

## PERSISTENCE RESULTS FOR CHEMICAL REACTION NETWORKS WITH TIME-DEPENDENT KINETICS AND NO GLOBAL CONSERVATION LAWS\*

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**Abstract.** New checkable criteria for persistence of chemical reaction networks are proposed, which extend and complement existing ones. 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). For time-invariant networks, parameter-dependent conditions for persistence of certain classes of networks are provided. As an illustration, two networks arising in the systems biology literature are analyzed, namely a hypoxia network and an apoptosis network.

**Key words.** chemical reaction networks, persistence, Petri nets

**AMS subject classifications.** 92C40, 92C42, 37C75, 93D99

**DOI.** 10.1137/090779401

**1. Introduction.** For differential equations evolving in Euclidean space, “persistence” is the property that 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 “nonextinction 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 [5]. Persistence is thus a property of central practical interest. In addition, and as remarked in our previous work [4], it is also of major theoretical interest in itself when coupled with work on chemical reaction networks by Horn, Jackson, and Feinberg (see, e.g., [15, 14, 11, 9, 10, 18, 19]) which guarantees global convergence to equilibria in “zero deficiency and weakly reversible” networks (the so-called global attractor conjecture), provided only that persistence is satisfied.

In [4], we presented criteria for checking persistence in closed chemical reaction networks, couched in the language of graph theory and Petri nets. One of the main results was that a time-invariant, conservative chemical network is persistent, provided that each siphon contains the support of a  $P$ -semiflow, regardless of the analytic reaction kinetics underlying the chemical reactions or the values of parameters such as rate constants. These results triggered further research by several other authors, leading to the derivation of algebraic approaches to the identification of siphons in [17], to the development of further connections between persistence and the global attractor conjecture in [2, 7, 12], and to the relaxation of the analyticity assumption by allowing Lipschitz reaction rates in [1].

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\*Received by the editors December 7, 2009; accepted for publication (in revised form) November 15, 2010; published electronically February 1, 2011.

<http://www.siam.org/journals/siap/71-1/77940.html>

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In the present paper, we extend the previous results in several directions, all of them of great relevance to applications:

- Kinetic coefficients are now allowed to be time-varying.
- No conservation assumption is made (so solutions are potentially unbounded).
- The case in which there are critical siphons (i.e., those that do not contain the support of any  $P$ -semiflow) is studied, and a sufficient condition for persistence is provided in that case. This latter condition is parameter-dependent.

The motivation for considering time-dependent coefficients is that these may be used to represent the effect of external inputs to the network, which allows one to include effects such as ligand concentrations, external electrical charge effects, and other environmental factors. Not making a conservation hypothesis is also necessary when considering models with external inputs such as inflows and outflows or when incorporating production and degradation processes. Finally, critical siphons arise naturally in many biological examples. The technical difficulties in the extensions include the need to generalize tools for autonomous systems that were based on analysis of omega limit sets and the need to carefully take into account the fact that trajectories are not now a priori assumed to be bounded. The key property that “extinction sets” must be siphons required a proof very different from an earlier one, and the necessity condition for persistence now requires a time-averaging argument.

We provide two concrete examples of applications of the new results. The first one 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. The second example is an apoptosis (programmed cell death) network. We characterize persistence using our result for critical siphons.

**2. Background on chemical reaction networks.** A *chemical reaction network* (CRN) is specified by two sets of positive integers  $\mathcal{R} := \{1, 2, \dots, n_r\}$  and  $\mathcal{S} := \{1, 2, \dots, n_s\}$ , and nonnegative integers (“stoichiometry coefficients”)  $\alpha_{ij}$  and  $\beta_{ij}$ ,  $i \in \mathcal{R}$ ,  $j \in \mathcal{S}$ , together with a vector of reaction rates as discussed below. One thinks of the elements of  $\mathcal{R}$  as reactions “ $R_i : \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \rightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j$ ” involving the “species”  $S_j$ . We allow the right- or left-hand sides to be empty (though not at the same time), representing, physically, *inflows* and *outflows* of the chemical reaction. Those species  $j$  for which  $\alpha_{ij} > 0$  are called *reactants* and those for which  $\beta_{ij} > 0$  the *products* of the reaction. Informally speaking, the forward arrow means that the transformation of reactants into products happens only in the direction of the arrow. If the converse transformation also occurs, then the reaction is reversible and we need to also list its inverse in the network as a separate reaction. It is convenient to arrange the stoichiometry coefficients into integer column vectors  $\alpha_i$  and  $\beta_i$ . The *stoichiometry matrix*  $\Gamma$  is an  $n_s \times n_r$  matrix with entries  $[\Gamma]_{ji} = \beta_{ij} - \alpha_{ij}$  (notice the reversal of indices). This will be used later in order to synthetically write the differential equation associated with a given chemical network. The reaction rates quantify the speed of reactions as a function of the species concentration vector  $S = [S_1, S_2, \dots, S_{n_s}]'$  and are arranged into a vector

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

where  $t \in [0, \infty)$  denotes time. We explicitly allow time dependence since we wish 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. We impose a mild uniformity requirement for technical reasons: there are nonnegative, continuous functions  $\underline{R}_i(S), \bar{R}_i(S)$  satisfying the following monotonicity constraint:

$$(1) \quad S \gg_{R_i} \hat{S} \Rightarrow \bar{R}_i(S) > \bar{R}_i(\hat{S})$$

for all  $i \in \mathcal{R}$  (and 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  $j = 1, \dots, n_s$ ) such that, for all  $i \in \mathcal{R}$ , for all  $S$ , and for all  $t \geq 0$ ,

$$(2) \quad R_i(0, t) = 0 \quad \text{and} \quad \underline{R}_i(S) \leq R_i(S, t) \leq \bar{R}_i(S).$$

We also 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:  $R_i(S, t) = k_i(t) \prod_{j=1}^{n_s} S_j^{\alpha_{ij}}$  for all  $i = 1, \dots, n_r$  (interpreting  $S^0 = 1$  for all  $S$ ); that is, the speed of each reaction is proportional to the concentration of its reagents. 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  $k_{\text{inf}}^i > 0$  and  $k_{\text{sup}}^i > 0$  such that  $k_{\text{inf}}^i \leq k(t) \leq k_{\text{sup}}^i$  for all  $t \geq 0$ .

