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# On persistence of chemical reaction networks with time-dependent kinetics and no global conservation laws

David Angeli, Patrick De Leenheer and Eduardo D. Sontag

*Abstract*— New checkable criteria for persistence of chemical reaction networks are proposed, which extend those obtained by the authors in previous work. The new results allow the consideration of reaction rates which are time-varying, thus incorporating the effects of external signals, and also relax the assumption of existence of global conservation laws, thus allowing for inflows (production) and outflows (degradation). As an illustration, a hypoxia network is analyzed.

## I. INTRODUCTION

For differential equations evolving in Euclidean space, "persistence" is the property that all solutions starting in the positive orthant do not approach the boundary of the orthant. Interpreted for chemical reactions and population models, this translates into a "non-extinction property" that states that no species will tend to be completely eliminated in the course of the reaction, provided that every species was present at the start of the reaction. In the previous work [4], the authors presented criteria for checking persistence in closed chemical reaction networks, couched in the language of graph theory and Petri nets.

In the present paper, we extend the previous results in two directions: (1) allowing the consideration of kinetic coefficients which are time-varying, and (2) dropping the assumption of conservation, thus allowing potentially unbounded solutions. Time-dependent coefficients represent the effect of external inputs, while inflows and outflows naturally give rise to non-conservative reactions networks. Production and degradation processes are, mathematically, seen as inflows and outflows respectively.

As an example of an application of the new results, this paper analyzes a model of the common core subsystem responsible for the hypoxia control network in *C.elegans*, *Drosophila*, and humans. Hypoxia (deprivation of adequate oxygen supply) results in the expression of specific genes in response to stress caused by low concentration of available oxygen. This particular example was picked for two reasons. First of all, viewing oxygen concentration as an external input gives rise to a network with time-dependent kinetic coefficients. Second, in this model there are no conservation laws that guarantee boundedness of solutions, and so the same example serves to illustrate the role of the new concepts of conditional persistence introduced in this work.

## II. BACKGROUND ON CHEMICAL REACTION NETWORKS

A *chemical reaction network* ("CRN", for short) is a list of chemical reactions $R_i$, taking place among species $S_j$, where the indices $i$ and $j$ take values in $\mathcal{R} := \{1, 2, \ldots, n_r\}$

and $\mathcal{S} := \{1, 2, \ldots n_s\}$ respectively. Individual reactions are then denoted as follows:

$$R_i : \quad \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \to \sum_{j \in \mathcal{S}} \beta_{ij} S_j \qquad (1)$$
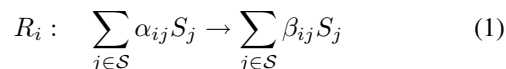
where the $\alpha_{ij}$ and $\beta_{ij}$ are nonnegative integers called the *stoichiometry coefficients*. The species $j$ on the left-hand side for which $\alpha_{ij} > 0$ are called *reactants* and the ones on the right-hand side for which $\beta > 0$ the *products*, of the reaction. Informally speaking, the forward arrow means that the transformation of reactants into products only happens in the direction of the arrow. If also the converse transformation occurs, then, the reaction is reversible and we need to also list its inverse in the network as a separate reaction. Sometimes, for convenience of notation, we will associate to a reaction the two integer column vectors $\alpha_i$ and $\beta_i$, whose entries are defined by the stoichiometry coefficients. It is worth pointing out that we allow chemical reactions in which both the right and left hand sides are actually empty (though not at the same time). This case corresponds, from a physical point of view, to *inflows* and *outflows* of the chemical reaction.

As usually done, we arrange the stoichiometry coefficients into an $n_s \times n_r$ matrix, called the *stoichiometry matrix* $\Gamma$, defined as follows:

$$[\Gamma]_{ji} = \beta_{ij} - \alpha_{ij}, \qquad (2)$$

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

We discuss, next, how the speed of reactions is affected by the concentrations of the different species. Each chemical reaction takes place continuously in time, at its own rate, which is assumed to be only a function of the concentration of the species taking part in it. In order to make this more precise, we define the vector $S = [S_1, S_2, \ldots S_{n_s}]'$ of species concentrations and, as a function of it, the vector of reaction rates

$$R(S, t) := [R_1(S, t), R_2(S, t), \ldots R_{n_r}(S, t)]'$$

where $t \in [0, \infty)$ denotes time. Notice that we explicitly allow time-dependence, as we wish to to consider the effect of external inputs to the system. Such inputs may represent chemical species which are not explicitly considered as part of the state variables but which, nevertheless, influence the reaction rates.

