ , which is a subset of  $\mathbb{R}^n$ , is called the *stoichiometric subspace* of  $G$ , and denoted by  $\Gamma$ . We denote

$$Q_j := \{i \in [n] \mid \alpha_{ij} > 0\}, \quad j = 1, \dots, m;$$

in other terms,  $Q_j$  is the subset of indices corresponding to the reactants of  $R_j$ .

The system of differential equations governing the evolution of the concentrations of the species of the network is given by

$$\frac{ds}{dt} = Nr(s(t)), \quad t \geq 0, \quad s \geq 0, \quad (3)$$

where  $r: \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}_{\geq 0}^m$  is a vector-valued function modeling the kinetic rates of each reaction as functions of the reactant species, henceforth referred to simply as the *vector of reaction rates*. We shall assume throughout this work that the vector of reaction rates satisfies the following hypotheses:

(r1)  $r = (r_1, \dots, r_m): \mathcal{O} \rightarrow \mathbb{R}^m$  is continuously differentiable on a neighborhood  $\mathcal{O}$  of  $\mathbb{R}_{\geq 0}^n$ , and  $r(s) \geq 0$  for every  $s \geq 0$ .

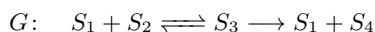
(r2) For each  $j \in [m]$ , and for each  $s = (s_1, \dots, s_n) \in \mathbb{R}_{\geq 0}^n$ ,

$$r_j(s) = 0 \quad \Leftrightarrow \quad s_i = 0 \quad \text{for some } i \in Q_j.$$

(r3) The flow of (3) is forward-complete; in other words, for any initial state, the (unique) maximal solution of the corresponding initial value problem in (3) is defined for all  $t \geq 0$ .

We note that (r1)–(r3) are satisfied under the most common kinetic assumptions in the literature, namely, mass-action, or more general power-law kinetics, Michaelis-Menten kinetics, or Hill kinetics, as well as combinations of these [4, pages 585–586]. We also note that it follows from (r2) and [20, Theorem 5.6] that  $\mathbb{R}_{\geq 0}^n$  is forward invariant for the flow of (3).

We will often give a reaction network by simply listing all the reactions in the network. When we do so, the sets of species and complexes will be tacitly implied. For instance,



is the reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  obtained by setting

$$\mathcal{S} := \{S_1, S_2, S_3, S_4\},$$

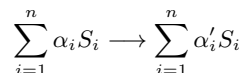
$$\mathcal{C} := \{S_1 + S_2, S_3, S_1 + S_4\}$$

and

$$\mathcal{R} := \{S_1 + S_2 \rightarrow S_3, S_3 \rightarrow S_1 + S_3, S_3 \rightarrow S_1 + S_4\}$$

in the formalism above.

**Definition 1** (Implied Subnetworks). Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network, and  $\mathcal{E} \subseteq \mathcal{S}$  be a subset of species. We define the *subnetwork implied by  $\mathcal{E}$*  as the network  $G_{\mathcal{E}} = (\mathcal{S}_{\mathcal{E}}, \mathcal{C}_{\mathcal{E}}, \mathcal{R}_{\mathcal{E}})$  consisting of reactions of  $G$  which involve exclusively species in  $\mathcal{E}$ . More precisely,  $\mathcal{R}_{\mathcal{E}} \subseteq \mathcal{R}$  is the subset of reactions



such that  $\alpha_i > 0$  or  $\alpha'_i > 0$  implies  $S_i \in \mathcal{E}$ . We then define  $\mathcal{C}_{\mathcal{E}} \subseteq \mathcal{C}$  to be the subset of complexes that appear as reactant or product of some reaction in  $\mathcal{R}_{\mathcal{E}}$ . Finally,  $\mathcal{S}_{\mathcal{E}} \subseteq \mathcal{S}$  is defined as the subset of species which are part of some complex in  $\mathcal{C}_{\mathcal{E}}$ .  $\triangle$

Note that it is not always true that  $\mathcal{S}_{\mathcal{E}} = \mathcal{E}$ .

## 2.2 Siphons and P-Semiflows

A few more concepts pertaining to reaction networks are needed. The terminology below is adapted from Petri net theory. See [3] for the context. But since no results from Petri net theory itself are needed, we chose to define these concepts as directly pertaining to their respective reaction networks, rather than the Petri nets associated with them.

Given a vector  $\omega = (\omega_1, \dots, \omega_n) \in \mathbb{R}_{\geq 0}^n$  associated with the species set  $\mathcal{S}$  of a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ , its *support* is defined to be the subset of species  $\text{supp } \omega := \{S_i \in \mathcal{S} \mid \omega_i > 0\}$ . Similarly, given a vector  $v = (v_1, \dots, v_m) \in \mathbb{R}_{\geq 0}^m$  associated with the reaction set  $\mathcal{R}$  of  $G$ , its *support* is defined to be the subset of reactions  $\text{supp } v := \{R_j \in \mathcal{R} \mid v_j > 0\}$ . Although we use the same notation in both cases, it will be clear from the context whether the underlying vector is associated with the species or the reaction set.

**Definition 2** (P-Semiflows). A *P-semiflow* or *positive conservation law* of a reaction network is any nonzero vector  $\omega \in \mathbb{R}_{\geq 0}^n$  such that  $\omega^T N = 0$ . We say that a reaction network is *conservative* if it has a strictly positive P-semiflow  $\omega \gg 0$ , that is,  $\text{supp } \omega = \mathcal{S}$ .  $\triangle$

**Definition 3** (Stoichiometric Compatibility Classes). The *stoichiometric subspace*  $S$  of a reaction network  $G$  is the left-kernel of  $N$ ,

$$S := \{c \in \mathbb{R}^n \mid c^T N = 0\}.$$

The *stoichiometric compatibility classes* of  $G$  are the subsets  $(s_0 + S) \cap \mathbb{R}_{\geq 0}^n$ ,  $s_0 \in \mathbb{R}_{\geq 0}^n$ .  $\triangle$

**Definition 4** (T-Semiflows). A *T-semiflow* of a reaction network is any nonzero vector  $v \in \mathbb{R}_{\geq 0}^m$  such that  $Nv = 0$ . We say that a reaction network is *consistent* if it has a strictly positive T-semiflow  $v \gg 0$ , that is, if  $\text{supp } v = \mathcal{R}$ .  $\triangle$

**Definition 5** (Siphons). A nonempty subset of species  $\Sigma \subseteq \mathcal{S}$  is called a *siphon* if every reaction which has a product in  $\Sigma$  also has a reactant in  $\Sigma$ . A siphon is said to be *minimal* if it does not properly contain any other siphon.  $\triangle$

*Example 6* (Single Phosphorylation Mechanism). The minimal siphons of the single phosphorylation mechanism from the Introduction (1) are  $\{E\}$ ,  $\{F\}$ , and  $\{S_0, S_1, ES_0, FS_1\}$ .  $\diamond$

*Remark 7.* Let  $y \rightarrow y_1 \rightarrow \dots \rightarrow y_k \rightarrow y'$  be a reaction path in a reaction network  $G$ , and suppose  $\Sigma$  is a siphon containing some species  $S'$  which is part of  $y'$ . Then  $\Sigma$  must contain some species  $S$  which is part of  $y$ .  $\square$

**Definition 8** (Siphon/P-Semiflow Property). A reaction network is said to have the *siphon/P-semiflow property*, or satisfy the *siphon/P-semiflow condition*, if every siphon contains the support of a P-semiflow.  $\triangle$

Note that, since every siphon is either itself minimal, or else contain a minimal siphon, we need only check the condition holds for minimal siphons.

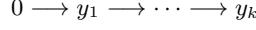
We give a couple more trivial examples. Besides further illustrating the scope of the concepts just introduced, they will be used several times in the analysis of more elaborate examples further down.

*Example 9* (Empty Networks). Our formalism allows for reaction networks to be empty. Any such network is vacuously conservative, consistent, and has the siphon/P-semiflow property.  $\diamond$

*Example 10 (Inflows).* Consider a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ , and suppose that



Then none of the species in  $\{S_i \in \mathcal{S}; \alpha_i > 0\}$  can belong to a siphon of  $G$ . Consequently, if one can find a chain of reactions



in  $G$ , then indeed none of the species which are a part of any of the complexes  $y_1, \dots, y_k$  can belong to a siphon. This observation may drastically reduce the number of species one is concerned about in checking the siphon/P-semiflow property.

In particular, if  $G$  is such that  $0 \rightarrow S \in \mathcal{R}$  for each  $S \in \mathcal{S}$ , then  $G$  has no siphons. In this case, the siphon/P-semiflow is vacuously satisfied.  $\diamond$

### 2.3 Persistence and Boundary Steady States

Intuitively, persistence (of a reaction network) is the property that no species concentration goes below a certain threshold as the system evolves, as long as they were initially all positive. This threshold may depend on the initial conditions though. In order to formulate this more precisely, let  $\sigma: \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}_{\geq 0}^n$  be the flow of (3). In other words, for each initial state  $s_0 \in \mathbb{R}_{\geq 0}^n$ ,  $\sigma(\cdot, s_0): \mathbb{R}_{\geq 0} \rightarrow \mathbb{R}_{\geq 0}^n$  is the unique, global solution of (3). The solution is unique in virtue of (r1), and defined for all  $t \geq 0$  on account of (r3).

**Definition 11 (Persistence).** A reaction network (3) is said to be *persistent* if

$$\liminf_{t \rightarrow \infty} \sigma_i(t, s_0) > 0, \quad \forall i \in [n], \quad (4)$$

for every initial state  $s_0 \gg 0$ .  $\triangle$

We also introduce a weaker notion of persistence. First, recall that, for each  $s_0 \geq 0$ , the  $\omega$ -limit set of  $s_0$  is the set

$$\omega(s_0) := \bigcap_{\tau \geq 0} \overline{\bigcup_{t \geq \tau} \{\sigma(t, s_0)\}}.$$

Note that  $s \in \omega(s_0)$  if, and only if there exists a sequence  $(t_k)_{k \in \mathbb{N}}$  going to infinity in  $\mathbb{R}_{\geq 0}$  such that

$$\lim_{k \rightarrow \infty} \sigma(t_k, s_0) = s.$$

We denote the boundary of the nonnegative orthant by

$$\partial \mathbb{R}_{\geq 0}^n := \{(x_1, \dots, x_n) \in \mathbb{R}_{\geq 0}^n \mid x_i = 0 \text{ for some } i \in [n]\}.$$

**Definition 12 (Bounded-Persistence).** A reaction network (3) is said to be *bounded-persistent* if  $\omega(s_0) \cap \partial \mathbb{R}_{\geq 0}^n = \emptyset$  for each  $s_0 \gg 0$ .  $\triangle$

Although we eventually state and prove our results in this work in terms of bounded-persistence, we would like to interpret them in light of persistence. This is done in the next two lemmas.

**Lemma 13.** *If a reaction network is persistent, then it is bounded-persistent.*



*Proof.* Take any  $s_0 \gg 0$ . If  $\omega(s_0) = \emptyset$ , then we have nothing to prove. So, suppose  $\omega(s_0) \neq \emptyset$ . Choose any  $s \in \omega(s_0)$ , and a sequence  $(t_k)_{k \in \mathbb{N}}$  going to infinity in  $\mathbb{R}_{\geq 0}$  such that

$$\lim_{k \rightarrow \infty} \sigma(t_k, s_0) = s.$$

Then

$$s_i = \liminf_{k \rightarrow \infty} \sigma_i(t_k, s_0) \geq \liminf_{t \rightarrow \infty} \sigma_i(t, s_0) > 0, \quad \forall i \in [n].$$

In particular,  $s \notin \partial \mathbb{R}_{\geq 0}^n$ . Thus,  $\omega(s_0) \cap \partial \mathbb{R}_{\geq 0}^n = \emptyset$ .  $\square$

The converse of Lemma 13 is not true. However, (4) holds for bounded trajectories of bounded-persistent networks—hence the terminology.

**Lemma 14.** *Suppose a solution  $\sigma(\cdot, s_0): \mathbb{R}_{\geq 0} \rightarrow \mathbb{R}_{\geq 0}^n$  of a bounded-persistent reaction network is bounded. Then*

$$\liminf_{t \rightarrow \infty} \sigma_i(t, s_0) > 0, \quad \forall i \in [n]. \quad (5)$$

*Proof.* Suppose on contrary that

$$\liminf_{t \rightarrow \infty} \sigma_{i_0}(t, s_0) = 0$$

for some  $i_0 \in [n]$ . Then

$$\lim_{k \rightarrow \infty} \sigma_{i_0}(t_k, s_0) = 0$$

along some sequence  $(t_k)_{k \in \mathbb{N}}$  going to infinity in  $\mathbb{R}_{\geq 0}$ . In virtue of boundedness, by passing into a subsequence, if necessary, we may assume without loss of generality that  $(\sigma(t_k, s_0))_{k \in \mathbb{N}}$  converges, say,

$$\lim_{k \rightarrow \infty} \sigma(t_k, s_0) = s_\infty.$$

We have  $s_\infty \in \omega(s_0)$  by definition. But since the  $i_0^{\text{th}}$  coordinate of  $s_\infty$  is zero, we conclude that  $s_\infty \in \partial \mathbb{R}_{\geq 0}^n$  also. This contradicts the bounded-persistence hypothesis that  $\omega(s_0) \cap \partial \mathbb{R}_{\geq 0}^n = \emptyset$ . Thus, (5) must hold.  $\square$

**Corollary 15.** *If a conservative reaction network is bounded-persistent, then it is persistent.*

*Proof.* Each stoichiometric compatibility class of a conservative network is compact [14, Appendix 1]. So, every solution of (3) is bounded.  $\square$

Conservative networks are a special case of dissipative networks (Definition 48), for which bounded-persistence is also equivalent to persistence. These will be discussed in Subsection 4.2.

Sufficient conditions for bounded-persistence of reaction networks were given in terms of siphons and P-semiflows in [3]. Further developments include a more direct proof of their result, which appeared shortly after in [1], and the treatment of time-varying reaction rates, given in [5]. The next proposition presents the result in the form in which we shall use it in this work.

**Proposition 16.** *If a reaction network has the siphon/P-semiflow property, then it is bounded-persistent.*

*Proof.* The same argument as in [3, Section 5] or the direct proof in [1, Theorem 2.5] still work under our weaker assumptions on the reaction rates.  $\square$

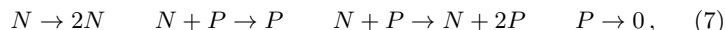
The next example shows that the siphon/P-semiflow property is not in general necessary for persistence.

*Example 17* (Lotka-Volterra Predator-Prey Model). The Lotka-Volterra equations,

$$\begin{cases} \frac{dN}{dt} = N(t)(a - bP(t)) \\ \frac{dP}{dt} = P(t)(cN(t) - d), \end{cases} \quad (6)$$

where  $a, b, c, d$  are positive parameters, model the population sizes at time  $t \geq 0$  of a predator species,  $P(t)$ , and its prey,  $N(t)$ , under the assumptions that  $N(t)$  grows exponentially in the absence of predators,  $P(t)$  decays exponentially in the absence of prey, and that both the growth rate of  $P(t)$  and the depletion rate of  $N(t)$  on account of predation are directly proportional to the population counts  $N(t)$  and  $P(t)$ . Solutions of (6) are known to be persistent. In fact, they are periodic [16, Section 3.1].

The equations in (6) could be alternatively derived as (3) from the reaction network



under mass-action kinetics (see, for instance, [13] for an account of mass-action kinetics). The minimal siphons of (7) are  $\{N\}$  and  $\{P\}$ . But the reaction network has no conservation laws. So, it does not have the siphon/P-semiflow property.  $\diamond$

Necessary conditions for a network to be persistent are known for conservative networks, and were also given in [3].

**Proposition 18.** *If a conservative reaction network is persistent, then it is consistent.*

*Proof.* See [3, Theorem 1].  $\square$

In Subsection 4.1 we discuss a class of conservative post-translational modification systems for which the siphon/P-semiflow condition and consistency are equivalent, and thus necessary and sufficient for persistence.

### 2.3.1 Boundary Steady States

A *steady state* of a reaction network  $G$  is any point  $s_0 \in \mathbb{R}_{\geq 0}^n$  such that  $Nr(s_0) = 0$ . A *boundary steady state* (of a reaction network  $G$ ) is then defined in our context to be any point  $s_0 \in \partial\mathbb{R}_{\geq 0}^n$  such that  $Nr(s_0) = 0$ , in other words, a steady state that lies in the boundary. The *zero coordinate set* of a point  $s \in \mathbb{R}_{\geq 0}^n$ , with respect to some given reaction network  $G$ , is the set

$$Z(s) := \{S_i \in \mathcal{S} \mid s_i = 0\} = \mathcal{S} \setminus \text{supp } s.$$

Thus, a point  $s \in \mathbb{R}_{\geq 0}^n$  is a boundary steady state if, and only if  $Z(s) \neq \emptyset$ .