With the above notation, the CRN is described by the system of differential equations

$$(3) \quad \dot{S}(t) = \Gamma R(S(t), t),$$

where  $S = S(t)$  evolves in  $\mathbb{R}_{\geq 0}^{n_s}$  and represents the vector of all species concentrations at time  $t$ , and  $\Gamma$  is the stoichiometry matrix. For systems with mass-action kinetics, one could write  $\dot{S}(t) = \sum_{i \in \mathcal{R}} (\beta_i - \alpha_i) k_i(t) S^{\alpha_i}(t)$ , where  $\beta_i = \beta_{i*}$  is the column vector  $\text{col}(\beta_{i1}, \dots, \beta_{in_s})$ ,  $\alpha_i = \alpha_{i*}$  is the column vector  $\text{col}(\alpha_{i1}, \dots, \alpha_{in_s})$ , and  $S^\gamma = S_1^{\gamma_1} \dots S_{n_s}^{\gamma_{n_s}}$  for any nonnegative vector  $\gamma = (\gamma_1, \dots, \gamma_{n_s})$ . It is straightforward to verify that the positive orthant is positively invariant for system (3). Moreover, for each  $S_0 \in \mathbb{R}_{\geq 0}^{n_s}$ , the affine subspace defined by  $S_0 + \text{Im}[\Gamma]$  is also invariant, regardless of the specific expression of reaction rates; its intersection with the positive orthant (which is therefore a forward invariant set) is called the *stoichiometry class* of  $S_0$ .

**3. Petri nets and structural invariants.** It is convenient to employ some terminology borrowed from graph theory, specifically Petri nets. We associate with a CRN a bipartite directed graph with weighted edges, and the *species-reaction Petri net*, or SR net, is a quadruple  $(V_S, V_R, E, W)$ , where  $V_S$  is a finite set of nodes, each one associated with a species,  $V_R$  is a finite set of nodes disjoint from  $V_S$ , corresponding to reactions, and  $E$  is a set of edges. (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. For each reaction  $R_i: \sum_{j \in \mathcal{S}} \alpha_{ij} S_j \rightarrow \sum_{j \in \mathcal{S}} \beta_{ij} S_j$ , we have an edge from  $S_j \in V_S$  to  $R_i \in V_R$  for all  $S_j$ 's if  $\alpha_{ij} > 0$ , and we say that  $R_i$  is an *output reaction for  $S_j$* . Similarly, we draw an edge

from  $R_i \in V_R$  to every  $S_j \in V_S$  if  $\beta_{ij} > 0$ , and we say that  $R_i$  is an *input reaction* for  $S_j$ . 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  $\Sigma$ . An entrywise nonnegative vector  $v$  is denoted by  $v \succeq 0$ . We write  $v \succ 0$  if  $v \succeq 0$  and  $v \neq 0$  and  $v \gg 0$  is  $v_i > 0$  for all  $i$ . A *P-semiflow* is a row vector  $c \succ 0$  such that  $c\Gamma = 0$ , and its *support* 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 3.1. 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.

An interpretation of *P-semiflows* for (3) is as nonnegative linear first integrals, that is, linear functions  $S \mapsto cS$  such that  $(d/dt)cS(t) \equiv 0$  along all solutions of (3). Finally, 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 3.2. 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 [21]. (In Petri net theory, the terminology is “*T*-invariant,” and the fluxes are flows of tokens.)

A vector  $v = (0, 0, \dots, 0, 1, 0, \dots, 0)$  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* because 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 3.3. 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.

The terminology “siphon” arises from Petri net theory. However, the concept of siphon was used (without giving it a name) by Feinberg in [9], in precisely the same context of CRNs as in [4] (more details are given below). At the time that we wrote [4], we were not aware of the previous introduction of the concept in [9].

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

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

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  $\Sigma$ .

**4. 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

$$(4) \quad \liminf_{t \rightarrow \infty} S_j(t) > 0 \quad \forall j \in \Sigma$$

(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 (4) 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 5.

THEOREM 1. Every conditionally persistent CRN is consistent.

Theorem 2 in [4] says that, for systems with constant rates, bounded persistence holds, provided that every siphon is stoichiometrically constrained. The following result extends this sufficient condition to systems with time-varying rates and is proved in section 5. A special case of this result, for constant rates, is implicitly given in [9]. Specifically, for trajectories whose limit sets are either single points or periodic orbits, Propositions 5.3.1 and 5.3.2 and Remark 6.1.E in [9] may be combined to provide the persistence result; the proofs in [9] are also based on siphon analysis.

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

## 5. 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

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

The first key technical fact that we need is the following; it is proved in section 7.

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 exist a bounded solution  $S(\cdot)$  with  $S(0) \gg 0$  and a sequence  $t_n \rightarrow \infty$  such that

$$(5) \quad \lim_{n \rightarrow \infty} S_j(t_n) = 0 \quad \Leftrightarrow \quad j \in \Sigma.$$

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

The second key technical fact, proved in section 6, is the following.

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 over only the indices  $j \in \Sigma$ . Thus (5) cannot hold.  $\square$

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

LEMMA 5.5. If  $\Sigma$  is conditionally persistent, then  $\Lambda(\Sigma)$  is conditionally persistent.

*Proof.* Suppose that we have a bounded solution such that  $\liminf_{t \rightarrow \infty} S_j(t) \geq s > 0$  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 (1),  $\underline{R}_i(S) > 0$  for all  $S \in K$ . Therefore  $\liminf_{t \rightarrow \infty} R_i(S(t), t) > 0$  for this same trajectory, which proves that  $\Lambda(\Sigma)$  is conditionally persistent.  $\square$

COROLLARY 5.6. If  $\Sigma$  is conditionally persistent, then  $\Lambda(\Sigma)$  is consistent.

*Proof.* This follows immediately from Lemma 5.5 and Theorem 3.  $\square$

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 the following result.

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  $t_n \rightarrow \infty$  such that  $\lim_{n \rightarrow \infty} S_{j_0}(t_n) = 0$ . 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  $\lim_{n \rightarrow \infty} S_j(t_n) = 0$ . Since  $j_0 \in \Sigma$ ,  $\Sigma$  is nonempty, and it is an extinction set by definition.  $\square$

Now Theorem 2 follows from the next result.