Some mild uniformity requirements are needed for technical reasons as far as time time-dependence is concerned. We assume, in particular, that for all $i \in \mathcal{R}$ for all $S$ and all $t \geq 0$

$$R_i(0, t) = 0 \quad \text{and} \quad \underline{R}_i(S) \leq R_i(S, t) \leq \bar{R}_i(S), \qquad (3)$$

where the $\underline{R}_i(S), \bar{R}_i(S)$ are non-negative, continuous functions of $S$, satisfying the following monotonicity constraint:

$$S \gg_{R_i} \hat{S} \Rightarrow \bar{R}_i(S) > \bar{R}_i(\hat{S}) \qquad (4)$$

for all $i \in \mathcal{R}$ (and a similarly for $\underline{R}_i$), where the notation $S \gg_{R_i} \hat{S}$ means that we have a strict inequality $S_j > \hat{S}_j$ whenever species $j$ is a reactant in reaction $i$. (We also write, more generally, $S \gg \hat{S}$ for any two vectors of species concentrations, if $S_j > \hat{S}_j$ for all $j = 1, \ldots, n_s$.)

Furthermore, we assume standard regularity assumptions of $R_i(S, t)$ in order to ensure local existence and uniqueness of solutions.

A special form of reaction rates are *mass-action kinetics*, which correspond to the following expression:

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

(interpreting $S^0 = 1$ for all $S$), that is, the speed of each reaction is proportional to the concentration of its reagents. Notice that we allow a time-varying kinetic rate $k_i(t)$, which may account for the effect of external species not explicitly included in the network under consideration. In the case of mass-action kinetics, a uniform lower and upper bound on $R_i(S, t)$ exists if and only if there exist constants $k_{\inf}^i > 0$ and $k_{\sup}^i > 0$ such that

$$k_{\inf}^i \leq k(t) \leq k_{\sup} \quad \forall t \geq 0.$$

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

$$\dot{S}(t) = \Gamma R(S(t), t). \qquad (5)$$

where $S = S(t)$ evolves in $\mathbb{R}_{\geq 0}^n$ and represents the vector of all species concentrations at time $t$, and $\Gamma$ is the stoichiometry matrix. For systems with mass-action kinetics the following alternative expression is valid:

$$\dot{S}(t) = \sum_{i \in \mathcal{R}} (\beta_i - \alpha_i) k_i(t) S^{\alpha_i}(t) \qquad (6)$$

where $\beta_i = \beta_{i*}$ is the column vector $\mathrm{col}(\beta_{i1}, \ldots, \beta_{in_s})$, $\alpha_i = \alpha_{i*}$ is the column vector $\mathrm{col}(\alpha_{i1}, \ldots, \alpha_{in_s})$, and $S^\gamma = S_1^{\gamma_1} \ldots S_{n_s}^{\gamma_{n_s}}$ for any nonnegative vector $\gamma = (\gamma_1, \ldots, \gamma_{n_s})$.

It is straightforward to verify that the positive orthant is positively invariant for system (5).

## III. Petri nets and structural invariants

In stating our results, we will employ some terminology borrowed from the graph theory, and specifically Petri nets. Although arising from the study of discrete processes, Petri nets provide a useful language and graphical representation for CRN's, and a number of structural and analytical tools

developed for them can be easily adapted to the continuous context of chemical reactions. In what follows, we associate to a CRN a bipartite directed graph (i.e., a directed graph with two types of nodes) with weighted edges, called the *species-reaction Petri net*, or SR-net for short. Mathematically, this is a quadruple

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

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

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

$$\sum_{j \in \mathcal{S}} \alpha_{ij} S_j \quad \rightarrow \quad \sum_{j \in \mathcal{S}} \beta_{ij} S_j, \qquad (7)$$

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

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

More generally, given a nonempty subset $\Sigma \subseteq \mathcal{S}$ of species, we say that a reaction $R_i$ is an *output (input) reaction for* $\Sigma$ if it is an output (input) reaction to some species of $\Sigma$.

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

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

The stoichiometry matrix $\Gamma$, previously introduced, is usually referred to as *incidence matrix* in the Petri Net literature.

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

*Definition 3.1:* A *P-semiflow* is a row vector $c \succ 0$ such that $c\,\Gamma = 0$. The *support* of a P-semiflow is the set of indexes $\{i \in V_S : c_i > 0\}$.