The next lemma was proved in [18] for mass-action kinetics. The same argument holds under (r2), and we provide the details for the sake of completeness.

**Lemma 19.** *Let  $G$  be a reaction network. If  $s_0$  is a boundary steady state, then  $Z(s_0)$  is a siphon.*

*Proof.* Pick any  $S_i \in Z(s_0)$ . We need to show that every reaction having  $S_i$  as one of its products also has a species in  $Z(s_0)$  as one of its reactants. Note that the condition is trivially fulfilled for every reaction having  $S_i$  appearing as both a reactant and a product.

Now consider the set  $\mathcal{J}_i$  of indices  $j \in [m]$  such that  $R_j$  is a reaction having  $S_i$  as one of its products, but not one of its reactants; that is,

$$\mathcal{J}_i := \{j \in [m] \mid \alpha'_{ij} > 0 \text{ and } \alpha_{ij} = 0\}.$$

If  $\mathcal{J}_i = \emptyset$ , then we have nothing left to prove. Thus, we may assume that  $\mathcal{J}_i \neq \emptyset$ . Since  $s_0$  is a steady state, we have

$$\frac{ds_i}{dt} = \sum_{j=1}^m (\alpha'_{ij} - \alpha_{ij}) r_j(s_0) = 0. \quad (8)$$

For each  $j \notin \mathcal{J}_i$ , we have  $\alpha'_{ij} = 0$  or  $\alpha_{ij} > 0$ . If  $\alpha_{ij} > 0$ , then  $r_j(s_0) = 0$  by (r2). So, the sum in (8) can be simplified as

$$\sum_{j \in \mathcal{J}_i} \alpha'_{ij} r_j(s_0) = 0,$$

from which we conclude that

$$r_j(s_0) = 0, \quad \forall j \in \mathcal{J}_i.$$

It then follows from (r2) that one of the reactants of  $R_j$  belongs to  $Z(s_0)$  for each  $j \in \mathcal{J}_i$ . This completes the proof that  $Z(s_0)$  is a siphon.  $\square$

**Proposition 20.** *Let  $G$  be a reaction network with the siphon/P-semiflow property. Then the stoichiometric compatibility classes of  $G$  which are not entirely contained in the boundary do not contain any boundary steady states.*

*Proof.* Let  $s_0$  be a boundary steady state of  $G$ . We want to show that the stoichiometric compatibility class of  $G$  containing  $s_0$  is contained in the boundary, in other words, we want to show that

$$(s_0 + S) \cap \mathbb{R}_{\geq 0}^n \subseteq \partial \mathbb{R}_{\geq 0}^n.$$

By Lemma 19, the zero set  $Z(s_0)$  of  $s_0$  is a siphon. By the siphon/P-semiflow property, there exists a P-semiflow  $\omega > 0$  such that  $\text{supp } \omega \subseteq Z(s_0)$ . In particular,  $\omega \cdot s_0 = 0$ . Now for any  $s_1 \in (s_0 + S) \cap \mathbb{R}_{\geq 0}^n$ , we must have  $\omega \cdot s_1 = 0$  also. In particular, it must be the case that  $\text{supp } \omega \subseteq Z(s_1) \neq \emptyset$ , meaning that  $s_1 \in \partial \mathbb{R}_{\geq 0}^n$ .  $\square$

## 2.4 Monomolecular Networks

Iterating the simplification procedures discussed in this work will often result in what we shall refer to as monomolecular networks. Intuitively, these are reaction networks in which each reactant or product consists of at most a single species. The precise definition is given below in Definition 21. For such networks, the siphon/P-semiflow can be checked by simply showing that each of their connected components is strongly connected (Lemma 22). For conservative monomolecular networks, the necessary and sufficient conditions for persistence given in Propositions 16 and 18 are actually equivalent, and characterized by this strong connectedness property (Proposition 24).

**Definition 21** (Monomolecular Networks). A reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  is said to be *monomolecular* if, for each  $y \in \mathcal{C}$ , either  $y = 0$  or  $y = S_i$  for some  $i \in [n]$ . In this case, we identify the nonzero complexes of  $G$  with the corresponding species.  $\triangle$

**Lemma 22.** *If a monomolecular reaction network is such that each of its connected components is strongly connected, then it has the siphon/P-semiflow property.*

*Proof.* Let  $(\mathcal{C}_1, \mathcal{R}_1), \dots, (\mathcal{C}_J, \mathcal{R}_J)$  be the connected components of  $(\mathcal{C}, \mathcal{R})$ . Let  $\{\mathbf{e}_1, \dots, \mathbf{e}_n\}$  be the canonical basis of  $\mathbb{R}^n$ . Fix arbitrarily  $j \in [J]$ . We have two possibilities.

If  $0 \notin \mathcal{C}_j$ , then any siphon of  $G$  containing some species  $S' \in \mathcal{C}_j$  contains  $\mathcal{C}_j$ . Indeed, for any other  $S \in \mathcal{C}_j$ , there exists a path in  $(\mathcal{C}, \mathcal{R})$  connecting  $S$  to  $S'$ . Thus,  $S$  belongs to any siphon containing  $S'$ . Furthermore,

$$\sum_{i: S_i \in \mathcal{C}_j} \mathbf{e}_i$$

is a P-semiflow of  $G$ . This follows from the fact that, for each reaction  $S \rightarrow S' \in \mathcal{R}_j$ , the column of  $N$  corresponding to  $S \rightarrow S'$  has exactly two nonzero entries, namely, a 1 in the row corresponding to  $S'$ , and a  $-1$  in the row corresponding to  $S$ .

If  $0 \in \mathcal{C}_j$ , then  $\mathcal{C}_j$  contains no siphons of  $G$ . Indeed, by strong connectedness, any species  $S \in \mathcal{C}_j$  appears in a path starting at  $0$  and ending at  $S$ . It then follows as argued in Example 10 that  $S$  cannot belong to any siphon of  $G$ .

We conclude that every siphon of  $G$  contains the support of a P-semiflow.  $\square$

**Lemma 23.** *If  $v$  is a T-semiflow of a conservative monomolecular network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ , then the reaction graph  $(\mathcal{C}, \mathcal{R})$  of  $G$  has a cycle  $(\mathcal{C}', \mathcal{R}')$  such that  $\mathcal{R}' \subseteq \text{supp } v$ .*

*Proof.* Choose any reaction  $R_{j_1} = S_{i_1} \rightarrow S_{i_2} \in \text{supp } v$ . So,  $N_{i_2 j_1} = 1 > 0$ . Since  $v$  is a T-semiflow of  $G$ , we have

$$N_{i_1} v_1 + \dots + N_{i_m} v_m = 0, \quad \forall i \in [n]. \quad (9)$$

Thus, there must exist a  $j_2 \in [m]$  such that  $v_{j_2} > 0$  and  $N_{i_2 j_2} = -1 < 0$ . Now since  $G$  is conservative, there must exist an  $\omega = (\omega_1, \dots, \omega_n) \gg 0$  such that

$$\omega_1 N_{1 j_1} + \dots + \omega_n N_{n j_1} = 0, \quad \forall j \in [m]. \quad (10)$$

Hence, there exists an  $i_3 \in [n]$  such that  $N_{i_3 j_2} = 1 > 0$ . Now  $R_{j_2} = S_{i_2} \rightarrow S_{i_3} \in \mathcal{R}$ . If  $i_3 = i_1$ , then  $S_{i_1} \rightarrow S_{i_2} \rightarrow S_{i_1}$  is a cycle in  $(\mathcal{C}, \mathcal{R})$ , and we are done. If that is not the case, then we can iterate the procedure until we find  $i_1, \dots, i_k \in [n]$  such that  $S_{i_\ell} \rightarrow S_{i_{\ell+1}} \rightarrow \dots \rightarrow S_{i_k} \rightarrow S_{i_\ell}$  is a cycle for some  $\ell \in [k-1]$ . This will have to happen eventually, since the number of species is finite.  $\square$

**Proposition 24.** *Suppose that a monomolecular reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  is conservative. Then the following four properties are equivalent.*

- (i)  $G$  is consistent.
- (ii) Each connected component of  $G$  is strongly connected.
- (iii)  $G$  has the siphon/P-semiflow property.

(iv)  $G$  is persistent.

*Proof.* It is enough to show that (i)  $\Rightarrow$  (ii)  $\Rightarrow$  (iii)  $\Rightarrow$  (iv)  $\Rightarrow$  (i). Note that we have (ii)  $\Rightarrow$  (iii) from Lemma 22, (iii)  $\Rightarrow$  (iv) from Proposition 16 and Corollary 15, and (iv)  $\Rightarrow$  (i) from Proposition 18. Thus, it remains to show that (i)  $\Rightarrow$  (ii).

First note that it suffices to show that each reaction of  $(\mathcal{C}, \mathcal{R})$  is in a directed cycle. Indeed, suppose that to be the case, and consider an undirected reaction path

$$S^{(1)} - \dots - S^{(k)} \quad (11)$$

connecting any two species in the same connected component of  $(\mathcal{C}, \mathcal{R})$ . To create a directed reaction path from  $S^{(1)}$  to  $S^{(k)}$ , simply replace any reaction in (11) that is of the form  $S^{(j)} \leftarrow S^{(j+1)}$  for some  $j \in [k-1]$  by the directed path linking  $S^{(j)}$  to  $S^{(j+1)}$  obtained from some directed cycle containing  $S^{(j)} \leftarrow S^{(j+1)}$ .

Now to state that each reaction of  $(\mathcal{C}, \mathcal{R})$  is in a directed cycle is equivalent to say that there are directed cycles  $(\mathcal{C}_1^\circ, \mathcal{R}_1^\circ), \dots, (\mathcal{C}_k^\circ, \mathcal{R}_k^\circ)$  in  $(\mathcal{C}, \mathcal{R})$  such that

$$\mathcal{R} = \bigcup_{i=1}^k \mathcal{R}_i^\circ. \quad (12)$$

We shall construct vectors  $v_0, v_1, \dots, v_k \in \mathbb{R}_{\geq 0}^m$  and cycles  $(\mathcal{C}_1^\circ, \mathcal{R}_1^\circ), \dots, (\mathcal{C}_k^\circ, \mathcal{R}_k^\circ)$  in  $(\mathcal{C}, \mathcal{R})$  such that,

- (a)  $v_0, v_1, \dots, v_{k-1}$  are T-semiflows of  $G$ ,
- (b)  $\text{supp } v_0 = \mathcal{R}$ ,  $\text{supp } v_k = \emptyset$ , and  $\text{supp } v_i \subsetneq \text{supp } v_{i-1}$ ,  $i = 1, \dots, k$ , and
- (c)  $(\text{supp } v_{i-1} \setminus \text{supp } v_i) \subseteq \mathcal{R}_i^\circ$ ,  $i = 1, \dots, k$ .

Note that it follows from (b) that

$$\bigcup_{i=1}^k (\text{supp } v_{i-1} \setminus \text{supp } v_i) = \mathcal{R},$$

and then from (c) that (12) holds, thus completing the proof.

Suppose that T-semiflows  $v_0, v_1, \dots, v_{k-1}$  of  $G$  and cycles  $(\mathcal{C}_1^\circ, \mathcal{R}_1^\circ), \dots, (\mathcal{C}_{k-1}^\circ, \mathcal{R}_{k-1}^\circ)$  in  $(\mathcal{C}, \mathcal{R})$  have been constructed such that  $v_0 \gg 0$ ,  $\text{supp } v_i \subsetneq \text{supp } v_{i-1}$ , and  $(\text{supp } v_{i-1} \setminus \text{supp } v_i) \subseteq \mathcal{R}_i^\circ$ , for  $i = 1, \dots, k-1$ . By Lemma 23, there exists a cycle  $(\mathcal{C}_k^\circ, \mathcal{R}_k^\circ)$  in  $(\mathcal{C}, \mathcal{R})$  such that  $\mathcal{R}_k^\circ \subseteq \text{supp } v_{k-1}$ . Let  $w_k \in \mathbb{R}_{\geq 0}^m$  be the vector defined by

$$(w_k)_j = \begin{cases} 1, & \text{if } R_j \in \mathcal{R}_k^\circ \\ 0, & \text{if } R_j \notin \mathcal{R}_k^\circ, \end{cases}$$

let

$$\delta_k := \min\{(v_{k-1})_j; (w_k)_j \neq 0\},$$

and set

$$v_k := v_{k-1} - \delta_k w_k.$$

Then  $v_k \geq 0$ ,  $Nv_k = 0$ ,  $\text{supp } v_k \subsetneq \text{supp } v_{k-1}$ , and  $(\text{supp } v_{k-1} \setminus \text{supp } v_k) \subseteq \mathcal{R}_k^\circ$ . If  $v_k = 0$ , then we are done. Otherwise, we may proceed with the construction. In view of the proper inclusion in (b), we must eventually have  $v_k = 0$ . This gives the inductive step. Since  $G$  is consistent by hypothesis, there exists a strictly positive T-semiflow  $v_0$ , giving the starting step.  $\square$

The property that every connected component of the reaction graph is strongly connected is also known in the literature as *weak reversibility* (see [13, Definition 6.1]). Thus, Lemma 22 or Proposition 24, as well as other results further down, could well have been stated in these terms. In this work, the property of weak reversibility only comes up in the context of monomolecular networks. Thus, we chose to use the more informative, explicit description in terms of strong connectivity of the connected components.

### 3 Intermediates and Catalysts

In this section we define the concepts of intermediate and catalyst of a reaction network. We also describe the reaction networks that are obtained from their removal. After establishing these concepts and underlying terminology in Subsections 3.1 and 3.2, we state our main results in Subsection 3.3.

#### 3.1 Intermediates

Consider a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ . Let  $\mathcal{Y}$  be a nonempty subset of  $\mathcal{S}$ , and write

$$\mathcal{Y} = \{Y_1, \dots, Y_p\},$$

and

$$\mathcal{S} \setminus \mathcal{Y} = \{S_1, \dots, S_q\}.$$

Consider the following two properties.

(I1) For each complex

$$\sum_{i=1}^q \alpha_i S_i + \sum_{i=1}^p \beta_i Y_i$$

in  $\mathcal{C}$ , if  $\beta_{i_0} > 0$  for some  $i_0 \in [p]$ , then

$$\sum_{i=1}^q \alpha_i S_i + \sum_{i=1}^p \beta_i Y_i = Y_{i_0}.$$

In this case, we identify the ‘complexes’ and ‘species’  $Y_1, \dots, Y_p$ . (See also Definition 21.)

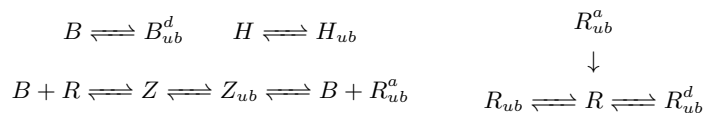
(I2) For each  $Y \in \mathcal{Y}$ , there exist  $y, y' \in \mathcal{C} \setminus \mathcal{Y}$ , and reaction paths from  $y$  to  $Y$  and from  $Y$  to  $y'$  such that all their non-endpoints are in  $\mathcal{Y}$ .

If (I1) and (I2) hold, then we may construct a reaction network  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  as follows. The reactions set  $\mathcal{R}^*$  is comprised of two kinds of reactions, namely, reactions  $y \rightarrow y'$  such that  $y \rightarrow y' \in \mathcal{R}$  for some  $y, y' \in \mathcal{C} \setminus \mathcal{Y}$ , and reactions  $y \rightarrow y'$  such that  $y \neq y'$ , and there is a reaction path connecting  $y$  to  $y'$  such that all their non-endpoints are in  $\mathcal{Y}$ . We set  $\mathcal{C}^*$  to be the set of reactant and product complexes in the reactions in  $\mathcal{R}^*$ , and we set  $\mathcal{S}^*$  to be the set of species that are part of some complex in  $\mathcal{C}^*$ . We observe that  $\mathcal{S}^*$  does not always coincide with  $\mathcal{S} \setminus \mathcal{Y}$ , as illustrated in Example 26 below.

**Definition 25** (Intermediates). Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network and  $\mathcal{Y}$  be a nonempty subset of  $\mathcal{S}$ . We call  $\mathcal{Y}$  a *set of intermediate species* of  $G$ , if (I1) and (I2) hold. In this case, the reaction network  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  defined as above is called the *reduction of  $G$  by the removal of the set of intermediates  $\mathcal{Y}$* . The elements of  $\mathcal{Y}$  are then referred to as the *intermediate species* of  $G$ .  $\triangle$

For brevity, we will often write simply intermediates instead of intermediate species.

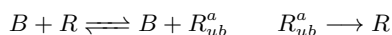
*Example 26* (A Ubiquitination Model). Consider the reaction network model for Ring1B/Bmi1 ubiquitination below [17].



Note that

$$\mathcal{Y} := \{B_{ub}^d, H, R_{ub}, R_{ub}^d, Z, Z_{ub}\}$$

is a set of intermediate species/complexes of the network. This network can be reduced to



by removing these intermediates and collapsing the paths in which they appear, as described above.