COROLLARY 5.8. If a CRN is not bounded-persistent, then there is a nonstoichiometrically 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.  $\square$

**6. 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  $L_\Sigma \cap \omega(S(0)) \neq \emptyset$ . 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 that  $y_l > 0$ . By property (2), 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 since 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  $\bar{R}(S) = (\bar{R}_1(S), \dots, \bar{R}_r(S))$  and its continuity, we know that there exists  $M > 0$  so that  $|\Gamma R(z, t)| \leq M$  for all  $z$  as before. Hence,

$$(6) \quad |S(t_b) - S(t_a)| = \left| \int_{t_a}^{t_b} \Gamma R(S(t), t) dt \right| \leq (t_b - t_a)M$$

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 because of 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 (6), 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 that

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

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 that  $y \notin \omega(S(0))$ , contradicting our hypothesis. Hence,  $\Sigma$  must be a siphon.  $\square$

**7. Proof of Theorem 3.** Suppose  $\Lambda \subseteq \mathcal{R}$  is a conditionally persistent set of reactions, and pick a bounded solution  $S(\cdot)$  with  $S(0) \gg 0$  such that  $\liminf_{t \rightarrow \infty} R_i(S(t), t) > 0$  for each  $i \in \Lambda$ . We need to show that  $\Lambda$  is consistent.

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

$$(7) \quad S(t, t_0, S_0) - S_0 = \int_{t_0}^t \dot{S}(t) dt = \Gamma \int_{t_0}^t R(S(t), t) dt.$$

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  $t_n \rightarrow +\infty$  such that

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

also admits a limit  $\tilde{R} \succeq 0$  as  $n \rightarrow +\infty$ . Now, taking limits along this subsequence in both sides of (7), after dividing by  $t$  we have that

$$(8) \quad 0 = \lim_{n \rightarrow +\infty} \frac{1}{t_n} (S(t_n, t_0, S_0) - S_0) = \Gamma \tilde{R}.$$

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

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

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

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

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

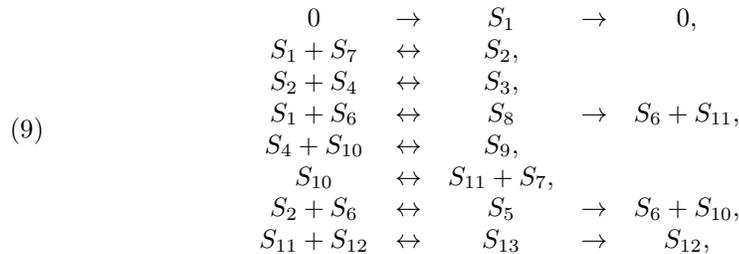
TABLE 1  
The various species in the hypoxia network (9).

$S_1$	HIF $\alpha$
$S_2$	HIF $\alpha$ :ARNT
$S_3$	HIF $\alpha$ :ARNT:HRE
$S_4$	HRE
$S_5$	HIF $\alpha$ :ARNT:PHD
$S_6$	PHD
$S_7$	ARNT
$S_8$	HIF $\alpha$ :PHD
$S_9$	HIF $\alpha$ OH:ARNT:HRE
$S_{10}$	HIF $\alpha$ OH:ARNT
$S_{11}$	HIF $\alpha$ OH
$S_{12}$	VHL
$S_{13}$	HIF $\alpha$ OH:VHL

**8. An example: Hypoxia network.** As discussed in the introduction, we analyze a model of the hypoxia control network. Starting from the model given in [16] for the core subsystem of the *hypoxia* control network in *C. elegans*, *Drosophila*, and humans, with 23 species and 32 reactions, the authors of [20] 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:



where the meaning of the various biochemical species is in Table 1.

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 [16], 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 [16] and the analysis of the model in [20] 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. Our results implicitly assume that the oxygen levels stay bounded away from zero (because of the assumption that all kinetic coefficients have a lower bound  $k_{\text{inf}}^i > 0$ ). It would be an interesting extension to study the behavior of this network when this assumption fails.

The associated reaction network, represented as a Petri net, is shown in Figure 1. This network admits four linearly independent  $P$ -semiflows, which are associated with

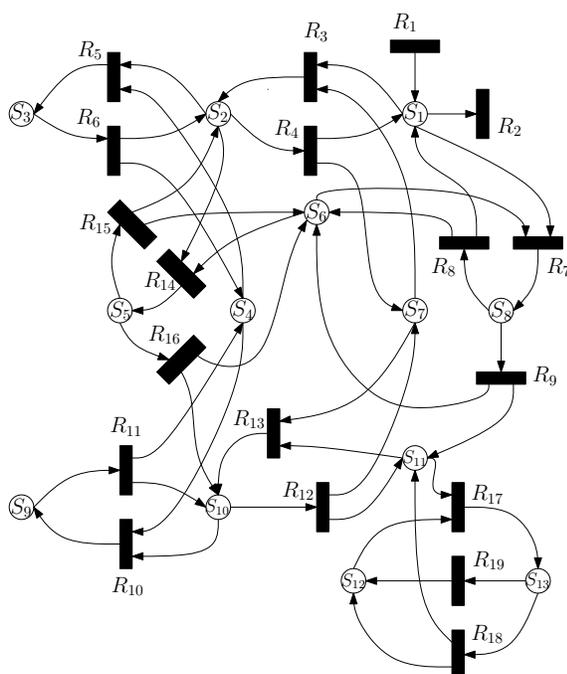


FIG. 1. Hypoxia network.

the conservation laws

$$(10) \quad \begin{aligned} S_{12} + S_{13} &= \text{const}_1, \\ S_3 + S_4 + S_9 &= \text{const}_2, \\ S_5 + S_6 + S_8 &= \text{const}_3, \\ S_2 + S_3 + S_5 + S_7 + S_9 + S_{1,0} &= \text{const}_4, \end{aligned}$$

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  $R_{17} + R_{18}$ .

In addition to these, one can find three nontrivial independent  $T$ -semiflows:

$$(11) \quad \begin{aligned} R_1 + R_2, \\ R_1 + R_7 + R_9 + R_{17} + R_{19}, \\ R_1 + R_3 + R_{14} + R_{16} + R_{12} + R_{17} + R_{19}. \end{aligned}$$

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 it shows that the property is not ruled out by the structure of the network.

Next, we find a set of minimal siphons:

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

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.* In other words, we have proved that for every bounded solution  $S(\cdot)$  with  $S(0) \gg 0$ , (4) holds, i.e.,  $\liminf_{t \rightarrow \infty} S_j(t) > 0$  for all  $j \in \Sigma$ .