Using the fact that the entries of $\Gamma$ are integers, it is easy to show that, given any P-semiflow $c$, there is always a P-semiflow with integer components which has the same support as $c$.

*Definition 3.2:* A nonempty subset $\Sigma \subseteq \mathcal{S}$ of species is *stoichiometrically constrained* if there is a P-semiflow whose support is included in $\Sigma$. When $\Sigma = \mathcal{S}$, that is, if there is some P-semiflow $c \gg 0$, we simply say that the

CRN (or the corresponding Petri net) is stoichiometrically constrained.

$P$-semiflows for the system (5) correspond to non-negative linear first integrals, that is, linear functions $S \mapsto cS$ such that $(d/dt)cS(t) \equiv 0$ along all solutions of (5). In particular, a Petri net is stoichiometrically constrained if and only if there is a positive linear conserved quantity for the system. (Petri net theory views Petri nets as "token-passing" systems, and, in that context, $P$-semiflows, also called *place-invariants*, amount to conservation relations for the "place markings" of the network, that show how many tokens there are in each "place," the nodes associated to species in SR-nets. We do not make use of this interpretation in this paper.)

*Definition 3.3:* A *T-semiflow* is a column vector $v \succ 0$ such that $\Gamma v = 0$.

Once again, one can assume without loss of generality that such a $v$ has integer entries.

*Definition 3.4:* A nonempty subset $\Lambda \subseteq \mathcal{R}$ of reactions is *consistent* if there is a $T$-semiflow whose support includes $\Lambda$. When $\Lambda = \mathcal{R}$, we also say that the CRN, or its associated Petri net, is consistent.

The notion of $T$-semiflow corresponds to the existence of a collection of positive reaction rates which do not produce any variation in the concentrations of the species. In other words, $v$ can be viewed as a set of *fluxes* that is in equilibrium [6]. (In Petri net theory, the terminology is "T-invariant," and the fluxes are flows of tokens.)

A vector $v = (0, 0, \ldots, 0, 1, 0, \ldots, 0)$ with a "1" in the $i$th position and 0's elsewhere represents the $i$th reaction; thus we may label such a unit vector as "$R_i$". With this notational convention, the following fact holds. Suppose that $R_k$ and $R_\ell$ are reactions that are reverses of each other, that is, $\alpha_{kj} = \beta_{\ell j}$ and $\beta_{kj} = \alpha_{\ell j}$ for every species $j \in \mathcal{S}$. Then, the vector $R_k + R_\ell$ is a $T$-semiflow, becaus the $k$th and $\ell$th columns of $\Gamma$ are opposites of each other. In chemical network models of biological systems, it is common for several of the reactions to be considered as reversible. This gives rise to many such "trivial" $T$-semiflows.

*Definition 3.5:* A nonempty set $\Sigma \subset V_S$ is called a *siphon* if each input reaction for $\Sigma$ is also an output reaction for $\Sigma$. A siphon is *minimal* if it does not contain (strictly) any other siphons.

For later use, we associate a particular set to a siphon $\Sigma$ as follows:

$$L_\Sigma = \{x \in \mathbb{R}^{n_s}_{\geq 0} \mid x_i = 0 \iff i \in \Sigma\}.$$

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

## IV. PERSISTENCE AND CONSISTENCY

Our main interest is the study of persistence: when do species remain nonzero, if they start nonzero? We will study two variants of this concept, and will provide a necessary characterization for one and a sufficient characterization for the other.

*Definition 4.1:* A nonempty subset $\Sigma \subseteq \mathcal{S}$ of species is *conditionally persistent* (respectively, bounded-persistent) if there exists a bounded solution $S(\cdot)$ with $S(0) \gg 0$ such that

$$\liminf_{t \to \infty} S_j(t) > 0 \quad \forall j \in \Sigma \tag{8}$$

(respectively, if this property holds for all bounded solutions). When $\Sigma = \mathcal{S}$, we say simply that the corresponding CRN is conditionally persistent or bounded-persistent respectively.

Note that in case $\Sigma = \mathcal{S}$ condition (8) amounts to the requirement that the omega-limit set $\omega(S(0))$ should not intersect the boundary of the main orthant.

The following result generalizes Theorem 1 in [4] to systems with time-varying rates, and provides a necessary condition for persistence. It is proved in Section V.

*Theorem 1:* Every conditionally persistent CRN is consistent.

The following result gives a sufficient condition for persistence, and generalizes Theorem 2 in [4] and [1] to systems with time-varying rates: It is proved in Section V.