We emphasize that  $\mathcal{S}^*$  does not always coincide with  $\mathcal{S} \setminus \mathcal{Y}$ . In this example,  $H_{ub}$  is in  $\mathcal{S} \setminus \mathcal{Y}$ , but not in  $\mathcal{S}^*$ . We also note that the same network  $G^*$  may arise from removing a different set of intermediates. For instance, in this example, we could have set  $H_{ub}$  as an intermediate in place of  $H$ .  $\diamond$

### 3.1.1 Removing One Intermediate at A Time

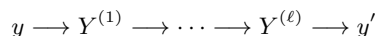
In part of the proof of our main result about the removal of intermediates, we use induction on the number of intermediates removed. Thus, a discussion of how the intermediates in a set of intermediates may be iteratively removed, one at a time, is warranted.

Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network, and suppose  $\mathcal{Y} = \{Y_1, \dots, Y_p\}$  is a set of intermediates of  $G$ . Set  $G_p := G$ . It follows directly from the definition that any nonempty subset of  $\mathcal{Y}$  is a set of intermediates of  $G$ . In particular,  $\{Y_p\}$  is a set of intermediates of  $G_p$ . Let  $G_{p-1}$  be the reduction of  $G_p$  by the removal of the set of intermediates  $\{Y_p\}$ . Now  $\{Y_1, \dots, Y_{p-1}\}$  is a set of intermediates of  $G_{p-1}$ . In particular,  $\{Y_{p-1}\}$  is a set of intermediates of  $G_{p-1}$ . We define  $G_{p-2}$  to be the reduction of  $G_{p-1}$  by the removal of the set of intermediates  $\{Y_{p-1}\}$ . Iterating this process  $p$  times, we obtain a sequence  $G_p, \dots, G_1, G_0$  such that  $G_p = G$ , and  $G_{i-1}$  is the reduction of  $G_i$  by the removal of the set of intermediates  $\{Y_i\}$ ,  $i = p, \dots, 1$ .

**Lemma 27.** *If  $G$ ,  $\mathcal{Y}$ , and  $G_p, \dots, G_1, G_0$  are like in the above construction, and  $G^*$  is the reduction of  $G$  by the removal of the set of intermediates  $\mathcal{Y}$ , then  $G_0 = G^*$ .*

*Proof.* We use induction on  $p$ . The claim is trivial for  $p = 1$ . So, suppose it has been proven to be true for the removal of up to  $p$  intermediates, for some  $p \geq 1$ . Let  $\mathcal{Y} = \{Y_1, \dots, Y_p, Y_{p+1}\}$  be a set of intermediates of  $G$ . As noted above,  $\{Y_2, \dots, Y_{p+1}\}$  is a set of intermediates of  $G$ . Let  $G_1^*$  be the reduction of  $G$  obtained by their removal. By the induction hypothesis,  $G_1^* = G_1$ , and so  $\mathcal{R}_1^* = \mathcal{R}_1$ . We want to show that  $\mathcal{R}_0 = \mathcal{R}^*$ .

$\mathcal{R}^* \subseteq \mathcal{R}_0$ . Let  $y \rightarrow y'$  be any reaction in  $\mathcal{R}^*$ . If  $y \rightarrow y' \in \mathcal{R}$ , then  $y \rightarrow y' \in \mathcal{R}_1^*$ , and so  $y \rightarrow y' \in \mathcal{R}_0$ . So, suppose  $y \rightarrow y' \notin \mathcal{R}$ . Then there exist  $Y^{(1)}, \dots, Y^{(\ell)} \in \mathcal{Y}$  such that



is a reaction path in  $G$ . We may assume without loss of generality that  $Y^{(1)}, \dots, Y^{(\ell)}$  are pairwise distinct. If  $Y^{(1)}, \dots, Y^{(\ell)} \in \{Y_2, \dots, Y_{p+1}\}$ , then  $y \rightarrow y' \in \mathcal{R}_1^*$ , and so  $y \rightarrow y' \in \mathcal{R}_0$  like in the previous case. Otherwise, we have  $Y_1 = Y^{(i)}$  for some  $i \in [\ell]$ . But now



is a reaction path in  $G_1^*$ , and so  $y \rightarrow y' \in \mathcal{R}_0$  once again.

$\mathcal{R}_0 \subseteq \mathcal{R}^*$ . Let  $y \rightarrow y'$  be any reaction in  $\mathcal{R}_0$ . If  $y \rightarrow y' \in \mathcal{R}_1^*$ , then there exists a reaction path connecting  $y$  to  $y'$  in  $G$  such that all its non-endpoints belong to  $\{Y_2, \dots, Y_{p+1}\}$ . In this case,  $y \rightarrow y' \in \mathcal{R}^*$ . If  $y \rightarrow y' \notin \mathcal{R}_1^*$ , then (13) is a reaction path in  $G_1^*$ . In this case there are reaction paths in  $G$  connecting  $y$  to  $Y_1$  and  $Y_1$  to  $y'$ , all non-endpoints of which belong to  $\{Y_2, \dots, Y_{p+1}\}$ . Concatenating these two reaction paths we obtain a reaction path in  $G$  connecting  $y$  and  $y'$  such that all its non-endpoints belong to  $\mathcal{Y}$ . It follows once again that  $y \rightarrow y' \in \mathcal{R}^*$ .  $\square$

### 3.2 Catalysts

Consider a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ . Let  $\mathcal{E}$  be a nonempty subset of  $\mathcal{S}$ , and write

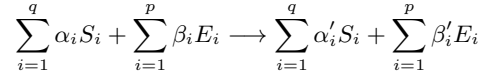
$$\mathcal{E} = \{E_1, \dots, E_p\},$$

and

$$\mathcal{S} \setminus \mathcal{E} = \{S_1, \dots, S_q\}.$$

Consider the following two properties.

(C1) For each reaction



in  $\mathcal{R}$ , if  $\beta_{i_0} > 0$  or  $\beta'_{i_0} > 0$  for some  $i_0 \in [p]$ , then

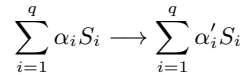
$$\sum_{i=1}^p \beta_i E_i = \sum_{i=1}^p \beta'_i E_i$$

or

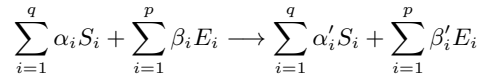
$$\alpha_1 = \alpha'_1 = \dots = \alpha_q = \alpha'_q = 0.$$

(C2) The subnetwork  $G_{\mathcal{E}} = (\mathcal{S}_{\mathcal{E}}, \mathcal{C}_{\mathcal{E}}, \mathcal{R}_{\mathcal{E}})$  implied by  $\mathcal{E}$  (Definition 1) has the siphon/P-semiflow property.

If (C1) and (C2) hold, then we may construct a reaction network  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  as follows. We set  $\mathcal{R}^*$  to be the set of reactions



such that

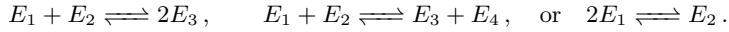


belongs to  $\mathcal{R}$ , and  $\alpha_{i_0} > 0$  or  $\alpha'_{i_0} > 0$  for some  $i_0 \in [q]$ . We then set  $\mathcal{C}^*$  to be the set of reactants and products in these reactions, and set  $\mathcal{S}^*$  to be the set of species that are part of some complex in  $\mathcal{C}^*$ . Contrary to what happened with intermediates,  $\mathcal{S}^*$  always agrees with  $\mathcal{S} \setminus \mathcal{E}$ .



**Definition 28** (Catalysts). Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network and  $\mathcal{E}$  be a nonempty subset of  $\mathcal{S}$ . We call  $\mathcal{E}$  a *set of catalysts of  $G$*  if (C1) and (C2) hold. In this case, the reaction network  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  defined as above is called a *reduction of  $G$  by the removal of the set of catalysts  $\mathcal{E}$* . The elements of  $\mathcal{E}$  are then referred to as the *catalysts of  $G$* .  $\triangle$

Typically (C2) is checked via Proposition 24, by showing that  $G_{\mathcal{E}}$  is a monomolecular network and each connected component of its reaction graph is strongly connected, as we shall see in some of the examples in Section 4. However, the theory allows for catalysts to interact in more complex, yet still biologically meaningful ways, for instance, in reversible reactions of the forms



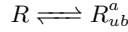
*Example 29* (A Ubiquitination Model (Continued)). Consider the network



obtained from the ubiquitination model in Example 26 after intermediates were removed. Note that

$$\mathcal{E} := \{B\}$$

is a set of catalysts. Thus, this network can be further reduced to



by removing  $B$  and projecting the reactions as described above.  $\diamond$

### 3.3 Main Results

We are now ready to state our main results.

**Theorem 30** (Removal of Intermediates). *Suppose a reaction network  $G^*$  is obtained from a reaction network  $G$  by the removal of a set of intermediates. Then,*

- (i)  $G$  has the siphon/ $P$ -semiflow property if, and only if  $G^*$  has the siphon/ $P$ -semiflow property, and
- (ii)  $G$  is consistent if, and only if  $G^*$  is consistent.

**Theorem 31** (Removal of Catalysts). *Suppose a reaction network  $G^*$  is obtained from a reaction network  $G$  by the removal of a set of catalysts  $\mathcal{E}$ . Then,*

- (i)  $G$  has the siphon/ $P$ -semiflow property if, and only if  $G^*$  has the siphon/ $P$ -semiflow property, and
- (ii) if  $G$  is consistent, then  $G^*$  is consistent; conversely, if  $G^*$  is consistent and  $G_{\mathcal{E}}$  is conservative, then  $G$  is consistent.

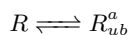
**Definition 32** (Primitive Networks). A reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  is said to be *primitive* (with respect to the removal of catalysts or intermediates) if no subset of  $\mathcal{S}$  is a set of catalysts or intermediates of  $G$ . If iteratively removing sets of intermediates and catalysts of a reaction network  $G$  results in a primitive reaction network  $G^*$ , then we refer to  $G^*$  as a *primitive reduction of  $G$* .  $\triangle$

**Theorem 33** (Uniqueness of The Primitive Reduction). *Let  $G$  be a reaction network, and suppose  $G_1^*$  and  $G_2^*$  are primitive reductions of  $G$ . Then  $G_1^* = G_2^*$ .*

Observe that Theorem 33 is more than just a theoretical curiosity. As noted in Example 26, choosing a set of intermediates or catalysts to remove is not something which can always be done in a unique way at each stage of the simplification process. Thus, knowing that one would always obtain the same minimally simplified reaction network regardless of the order in which catalysts and intermediates are removed has also practical relevance.

The proofs of Theorems 30, 31 and 33 will be given in Section 5. We first illustrate the results with a few examples worked out in detail.

*Example 34* (A Ubiquitination Model (Concluded)). The network

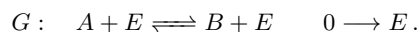


is a strongly connected monomolecular network. By Proposition 24, it has the siphon/P-semiflow property. By Theorems 30(*i*) and 31(*i*), the ubiquitination model from Example 26 also has the siphon/P-semiflow property. So long as the reaction rates satisfy our hypothesis, it will follow from Proposition 16 that the network is bounded-persistent. But because the network is also conservative, we may conclude that it is in fact persistent (Corollary 15).  $\diamond$

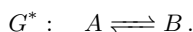
We emphasize that the procedures of removal of intermediates and catalysts carried out in Examples 26 and 29, as well as the analysis of the emerging underlying substrate network for strong connectedness in Example 34, are essentially graphical. More specifically, one need not do any calculations with the stoichiometric matrix.

In Theorem 31(*ii*), the hypothesis that  $G_{\mathcal{E}}$  be conservative is not superfluous. If that is not the case, then it might happen that  $G^*$  is consistent and  $G$  is not, as shown in Example 35 below. However, if  $G$  is consistent, then  $G^*$  is consistent regardless of whether  $G_{\mathcal{E}}$  is conservative or not, as shown in Lemma 61.

*Example 35* (Non-Conservative  $G_{\mathcal{E}}$ ). Consider the reaction network



The singleton  $\mathcal{E} := \{E\}$  is a set of catalysts of  $G$ , the removal of which yields the reaction network



The stoichiometric matrices of  $G$  and  $G^*$  are, respectively,

$$N = \begin{bmatrix} -1 & 1 & 0 \\ 1 & -1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad \text{and} \quad N^* = \begin{bmatrix} -1 & 1 \\ 1 & -1 \end{bmatrix}.$$

One can readily see that  $G^*$  has the strictly positive T-semiflow  $(1, 1)$ , and is, therefore, consistent. On the other hand, any T-semiflow of  $G$  must have its third coordinate equal to zero, so  $G$  is not consistent.  $\diamond$

## 4 Examples

We shall apply Theorems 30 and 31 to two main classes of reaction networks. In Subsection 4.1, we give necessary and sufficient conditions for cascades of a class of post-translational modification (PTM) systems to be persistent. The reaction network in the introduction, as well as the

ubiquitination model discussed in Examples 26, 29 and 34, will turn out to be special cases of PTM systems. In Subsection 4.2, we argue that a nonconservative reaction network may still be shown to be persistent via the siphon/P-semiflow property as long as it can be shown to be dissipative. Finally, in Subsection 4.3, we consider the relationship between the siphon/P-semiflow property and boundary steady states discussed in [18] in light of our model simplification results.

## 4.1 Cascades of PTM Systems

In this subsection, we study the persistence of a class PTM systems. Combining Theorems 30 and 31 with Propositions 16, 18, and 24, we will achieve necessary and sufficient conditions for persistence of cascades of PTM systems in terms of strong connectedness of the connected components of the underlying substrate network of each layer. Our results are related to [11], where a class of PTM systems (there called *binary enzymatic networks*) is studied under mass-action kinetics, and characterized for a weaker concept of persistence (*vacuous persistence*) in terms of the *futility* of the enzymes.

### 4.1.1 PTM Systems

Consider a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ . Let

$$\mathcal{S} = \text{Enz} \cup \text{Sub} \cup \text{Int}$$

be a partition of the species set. Thus, Enz, Sub, and Int are pairwise disjoint. Consider the following properties.

- (M1) The reactions set  $\mathcal{R}$  can be partitioned into a disjoint union of subsets

$$\mathcal{R} = \mathcal{R}_{S \rightarrow S'} \cup \mathcal{R}_{S+E \rightarrow S'+E} \cup \mathcal{R}_{S+E \rightarrow Y'} \cup \mathcal{R}_{Y \rightarrow S'+E} \cup \mathcal{R}_{Y \rightarrow Y'},$$

which are uniquely determined from the partition  $\mathcal{S} = \text{Enz} \cup \text{Sub} \cup \text{Int}$  by the inclusions

$$\mathcal{R}_{S \rightarrow S'} \subseteq \{S \rightarrow S'; S, S' \in \text{Sub}\},$$

$$\mathcal{R}_{S+E \rightarrow S'+E} \subseteq \{S + E \rightarrow S' + E; E \in \text{Enz}, \text{ and } S, S' \in \text{Sub}\},$$

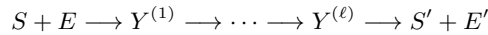
$$\mathcal{R}_{S+E \rightarrow Y'} \subseteq \{S + E \rightarrow Y'; E \in \text{Enz}, S \in \text{Sub}, \text{ and } Y' \in \text{Int}\},$$

$$\mathcal{R}_{Y \rightarrow S'+E} \subseteq \{Y \rightarrow S' + E; E \in \text{Enz}, S' \in \text{Sub}, \text{ and } Y \in \text{Int}\},$$

$$\mathcal{R}_{Y \rightarrow Y'} \subseteq \{Y \rightarrow Y'; Y, Y' \in \text{Int}\}.$$

- (M2) Int is either empty or a set of intermediates of  $G$ .

- (M3) If



is a reaction path in  $G$  for some  $E, E' \in \text{Enz}$ , some  $S, S' \in \text{Sub}$ , and some  $Y^{(1)}, \dots, Y^{(\ell)} \in \text{Int}$ , then  $E = E'$ .

**Definition 36** (PTM Systems). Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network, and let

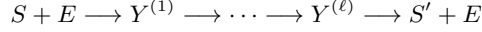
$$\mathcal{S} = \text{Enz} \cup \text{Sub} \cup \text{Int}$$

be a partition of the species set. We say that  $G$  is a *PTM system* with *enzyme* set Enz, *substrate* set Sub, and *intermediate enzyme-substrate complex* set Int if it has properties (M1)–(M3) above.  $\triangle$

Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a PTM system. If  $\text{Int} = \emptyset$ , then set  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*) := G$ . Otherwise, let  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  be the network obtained from  $G$  by the removal of the set of intermediates  $\text{Int}$ . Thus,

$$\mathcal{R}^* = \mathcal{R}_{S \rightarrow S'} \cup \mathcal{R}_{S+E \rightarrow S'+E} \cup \mathcal{R}_{S+E \rightarrow S'+E}^Y,$$

where  $\mathcal{R}_{S+E \rightarrow S'+E}^Y$  is the set of reactions of the form  $S + E \rightarrow S' + E$  such that



is a reaction path in  $G$  for some  $E \in \text{Enz}$ , some  $S, S' \in \text{Sub}$  such that  $S \neq S'$ , and some  $Y^{(1)}, \dots, Y^{(\ell)} \in \text{Int}$ . Regardless of whether  $\text{Int}$  is empty or nonempty, we shall abuse the terminology and refer to the reaction network  $G^*$  defined as above as the reaction network obtained from  $G$  by the removal of the set of intermediates  $\text{Int}$ , for simplicity.