Of course, this statement is a conditional statement, and its usefulness relies upon actually showing first that a solution of interest is indeed bounded. This is hard to do without a more careful study of the equations. In our example, all species except for  $S_{11}$  and  $S_1$  belong to the support of a  $P$ -semiflow, and thus their boundedness trivially follows by conservation of the associated linear functional  $v'S$  along solutions of the CRN. In addition,  $S_1$  is also bounded. This follows because

$$(13) \quad \dot{S}_1 = R_1 - k_2 S_1 - R_3 - R_7 + R_4 + R_8 \leq -k_2 S_1 + R_1 + R_4 + R_8$$

and  $R_1$ ,  $R_4$ , and  $R_8$  are bounded (being continuous functions of variables already known to be bounded). Boundedness of  $S_{11}$  is more challenging to prove, and indeed simulations show that this variable has potential for diverging to infinity. However, for appropriate ranges of kinetic constants  $k_i$ , and for appropriate subsets of initial conditions, as represented by the constants appearing in (12), one can prove boundedness; an example is the following.

LEMMA 8.1. *Suppose that kinetic constants and initial conditions satisfy*

$$L = 2k_{12}\text{const}_4 + 2k_9\text{const}_3 - (k_{19} - k_{18})\text{const}_1 < 0.$$

*Then,  $S(t)$  is bounded.*

*Proof.* We introduce the function  $V(S) := 2S_{11} + S_{13}$  evaluated along trajectories. We have that

$$\begin{aligned} \dot{V} &= 2\dot{S}_{11} + \dot{S}_{13} = 2R_{12} + 2R_9 + R_{18} - R_{17} - R_{19} - 2R_{13} \\ &\leq 2k_{12}\text{const}_4 + 2k_9\text{const}_3 + (k_{18} - k_{19})S_{13} - k_{17}S_{11}S_{12} \\ &= 2k_{12}\text{const}_4 + 2k_9\text{const}_3 + (k_{18} - k_{19})S_{13} - k_{17}S_{11}(\text{const}_1 - S_{13}) \\ &= K - [ay + bx(c - y)], \end{aligned}$$

with  $K = 2k_{12}\text{const}_4 + 2k_9\text{const}_3$ ,  $a = k_{19} - k_{18}$ ,  $b = k_{17}$ ,  $c = \text{const}_1$ ,  $x = S_{11}$ , and  $y = S_{13}$ , and we note that  $y \leq c$  (because  $S_{12} + y = c$ ). In general,

$$ay + bx(c - y) = ac + \frac{b}{2}(c - y) \left[ (2x + y) - \left( y + \frac{2a}{b} \right) \right] > ac,$$

the inequality holding as long as  $V = 2x + y > c + 2a/b$ . In summary,  $\dot{V} < L < 0$  whenever  $V > c + 2a/b$ , which means that  $V$  is bounded, so also  $S_{11}$  is bounded.  $\square$

The above sufficient condition for boundedness, and hence persistence, will be satisfied if the initial concentrations of either  $S_{12}$  or  $S_{13}$  (VHL and HIF $\alpha$  OH:VHL, respectively) are large enough, provided that the rate of reaction 19 is larger than that

of reaction 18. VHL is the von Hippel–Lindau protein, a component of a ubiquitin-dependent degradation pathway which degrades hydroxylated HIF $\alpha$  by binding to it. An interpretation of our conditions is as follows. Via an intermediate pathway, HIF $\alpha$  ( $S_1$ ) is first hydroxylated into HIF $\alpha$ -OH ( $S_{11}$ ), and this step is activated by increasing oxygen levels (this may be one of the reasons why low oxygen levels lead to the experimentally observed sharp rise in HIF $\alpha$ : hydroxylation of HIF $\alpha$  becomes less efficient, allowing HIF $\alpha$  to build up). Then, hydroxylated HIF $\alpha$  is degraded, after it first forms a complex ( $S_{13}$ ) with the protein VHL ( $S_{12}$ ). But this complex also dissociates, yielding back hydroxylated HIF $\alpha$ , thereby preventing the degradation. The conditions of Lemma 8.1 guaranteeing boundedness of  $S_{11}$  require that the rate of complex degradation should be faster than the dissociation rate and that there should be enough VHL, or enough complex  $S_{12}$ . In other words, these conditions require that the final part of the pathway in the degradation of HIF $\alpha$  be operating efficiently.

The above lemma ensures that solutions are bounded (and hence persistent) for appropriate initial conditions and parameters. More generally, however, one could ask which *individual variables* have the potential for becoming unbounded. An algorithm developed for this purpose is illustrated in [3]. 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 mass-action kinetics. A restriction of the algorithm is that it requires the knowledge that each individual species either (1) converges to 0, (2) is bounded and bounded away of 0, or (3) diverges to infinity. If these requirements are satisfied, scenarios are described by labeling each species with a symbol in  $\{0, 1, \omega\}$  depending on its asymptotic behavior. Necessary conditions are provided which allow one to check whether a particular scenario is compatible with the network and the prescribed assumptions. While such a classification of behaviors does not cover all the potential asymptotic dynamics of general systems (for instance, a species might tend to zero along a particular subsequence of times but diverge to infinity along some other), it appears to be, in practical situations, a fairly mild restriction.

Based on the analysis presented in [3], and under the assumptions therein, only two scenarios are possible for the hypoxia network. These are listed in the table below:

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
	$S_8$	$S_9$	$S_{10}$	$S_{11}$	$S_{12}$	$S_{13}$	
I	1	1	1	1	1	1	
II	1	1	1	$\omega$	0	1	

There seems to be no theoretical way to decide for which parameters scenario I holds and for which parameters scenario II holds. Indeed, simulations showed that scenarios I and II are both possible for different values of the kinetic constants. In the case that scenario I holds, we have persistence since solutions are bounded. Notice that persistence is violated in scenario II since several species (namely  $S_2, S_3, S_5, S_7, S_{12}$ ) vanish asymptotically. This does not contradict our theoretical results, as species  $S_{11}$  in this case gets accumulated and diverges to infinity, thus violating the boundedness assumption which is crucial to Theorem 2. Notice that the set of species tending to zero along a particular sequence of times (the latter chosen in order for

all species to admit a limit either finite or infinite) need not be a siphon in the case of unbounded solutions; this is in contrast to the bounded case treated in Theorem 4.