*Theorem 2:* If a CNR has the property that every siphon is stoichiometrically constrained, then it is bounded-persistent.

## V. KEY TECHNICAL RESULTS

*Definition 5.1:* A nonempty subset $\Lambda \subseteq \mathcal{R}$ of reactions is *conditionally persistent* if there exists a bounded solution $S(\cdot)$ with $S(0) \gg 0$ such that

$$\liminf_{t \to \infty} R_i(S(t), t) > 0 \quad \forall i \in \Lambda.$$

The first key technical fact that we need is as follows; it is proved in Section VII.

*Theorem 3:* Every conditionally persistent subset $\Lambda$ of reactions is consistent.

*Definition 5.2:* A nonempty subset $\Sigma \subseteq \mathcal{S}$ of species is an *extinction set* if there exists a bounded solution $S(\cdot)$ with $S(0) \gg 0$ and a sequence $t_n \to \infty$ such that

$$\lim_{n \to \infty} S_j(t_n) = 0 \quad \Leftrightarrow \quad j \in \Sigma. \tag{9}$$

Equivalently, $\Sigma$ is an extinction set if and only if $L_\Sigma \bigcap \omega(S(0)) \neq \emptyset$ for some bounded solution $S(\cdot)$.

The second key technical fact, proved in Section VI, is as follows.

*Theorem 4:* Every extinction set of species is a siphon.

*Lemma 5.3:* An extinction set cannot be stoichiometrically constrained.

*Proof:* Let $\Sigma$ be an extinction set, and pick a bounded solution $S(\cdot)$ as in the definition of extinction. Suppose that $\Sigma \subseteq \mathcal{S}$ is stoichiometrically constrained. Let $c$ be a $P$-semiflow whose support is included in $\Sigma$. Since $c$ is a $P$-semiflow, $cS(t) = cS(0) > 0$ for all $t \geq 0$ (the last inequality because $S(0) \gg 0$ and $c > 0$). Since the support of $c$ is a subset of $\Sigma$, it follows that $cS(t) = \sum c_j S_j(t)$, with the sum only over the indices $j \in \Sigma$. Thus (9) cannot hold. ∎

We defined what it means for a reaction $R_i$ to be an output reaction for a species $S_j$, namely that $S_j$ should be a reactant of $R_i$. More generally, we use the following concept.

*Definition 5.4:* Consider a nonempty subset $\Sigma \subseteq \mathcal{S}$ of species. A reaction $R_i$ is said to be a *sink* for $\Sigma$ if the set

**4561**

of reactants of $R_i$ is a subset of $\Sigma$. The set of all sinks for $\Sigma$ is denoted as $\Lambda(\Sigma)$.

*Lemma 5.5:* If $\Sigma$ is conditionally persistent, then $\Lambda(\Sigma)$ is conditionally persistent.

*Proof:* Suppose that we have a bounded solution such that $\liminf_{t\to\infty} S_j(t) \geq s > 0$ for every $j \in \Sigma$. Since the solution $S(\cdot)$ is bounded, this means its closure is a compact subset $K$ of the (closed) positive orthant. Pick any sink $R_i$ for $\Sigma$. By Property (4), $\underline{R}_i(S) > 0$ for all $S \in K$. Therefore $\liminf_{t\to\infty} R_i(S(t),t) > 0$ for this same trajectory, which proves that $\Lambda(\Sigma)$ is conditionally persistent. ∎

*Corollary 5.6:* If $\Sigma$ is conditionally persistent, then $\Lambda(\Sigma)$ is consistent.

*Proof:* This follows immediately from Lemma 5.5 and Theorem 3. ∎

This completes the proof of Theorem 1, because the hypothesis of the Theorem says that $\Sigma = \mathcal{S}$ is conditionally persistent. By Corollary 5.6, $\Lambda(\mathcal{S}) = \mathcal{R}$ is consistent, which means that the CRN is consistent, as claimed.

To prove Theorem 2, we observe:

*Lemma 5.7:* If a CRN is not bounded-persistent, then there is some extinction set.

*Proof:* Suppose that there is some bounded solution $S(\cdot)$ with $S(0) \gg 0$, some species $j_0 \in \mathcal{S}$, and some sequence $t_n \to \infty$, such that $\lim_{n\to\infty} S_j(t_n) = 0$. For this solution, and for this same sequence $\{t_n\}$, let $\Sigma$ be defined as the set of species $j \in \mathcal{S}$ such that $\lim_{n\to\infty} S_j(t_n) = 0$. Since $j_0 \in \Sigma$, $\Sigma$ is nonempty, and it is an extinction set by definition. ∎

Now Theorem 2 follows from:

*Corollary 5.8:* If a CRN is not bounded-persistent, then there is a non-stoichiometrically constrained siphon.