Now  $\mathcal{S}^* \subseteq \text{Enz} \cup \text{Sub}$ , and  $\text{Enz}^* := \text{Enz} \cap \mathcal{S}^*$ , if nonempty, is a set of catalysts of  $G^*$ . Indeed, it follows directly from the form of the reactions that (C1) holds, and the subnetwork of  $G^*$  implied by  $\text{Enz}^*$  is the empty network, so (C2) also holds. If  $\text{Enz}^* = \emptyset$ , then we set  $G^{**} = (\mathcal{S}^{**}, \mathcal{C}^{**}, \mathcal{R}^{**}) := G^*$ . Otherwise, let  $G^{**} = (\mathcal{S}^{**}, \mathcal{C}^{**}, \mathcal{R}^{**})$  be the network obtained from  $G^*$  by the removal of the set of catalysts  $\text{Enz}^*$ . Then  $G^{**}$  is a monomolecular network consisting of the reactions  $S \rightarrow S'$  such that  $S + \alpha E \rightarrow S' + \alpha E \in \mathcal{R}^*$  for some  $E \in \text{Enz}^*$ , some  $\alpha \in \{0, 1\}$ , and some  $S, S' \in \text{Sub}$  such that  $S \neq S'$ . We refer to  $G^{**}$  as the *underlying substrate network* of  $G$ . Once again we abuse the terminology and refer to the reaction network  $G^{**}$  constructed as above simply as the reaction network obtained from  $G^*$  by the removal of the set of catalysts  $\text{Enz}^*$ , even when  $\text{Enz}^*$  happens to be empty.

The proof of the next result uses Corollaries 56 and 64, which appear in the proofs of Theorems 30 and 31 in the next section.

**Proposition 37.** *Let  $G$  be a PTM system. Then the following properties are equivalent.*

- (i)  $G$  is consistent.
- (ii) Each connected component of the underlying substrate network of  $G$  is strongly connected.
- (iii)  $G$  has the siphon/P-semiflow property.
- (iv)  $G$  is persistent.

*Proof.* We show that (i)  $\Rightarrow$  (ii)  $\Rightarrow$  (iii)  $\Rightarrow$  (iv)  $\Rightarrow$  (i).

By [23, Equations (16) and (17)],  $G$  is conservative. (See also Lemma 43 below.) Therefore (iii)  $\Rightarrow$  (iv) follows from Proposition 16 and Corollary 15, and (iv)  $\Rightarrow$  (i) follows directly from Proposition 18. It remains to show the other two implications.

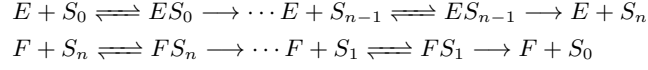
(i)  $\Rightarrow$  (ii). By Corollary 56,  $G^*$  is also conservative, and so it follows from Corollary 64 that  $G^{**}$ , the underlying substrate network of  $G$ , is conservative as well. So, it follows from Theorems 30(ii) and 31(ii) that  $G^{**}$  is consistent. It then follows by Proposition 24 that each connected component of  $G^{**}$  is strongly connected.

(ii)  $\Rightarrow$  (iii). By Proposition 24,  $G^{**}$  has the siphon/P-semiflow property. It then follows by Theorems 31(i) and 30(i), respectively, that  $G^*$  and, consequently,  $G$  have the siphon/P-semiflow property.  $\square$

*Remark 38.* In view of Proposition 24, statement (ii) in Proposition 37 is equivalent to each of the statements that the underlying substrate network

$G^{**}$  of  $G$  is consistent, has the siphon/P-semiflow property, or is persistent. Thus, either of these properties could also be checked to establish the persistence of  $G$ .  $\square$

*Example 39* (An  $n$ -Site Phosphorylation Mechanism). The sequential and distributive  $n$ -site phosphorylation mechanism given by



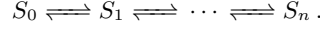
is a PTM system with

$$\begin{aligned} \text{Enz} &= \{E, F\}, \\ \text{Sub} &= \{S_0, S_1, \dots, S_n\}, \end{aligned}$$

and

$$\text{Int} = \{ES_0, ES_1, \dots, ES_{n-1}, FS_n, FS_{n-1}, \dots, FS_1\}.$$

The underlying substrate network obtained by removing the set of intermediates  $\text{Int}$ , then the set of enzymes  $\text{Enz}$  is



It consists of a single strongly connected component, so the PTM system is persistent by Proposition 37.  $\diamond$

#### 4.1.2 Signaling Cascades of PTM Systems

We now discuss a formalism for cascades of PTM systems. Intuitively, a signaling cascade of PTM systems is a reaction network which can be decomposed into a hierarchy of PTM systems in such a way that substrates at a certain level, or layer, may act as catalysts in lower levels (but not in higher levels).

Consider a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ , and write the species, complex and reaction sets of the network as (not necessarily disjoint) unions,

$$\mathcal{S} = \bigcup_{i=1}^T \mathcal{S}_i, \quad \mathcal{C} = \bigcup_{i=1}^T \mathcal{C}_i, \quad \text{and} \quad \mathcal{R} = \bigcup_{i=1}^T \mathcal{R}_i.$$

Consider the following properties.

(F1) For each  $i \in [T]$ ,  $G_i := (\mathcal{S}_i, \mathcal{C}_i, \mathcal{R}_i)$  is a PTM system with enzyme, substrate, and intermediate enzyme-substrate complex sets, respectively,  $\text{Enz}_i$ ,  $\text{Sub}_i$ , and  $\text{Int}_i$ .

$$(F2) \quad \text{Sub}_j \cap \left( \bigcup_{i=1}^{j-1} \text{Sub}_i \right) = \emptyset, \quad j = 2, \dots, T.$$

$$(F3) \quad \text{Enz}_j \cap \left( \bigcup_{i=1}^j \text{Sub}_i \right) = \emptyset, \quad j = 1, \dots, T.$$

$$(F4) \quad \left( \bigcup_{i=1}^T \text{Int}_i \right) \cap \left( \bigcup_{i=1}^T (\text{Enz}_i \cup \text{Sub}_i) \right) = \emptyset.$$

**Definition 40** (Signaling Cascades of PTM Systems). Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network, and write  $\mathcal{S}$ ,  $\mathcal{C}$ , and  $\mathcal{R}$  as (not necessarily disjoint) unions,

$$\mathcal{S} = \bigcup_{i=1}^T \mathcal{S}_i, \quad \mathcal{C} = \bigcup_{i=1}^T \mathcal{C}_i, \quad \text{and} \quad \mathcal{R} = \bigcup_{i=1}^T \mathcal{R}_i.$$

We say that  $G$  is a *signaling cascade of PTM systems* if it satisfies properties (F1)–(F4). In this case, the PTM systems  $G_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1), \dots, G_T = (\mathcal{S}_T, \mathcal{C}_T, \mathcal{R}_T)$  are referred to as the *layers* of the cascade.  $\triangle$

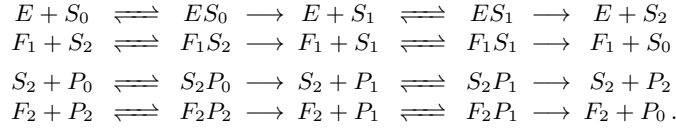
Observe that (F3) implies that any enzyme which is a substrate in some layer may appear in any layer below it, and not just the one immediately below the layer where it acts as a substrate. Thus, the layer hierarchy implied in the definition of signaling cascades of PTM systems may be a tree, in other words, it is not constrained to linear, sequential relationships where each layer can only provide the layer immediately after with enzymes.

**Proposition 41.** *Let  $G$  be a signaling cascade of PTM systems. Then the following properties are equivalent.*

- (i)  $G$  is consistent.
- (ii) The connected components of the underlying substrate network of each layer of  $G$  are strongly connected.
- (iii)  $G$  has the siphon/ $P$ -semiflow property.
- (iv)  $G$  is persistent.

The proof of Proposition 41 will be given in the next subsection.

*Example 42 (Double Phosphorylation Cascade).* Consider the concatenation of double phosphorylation mechanisms from Example 39 given by the reaction network



The double phosphorylation of a substrate  $S_0$  is catalyzed by a kinase  $E$ , and the dephosphorylation of its singly and doubly phosphorylated forms is catalyzed by a phosphatase  $F_1$ . The doubly phosphorylated form  $S_2$  of  $S_0$  then acts as a kinase in a similar double phosphorylation/dephosphorylation mechanism for another substrate  $P_0$ . This is a signaling cascade of PTM systems with

$$\begin{aligned}
\text{Enz}_1 &= \{S_2, F_2\}, \\
\text{Sub}_1 &= \{P_0, P_1, P_2\}, \\
\text{Int}_1 &= \{S_2P_0, S_2P_1, F_2P_2, F_2P_1\}, \\
\text{Enz}_2 &= \{E, F_1\}, \\
\text{Sub}_2 &= \{S_0, S_1, S_2\},
\end{aligned}$$

and

$$\text{Int}_2 = \{ES_0, ES_1, F_1S_2, F_1S_1\}.$$

Each of the layers of the cascade coincides with the double phosphorylation mechanism in Example 39. In particular, they have the property that each connected component of their underlying substrate networks is strongly connected. Persistence then follows from Proposition 41.  $\diamond$

It is also important to note that signaling cascades of PTM systems are conservative.

**Lemma 43.** *Any signaling cascade of PTM systems is conservative.*

*Proof.* Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a cascade of PTM systems with  $T$  layers. Write

$$\mathcal{S} = \{S_1, \dots, S_n\},$$

and set

$$\text{Enz} := \bigcup_{i=1}^T \text{Enz}_i, \quad \text{Sub} := \bigcup_{i=1}^T \text{Sub}_i, \quad \text{and} \quad \text{Int} := \bigcup_{i=1}^T \text{Int}_i.$$

By (F1),  $(\text{Enz} \cup \text{Sub}) \cup \text{Int} = \mathcal{S}$ , and by (F4),  $(\text{Enz} \cup \text{Sub}) \cap \text{Int} = \emptyset$ . For each  $i \in [n]$ , set

$$\omega_i := \begin{cases} 1, & \text{if } S_i \in \text{Enz} \cup \text{Sub} \\ 2, & \text{if } S_i \in \text{Int}. \end{cases}$$

Then  $\omega := (\omega_1, \dots, \omega_n)$  is a conservation law of  $G$ . This can be readily seen from the possible forms a reaction in  $\mathcal{R}$  may take. Since every entry of  $\omega$  is strictly positive, this means  $G$  is conservative.  $\square$

### 4.1.3 Proof of Proposition 41

Note that  $(iv) \Rightarrow (i)$  follows directly from Proposition 18, and, since a signaling cascade of PTM systems is always conservative by Lemma 43,  $(iii) \Rightarrow (iv)$  follows from Proposition 16 and Corollary 15. It remains to prove  $(i) \Rightarrow (ii)$ , which is the content of Lemma 46 below, and  $(ii) \Rightarrow (iii)$ , which is done in Lemma 47.

We begin with a few simple observations about signaling cascades of PTM systems.

We first notice that we may assume without loss of generality that the cascade has no intermediate enzyme-substrate complexes. Indeed, let  $G$  be a signaling cascade of PTM systems with layers  $G_1, \dots, G_T$ . By (F1) and (F4),

$$\text{Int} := \bigcup_{i=1}^T \text{Int}_i$$

is a set of intermediates of  $G$ , provided that it is nonempty. Assume this to be the case, and let  $G^*$  be the reaction network obtained by the removal of the set of intermediates  $\text{Int}$ . For each  $i \in [T]$ , let  $G_i^*$  be the reaction network obtained from  $G_i$  by the removal of the set of intermediates  $\text{Int}_i$ .

**Lemma 44.** *In the construction above,  $G^*$  is a signaling cascade of PTM systems with layers  $G_1^*, \dots, G_T^*$ . Furthermore,*

- (i)  $G^*$  has the siphon/ $P$ -semiflow property if, and only if  $G$  does also, and
- (ii)  $G^*$  is consistent if, and only if  $G$  is also.

*Proof.* For each  $i \in [T]$ , set  $\text{Sub}_i^* := \text{Sub}_i \cap \mathcal{S}_i^*$ ,  $\text{Enz}_i^* := \text{Enz}_i \cap \mathcal{S}_i^*$ , and  $\text{Int}_i^* := \emptyset$ . Then  $G_i^*$  is a PTM system with enzyme, substrate, and intermediate enzyme-substrate complex sets, respectively,  $\text{Enz}_i^*$ ,  $\text{Sub}_i^*$ , and  $\text{Int}_i^*$ , thus satisfying (F1). Properties (F2) and (F3) are inherited directly from  $G$ , and (F4) is trivial. This proves the first statement. Statements (i) and (ii) follow directly from Theorem 30.  $\square$

Throughout the rest of this subsection,  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  will be assumed to be a signaling cascade of PTM systems with an empty set of intermediates.

Next, let  $G^- = (\mathcal{S}^-, \mathcal{C}^-, \mathcal{R}^-)$  be the reaction network determined by

$$\mathcal{R}^- := \bigcup_{i=1}^{T-1} \mathcal{R}_i.$$

Hence,

$$\mathcal{S}^- := \bigcup_{i=1}^{T-1} \mathcal{S}_i \quad \text{and} \quad \mathcal{C}^- := \bigcup_{i=1}^{T-1} \mathcal{C}_i.$$

Set

$$\text{Enz}'_T := \text{Enz}_T \cap \left( \bigcup_{i=1}^{T-1} \text{Enz}_i \right).$$

If  $\text{Enz}'_T \neq \emptyset$ , then it is a set of catalysts of  $G^-$ . So, define  $G'$  to be the network obtained from  $G^-$  by the removal of the set of catalysts  $\text{Enz}'_T$ .

**Lemma 45.** *In the construction above,  $G'$  is a cascade of PTM systems with  $T - 1$  layers  $G'_1, \dots, G'_T$ . Furthermore, for each  $i \in [T - 1]$ , the underlying substrate networks of  $G'_i$  and  $G_i$  coincide.*

*Proof.* For each  $i \in [T - 1]$ , define  $\mathcal{R}'_i$  to be the set of reactions  $S + \alpha E \rightarrow S' + \alpha E \in \mathcal{R}_i$  such that  $S, S' \in \text{Sub}_i$ ,  $S \neq S'$ ,  $E \in \text{Enz}_i \setminus \text{Enz}_T$ , and  $\alpha \in \{0, 1\}$ , plus the reactions  $S \rightarrow S'$  such that  $S + E \rightarrow S' + E \in \mathcal{R}_i$  for some  $S, S' \in \text{Sub}_i$ ,  $S \neq S'$ , and  $E \in \text{Enz}_T$ . Then define  $G'_i = (\mathcal{S}'_i, \mathcal{C}'_i, \mathcal{R}'_i)$  to be the reaction network determined by  $\mathcal{R}'_i$ . We then have

$$\mathcal{S}' = \bigcup_{i=1}^{T-1} \mathcal{S}'_i, \quad \mathcal{C}' = \bigcup_{i=1}^{T-1} \mathcal{C}'_i, \quad \text{and} \quad \mathcal{R}' = \bigcup_{i=1}^{T-1} \mathcal{R}'_i.$$

Now  $G'_i$  is a PTM system with  $\text{Enz}'_i = \text{Enz}_i \setminus \text{Enz}_T$ ,  $\text{Sub}'_i = \text{Sub}_i$ , and  $\text{Int}'_i = \emptyset$ ,  $i = 1, \dots, T - 1$ . Indeed, (M1) is fulfilled by construction, and (M2) and (M3) hold vacuously. Thus, (F1) holds. Furthermore, properties (F2) and (F3) are inherited from  $G$ , and (F4) is fulfilled vacuously. This shows  $G'$  is a signaling cascade of PTM systems with layers  $G'_1, \dots, G'_{T-1}$ .