**9. Cascade decompositions and siphons.** One of the main results in [4] states that a time-invariant, conservative CRN is persistent if each siphon contains the support of a  $P$ -semiflow, regardless of the reaction kinetics underlying the chemical reactions or the values of parameters such as rate constants. In the previous sections we have extended this result to CRNs with time-varying reaction kinetics which may have inflows and outflows. In this section, we return to the time-independent case, and we explore the scenario of a CRN that has a siphon which does not contain the support of a  $P$ -semiflow (using the terminology of [4], we say that this siphon is critical). We will see that a simple sufficient condition based on a linearization argument can be formulated that still guarantees persistence of the CRN. The linearization condition is related to, though different from, other conditions imposed on CRNs in order to obtain persistence, notably that given in section 7.2.1 in [6].

Let  $\Sigma$  be a critical siphon. We partition the state  $S$  according to species which do not belong to  $\Sigma$ , denoted by  $\zeta$ , and species which belong to  $\Sigma$ , denoted by  $\sigma$ . As shown in [4], siphons have the forward invariance property stated below:

$$S_0 \in L_\Sigma \Rightarrow S(t, S_0) \in L_\Sigma \quad \forall t \geq 0.$$

If the system equations of the CRN are

$$(14) \quad \begin{bmatrix} \dot{\zeta} \\ \dot{\sigma} \end{bmatrix} = \begin{bmatrix} f_\zeta(\zeta, \sigma) \\ f_\sigma(\zeta, \sigma) \end{bmatrix},$$

then the forward invariance property implies the following condition:

$$(15) \quad f_\sigma(\zeta, 0) = 0 \quad \forall \zeta \succeq 0.$$

Consequently, the dynamics on the closure of  $L_\Sigma$  (which is a forward invariant orthant of lower dimension, as proved in [4]) is completely determined by the equations:

$$(16) \quad \dot{\zeta}(t) = f_\zeta(\zeta(t), 0).$$

If  $\omega(S_0) \cap L_\Sigma$  is nonempty for some  $S_0 \gg 0$ , then it is also a forward invariant set, and so is its closure, and the dynamics on the latter set are described by the evolution of the  $\zeta$  components, as given by (16). Checking nonemptiness of such a set may in general be a rather challenging task. However, in some cases it turns out that this is indeed possible by analysis of a reduced variational equation. Since  $\zeta$  in (15) is arbitrary, we have the following further fact:

$$(17) \quad \left. \frac{\partial f_\sigma(\zeta, \sigma)}{\partial \zeta} \right|_{\sigma=0} = 0 \quad \forall \zeta \succeq 0.$$

Therefore, the variational equation associated with solutions corresponding with any initial condition  $S(0) = [\zeta(0)', 0]'$  takes the following block triangular structure:

$$(18) \quad \begin{bmatrix} \dot{\delta\zeta}(t) \\ \dot{\delta\sigma}(t) \end{bmatrix} = \begin{bmatrix} \frac{\partial f_\zeta}{\partial \zeta}(\zeta(t), 0) & \frac{\partial f_\zeta}{\partial \sigma}(\zeta(t), 0) \\ 0 & \frac{\partial f_\sigma}{\partial \sigma}(\zeta(t), 0) \end{bmatrix} \begin{bmatrix} \delta\zeta(t) \\ \delta\sigma(t) \end{bmatrix}.$$

These observations suggest that persistence may be understood by examining the stability properties of a reduced-order variational equation

$$(19) \quad \dot{\delta\sigma}(t) = \frac{\partial f_\sigma}{\partial \sigma}(\zeta(t), 0) \delta\sigma(t)$$

whose dynamics may determine whether the part of the boundary where  $\sigma = 0$  is repelling or attracting to interior solutions. We claim that (19) is a time-varying positive system because the matrix

$$(20) \quad \frac{\partial f_\sigma}{\partial \sigma}(\zeta, 0)$$

is Metzler for all  $\zeta \succeq 0$ . This will follow from the forward invariance of the nonnegative orthant for system (14). Indeed, a standard first-order Taylor expansion yields

$$(21) \quad \begin{aligned} f_\sigma(\zeta, \sigma) &= f_\sigma(\zeta, 0) + \frac{\partial f_\sigma}{\partial \sigma}(\zeta, 0)\sigma + o(|\sigma|) \\ &= \frac{\partial f_\sigma}{\partial \sigma}(\zeta, 0)\sigma + o(|\sigma|). \end{aligned}$$

If  $[\frac{\partial f_\sigma}{\partial \sigma}(\zeta, 0)]_{i,j}$  is negative for some integers  $i \neq j$  and some  $\zeta$  and we let  $\sigma = \varepsilon e_j$  ( $e_j$  is the  $j$ th element of the canonical basis of the Euclidean space of compatible dimension), this yields, thanks to (21),  $f_\sigma(\zeta, \varepsilon e_j)_i < 0$  for all sufficiently small  $\varepsilon > 0$ . This violates forward invariance of the positive orthant (since, clearly,  $[\varepsilon e_j]_i = 0$ ).

Assume that closed invariant sets of (16) are equilibria; let us denote them by  $\zeta_e$ . In view of what has been said so far, one is led naturally to considering the following implications concerning persistence:

1.  $\frac{\partial f_\sigma}{\partial \sigma}(\zeta_e, 0)$  is Hurwitz  $\Rightarrow$  CRN is not persistent.
2.  $\frac{\partial f_\sigma}{\partial \sigma}(\zeta_e, 0)$  has a positive dominant eigenvalue and is irreducible  $\Rightarrow \zeta_e \notin \omega(S_0)$ .

This suggests that persistence can sometimes be analyzed by simple linearization techniques around boundary equilibria. Item 1 of the above claim is obvious, as the existence of a boundary equilibrium point with a nontrivial stable manifold clearly violates persistence of a CRN.

We proceed next to a formal statement and proof of item 2. The proof relies on a few auxiliary results which are deferred to the appendix.