*Proof:* Assume that the given CNR is not bounded-persistent. By Lemma 5.7, there is an extinction set $\Sigma$. By Theorem 4, $\Sigma$ is a siphon. By Lemma 5.3, $\Sigma$ is not stoichiometrically constrained. ∎

## VI. PROOF OF THEOREM 4

Let the nonempty subset $\Sigma \subseteq \mathcal{S}$ of species be an extinction set. Pick a bounded solution $S(\cdot)$ with $S(0) \gg 0$ such that $L_\Sigma \bigcap \omega(S(0)) \neq \emptyset$. We need to prove that $\Sigma$ is a siphon.

Assume that $y \in L_\Sigma \cap \omega(S(0))$ but that $\Sigma$ is not a siphon. Hence, there exists a species $S_j \in \Sigma$ so that for at least one of its input reactions $R_k$ and all of $R_k$'s reactant species $S_l$, it holds $y_l > 0$. By Property (3), we have that

$$R_k(y,t) \geq \underline{R}_k(y) \doteq \bar{r} > 0$$

for some positive value $\bar{r}$ and all $t \geq t_0$.

Therefore, since all output reactions of $S_j$ have zero rate at $y$ (no matter what the value of $t$ is), and at least some incoming reaction is strictly positive, it follows by continuity of each of the $\underline{R}_k(S)$'s that there is some $\varepsilon > 0$, so that:

$$\dot{S}_j(t) = [\Gamma R(z(t),t)]_j \geq \bar{r}/2$$

whenever $z(t) \in \mathcal{B}_\varepsilon(y) \doteq \{z \succeq 0 : |z - y| \leq \varepsilon\}$ and $t \geq t_0$. Now, using the uniform upper bound $\bar{R}(S) = (\bar{R}_1(S),\ldots,\bar{R}_r(S))$ and its continuity, we know that there

exists $M > 0$ so that $|\Gamma R(z,t)| \leq M$ for all $z$ as before. Hence,

$$|S(t_b) - S(t_a)| = \left| \int_{t_a}^{t_b} \Gamma R(S(t),t)\,dt \right| \leq (t_b - t_a)M \tag{10}$$

whenever $S(t) \in \mathcal{B}_\varepsilon(y)$ for $t \in [t_a, t_b]$.

Assume without loss of generality (choosing a smaller $\varepsilon$ if necessary) that $\varepsilon$ is such that $S(0) \notin \mathcal{B}_\varepsilon(y)$. Consider now any partial trajectory crossing the boundary of $\mathcal{B}_\varepsilon(y)$ at time $t_\varepsilon$, and hitting the boundary of $\mathcal{B}_{\varepsilon/2}(y)$ at time $t_{\varepsilon/2}$, where $t_{\varepsilon/2}$ is picked as the first time after $t_\varepsilon$ when this happens. Notice that such a partial trajectory exists, because $S(0) \notin \mathcal{B}_\varepsilon(y)$ and by our assumption that $y \in \omega(S(0))$.

Since $\dot{S}_j(t) \geq \bar{r}/2$ for all $t \geq t_0$ whenever $z(t)$ belongs to $\mathcal{B}_\varepsilon(y)$, it follows that necessarily we must exit $\mathcal{B}_\varepsilon(y)$ an infinite number of times, hence infinitely many such partial trajectories exist.

By the estimate in (10), the time it takes to get from the boundary of $\mathcal{B}_\varepsilon(y)$ to $\mathcal{B}_{\varepsilon/2}(y)$ is at least $\varepsilon/2M$. Moreover, since $\dot{S}_j(t) \geq \bar{r}/2$ we have:

$$\begin{aligned} S_j(t_{\varepsilon/2}) = S_j(t_\varepsilon) + \int_{t_\varepsilon}^{t_{\varepsilon/2}} \dot{S}_j(t)\,dt &\geq \\ S_j(t_\varepsilon) + \varepsilon\bar{r}/4M &\geq \varepsilon\bar{r}/4M. \end{aligned}$$

Obviously, for $t \geq t_{\varepsilon/2}$, and as long as $S(t) \in \mathcal{B}_\varepsilon(y)$, we also have: $S_j(t) \geq S_j(t_{\varepsilon/2}) \geq \varepsilon\bar{r}/4M$. This shows indeed $y \notin \omega(S(0))$, contradicting our hypothesis. Hence, $\Sigma$ must be a siphon. ∎

## VII. PROOF OF THEOREM 3

Suppose that $\Lambda \subseteq \mathcal{R}$ is a conditionally persistent set of reactions, and pick a bounded solution $S(\cdot)$ with $S(0) \gg 0$ such that $\liminf_{t\to\infty} R_i(S(t),t) > 0$ for each $i \in \Lambda$. We need to show that $\Lambda$ is consistent.