To establish the second statement, it is enough to show that  $(\mathcal{R}'_i)^{**} = \mathcal{R}_i^{**}$ ,  $i = 1, \dots, T - 1$ . Let  $i \in [T - 1]$ , and  $S \rightarrow S' \in (\mathcal{R}'_i)^{**}$ . Then, by construction,  $S + \alpha E \rightarrow S' + \alpha E \in \mathcal{R}_i$  for some  $S, S' \in \text{Sub}_i$ ,  $S \neq S'$ ,  $E \in \text{Enz}_i$ , and  $\alpha \in \{0, 1\}$ , and so  $S \rightarrow S' \in \mathcal{R}_i^{**}$ . Conversely, if  $S \rightarrow S' \in \mathcal{R}_i^{**}$ , then  $S + \alpha E \rightarrow S' + \alpha E \in \mathcal{R}_i$  for some  $S, S' \in \text{Sub}_i$ ,  $S \neq S'$ ,  $E \in \text{Enz}_i$ , and  $\alpha \in \{0, 1\}$ . If  $E \in \text{Enz}_T$  and  $\alpha = 1$ , then we get  $S \rightarrow S' \in \mathcal{R}'_i$  by construction, and so  $S \rightarrow S' \in (\mathcal{R}'_i)^{**}$ . Otherwise,  $S + \alpha E \rightarrow S' + \alpha E \in \mathcal{R}'_i$ , and so  $S \rightarrow S' \in (\mathcal{R}'_i)^{**}$  after the removal of catalysts.  $\square$

Finally, let  $\widehat{G}_T$  be the reaction network obtained from  $G$  by the removal of the set of catalysts  $\text{Enz}_T$ , and let  $G_T^{**}$  be the underlying substrate network of  $G_T$ . Upon ordering the species and reactions of  $\widehat{G}_T$  in such a way that all species belonging to  $\text{Sub}_T$  correspond to the bottom-most rows, and all monomolecular reactions between species in  $\text{Sub}_T$  correspond to the right-most columns, the stoichiometric matrix of  $\widehat{G}_T$  may be written as

$$\widehat{N}_T = \begin{bmatrix} N' & \vdots & 0 \\ \cdots & \vdots & \cdots \\ 0 & \vdots & N_T^{**} \end{bmatrix}, \quad (14)$$

where  $N'$  is the stoichiometric matrix of the network  $G'$  introduced above, and  $N_T^{**}$  is the stoichiometric matrix of  $G_T^{**}$ . This decomposition will be used in the proofs of the next two results.



**Lemma 46.** *Let  $G$  be a signaling cascade of PTM systems. If  $G$  is consistent, then the connected components of the underlying substrate network of each layer of  $G$  are strongly connected.*

*Proof.* We use induction on the number  $T$  of layers.

For  $T = 1$ , this follows from Proposition 37.

Now suppose the result holds for cascades of PTM systems with  $T - 1$  layers for some  $T \geq 2$ , and let  $G$  be a cascade with  $T$  layers. By Lemma 43 and Corollary 64,  $\widehat{G}_T$  is conservative. Thus,  $\widehat{G}_T$  is consistent by Theorem 31(ii). So, there exists a  $\widehat{v}_T \gg 0$  such that  $\widehat{N}_T \widehat{v}_T = 0$ . We may write  $\widehat{v}_T = (v', v_T^{**})$ , where  $v'$  corresponds to the reactions of  $G'$ , and  $v_T^{**}$  corresponds to the reactions of  $G_T^{**}$ . From (14), we obtain

$$N'v' = 0 \quad \text{and} \quad N_T^{**}v_T^{**} = 0,$$

concluding that  $G'$  and  $G_T^{**}$  are consistent. It follows by the inductive hypothesis, Lemma 45, and Proposition 24 that  $G_1^{**}, \dots, G_{T-1}^{**}, G_T^{**}$ , the underlying substrate networks of  $G_1, \dots, G_{T-1}, G_T$ , respectively, are such that their connected components are strongly connected. This establishes the inductive step, proving the result.  $\square$

**Lemma 47.** *Let  $G$  be a signaling cascade of PTM systems. If the connected components of the underlying substrate network of each layer of  $G$  are strongly connected, then  $G$  has the siphon/P-semiflow property.*

*Proof.* We use induction on the number  $T$  of layers.

For  $T = 1$ , this follows from Proposition 37.

Now suppose the result holds for signaling cascades of PTM systems with  $T - 1$  layers for some  $T \geq 2$ , and let  $G$  be a cascade with  $T$  layers. Since  $\widehat{G}_T$  is obtained from  $G$  by the removal of a set of catalysts, it is enough to show that  $\widehat{G}_T$  has the siphon/P-semiflow property, and this will then be true of  $G$  as well in virtue of Theorem 31(i).

By construction, the species set  $\widehat{S}_T$  of  $\widehat{G}_T$  can be partitioned as the disjoint union  $\widehat{S}_T = \mathcal{S}' \cup \mathcal{S}_T^{**}$  of the species sets of  $G'$  and  $G_T^{**}$ . We claim that every minimal siphon of  $\widehat{G}_T$  is entirely contained in either  $\mathcal{S}'$  or  $\mathcal{S}_T^{**}$ . To see this, let  $\widehat{\Sigma}_T$  be any minimal siphon of  $\widehat{G}_T$ , and suppose it is not entirely contained in  $\mathcal{S}'$ . So,  $\widehat{\Sigma}_T \cap \mathcal{S}_T^{**} \neq \emptyset$ . By hypothesis,  $G_T^{**}$  is a monomolecular network with the property that each of its connected components is strongly connected. Thus, each of its connected components is a minimal siphon. We conclude that  $\widehat{\Sigma}_T$  contains one of the connected components of  $G_T^{**}$  and, by minimality, must be actually equal to it.

By the inductive hypothesis and Lemma 45,  $G'$  has the siphon/P-semiflow property. By Proposition 24,  $G_T^{**}$  also has the siphon/P-semiflow property. We conclude from the block-diagonal decomposition in (14) and the claim above that  $\widehat{G}_T$  has the siphon/P-semiflow property.  $\square$

## 4.2 Dissipative Networks

In the next definition, we use the same notation as in Subsection 2.3.

**Definition 48** (Dissipative Networks). A reaction network (3) is said to be *dissipative* if its solutions are eventually uniformly bounded. More precisely, if there exists a constant  $K \geq 0$  such that

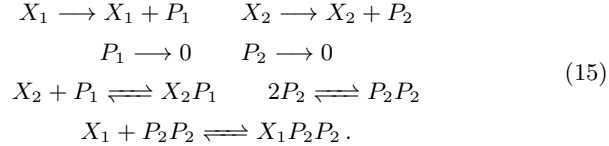
$$\limsup_{t \rightarrow \infty} |\sigma(t, s_0)| \leq K,$$

for each initial state  $s_0 \geq 0$ .  $\triangle$

**Corollary 49.** *If a dissipative reaction network is bounded-persistent, then it is persistent.*

*Proof.* Indeed, every solution of a dissipative reaction network is bounded. The conclusion then follows from Lemma 13.  $\square$

*Example 50 (Monomer-Dimer Toggle).* Consider the monomer-dimer toggle model given by the reaction network

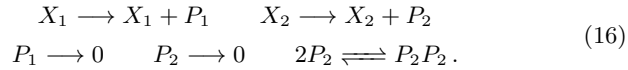


The four reactions in the top row model basal protein production and degradation. The  $P_2P_2$  represents a dimeric species, while  $X_2P_1$  and  $X_1P_2P_2$  represent, respectively, monomers and dimers bound to gene promoters. See [19, Page S1] for further contextualization.

By removing the set of intermediates

$$\{X_2P_1, X_1P_2P_2\},$$

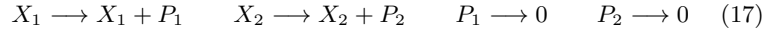
we obtain the network



Now

$$\{P_2P_2\}$$

constitutes a set of intermediates of (16), and so a second round of removal of intermediates is allowed. This yields



Now

$$\{X_1, X_2\}$$

is a set of catalysts of (17). Their removal leaves us with

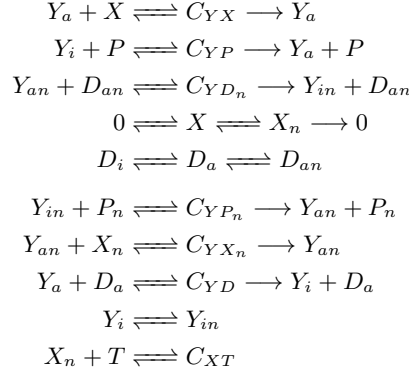


This is a strongly connected monomolecular network. It follows from Proposition 24 that it has the siphon/P-semiflow property, and then from Theorem 30 that (15) also has the siphon/P-semiflow property. It then follows from 16 that (15) is bounded-persistent.

Under mass-action kinetics, (15) is dissipative [19, Pages S7–S8]. Thus, it is also persistent by Corollary 49.  $\diamond$

### 4.3 Boundary Steady States

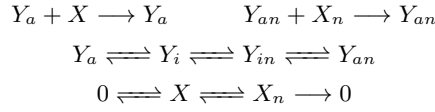
*Example 51 (A Shuttling and Degradation Focused Wnt Model).* The following reaction network model for the Wnt pathway was proposed in [15].



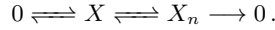
Note that  $\{C_{YX}, C_{YP_n}, C_{YP}, C_{YX_n}, C_{YD_n}, C_{YD}, C_{XT}, D_i\}$  is a set of intermediates. Their removal yields the reaction network



Now  $\{D_a, D_{an}, P_n, P\}$  constitutes a set of catalysts. After their removal, we obtain the reaction network



We may now remove  $\{Y_i, Y_{in}\}$  as a set of intermediates, and then remove  $\{Y_a, Y_{an}\}$  as a set of catalysts, thus obtaining



This is a strongly connected monomolecular network. So, by Lemma 22 and Theorems 30 and 31, the original network has the siphon/P-semiflow property. Thus, by Proposition 16, the Wnt pathway is bounded-persistent. And by Proposition 20, it has no boundary steady states in any stoichiometric compatibility class that is not already contained in the boundary of the positive orthant.  $\diamond$

## 5 Proofs of Theorems 30, 31 and 33

In Subsection 5.1 we prove Theorem 30. The two statements in the theorem will be proven separately as Lemma 60 and Corollary 62, respectively. Likewise, the proof of Theorem 31 is carried out in Subsection 5.2, and split into Lemmas 66 and 67, respectively. The structure is the same in each case. We start with a description of the relationship between the conservation laws of original and reduced models, continue with a similar analysis of their siphons, then conclude that the siphon/P-semiflow property is simultaneously either present or absent in both models. We then discuss consistency.

## 5.1 Intermediates

We begin with a general fact about reaction networks. Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network, and let  $(\mathcal{C}_1, \mathcal{R}_1), \dots, (\mathcal{C}_J, \mathcal{R}_J)$  be the connected components of its reaction graph  $(\mathcal{C}, \mathcal{R})$ .

**Lemma 52.** *For each  $j \in [J]$ ,*

$$y' - y \in \Gamma, \quad \forall y, y' \in \mathcal{C}_j.$$

*Proof.* Since  $y$  and  $y'$  are in the same connected component of  $(\mathcal{C}, \mathcal{R})$ , there exists an undirected path

$$y - y_1 - \dots - y_k - y'$$

in  $(\mathcal{C}, \mathcal{R})$  connecting  $y$  and  $y'$ . In the above, each dash, ‘—,’ represents a forward arrow, ‘→,’ or a backward arrow, ‘←,’ depending on which is the case. Now

$$y' - y = (y' - y_1) + \sum_{i=2}^k (y_{i-1} - y_i) + (y_k - y),$$

so that  $y' - y \in \Gamma$ . □

Now suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G$  by the removal of a set of intermediates  $\mathcal{Y}$ . Recall that  $\mathcal{S}^*$  does not always agree with  $\mathcal{S} \setminus \mathcal{Y}$ . Let

$$\mathcal{X} := (\mathcal{S} \setminus \mathcal{Y}) \setminus \mathcal{S}^*,$$

and write

$$\mathcal{X} = \{X_1, \dots, X_\ell\},$$

and

$$\mathcal{S}^* = \{S_1^*, \dots, S_n^*\}.$$

Thus,

$$\mathcal{S} = \mathcal{S}^* \cup \mathcal{X} \cup \mathcal{Y} = \{S_1^*, \dots, S_n^*, X_1, \dots, X_\ell, Y_1, \dots, Y_p\}.$$

This is the ordering we shall assume whenever working with the stoichiometric matrix or the stoichiometric subspace of  $G$ . Given a complex

$$y = (\alpha_1, \dots, \alpha_n, \gamma_1, \dots, \gamma_\ell, \beta_1, \dots, \beta_p) = \sum_{i=1}^n \alpha_i S_i^* + \sum_{i=1}^{\ell} \gamma_i X_i + \sum_{i=1}^p \beta_i Y_i,$$

in  $\mathcal{C}$ , we will denote its projection over the first  $n$  coordinates by

$$\hat{y} := (\alpha_1, \dots, \alpha_n) = \sum_{i=1}^n \alpha_i S_i^*.$$

Conversely, given a complex

$$\hat{y} = (\alpha_1, \dots, \alpha_n) = \sum_{i=1}^n \alpha_i S_i^*,$$

we denote its embedding in  $\mathbb{R}^{n+\ell+p}$  by

$$y := (\alpha_1, \dots, \alpha_n, 0, \dots, 0) = \sum_{i=1}^n \alpha_i S_i^*.$$

**Lemma 53.** For each  $j \in [J]$ , if  $y, y' \in \mathcal{C}_j \setminus \mathcal{Y}$  and  $y \neq y'$ , then  $\widehat{y}$  and  $\widehat{y}'$  are in the same connected component of  $(\mathcal{C}^*, \mathcal{R}^*)$ .

*Proof.* There exists an undirected path

$$y - y_1 - \cdots - y_k - y'$$

in  $(\mathcal{C}, \mathcal{R})$  connecting  $y$  and  $y'$ , where each dash, ‘—,’ represents a forward arrow, ‘ $\rightarrow$ ,’ or a backward arrow, ‘ $\leftarrow$ ,’ depending on which is the case. Let  $i_1, \dots, i_d \in [k]$  be the indices such that  $y_{i_1}, \dots, y_{i_d} \in \mathcal{C} \setminus \mathcal{Y}$ , so that each non-endpoint in each of the paths

$$\begin{aligned} & y - y_1 - \cdots - y_{i_1-1} - y_{i_1}, \\ & y_{i_1} - y_{i_1+1} - \cdots - y_{i_2-1} - y_{i_2}, \\ & \quad \vdots \\ & y_{i_d} - y_{i_d+1} - \cdots - y_k - y', \end{aligned} \tag{19}$$

is an intermediate.

We first note that we may assume without loss of generality that, within each path, all arrows point in the same direction. To see this, suppose that is not the case for, say, the first path. Suppose  $y \rightarrow y_1$ , and let  $q \in [i_1 - 1]$  be the index corresponding to the first (intermediate) complex where the arrows switch directions. So,

$$y \rightarrow y_1 \rightarrow \cdots \rightarrow y_{q_1} \leftarrow y_{q_1+1} \cdots \tag{20}$$

By (I2), there exists  $y^{(1)} \in \mathcal{C} \setminus \mathcal{Y}$ , and  $Y^{(1)}, \dots, Y^{(p_1)} \in \mathcal{Y}$  such that,

$$y_{q_1} \rightarrow Y^{(1)} \rightarrow \cdots \rightarrow Y^{(p_1)} \rightarrow y^{(1)}$$

is a reaction path in  $G$ . We may then replace  $y_{q_1}$  in (20) by the (undirected) path

$$y_{q_1} \rightarrow Y^{(1)} \rightarrow \cdots \rightarrow Y^{(p_1)} \rightarrow y^{(1)} \leftarrow Y^{(p_1)} \leftarrow \cdots \leftarrow Y^{(1)} \leftarrow y_{q_1},$$

and then split the first path in (19) into the two paths

$$\begin{aligned} & y \rightarrow y_1 \rightarrow \cdots \rightarrow y_{q_1} \rightarrow Y^{(1)} \rightarrow \cdots \rightarrow Y^{(p_1)} \rightarrow y^{(1)}, \\ & y^{(1)} \leftarrow Y^{(p_1)} \leftarrow \cdots \leftarrow Y^{(1)} \leftarrow y_{q_1} \leftarrow y_{q_1+1} \cdots \end{aligned}$$

If there are other changes of direction between  $y_{q_1}$  and  $y_{i_1}$ , we may employ the same construction as many times as needed, splitting each segment into two paths as just described. If  $y \leftarrow y_1$  instead, the argument is analogous, and the same construction applies also to any other path not having the property that all arrows point in the same direction.

Next, we may assume without loss of generality that  $y, y_{i_1}, \dots, y_{i_d}, y'$  are pairwise distinct. Otherwise, we may simply collapse all loops starting and ending at non-intermediate complexes. Now

$$\widehat{y} - \widehat{y}_{i_1} - \cdots - \widehat{y}_{i_d} - \widehat{y}'$$

is an undirected path in  $(\mathcal{C}^*, \mathcal{R}^*)$ . We conclude that  $\widehat{y}$  and  $\widehat{y}'$  are in the same connected component of  $(\mathcal{C}^*, \mathcal{R}^*)$ .  $\square$

## Conservation Laws

In what follows,  $\Gamma^* \subseteq \mathbb{R}^n$  is the stoichiometric subspace of  $G^*$ . Thus, its orthogonal complement  $(\Gamma^*)^\perp$  is taken in  $\mathbb{R}^n$ .