**THEOREM 5.** *Consider a time-invariant CRN whose associated Petri net is conservative. Let  $\Sigma$  be a minimal siphon, and let the state  $S(t)$  be partitioned accordingly:  $[\zeta(t), \sigma(t)]$ . Let  $\zeta_e$  be an equilibrium of (16), globally asymptotically stable relative to its stoichiometry class. Assume, moreover, that*

$$(22) \quad \frac{\partial f_\sigma}{\partial \sigma}(\zeta_e, 0) \text{ is irreducible and } \lambda_{PF} \left( \frac{\partial f_\sigma}{\partial \sigma}(\zeta_e, 0) \right) > 0.$$

*Then, for any initial condition  $S_0 = [\zeta_0, \sigma_0]$  in the interior of the positive orthant and denoting by  $\omega(S_0)$  the  $\omega$ -limit set of the corresponding solution  $S(t, S_0)$ , there holds that  $S_e \doteq [\zeta_e, 0] \notin \omega(S_0)$ .*

*Proof.* The proof is by contradiction. Let  $S_0$  in the interior of the positive orthant be such that  $S(t_n, S_0) \rightarrow S_e$  along some increasing sequence  $t_n \rightarrow +\infty$  as  $n \rightarrow +\infty$ . Let  $c \gg 0$  be a left eigenvector of  $\frac{\partial f_\sigma}{\partial \sigma}(\zeta_e, 0)$ :

$$c \frac{\partial f_\sigma}{\partial \sigma}(\zeta_e, 0) = \lambda_{PF} \left( \frac{\partial f_\sigma}{\partial \sigma}(\zeta_e, 0) \right) c,$$

and define the following function:

$$V(S) := [0, c]S.$$

Then,  $V(S_e) = 0$  (since  $S_e$  is of the form  $[\ast, 0]'$ ), and  $\dot{V}(S) > 0$  for all  $S \in Q \cap \text{int}(\mathbb{R}_{\geq 0}^n)$ , where  $Q$  is a neighborhood of  $S_e$  chosen as in the proof of Lemma A.1, applied to the vector function  $f_\sigma(\zeta, \sigma)$ . Furthermore, we assume without loss of generality that  $Q$  is relatively open in  $\mathbb{R}_{\geq 0}^n$ . Returning to the solution starting at  $S_0$ , we claim the following:

*There is some  $p \in \omega(S_0) \cap L_\Sigma$  with  $p \neq S_e$ .*

By passing to a subsequence if necessary we assume that  $S(t_n, S_0) \in Q$  for all  $n$ . There is some sufficiently large  $n^\ast$  such that for all  $n > n^\ast$  we can define

$$\tau_n := \sup\{t \mid t < t_n \text{ and } S(t, S_0) \notin Q\},$$

which can be thought of as the most recent entry time (into  $Q$ ) before time  $t_n$ . Notice that  $n^\ast$  exists, so that the definition of the  $\tau_n$ 's makes sense. Indeed, if  $n^\ast$  did not exist, then  $S(t, S_0)$  would belong to the open set  $Q \cap \text{int}(\mathbb{R}_{\geq 0}^n)$  for all  $t > 0$ . In that set, the function  $V$  is increasing along solutions as we have remarked earlier, and thus  $\lim_{t \rightarrow +\infty} V(S(t, S_0)) > V(S_0) > 0$ , contradicting that  $\liminf_{t \rightarrow +\infty} V(S(t, S_0)) = 0$  since  $S(t_n, S_0) \rightarrow S_e$  and  $V(S_e) = 0$ . A similar argument shows that  $\tau_n \rightarrow +\infty$  as  $n \rightarrow +\infty$ .

For all  $n > n^\ast$  and  $t \in (\tau_n, t_n)$ , we have that  $S(t, S_0) \in Q \cap \text{int}(\mathbb{R}_{\geq 0}^n)$ , and hence  $V$  is increasing along this part of the solution. In particular, for all  $n > n^\ast$ ,

$$0 < V(S(\tau_n, S_0)) < V(S(t_n, S_0)),$$

where the quantity on the right-hand side tends to zero as  $n \rightarrow +\infty$ . Consequently,

$$V(S(\tau_n, S_0)) \rightarrow 0 \text{ as } n \rightarrow +\infty.$$

By the definition of  $V$ , it follows that  $V(S(\tau_n, S_0)) = c p_n$ , where  $S(\tau_n, S_0) = [\ast, p_n]'$  for all  $n > n^\ast$ . Since  $c \gg 0$ , it follows that  $p_n \rightarrow 0$  as  $n \rightarrow +\infty$ .

By continuity of solutions, the sequence  $S(\tau_n, S_0)$  belongs to  $Q^c$ , the complement of  $Q$ , which is a closed set. Since the sequence  $S(\tau_n, S_0)$  is bounded, we can pass to a subsequence if necessary and assume that  $S(\tau_n, S_0) \rightarrow p \in Q^c$  as  $n \rightarrow +\infty$ . By the previous argument,  $p$  must belong to  $L_\Sigma$ , and  $p$  clearly belongs to  $\omega(S_0)$  as well. Finally, since  $p \in Q^c$ , there holds that  $p \neq S_e$ , which establishes the claim.

On the other hand, let  $C := \omega(S_0) \cap \text{cl}(L_\Sigma)$ . By definition,  $C$  is a compact set. We wish to show that  $C$  is invariant. First, any solution starting in  $C$  exists for all  $t \in \mathbb{R}$  since it belongs to the compact invariant set  $\omega(S_0)$ . Moreover, by (15) and denoting by  $\sigma(t)$  the  $\sigma$ -component of any such solution at time  $t$ , it holds that  $\sigma(t) = 0$  for all  $t \in \mathbb{R}$ , thus showing that such solutions remain in  $\text{cl}(L_\Sigma)$  for all  $t \in \mathbb{R}$ . It follows that  $C$  is indeed invariant. Hence, Corollary A.3, applied with  $C$  equal to the closure of  $\omega(S_0) \cap L_\Sigma$ , and  $x_0 = S_e$ , implies that  $C = \{S_e\}$ , which contradicts the fact that  $p$  belongs to  $C$ .  $\square$

**10. Example: Apoptosis regulation pathway.** We consider the model proposed in [8] of a network responsible for the regulation of apoptosis (cell death) as

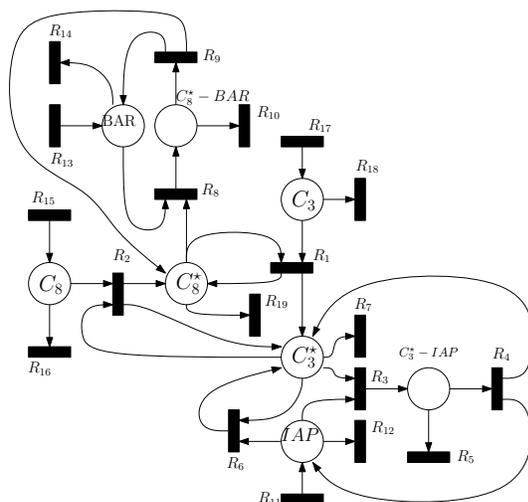
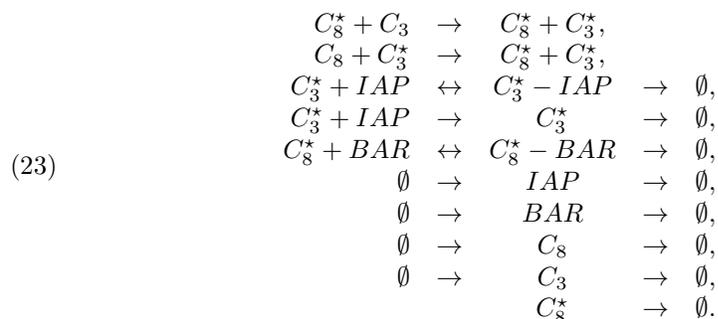