Pick an arbitrary $t \geq t_0$. Clearly:

$$S(t,t_0,S_0) - S_0 = \int_{t_0}^{t} \dot{S}(t)\,dt = \Gamma \int_{t_0}^{t} R(S(t),t)\,dt. \tag{11}$$

Since $S(t,t_0,S_0)$ is bounded, so are $R(S(t),t)$ and its average

$$\frac{1}{t} \int_{t_0}^{t} R(S(t),t)\,dt.$$

Hence, there exists a sequence $t_n \to +\infty$ such that

$$\frac{1}{t_n} \int_{t_0}^{t_n} R(S(t),t)\,dt$$

also admits a limit $\widetilde{R} \succeq 0$ as $n \to +\infty$. Now taking limits along this subsequence in both sides of (11), after dividing by $t$ yields:

$$0 = \lim_{n\to+\infty} \frac{1}{t_n} (S(t_n,t_0,S_0) - S_0) = \Gamma \widetilde{R}. \tag{12}$$

Moreover, for all $i \in \Lambda$ we have

$$\liminf_{t\to+\infty} R_i(S(t),t) = r_i > 0$$

**4562**

| $S1$ | HIF$\alpha$ |
|---|---|
| $S2$ | HIF$\alpha$:ARNT |
| $S3$ | HIF$\alpha$:ARNT:HRE |
| $S4$ | HRE |
| $S5$ | HIF$\alpha$:ARNT:PHD |
| $S6$ | PHD |
| $S7$ | ARNT |
| $S8$ | HIF$\alpha$:PHD |
| $S9$ | HIF$\alpha$ OH:ARNT:HRE |
| $S10$ | HIF$\alpha$ OH:ARNT |
| $S11$ | HIF$\alpha$ OH |
| $S12$ | VHL |
| $S13$ | HIF$\alpha$ OH:VHL |

TABLE I

THE VARIOUS SPECIES IN THE HYPOXIA NETWORK (13).



Fig. 1. Hypoxia network

and hence there exists $T > 0$ so that for all $t \geq T$, $R_i(S(t), t) \geq r_i/2$. Letting $r$ be the minimum of the $r_i$'s, we conclude that:

$$
\begin{aligned}
\widetilde{R}_i &= \lim_{n \to +\infty} \frac{1}{t_n} \int_{t_0}^{t_n} R_j(S(t), t)\, dt \\
&\geq \lim \frac{1}{t_n}(t_n - t_0)\frac{r}{2} = \frac{r}{2} > 0.
\end{aligned}
$$

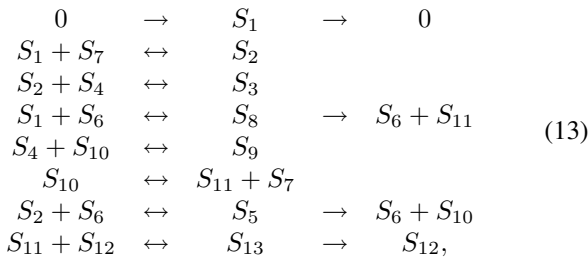So, $\widetilde{R}$ is a $T$-semiflow $v$ whose support contains $\Lambda$. ∎

## VIII. AN EXAMPLE: HYPOXIA NETWORK

As discussed in the Introduction, we analyze a model of the hypoxia control network. Starting from the model given in [5] for the core subsystem of the *hypoxia* control network in *C.elegans*, *Drosophila*, and humans, with 23 species and 32 reactions, the authors of [7] picked a subsystem consisting of 13 species and 19 reactions which constitute the key components explaining experimentally observed behaviors. We analyze this simplified model.

One of the species, $S_1$, which represents the transcription factor HIF$\alpha$, is subject to production and degradation (or, in formal terms, "inflows" and "outflows").