**Lemma 54.** *For each  $j \in [J]$ ,*

$$(\omega^*, x, 0) \cdot y = (\omega^*, x, 0) \cdot y', \quad \forall y, y' \in \mathcal{C}_j \setminus \mathcal{Y}, \quad \forall (\omega^*, x) \in (\Gamma^*)^\perp \times \mathbb{R}^\ell.$$

*Proof.* Fix arbitrarily  $\omega^* \in (\Gamma^*)^\perp$ ,  $x \in \mathbb{R}^\ell$ ,  $j \in [J]$ , and  $y'_j \in \mathcal{C}_j \setminus \mathcal{Y}$ . The equality is trivial if  $y = y'$ , so, assume  $y \neq y'$ . By Lemma 53,  $\widehat{y}$  and  $\widehat{y}'$  are in the same connected component of  $(\mathcal{C}^*, \mathcal{R}^*)$ . By Lemma 52, we conclude that  $\widehat{y} - \widehat{y}' \in \Gamma^*$ . In particular,  $\omega^* \cdot (\widehat{y} - \widehat{y}') = 0$ , yielding the result.  $\square$

For each  $j \in [J]$ , fix arbitrarily a complex  $y_j \in \mathcal{C}_j \setminus \mathcal{Y}$ . Property (I2) in the definition of intermediates ensures that  $\mathcal{C}_j \setminus \mathcal{Y}$  is always nonempty. For each  $i \in [p]$ , let  $j_i \in [J]$  be the index uniquely defined by the property that  $Y_i \in \mathcal{C}_{j_i}$ . Define

$$\begin{aligned} a: (\Gamma^*)^\perp \times \mathbb{R}^\ell &\longrightarrow \mathbb{R}^p \\ (\omega^*, x) &\longmapsto ((\omega^*, x, 0) \cdot y_{j_1}, \dots, (\omega^*, x, 0) \cdot y_{j_p}). \end{aligned}$$

Note that, by Lemma 54,  $a$  is independent of the chosen representatives  $y_j \in \mathcal{C}_j \setminus \mathcal{Y}$ ,  $j \in [J]$ .

**Lemma 55.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\mathcal{Y}$ . Then*

$$\Gamma^\perp = \{(\omega^*, x, a(\omega^*, x)); (\omega^*, x) \in (\Gamma^*)^\perp \times \mathbb{R}^\ell\}.$$

*Proof.* (I) We first show that

$$\{(\omega^*, x, a(\omega^*, x)); (\omega^*, x) \in (\Gamma^*)^\perp \times \mathbb{R}^\ell\} \subseteq \Gamma^\perp. \quad (21)$$

To this end, fix arbitrarily  $\omega^* \in (\Gamma^*)^\perp$ , and  $x \in \mathbb{R}^\ell$ . Denote

$$\omega := (\omega^*, x, a(\omega^*, x)).$$

Fix arbitrarily  $y \rightarrow y' \in \mathcal{R}$ . We want to show that

$$\omega \cdot (y' - y) = 0.$$

There are four possibilities.

(1) If  $\widehat{y} \rightarrow \widehat{y}' \in \mathcal{R}^*$ , then

$$\omega \cdot (y' - y) = \omega^* \cdot (\widehat{y}' - \widehat{y}) = 0.$$

(2) If  $y \rightarrow y' = y \rightarrow Y_i$  for some  $Y_i \in \mathcal{Y}$ , and some  $y \in \mathcal{C} \setminus \mathcal{Y}$ , then

$$\omega \cdot (y' - y) = \omega \cdot (Y_i - y) = (\omega^*, x, 0) \cdot y_{j_i} - (\omega^*, x, 0) \cdot y = 0,$$

as argued above, since  $y$  and  $y_{j_i}$  belong to the same connected component of  $(\mathcal{C}, \mathcal{R})$ .

(3) If  $y \rightarrow y' = Y_i \rightarrow y'$  for some  $Y_i \in \mathcal{Y}$ , and  $y \in \mathcal{C} \setminus \mathcal{Y}$ , then the argument is the same as in (2).

(4) If  $y \rightarrow y' = Y_i \rightarrow Y_k$  for some  $Y_i, Y_k \in \mathcal{Y}$ , then  $j_i = j_k$ , and so

$$\omega \cdot (y' - y) = \omega \cdot (Y_k - Y_i) = (\omega^*, x, 0) \cdot y_{j_i} - (\omega^*, x, 0) \cdot y_{j_k} = 0.$$

This establishes (21). In particular,

$$\dim((\Gamma^*)^\perp \times \mathbb{R}^\ell) = \dim(\Gamma^*)^\perp + \ell \leq \dim \Gamma^\perp.$$

(II) To finish the proof, it is now enough to show that

$$\dim \Gamma^\perp \leq \dim(\Gamma^*)^\perp + \ell. \quad (22)$$

We claim that

$$\dim \Gamma \geq \dim \Gamma^* + p. \quad (23)$$

On the one hand, for each reaction  $\hat{y} \rightarrow \hat{y}' \in \mathcal{R}^*$ , there exists a reaction path in  $G$  connecting  $y$  to  $y'$ , so  $y$  and  $y'$  are in the same connected component of  $(\mathcal{C}, \mathcal{R})$ . It follows by Lemma 52 that  $y' - y \in \Gamma$ . On the other hand, for each intermediate  $Y_i \in \mathcal{Y}$ , there exists a  $y^{(i)} \in \mathcal{C} \setminus \mathcal{Y}$  and a reaction path in  $G$  connecting  $Y_i$  to  $y^{(i)}$ . Again by Lemma 52, we have  $Y_i - y^{(i)} \in \Gamma$ . Furthermore,  $Y_1 - y^{(1)}, \dots, Y_p - y^{(p)}, (y' - y)$  are linearly independent for any  $\hat{y} \rightarrow \hat{y}' \in \mathcal{R}^*$ . This gives us (23).

Combining  $\dim \Gamma + \dim \Gamma^\perp = \dim \mathbb{R}^{n+\ell+p}$  and (23), we get

$$\dim \Gamma^\perp = n + \ell + p - \dim \Gamma \leq n - \dim \Gamma^* + \ell = \dim(\Gamma^*)^\perp + \ell.$$

This establishes (22), completing the proof.  $\square$

**Corollary 56.** *Suppose that  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\mathcal{Y}$ . If  $G$  is conservative, then  $G^*$  is also conservative.*

*Proof.* Indeed, if  $\omega = (\omega^*, x, a(\omega^*, x))$  is a strictly positive conservation law of  $G$ , then  $\omega^*$  is a strictly positive conservation law of  $G^*$ .  $\square$

## Siphons

**Lemma 57.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\mathcal{Y}$ . If  $\Sigma$  is a siphon of  $G$ , then*

$$\Sigma^* := \Sigma \cap \mathcal{S}^*$$

*is either the empty set, or a siphon of  $G^*$ . Furthermore, if  $\Sigma^*$  is empty, then  $\Sigma \cap \mathcal{X}$  is nonempty.*

*Proof.* First suppose  $\Sigma^* \neq \emptyset$ . Pick any  $S' \in \Sigma^*$ , and let  $\hat{y} \rightarrow \hat{y}' \in \mathcal{R}^*$  be any reaction having  $S'$  as one of its products. Then there exists a reaction path in  $G$  connecting  $y$  and  $y'$ . Since  $\Sigma$  is a siphon of  $G$ , we conclude that some species  $S$  constituting  $y$  belongs to  $\Sigma$ . Since  $\hat{y} \in \mathcal{C}^*$ , we must have  $S \in \Sigma^*$ . Thus,  $\Sigma^*$  is a siphon of  $G^*$ .

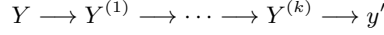
Now suppose  $\Sigma^* = \emptyset$ . Since  $\Sigma \neq \emptyset$  and  $\mathcal{S} = \mathcal{S}^* \cup \mathcal{X} \cup \mathcal{Y}$ , we must have  $\Sigma \cap \mathcal{X} \neq \emptyset$  or  $\Sigma \cap \mathcal{Y} \neq \emptyset$ . If  $\Sigma \cap \mathcal{X} \neq \emptyset$ , then we have nothing left to prove. So, assume  $\Sigma \cap \mathcal{Y} \neq \emptyset$ , and fix arbitrarily a  $Y \in \Sigma \cap \mathcal{Y}$ . By (I2), there exist  $y \in \mathcal{C} \setminus \mathcal{Y}$  and a reaction path in  $G$  connecting  $y$  to  $Y$ . By the siphon property, we conclude that one of the species in  $y$  belongs to  $\Sigma$ . Since  $y$  is supported in  $\mathcal{S}^* \cup \mathcal{X}$ , and since  $\Sigma \cap \mathcal{S}^* = \emptyset$  by hypothesis, we conclude that  $\Sigma \cap \mathcal{X} \neq \emptyset$ .  $\square$

In order to state the next result, we need to introduce a couple of new pieces of notation. Given a species  $S \in \mathcal{S}$ , we will denote by  $K(S)$  the

subset of  $\mathcal{C}$  of complexes where  $S$  appear as a product of some reaction. Given a subset  $\Sigma \subseteq \mathcal{S}$ , we define

$$K(\Sigma) := \bigcup_{S \in \Sigma} K(S).$$

We then define  $M(\Sigma)$  to be the subset of intermediates  $Y \in \mathcal{Y}$  which appear in a chain of reactions



for some  $y' \in K(\Sigma)$ , and some  $Y^{(1)}, \dots, Y^{(k)} \in \mathcal{Y}$ .

**Lemma 58.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\mathcal{Y}$ . If  $\Sigma^*$  is a siphon of  $G^*$ , then*

$$\Sigma := \Sigma^* \cup M(\Sigma^*)$$

*is a siphon of  $G$ . Furthermore, any siphon of  $G$  containing  $\Sigma^*$  must also contain  $M(\Sigma^*)$ .*

*Proof.* Pick any  $S' \in \Sigma$ , and let  $y \rightarrow y' \in \mathcal{R}$  be any reaction having  $S'$  as one of its products.

Suppose first  $S' \in \Sigma^*$ . If  $\widehat{y} \rightarrow \widehat{y}' \in \mathcal{R}^*$ , then  $\Sigma^*$  contains some reactant of  $y \rightarrow y'$ , and so does  $\Sigma$ . If  $\widehat{y} \rightarrow \widehat{y}' \notin \mathcal{R}^*$ , then  $y \rightarrow y' = Y \rightarrow y'$  for some  $Y \in \mathcal{Y}$ . By definition,  $Y \in M(\Sigma^*) \subseteq \Sigma$ .

Now suppose  $S' \notin \Sigma^*$ . Then  $S' \in M(\Sigma^*)$ , meaning that  $y' = S' \in \mathcal{Y}$ , and that there exists a reaction path



in  $G$  such that  $y'_0 \in K(\Sigma^*)$ , and  $Y^{(1)}, \dots, Y^{(k)} \in \mathcal{Y}$ . If  $y \in \mathcal{Y}$ , then it follows that  $y \in M(\Sigma^*)$ , and so  $y \rightarrow y'$  has a reactant in  $\Sigma$ . If  $y \notin \mathcal{Y}$ , we have  $\widehat{y} \rightarrow \widehat{y}'_0 \in \mathcal{R}^*$ , and so one of the species constituting  $\widehat{y}$  belongs to  $\Sigma^*$ . We conclude that one of the reactants of  $y \rightarrow y'$  belongs to  $\Sigma$ . This completes the proof that  $\Sigma$  is a siphon of  $G$ .

It follows straight from the construction of  $M(\Sigma^*)$  and the definition of siphon that any siphon of  $G$  which contains  $\Sigma^*$  must also contain all the intermediates in  $M(\Sigma^*)$ .  $\square$

### The Siphon/P-Semiflow Property

**Lemma 59.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\mathcal{Y}$ . If  $(\omega^*, x, 0) \geq 0$  and  $(\omega^*, x, 0) \cdot y_{j_i} > 0$ , then  $Y_i \in M(\text{supp}(\omega^*, x, 0))$ .*

*Proof.* By construction,  $Y_i$  and  $y_{j_i}$  are in the same connected component of  $(\mathcal{C}, \mathcal{R})$ . By (I2), there are  $y' \in \mathcal{C} \setminus \mathcal{Y}$  and a reaction path connecting  $Y_i$  to  $y'$  such that all its non-endpoints are intermediate complexes. Now  $y_{j_i}$  and  $y'$  are in the same connected component of  $(\mathcal{C}, \mathcal{R})$ , and so

$$(\omega^*, x, 0) \cdot y' = (\omega^*, x, 0) \cdot y_{j_i} > 0$$

by Lemma 54. In particular,

$$\text{supp}(\omega^*, x, 0) \cap \text{supp } y' \neq \emptyset,$$

and

$$Y_i \in M(\text{supp}(\omega^*, x, 0) \cap \text{supp } y') \subseteq M(\text{supp}(\omega^*, x, 0)),$$

completing the proof.  $\square$



**Lemma 60.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\mathcal{Y}$ . Then  $G$  has the siphon/P-semiflow property if, and only if  $G^*$  has the siphon/P-semiflow property.*

*Proof.* ( $\Rightarrow$ ) Suppose  $G$  has the siphon/P-semiflow property. Let  $\Sigma^*$  be any siphon of  $G^*$ . By Lemma 58,

$$\Sigma := \Sigma^* \cup M(\Sigma^*)$$

is a siphon of  $G$ . Let  $\omega \in \Gamma^\perp$  be a P-semiflow supported in  $\Sigma$ , and write

$$\omega = (\omega^*, x, a(\omega^*, x))$$

for some  $\omega^* \in (\Gamma^*)^\perp$ , and some  $x \in \mathbb{R}^\ell$  (Lemma 55). Since  $\Sigma^* \subseteq \mathcal{S}^*$  and  $M(\Sigma^*) \subseteq \mathcal{Y}$ , we conclude that  $\text{supp } \omega^* \subseteq \Sigma^*$ , and that  $x = 0$ . Furthermore,  $\omega^* > 0$ , for if  $\omega^* = 0$ , then  $a(\omega^*, x) = 0$ , and so  $\omega = 0$ , contradicting the assumption that  $\omega$  is a P-semiflow. This shows  $G^*$  has the siphon/P-semiflow property.

( $\Leftarrow$ ) Suppose  $G^*$  has the siphon/P-semiflow property. Let  $\Sigma$  be any siphon of  $G$ . Set

$$\Sigma^* := \Sigma \cap \mathcal{S}^*$$

By Lemma 57, there are two possibilities.

If  $\Sigma^* = \emptyset$ , then

$$\mathcal{P} := \Sigma \cap \mathcal{X}$$

is nonempty. Set

$$\omega := (0, x, a(0, x)) > 0,$$

where

$$x := \sum_{i: X_i \in \mathcal{P}} \mathbf{e}_i.$$

By Lemma 59,  $Y_i \in M(\mathcal{P}) = M(\text{supp}(0, x, 0))$  for each  $i \in [p]$  such that  $a_i(0, x) = (0, x, 0) \cdot y_{j_i} > 0$ . Since  $M(\mathcal{P}) \subseteq \Sigma$ , it follows that  $\text{supp } \omega \subseteq \mathcal{P} \cup M(\mathcal{P}) \subseteq \Sigma$ .