FIG. 2. Apoptosis network.

shown in Figure 2. This comprises the reactions listed below:



Due to the presence of many inflows as well as outflows (degradation of molecules), the network does not exhibit conserved moieties; in the language of Petri nets, there are no  $P$ -semiflows. Computation of  $T$ -semiflows yields, besides the obvious ones,  $R_3 + R_4$ ,  $R_8 + R_9$ ,  $R_{13} + R_{14}$ ,  $R_{15} + R_{16}$ ,  $R_{11} + R_{12}$ ,  $R_{17} + R_{18}$ , entailed by the presence of reversible reactions, the following vectors:

$$\begin{array}{c}
 \hline
 R_6 + R_{11}, \\
 R_2 + R_{15} + R_{19}, \\
 R_1 + R_7 + R_{17}, \\
 R_1 + R_3 + R_5 + R_{11} + R_{17}, \\
 R_2 + R_8 + R_{10} + R_{13} + R_{15} \\
 \hline
 \end{array}$$

Notice that every reaction belongs to at least one  $T$ -semiflow; hence the network is consistent and fulfills the necessary condition for persistence. Thus, it is interesting to look for sufficient conditions for persistence. Analysis of input-output stability of the network is made possible by the presence of outflows in every chemical species. Indeed, by letting

$$\begin{aligned}
 (24) \quad V = & [C_8] + [C_8^*] + [C_3] + [C_3^*] + [IAP] \\
 & + [BAR] + [C_8^* - BAR] + [C_3^* - IAP]
 \end{aligned}$$

and taking its derivative with respect to time, we have that

$$(25) \quad \begin{aligned} \dot{V} \leq & (k_{17} + k_{15} + k_{13} + k_{11}) - k_{16}[C_8] - k_{19}[C_8^*] \\ & - k_{18}[C_3] - k_7[C_3^*] - k_{12}[IAP] - k_{14}[BAR] \\ & - k_8[C_8^* - BAR] - k_5[C_3^* - IAP] \leq K_{in} - K_{out}V, \end{aligned}$$

where the last inequality follows by letting

$$(26) \quad \begin{aligned} K_{in} &= (k_{17} + k_{15} + k_{13} + k_{11}), \\ K_{out} &= \min\{k_{16}, k_{19}, k_{18}, k_7, k_{12}, k_{14}, k_8, k_5\}. \end{aligned}$$

Hence,  $\limsup_{t \rightarrow +\infty} V(t) \leq K_{in}/K_{out} < +\infty$ , which proves input-output stability of the network (this is actually true even for time-varying inflows, in which case the sup norm of  $K_{in}(t)$  is needed in constructing the estimate). To further analyze the network it is useful to investigate the presence of siphons. Indeed, there exists one minimal siphon:  $\Sigma = \{C_3^*, C_8^*, C_3^* - IAP, C_8^* - BAR\}$ . Due to the absence of  $P$ -semiflows this is indeed a critical siphon. Let  $\sigma = [C_3^*, C_8^*, C_3^* - IAP, C_8^* - BAR]'$  be the concentration vector of species belonging to  $\Sigma$ , and let  $\zeta = [IAP, C_8, C_3, BAR]'$ . Writing down explicit equations for  $\dot{\sigma}$  yields

$$(27) \quad \dot{\sigma} = \begin{bmatrix} -k_7\sigma_1 - k_3\sigma_1\zeta_1 + k_1\zeta_3\sigma_2 + k_4\sigma_3 \\ -k_{19}\sigma_2 - k_8\sigma_2\zeta_4 + k_9\sigma_4 + k_2\zeta_2\sigma_1 \\ -(k_4 + k_5)\sigma_3 + k_3\sigma_1\zeta_1 \\ -(k_9 + k_{10})\sigma_4 + k_8\sigma_2\zeta_4 \end{bmatrix}.$$

Taking the Jacobian of the vector field for  $\sigma = 0$  yields

$$J = \begin{bmatrix} -k_7 - k_3\zeta_1 & k_1\zeta_3 & k_4 & 0 \\ k_2\zeta_2 & -k_{19} - k_8\zeta_4 & 0 & k_9 \\ k_3\zeta_1 & 0 & -k_4 - k_5 & 0 \\ 0 & k_8\zeta_4 & 0 & -k_9 - k_{10} \end{bmatrix}.$$

The species not included in  $\Sigma$ , on the other hand, evolve according to the following simple equations (once each variable in  $\Sigma$  is set to 0):

$$(28) \quad \begin{aligned} \dot{\zeta}_1 &= k_{11} - k_{12}\zeta_1 - k_3\sigma_1\zeta_1 - k_6\sigma_1\zeta_1 + k_4\sigma_3, \\ \dot{\zeta}_2 &= k_{15} - k_{16}\zeta_2 - k_2\sigma_2\zeta_2, \\ \dot{\zeta}_3 &= k_{17} - k_{18}\zeta_3 - k_1\sigma_2\zeta_3, \\ \dot{\zeta}_4 &= k_{13} - k_{14}\zeta_4 - k_8\sigma_2\zeta_4 + k_9\sigma_4, \end{aligned}$$

which letting  $\sigma = 0$  yields the simple globally asymptotically stable embedded system

$$(29) \quad \begin{aligned} \dot{\zeta}_1 &= k_{11} - k_{12}\zeta_1, \\ \dot{\zeta}_2 &= k_{15} - k_{16}\zeta_2, \\ \dot{\zeta}_3 &= k_{17} - k_{18}\zeta_3, \\ \dot{\zeta}_4 &= k_{13} - k_{14}\zeta_4 \end{aligned}$$

whose equilibrium is located at  $[k_{11}/k_{12}, k_{15}/k_{16}, k_{17}/k_{18}, k_{13}/k_{14}]$ . Hence, we have a boundary equilibrium at  $[k_{11}/k_{12}, k_{15}/k_{16}, k_{17}/k_{18}, k_{13}/k_{14}, 0, 0, 0, 0]$ .