The reactions are as follows:

$$
\begin{aligned}
0 &\rightarrow S_1 \rightarrow 0 \\
S_1 + S_7 &\leftrightarrow S_2 \\
S_2 + S_4 &\leftrightarrow S_3 \\
S_1 + S_6 &\leftrightarrow S_8 \rightarrow S_6 + S_{11} \\
S_4 + S_{10} &\leftrightarrow S_9 \\
S_{10} &\leftrightarrow S_{11} + S_7 \\
S_2 + S_6 &\leftrightarrow S_5 \rightarrow S_6 + S_{10} \\
S_{11} + S_{12} &\leftrightarrow S_{13} \rightarrow S_{12},
\end{aligned}
\tag{13}
$$

where the meaning of the various biochemical species is in Table $I$.

External oxygen affects the dynamics of the system by scaling the rate constants for the reactions $S_8 \rightarrow S_6 + S_{11}$ and $S_5 \rightarrow S_6 + S_{10}$. Mathematically, this means that $k_i(t)$ is proportional to the oxygen concentration (and hence is potentially time-dependent) for each of these two reactions.

According to [5], when the oxygen level falls below a critical value, a sharp rise in HIF$\alpha$ is observed, while this protein is undetectable if the oxygen level is above the critical value. The modeling effort in [5] and the analysis of the model in [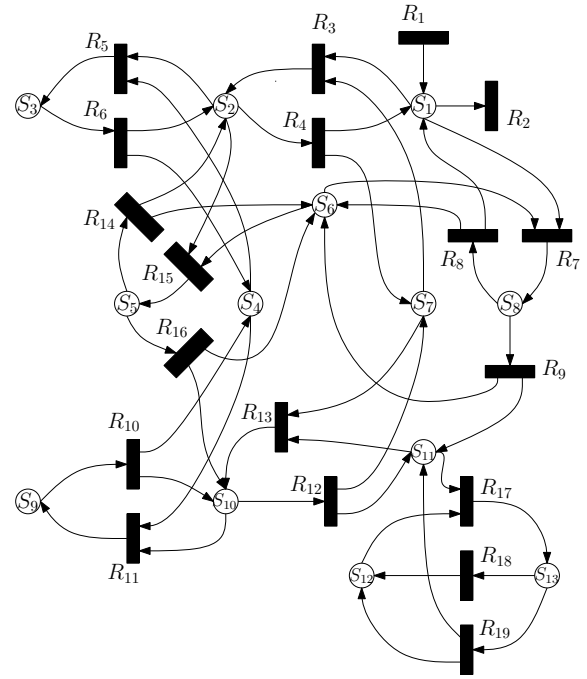7] were aimed at understanding this switch-like behavior. For simplicity, the oxygen level was kept constant, and the model was investigated over a range of values of this constant. Here we will investigate the persistence properties of this network under the assumption that oxygen levels are time-varying.

The associated reaction network, represented as a Petri Net, is shown in Fig. 1. This network admits 4 linearly independent $P$-semiflows, which are associated to the following conservation laws:

$$
\begin{aligned}
S_{12} + S_{13} &= \text{const}_1 \\
S_3 + S_4 + S_9 &= \text{const}_2 \\
S_5 + S_6 + S_8 &= \text{const}_3 \\
S_2 + S_3 + S_5 + S_7 + S_9 + S_{10} &= \text{const}_4
\end{aligned}
\tag{14}
$$

and it is clearly not conservative, due to the presence of outflows and inflows (equation for $S_1$). Not only are there no strictly positive conservation laws $v\Gamma = 0$, but there are not even "decreasing" semiflows satisfying $v\Gamma \leq 0$ which could be used as Lyapunov functions in order to establish boundedness of solutions. In addition, some kinetic rates are allowed to be time-varying. Thus, the techniques from [4] cannot be applied to study persistence.

We consider next the possible $T$-semiflows. There are several "trivial" ones, corresponding to the reversible reactions: $R_3+R_4$, $R_5+R_6$, $R_7+R_8$, $R_{10}+R_{11}$, $R_{12}+R_{13}$,$R_{14}+R_{15}$ and $R_{17} + R_{19}$.

In addition to these, one can find 3 non-trivial independent $T$-semiflows:

$$
\begin{aligned}
&R_1 + R_2 \\
&R_1 + R_7 + R_9 + R_{17} + R_{18} \\
&R_1 + R_3 + R_{15} + R_{16} + R_{12} + R_{17} + R_{18}.
\end{aligned}
\tag{15}
$$

**4563**

Since every reaction appears in at least some $T$-semiflow, the sum of the semiflows shown is also a semiflow which is strictly positive, and we can conclude that the network is consistent.