If  $\Sigma^* \neq \emptyset$ , then  $\Sigma^*$  is a siphon of  $G^*$ . Hence, there exists a P-semiflow  $\omega^* \in (\Gamma^*)^\perp$  supported in  $\Sigma^*$ . By Lemma 59,  $Y_i \in M(\text{supp}(\omega^*, 0, 0)) \subseteq M(\Sigma^*)$  for every  $i \in [p]$  such that  $a_i(\omega^*, 0) = (\omega^*, 0, 0) \cdot y_{j_i} > 0$ . Since  $M(\Sigma^*) \subseteq \Sigma$ , it follows that

$$\omega := (\omega^*, 0, a(\omega^*, 0))$$

is a P-semiflow of  $G$  supported in  $\Sigma$ . This establishes that  $G$  has the siphon/P-semiflow property.  $\square$

## Consistency

**Lemma 61.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\{Y\}$  containing a single intermediate  $Y$ . Then  $G^*$  is consistent if, and only if network  $G$  is consistent.*

*Proof.* ( $\Rightarrow$ ) Suppose  $G^*$  is consistent. This is equivalent to say that

$$\sum_{y \rightarrow y' \in \mathcal{R}^*} v_{y \rightarrow y'} (y' - y) = 0$$

for some  $v_{y \rightarrow y'} > 0$ ,  $y \rightarrow y' \in \mathcal{R}^*$ . Let  $\mathcal{R}_Y^* \subseteq \mathcal{R}^*$  be the subset of reactions  $y \rightarrow y' \in \mathcal{R}^*$  such that  $y \rightarrow Y, Y \rightarrow y' \in \mathcal{R}$ , and let  $\mathcal{R}_c^* \subseteq \mathcal{R}^*$  be the subset of all reactions  $y \rightarrow y' \in \mathcal{R}^*$  such that  $y \rightarrow y' \in \mathcal{R}$ . Note that

$$\mathcal{R}^* = \mathcal{R}_Y^* \cup \mathcal{R}_c^*,$$

and that the union need not be disjoint. Let  $\mathcal{C}_{\leftrightarrow} \subseteq \mathcal{C}$  be the subset of complexes  $y \in \mathcal{C}$  such that  $y \rightarrow Y, Y \rightarrow y \in \mathcal{R}$ . Then

$$\begin{aligned} 0 &= \left( \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \setminus \mathcal{R}_c^*} + \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} + \sum_{y \rightarrow y' \in \mathcal{R}_c^* \setminus \mathcal{R}_Y^*} \right) v_{y \rightarrow y'} (y' - y) \\ &\quad + \sum_{y \in \mathcal{C}_Y} (Y - y) + \sum_{y' \in \mathcal{C}_Y} (y' - Y) \\ &= \left( \frac{1}{2} \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} + \sum_{y \rightarrow y' \in \mathcal{R}_c^* \setminus \mathcal{R}_Y^*} \right) v_{y \rightarrow y'} (y' - y) \\ &\quad + \left( \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \setminus \mathcal{R}_c^*} v_{y \rightarrow y'} + \frac{1}{2} \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} v_{y \rightarrow y'} + \sum_{y \in \mathcal{C}_Y} \right) (Y - y) \\ &\quad + \left( \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \setminus \mathcal{R}_c^*} v_{y \rightarrow y'} + \frac{1}{2} \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} v_{y \rightarrow y'} + \sum_{y' \in \mathcal{C}_Y} \right) (y' - Y) \\ &= \sum_{y \rightarrow y' \in \mathcal{R}} w_{y \rightarrow y'} (y' - y), \end{aligned}$$

where

$$w_{y \rightarrow y'} = v_{y \rightarrow y'}, \quad \text{if } y \rightarrow y' \in \mathcal{R}_c^* \setminus \mathcal{R}_Y^*, \quad (24)$$

$$w_{y \rightarrow y'} = \frac{v_{y \rightarrow y'}}{2}, \quad \text{if } y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*, \quad (25)$$

$$w_{y \rightarrow Y} = \begin{cases} \left( \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \setminus \mathcal{R}_c^*} + \frac{1}{2} \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} \right) v_{y \rightarrow y'} + 1, & \text{if } y \in \mathcal{C}_Y \\ \left( \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \setminus \mathcal{R}_c^*} + \frac{1}{2} \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} \right) v_{y \rightarrow y'}, & \text{if } y \notin \mathcal{C}_Y, \end{cases} \quad (26)$$

and, similarly,

$$w_{Y \rightarrow y'} = \begin{cases} \left( \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \setminus \mathcal{R}_c^*} + \frac{1}{2} \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} \right) v_{y \rightarrow y'} + 1, & \text{if } y' \in \mathcal{C}_Y \\ \left( \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \setminus \mathcal{R}_c^*} + \frac{1}{2} \sum_{y \rightarrow y' \in \mathcal{R}_Y^* \cap \mathcal{R}_c^*} \right) v_{y \rightarrow y'}, & \text{if } y' \notin \mathcal{C}_Y. \end{cases} \quad (27)$$

Since  $G^*$  is obtained from  $G$  by the removal of a single intermediate  $Y$ , every reaction in  $\mathcal{R}$  is of the form  $y \rightarrow y'$ ,  $y \rightarrow Y$  or  $Y \rightarrow y'$  for some  $y \rightarrow y' \in \mathcal{R}^*$ , or of the form  $y \rightarrow Y$  or  $Y \rightarrow y$  for some  $y \in \mathcal{C}_Y$ . Thus, (24)–(27) above yield  $w_{y \rightarrow y'} > 0$  for every  $y \rightarrow y' \in \mathcal{R}$ , and we conclude that  $G$  is consistent.

( $\Leftarrow$ ) Now suppose  $G$  is consistent, so that there exist  $w_{y \rightarrow y'} > 0$ ,  $y \rightarrow y' \in \mathcal{R}$ , such that

$$\sum_{y \rightarrow y' \in \mathcal{R}} w_{y \rightarrow y'} (y' - y) = 0. \quad (28)$$

We partition the set  $\mathcal{R}$  of reactions of  $G$  as the (disjoint) union

$$\mathcal{R} = \mathcal{R}_c^* \cup \mathcal{R}_{\rightarrow Y} \cup \mathcal{R}_{Y \rightarrow},$$

where  $\mathcal{R}_c^*$  is defined as in the first part of the proof,  $\mathcal{R}_{\rightarrow Y}$  is the subset of  $\mathcal{R}$  of reactions having  $Y$  as a product, and  $\mathcal{R}_{Y \rightarrow}$  is the subset of  $\mathcal{R}$  of reactions having  $Y$  as a reactant. Observe that  $Y$  is linearly independent with each complex in  $\mathcal{C}^*$ . Combining all coefficients of  $Y$  in (28), we obtain

$$\sum_{y \rightarrow Y \in \mathcal{R}_{\rightarrow Y}} w_{y \rightarrow Y} - \sum_{Y \rightarrow y' \in \mathcal{R}_{Y \rightarrow}} w_{Y \rightarrow y'} = 0,$$

and so

$$\sum_{Y \rightarrow y' \in \mathcal{R}_{Y \rightarrow}} w_{Y \rightarrow y'} y' - \sum_{y \rightarrow Y \in \mathcal{R}_{\rightarrow Y}} w_{y \rightarrow Y} y + \sum_{y \rightarrow y' \in \mathcal{R}_c^*} w_{y \rightarrow y'} (y' - y) = 0. \quad (29)$$

Set

$$V := \sum_{y \rightarrow Y \in \mathcal{R}_{\rightarrow Y}} w_{y \rightarrow Y} = \sum_{Y \rightarrow y' \in \mathcal{R}_{Y \rightarrow}} w_{Y \rightarrow y'}.$$

We have

$$\sum_{Y \rightarrow y' \in \mathcal{R}_{Y \rightarrow}} w_{Y \rightarrow y'} y' = \sum_{y \rightarrow Y \in \mathcal{R}_{\rightarrow Y}} \sum_{Y \rightarrow y' \in \mathcal{R}_{Y \rightarrow}} \frac{w_{y \rightarrow Y} w_{Y \rightarrow y'}}{V} y'$$

and

$$\sum_{y \rightarrow Y \in \mathcal{R}_{\rightarrow Y}} w_{y \rightarrow Y} y = \sum_{y \rightarrow Y \in \mathcal{R}_{\rightarrow Y}} \sum_{Y \rightarrow y' \in \mathcal{R}_{Y \rightarrow}} \frac{w_{y \rightarrow Y} w_{Y \rightarrow y'}}{V} y.$$

Plugging these last two identities into (29), we may rewrite it as

$$\sum_{y \rightarrow y' \in \mathcal{R}^*} v_{y \rightarrow y'} (y' - y) = 0,$$

where

$$v_{y \rightarrow y'} := w_{y \rightarrow y'}, \quad \text{if } y \rightarrow y' \in \mathcal{R}_c^* \setminus \mathcal{R}_{\rightarrow},$$

$$v_{y \rightarrow y'} := w_{y \rightarrow y'} + \frac{w_{y \rightarrow Y} w_{Y \rightarrow y'}}{V}, \quad \text{if } y \rightarrow y' \in \mathcal{R}_c^* \cap \mathcal{R}_{\rightarrow},$$

and

$$v_{y \rightarrow y'} := \frac{w_{y \rightarrow Y} w_{Y \rightarrow y'}}{V}, \quad \text{if } \mathcal{R}_{\rightarrow} \setminus \mathcal{R}_c^*.$$

In particular,  $v_{y \rightarrow y'} > 0$  for every  $y \rightarrow y' \in \mathcal{R}^*$ , showing that  $G^*$  is consistent.  $\square$

**Corollary 62.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of intermediates  $\mathcal{Y} = \{Y_1, \dots, Y_p\}$ . Then  $G$  is consistent if, and only if  $G^*$  is consistent.*

*Proof.* Let  $G_p := G$  and, for  $i = p, \dots, 1$ , let  $G_{i-1}$  be the reaction network obtained from  $G_i$  by the removal of the set of intermediates  $\{Y_i\}$ . By Lemma 27,  $G_0 = G^*$ . Iterating Lemma 61, we conclude that  $G^*$  is consistent if, and only if  $G$  is consistent.  $\square$

## 5.2 Catalysts

Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of catalysts  $\mathcal{E}$ . Let  $G_{\mathcal{E}} = (\mathcal{S}_{\mathcal{E}}, \mathcal{C}_{\mathcal{E}}, \mathcal{R}_{\mathcal{E}})$  be the subnetwork of  $G$  implied by  $\mathcal{E}$ , and write

$$\begin{aligned}\mathcal{S}^* &= \{S_1^*, \dots, S_n^*\}, \\ \mathcal{S}_{\mathcal{E}} &= \{E_1^a, \dots, E_{q_a}^a\},\end{aligned}$$

and

$$\mathcal{E} \setminus \mathcal{S}_{\mathcal{E}} = \{E_1^u, \dots, E_{q_u}^u\}.$$

Thus

$$\mathcal{S} = \{S_1^*, \dots, S_n^*, E_1^a, \dots, E_{q_a}^a, E_1^u, \dots, E_{q_u}^u\}.$$

These are the orderings we shall assume on the species whenever working with the stoichiometric matrices or stoichiometric subspaces of  $G$ ,  $G^*$  or  $G_{\mathcal{E}}$ .

### Conservation Laws

**Lemma 63.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of catalysts  $\mathcal{E}$ . Then*

$$\Gamma^{\perp} = (\Gamma^*)^{\perp} \times \Gamma_{\mathcal{E}}^{\perp} \times \mathbb{R}^{q_u} \subseteq \mathbb{R}^{n+q_a+q_u}.$$

*Proof.* Write

$$\mathcal{R}^* = \{R_1^*, \dots, R_m^*\},$$

and set  $\mathcal{R}_S := \mathcal{R} \setminus \mathcal{R}_{\mathcal{E}}$ . For each  $j \in [m]$ , let  $R_1^{(j)}, \dots, R_{k_j}^{(j)} \in \mathcal{R}_S$  be the reactions of  $G$  from which  $R_j^*$  is obtained by removing the catalysts from both reactant and product in the construction of  $G^*$ . Write

$$\mathcal{R}_{\mathcal{E}} = \{R_1^{\mathcal{E}}, \dots, R_{m_{\mathcal{E}}}^{\mathcal{E}}\}.$$

Thus

$$\mathcal{R} = \{R_1^{(1)}, \dots, R_{k_1}^{(1)}, \dots, R_1^{(m)}, \dots, R_{k_m}^{(m)}, R_1^{\mathcal{E}}, \dots, R_{m_{\mathcal{E}}}^{\mathcal{E}}\}.$$

With these orderings on  $\mathcal{R}$ ,  $\mathcal{R}^*$  and  $\mathcal{R}_{\mathcal{E}}$ , we may express the stoichiometric matrix  $N$  of  $G$  as

$$N = \begin{bmatrix} N' & 0 \\ 0 & N_{\mathcal{E}} \\ 0 & 0 \end{bmatrix}, \quad (30)$$

where  $N'$  has  $n$  rows,  $k_1 + \dots + k_m$  columns, and has the property that the columns corresponding to  $R_1^{(j)}, \dots, R_{k_j}^{(j)}$  are equal to the  $j^{\text{th}}$  column of  $N^*$ , for  $j = 1, \dots, m$ , where  $N_{\mathcal{E}}$  is the stoichiometric matrix of  $G_{\mathcal{E}}$ , and where the bottom  $q_u$  rows are zero.

Given  $\omega^* \in \mathbb{R}^n$ , we have  $(\omega^*)^T N^* = 0$  if, and only if  $(\omega^*)^T N' = 0$ . Hence, given

$$\omega = (\omega^*, \omega_{\mathcal{E}}, x) \in \mathbb{R}^{n+q_a+q_u},$$

we have  $\omega^T N = 0$  if, and only if  $(\omega^*)^T N^* = 0$ , and  $\omega_{\mathcal{E}}^T N_{\mathcal{E}} = 0$ . This proves the lemma.  $\square$

**Corollary 64.** *Suppose that  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of catalysts  $\mathcal{E}$ . If  $G$  is conservative, then  $G^*$  is also conservative.*

*Proof.* Indeed, if  $\omega = (\omega^*, \omega_{\mathcal{E}}, x)$  is a strictly positive conservation law of  $G$ , then  $\omega^*$  is a strictly positive conservation law of  $G^*$ .  $\square$

## Siphons

**Lemma 65.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of catalysts  $\mathcal{E}$ . Let  $\Sigma$  be a minimal siphon of  $G$ . Then one of the three possibilities below is true.*

- (i)  $\Sigma \subseteq \mathcal{S}^*$ , and it is a minimal siphon of  $G^*$ .
- (ii)  $\Sigma \subseteq \mathcal{S}_{\mathcal{E}}$ , and it is a minimal siphon of  $G_{\mathcal{E}}$ .
- (iii)  $\Sigma = \{E\}$  for some  $E \in \mathcal{E} \setminus \mathcal{S}_{\mathcal{E}}$ .

*Proof.* Suppose  $\Sigma \cap (\mathcal{E} \setminus \mathcal{S}_{\mathcal{E}}) \neq \emptyset$ . Pick any  $E \in (\mathcal{E} \setminus \mathcal{S}_{\mathcal{E}})$ . Then  $E$  appears as a reactant in every reaction in which it also appears as a product. We conclude that  $\{E\}$  is a siphon, which must then be minimal. It follows that (iii) holds.

Now suppose  $\Sigma \cap (\mathcal{E} \setminus \mathcal{S}_{\mathcal{E}}) = \emptyset$ . We have two possibilities.

If  $\Sigma_{\mathcal{E}} := \Sigma \cap \mathcal{S}_{\mathcal{E}} \neq \emptyset$ , then it is a siphon of  $G$ . Indeed, pick any  $S' \in \Sigma_{\mathcal{E}}$ , and let  $y \rightarrow y' \in \mathcal{R}$  be any reaction having  $S'$  as one of its products. Since  $\Sigma$  is a siphon of  $G$ ,  $y \rightarrow y'$  must have one of its reactants  $S$  in  $\Sigma$ . If  $y \rightarrow y' \notin \mathcal{R}_{\mathcal{E}}$ , then  $S'$  is also a reactant in  $y \rightarrow y'$ , and we may assume without loss of generality that  $S = S'$ . If  $y \rightarrow y' \in \mathcal{R}_{\mathcal{E}}$ , then we have  $y = S$  and  $S \in \mathcal{S}_{\mathcal{E}}$ . In either case,  $y \rightarrow y'$  has a reactant  $S$  in  $\Sigma_{\mathcal{E}}$ . This shows  $\Sigma_{\mathcal{E}}$  is a siphon of  $G_{\mathcal{E}}$ . By the minimality assumption, we must have  $\Sigma = \Sigma_{\mathcal{E}} \subseteq \mathcal{S}_{\mathcal{E}}$ . Since every siphon of  $G_{\mathcal{E}}$  is also a siphon of  $G$ , we conclude that  $\Sigma_{\mathcal{E}}$  must be a minimal siphon of  $G_{\mathcal{E}}$ .

If  $\Sigma \cap \mathcal{E} = \emptyset$ , then  $\Sigma \subseteq \mathcal{S}^*$ . It follows from the construction of  $G^*$  that  $\Sigma$  is a minimal siphon of  $G^*$ .  $\square$

## The Siphon/P-Semiflow Property

**Lemma 66.** *Suppose  $G^* = (\mathcal{S}^*, \mathcal{C}^*, \mathcal{R}^*)$  is the reduction of  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  by the removal of a set of catalysts  $\mathcal{E}$ . Then  $G$  has the siphon/P-semiflow property if, and only if  $G^*$  has the siphon/P-semiflow property.*

*Proof.* ( $\Rightarrow$ ) Suppose  $G$  has the siphon/P-semiflow property. Let  $\Sigma^*$  be a minimal siphon of  $G^*$ . Note that  $\Sigma^*$  is also a minimal siphon of  $G$ . This is a direct consequence of the construction of  $G^*$ . Let  $\omega \in \Gamma^{\perp}$  be a P-semiflow of  $G$  supported in  $\Sigma^*$ . By Lemma 63, we may express  $\omega$  as

$$\omega = (\omega^*, \omega_{\mathcal{E}}, x)$$

for some  $\omega^* \in (\Gamma^*)^{\perp}$ , some  $\omega_{\mathcal{E}} \in \Gamma_{\mathcal{E}}^{\perp}$ , and some  $x \in \mathbb{R}^{q_u}$ . Since  $\omega$  is supported in  $\Sigma^*$ , we must have  $\omega_{\mathcal{E}} = 0$ ,  $x = 0$ , and  $\omega^* > 0$ . This shows  $\Sigma^*$  contains the support of a P-semiflow of  $G^*$ .