Applying Lemma A.4 from the appendix leads to the following.

LEMMA 10.1. *Let  $\zeta_i > 0$  be arbitrary. Then  $J$  is Hurwitz if and only if its determinant is positive.*

*Proof.* Necessity is trivial. To prove sufficiency we calculate the four leading principal minors:

$$\begin{aligned} M_1 &= -k_7 - k_3\zeta_1, \\ M_2 &= (k_7 + k_3\zeta_1)(k_{19} + k_8\zeta_4) - k_1k_2\zeta_2\zeta_3, \\ M_3 &= -(k_4 + k_5)(k_7(k_{19} + k_8\zeta_4) - k_1k_2\zeta_2\zeta_3) \\ &\quad - k_3k_5\zeta_1(k_{19} + k_8\zeta_4) \\ &= -(k_4 + k_5)M_2 + k_4k_3\zeta_1(k_{19} + k_8\zeta_4), \\ M_4 &= -(k_9 + k_{10})M_3 - k_9(k_7(k_4 + k_5) + k_3k_5\zeta_1)k_8\zeta_4. \end{aligned}$$

It follows that

$$\det(J) = M_4 > 0 \Rightarrow M_3 < 0 \Rightarrow M_2 > 0,$$

and  $M_1 < 0$  is immediately clear. Thus, by Lemma A.4 in the appendix,  $J$  is Hurwitz whenever its determinant is positive.  $\square$

Consequently, if the determinant of  $J$  is positive, then the CRN is not persistent. On the other hand, if the determinant of  $J$  is negative, then it follows from Theorem 5 that the CRN is persistent (notice that  $J$  is irreducible).

**11. Conclusions.** New checkable criteria for persistence of CRNs have been proposed, which apply even when kinetic rates are time-dependent. These “time-dependent” rates may represent inflows and outflows, as well as the effect of external inputs. Finally, the case when critical siphons are present is also studied. As an illustration, a hypoxia network and an apoptosis network are analyzed.

**Appendix.** In this appendix we state and prove a few results used in the proof of Theorem 5.

LEMMA A.1. *Assume that  $g : \mathbb{R}^n \times \mathbb{R}^m \rightarrow \mathbb{R}^m$  is a  $C^1$  vector function in some open set containing  $\mathbb{R}_{\geq 0}^{n+m}$ .*

*Suppose that there is some  $c \gg 0$  such that the following hold:*

1.  $g(x, 0) = 0$  for all  $x \gg 0$ .
2.  $c\partial g/\partial y(\bar{x}, 0) \gg 0$  at some  $\bar{x} \gg 0$ .

*Then  $cg(x, y) > 0$  for all  $x$  in a neighborhood of  $\bar{x}$  and all  $y \gg 0$  in a neighborhood of  $y = 0$ .*

*Proof.* For each  $(x, y)$ , consider  $m(t) = g(x, ty)$  as a function of  $t$  in  $[0, 1]$ . By the fundamental theorem of calculus and using  $g(x, 0) = 0$ ,

$$g(x, y) = g(x, 0) + \int_0^1 (dm/dt)(t)dt = F(x, y)y,$$

where  $F(x, y) = \int_0^1 (\partial g/\partial y)(x, ty)dt$ .

Pick a convex neighborhood  $Q$  of  $(\bar{x}, 0)$ , where  $c\partial g/\partial y(x, y) \gg 0$  for all  $(x, y)$  in  $Q$ . By convexity, also  $c\partial g/\partial y(x, ty) \gg 0$  for all  $t$  in  $[0, 1]$  and all  $(x, y)$  in  $Q$ ; therefore  $cF(x, y) \gg 0$  for all  $(x, y)$  in  $Q$ , and hence  $cg(x, y) > 0$  if  $(x, y)$  is in  $Q$  and  $y \gg 0$ .  $\square$

The following two lemmas are standard “folk” facts in dynamical systems theory, and we include proofs only in order to make the exposition self-contained.

LEMMA A.2. *Suppose that  $C$  is a compact, invariant set of a dynamical system generated by a system  $\dot{x} = F(x)$  defined on some set  $X$  in  $\mathbb{R}^n$  and that  $x_0$  is a point such that the following hold:*

1. *For every compact, forward invariant subset  $D$  of  $C$ ,  $x_0$  belongs to  $D$ .*
2. *For every  $x$  in  $C$  different from  $x_0$ ,  $x_0$  does not belong to  $\alpha(x)$ .*

*Then,  $C = \{x_0\}$ .*

*Proof.* Suppose that there is some  $x$  in  $C$  which is different from  $x_0$ . Consider  $D = \alpha(x)$ . Since  $C$  is compact and invariant,  $D$  is nonempty, compact, and invariant. Since  $D$  is, in particular, forward invariant, it follows from property 1 that  $x_0 \in D$ . A contradiction is obtained because property 2 asserts that  $x_0$  does not belong to  $D$ .  $\square$

COROLLARY A.3. *Suppose that  $C$  is a nonempty compact, invariant set of a dynamical system generated by a system  $\dot{x} = F(x)$  defined on some set  $X$  in  $\mathbb{R}^n$  and that  $x_0$  is globally asymptotically stable. Then,  $C = \{x_0\}$ .*

*Proof.* Denote the flow on  $C$  generated by the system by  $\phi(t, x)$ . We verify properties 1 and 2 from Lemma A.2. Property 1 is clear because of attractivity of  $x_0$ . Property 2 follows from stability of  $x_0$ . Indeed, pick a neighborhood  $U$  of  $x_0$  which does not contain  $x$  and a neighborhood  $V \subset U$  of  $x_0$  such that trajectories starting in  $V$  cannot exit  $U$  in positive time. If  $x_0$  belongs to  $\alpha(x)$ , then there is some  $t > 0$  such that  $\phi(-t, x) \in V$ . Then  $x = \phi(t, \phi(-t, x)) \in U$ , a contradiction.  $\square$

In the example of the regulation pathway for apoptosis, the following basic result from [13] is used.

LEMMA A.4. *A Metzler matrix is Hurwitz if and only if its leading principal minors alternate in sign, with the first one being negative.*

**Acknowledgments.** The authors would like to express their gratitude to the anonymous reviewers for comments and suggestions that have significantly contributed to enhancing the readability of the paper and to clarifying its technical contribution.

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