Thus, the necessary condition for persistence in Theorem 3 is satisfied. This does not quite prove conditional persistence, but shows that the property is not ruled out by the structure of the network.

Next, we find a set of minimal siphons:

$$
\begin{aligned}
&\{S_{12}, S_{13}\}\\
&\{S_3, S_4, S_9\}\\
&\{S_5, S_6, S_8\}\\
&\{S_2, S_3, S_5, S_7, S_9, S_{10}\}.
\end{aligned}
\tag{16}
$$

Notice that all of them coincide with the support of some $P$-semiflow, hence every siphon is stoichiometrically constrained. We conclude that the network is bounded-persistent, by Theorem 2. Next step is the investigation of which variables have the potential for becoming unbounded. An algorithm developed for this purpose is illustrated in [2]. It is based on a linear time-varying embedding of individual species equations and it carries out a consistency check in order to verify which scenarios are compatible with the topology of the network, assuming (for instance) mass-action kinetics. Scenarios are described by labeling each species with a symbol in $\{0, 1, \omega\}$ depending on its asymptotic behaviour, namely converging to 0, bounded and bounded away of 0 or diverging to infinity. While such a classification of behaviours does not cover all the potential asymptotic dynamics of general systems, it appears to be, in practical situations, a fairly mild restriction.

Running the algorithm on the hypoxia network yields 3 potential scenarios for the asymptotic behaviour of the network:

| Scenario | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ | $S_7$ |
|---|---|---|---|---|---|---|---|
| I | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| II | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| III | 1 | 1 | 1 | 1 | 1 | 1 | 0 |

| | $S_8$ | $S_9$ | $S_{10}$ | $S_{11}$ | $S_{12}$ | $S_{13}$ |
|---|---|---|---|---|---|---|
| I | 1 | 1 | 1 | 1 | 1 | 1 |
| II | 1 | 1 | 1 | $\infty$ | 0 | 1 |
| III | 1 | 1 | 1 | $\infty$ | 0 | 1 |

Moreover, see [2] further details, asymptotic invariant vector analysis allows to discard Scenario III, see Fig. 2). Indeed, the linear function $S_2 + S_3 + S_5$ is associated to a $P$-decreasing vector of the reduced net obtained by removing reaction $R_3$, which, according to the labeling is asymptotically switched off. Hence, in this scenario, the quantity $S_2 + S_3 + S_5$ gets asymptotically dissipated with a strictly positive rate; a clear contradiction.

Indeed, simulations showed that both scenarios I and II are possible for different values of the kinetic constants. Notice that persistence is violated in scenario II as several species (namely $S_2, S_3, S_5, S_7, S_{12}$ ) vanish asymptotically. This is not in contrast with our theoretical developments as species $S_{11}$ in this case gets accumulated and diverges to
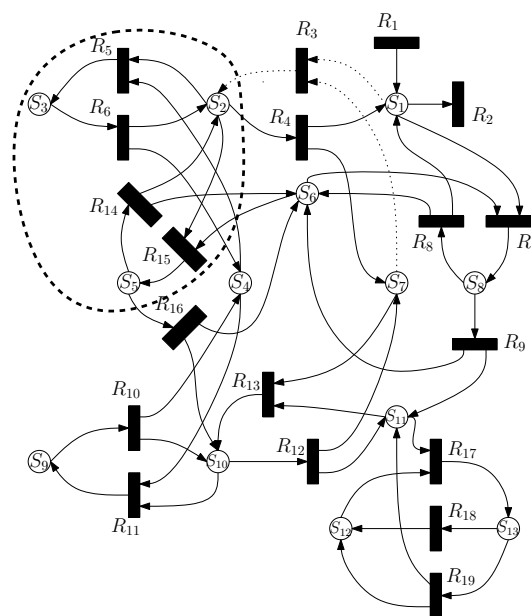


Fig. 2.   Reduced Petri Net and P-decreasing vector support

infinity, thus violating the boundedness assumption which is crucial to Theorem 2. This also shows that extinction sets for unbounded solutions need not be siphons.

## IX. CONCLUSIONS

Checkable criteria for persistence, both necessary and sufficient, have been extended to reactions with time-dependent rates. Such "time-dependent" rates may represent inflows and outflows as well as the effect of external inputs. As an illustration, a hypoxia network is analyzed.

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