( $\Leftarrow$ ) Suppose  $G^*$  has the siphon/P-semiflow property. Let  $\Sigma$  be a minimal siphon of  $G$ . By Lemma 65, we have three possibilities.

If  $\Sigma \subseteq \mathcal{S}^*$  and is a siphon of  $G^*$ , then there exists a P-semiflow  $\omega^* \in (\Gamma^*)^{\perp}$  of  $G^*$  supported in  $\Sigma$ . We conclude from Lemma 63 that

$$\omega := (\omega^*, 0, 0) \in (\Gamma^*)^{\perp} \times \Gamma_{\mathcal{E}} \times \mathbb{R}^{q_u}$$

is a P-semiflow of  $G$  which is supported in  $\Sigma$ .

If  $\Sigma \subseteq \mathcal{S}_{\mathcal{E}}$  and is a siphon of  $G_{\mathcal{E}}$ , then there exists a P-semiflow  $\omega_{\mathcal{E}} \in \Gamma_{\mathcal{E}}$  of  $G_{\mathcal{E}}$  supported in  $\Sigma$  by (C2). We conclude from Lemma 63 that

$$\omega := (0, \omega_{\mathcal{E}}, 0) \in (\Gamma^*)^{\perp} \times \Gamma_{\mathcal{E}} \times \mathbb{R}^{q_u}$$

is a P-semiflow of  $G$  which is supported in  $\Sigma$ .

If  $\Sigma = \{E_i\}$  for some  $E_i \in \mathcal{E} \setminus \mathcal{S}_\mathcal{E}$ , then it follows from Lemma 63 that

$$\omega := (0, 0, \mathbf{e}_i) \in (\Gamma^*)^\perp \times \Gamma_\mathcal{E} \times \mathbb{R}^{q_u}$$

is a P-semiflow of  $G$  which is supported in  $\Sigma$ .

In either case,  $\Sigma$  contains the support of a P-semiflow of  $G$ . This shows  $G$  has the siphon/P-semiflow property.  $\square$

### Consistency

**Lemma 67.** *Let  $G^*$  be the reduction of a reaction network  $G$  by the removal of a set of catalysts  $\mathcal{E}$ . If  $G$  is consistent, then  $G^*$  is consistent. Conversely, if  $G^*$  is consistent and  $G_\mathcal{E}$  is conservative, then  $G$  is consistent.*

*Proof.* We write the stoichiometric matrix  $N$  of  $G$  as in the proof of Lemma 63.

First suppose that  $G$  is consistent, and let  $v \gg 0$  be such that  $Nv = 0$ . Thus,  $N'v' = 0$ , where

$$v' := (v_1, \dots, v_{k_1+\dots+k_m}) \gg 0.$$

Defining  $v^* \in \mathbb{R}^m$  by setting

$$v_j^* := v_{k_1+\dots+k_{j-1}+1} + \dots + v_{k_1+\dots+k_{j-1}+k_j},$$

we then get  $v^* \gg 0$  and  $N^*v^* = 0$ , showing that  $G^*$  is consistent.

Now suppose  $G^*$  is consistent and  $G_\mathcal{E}$  is conservative. Let  $v^* \gg 0$  be any vector such that  $N^*v^* = 0$ . Set

$$v'_j := \frac{1}{k_j}(v_j, \dots, v_j) \in \mathbb{R}^{k_j}, \quad j = 1, \dots, m,$$

and then set

$$v' := (v'_1, \dots, v'_m) \in \mathbb{R}^{k_1+\dots+k_m}.$$

Then  $N'v' = 0$ . Since  $G_\mathcal{E}$  has the siphon/P-semiflow property by the definition of catalysts, it follows from the assumption that it is conservative and Propositions 16 and 18 that  $G_\mathcal{E}$  is consistent. Let  $v_\mathcal{E} \gg 0$  be such that  $N_\mathcal{E}v_\mathcal{E} = 0$ . Setting  $v := (v', v_\mathcal{E})$ , we have  $v \gg 0$ , and  $Nv = 0$ , proving that  $G$  is consistent.  $\square$

## 5.3 Uniqueness of The Primitive Reduction

To prove Theorem 33, we will use induction on the number of species. We start with a few observations and auxiliary results.

In this subsection we will use the following notation. Given a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  and a set  $\mathcal{A} \subseteq \mathcal{S}$  of intermediates or catalysts of  $G$ , we will denote by  $G^*_\mathcal{A} = (\mathcal{S}^*_\mathcal{A}, \mathcal{C}^*_\mathcal{A}, \mathcal{R}^*_\mathcal{A})$  the reaction network obtained from  $G$  by the removal of  $\mathcal{A}$  (as a set of intermediates or catalysts, whichever happens to be the case). Given another set  $\mathcal{B} \subseteq \mathcal{S}$  of intermediates (respectively, catalysts) of  $G$ , note that  $\mathcal{B} \setminus \mathcal{A}$  is either empty, or else also a set of intermediates (respectively, catalysts) of  $G^*_\mathcal{A}$ . We then denote by  $G^*_{\mathcal{A}\mathcal{B}} = (\mathcal{S}^*_{\mathcal{A}\mathcal{B}}, \mathcal{C}^*_{\mathcal{A}\mathcal{B}}, \mathcal{R}^*_{\mathcal{A}\mathcal{B}})$  the reaction network obtained from  $G^*_\mathcal{A}$  by the removal of  $\mathcal{B} \setminus \mathcal{A}$ .

**Lemma 68.** *Given a reaction network  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ , suppose  $\mathcal{A}, \mathcal{B} \subseteq \mathcal{S}$  are two sets of intermediates or two sets of catalysts of  $G$ . Let  $\mathcal{D} := \mathcal{A} \cup \mathcal{B}$ . Then  $G^*_\mathcal{D} = G^*_{\mathcal{A}\mathcal{B}} = G^*_{\mathcal{B}\mathcal{A}}$ .*

*Proof.* If  $\mathcal{A}$  and  $\mathcal{B}$  are both sets of intermediates, then the result follows from Lemma 27. Removing first the intermediates in  $\mathcal{A}$  one at a time, then removing the intermediates in  $\mathcal{B} \setminus \mathcal{A}$  yields  $G_{\mathcal{A}\mathcal{B}}^*$ . The analogue procedure starting with the intermediates in  $\mathcal{B}$  yields  $G_{\mathcal{B}\mathcal{A}}^*$ . One then concludes by the same lemma that  $G_{\mathcal{A}\mathcal{B}}^* = G_{\mathcal{B}\mathcal{A}}^* = G_{\mathcal{D}}^*$ .

Now suppose  $\mathcal{A}$  and  $\mathcal{B}$  are both sets of catalysts. Then both  $\mathcal{R}_{\mathcal{A}\mathcal{B}}^*$  and  $\mathcal{R}_{\mathcal{B}\mathcal{A}}^*$  consist of the reactions

$$\sum_{i: S_i \notin \mathcal{D}} \alpha_i S_i \longrightarrow \sum_{i: S_i \notin \mathcal{D}} \alpha'_i S_i$$

such that

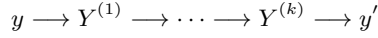
$$\sum_{i=1}^n \alpha_i S_i \longrightarrow \sum_{i=1}^n \alpha'_i S_i$$

belongs to  $\mathcal{R}$ , and  $\alpha_{i_0} > 0$  or  $\alpha'_{i_0} > 0$  for some  $i_0 \in [n]$  such that  $S_{i_0} \notin \mathcal{D}$ . This shows  $\mathcal{R}_{\mathcal{A}\mathcal{B}}^* = \mathcal{R}_{\mathcal{B}\mathcal{A}}^* = \mathcal{R}_{\mathcal{D}}^*$ , establishing the result.  $\square$

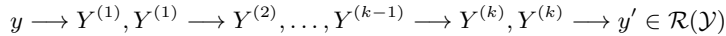
Finally, the removal of a set of catalysts also commutes with the removal of a set of intermediates, in the following sense.

**Lemma 69.** *Let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network,  $\mathcal{Y} \subseteq \mathcal{S}$  be a set of intermediates, and  $\mathcal{E} \subseteq \mathcal{S}$  be a set of catalysts. Then  $G_{\mathcal{Y}\mathcal{E}}^* = G_{\mathcal{E}\mathcal{Y}}^*$ .*

*Proof.* Let  $\mathcal{R}(\mathcal{Y})$  be the subset of reactions  $c \rightarrow c' \in \mathcal{R}$  having some intermediate in  $\mathcal{Y}$  as a reactant or product. It follows directly from property (I2) of intermediates that  $\mathcal{R}(\mathcal{Y})$  is the subset of reactions  $c \rightarrow c' \in \mathcal{R}$  which appear in some reaction path



such that  $y, y' \in \mathcal{C} \setminus \mathcal{Y}$  and  $Y^{(1)}, \dots, Y^{(k)} \in \mathcal{Y}$ . Let  $\mathcal{R}(\mathcal{E})$  be the subset of reactions  $c \rightarrow c' \in \mathcal{R}$  having some catalyst in  $\mathcal{E}$  as both reactant and product. Observe that  $\mathcal{R}(\mathcal{Y}) \cap \mathcal{R}(\mathcal{E}) = \emptyset$ . Thus, both  $\mathcal{R}_{\mathcal{Y}\mathcal{E}}^*$  and  $\mathcal{R}_{\mathcal{E}\mathcal{Y}}^*$  consist of the set of reactions  $y \rightarrow y'$  such that  $y \rightarrow y' \in \mathcal{R} \setminus (\mathcal{R}(\mathcal{Y}) \cup \mathcal{R}(\mathcal{E})) \cup \mathcal{R}_{\mathcal{E}}$ , or



for some  $y, y' \in \mathcal{C} \setminus \mathcal{Y}$  and  $Y^{(1)}, \dots, Y^{(k)} \in \mathcal{Y}$ , or

$$y \longrightarrow y' = \sum_{i: S_i \notin \mathcal{E}} \alpha_i S_i \longrightarrow \sum_{i: S_i \notin \mathcal{E}} \alpha'_i S_i$$

for some

$$\sum_{i=1}^n \alpha_i S_i \longrightarrow \sum_{i=1}^n \alpha'_i S_i$$

belonging to  $\mathcal{R}(\mathcal{E})$ .  $\square$

*Proof of Theorem 33.* We use induction on the number of species. A reaction network with zero species (the empty network) is already primitive, so, in this case, the result holds vacuously.

Now suppose the result holds for reaction networks with up to  $n \geq 0$  species, and let  $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$  be a reaction network with  $|\mathcal{S}| = n + 1$  species. If  $G$  is already primitive, then it is automatically its unique primitive reduction, in which case we have nothing left to prove. So, we may assume  $G$  is not primitive.

Let  $\mathcal{A}, \mathcal{B} \subseteq \mathcal{S}$  be sets of intermediates or catalysts of  $G$  such that  $\mathcal{A} \neq \mathcal{B}$ . By the induction hypothesis,  $G_{\mathcal{A}}^*$  and  $G_{\mathcal{B}}^*$  have unique primitive reductions, respectively,  $G_{\mathcal{A}}^{**}$  and  $G_{\mathcal{B}}^{**}$ . We want to show that  $G_{\mathcal{A}}^{**} = G_{\mathcal{B}}^{**}$ .

Let  $G_{\mathcal{A}\mathcal{B}}^{**}$  (respectively,  $G_{\mathcal{B}\mathcal{A}}^{**}$ ) be the primitive reduction of  $G_{\mathcal{A}\mathcal{B}}^*$  (respectively,  $G_{\mathcal{B}\mathcal{A}}^*$ ). Note that  $G_{\mathcal{A}\mathcal{B}}^{**} = G_{\mathcal{A}}^{**}$  and  $G_{\mathcal{B}\mathcal{A}}^{**} = G_{\mathcal{B}}^{**}$ . By Lemmas 68 and 69,  $G_{\mathcal{A}\mathcal{B}}^* = G_{\mathcal{B}\mathcal{A}}^*$ , and hence  $G_{\mathcal{A}}^{**} = G_{\mathcal{B}}^{**}$ .  $\square$

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## References

- [1] David F. Anderson. Global asymptotic stability for a class of nonlinear chemical equations. *SIAM J. Appl. Math.*, 68(5):1464–1476, 2008.
- [2] David F. Anderson. A proof of the global attractor conjecture in the single linkage class case. *SIAM J. Appl. Math.*, 71(4):1487–1508, 2011.
- [3] David Angeli, Patrick De Leenheer, and Eduardo D. Sontag. A Petri net approach to the study of persistence in chemical reaction networks. *Math. Biosci.*, 210(2):598–618, 2007.
- [4] David Angeli, Patrick De Leenheer, and Eduardo D. Sontag. Graph-theoretic characterizations of monotonicity of chemical networks in reaction coordinates. *J. Math. Biol.*, 61(4):581–616, 2010.
- [5] David Angeli, Patrick De Leenheer, and Eduardo D. Sontag. Persistence results for chemical reaction networks with time-dependent kinetics and no global conservation laws. *SIAM J. Appl. Math.*, 71(1):128–146, 2011.
- [6] Roberto Cordone, Luca Ferrarini, and Luigi Piroddi. Enumeration algorithms for minimal siphons in petri nets based on place constraints. *IEEE transactions on systems, man and cybernetics. Part A, Systems and humans*, 35(6):844–854, 2005.
- [7] Gheorghe Craciun, Fedor Nazarov, and Casian Pantea. Persistence and permanence of mass-action and power-law dynamical systems. *SIAM J. Appl. Math.*, 73(1):305–329, 2013.
- [8] Martin Feinberg. Lectures on chemical reaction networks. Available online at <http://www.crnt.osu.edu/LecturesOnReactionNetworks>, 1980. Notes of lectures given at the Mathematics Research Centre, University of Wisconsin-Madison, in the autumn of 1979.
- [9] Martin Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors. the deficiency zero and deficiency one theorems. *Chemical Engineering Science*, 42(10):2229 – 2268, 1987.



- [10] Elisenda Feliu and Carsten Wiuf. Simplifying biochemical models with intermediate species. *J. R. Soc. Interface*, 10(87):20130484, 2013.
- [11] Gilles Gnacadjá. Reachability, persistence, and constructive chemical reaction networks (part III): a mathematical formalism for binary enzymatic networks and application to persistence. *J. Math. Chem.*, 49(10):2158–2176, 2011.
- [12] Manoj Gopalkrishnan, Ezra Miller, and Anne Shiu. A geometric approach to the global attractor conjecture. *SIAM J. Appl. Dyn. Syst.*, 13(2):758–797, 2014.
- [13] Jeremy Gunawardena. Chemical reaction network theory for *in-silico* biologists. Available online at <http://vcp.med.harvard.edu/papers/crnt.pdf>, 2003.
- [14] F. Horn and Roy Jackson. General mass action kinetics. *Arch. Rational Mech. Anal.*, 47:81–116, 1972.
- [15] Adam L. MacLean, Zvi Rosen, Helen M. Byrne, and Heather A. Harrington. Parameter-free methods distinguish wnt pathway models and guide design of experiments. *PNAS*, 112(9):2652–2657, 2015.
- [16] James D. Murray. *Mathematical Biology*. Springer, 2002.
- [17] Lan K. Nguyen, Javier Muñoz-García, Helene Maccario, Aaron Ciechanover, Walter Kolch, and Boris N. Kholodenko. Switches, excitable responses and oscillations in the ring1b/bmi1 ubiquitination system. *PLoS Comput Biol*, 7(12), 2011.
- [18] Anne Shiu and Bernd Sturmfels. Siphons in chemical reaction networks. *Bull. Math. Biol.*, 72(6):1448–1463, 2010.
- [19] Dan Siegal-Gaskins, Elisa Franco, Tiffany Zhou, and Richard M. Murray. An analytical approach to bistable biological circuit discrimination using real algebraic geometry. *Journal of The Royal Society Interface*, 12(108), 2015.
- [20] Georgi V. Smirnov. *Introduction to the theory of differential inclusions*, volume 41 of *Graduate Studies in Mathematics*. American Mathematical Society, Providence, RI, 2002.
- [21] Hal L. Smith and Horst R. Thieme. *Dynamical systems and population persistence*, volume 118 of *Graduate Studies in Mathematics*. American Mathematical Society, Providence, RI, 2011.
- [22] Eduardo D. Sontag. Structure and stability of certain chemical networks and applications to the kinetic proofreading model of T-cell receptor signal transduction. *IEEE Trans. Automat. Control*, 46(7):1028–1047, 2001.
- [23] Matthew Thomson and Jeremy Gunawardena. The rational parameterisation theorem for multisite post-translational modification systems. *J. Theoret. Biol.*, 261(4):626–636, 2009.