SIAM J. APPL. MATH.
Vol. 72, No. 4, pp. 959–981

VARIABLE ELIMINATION IN CHEMICAL REACTION NETWORKS WITH MASS-ACTION KINETICS*

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Abstract. We consider chemical reaction networks taken with mass-action kinetics. The steady states of such a system are solutions to a system of polynomial equations. Even for small systems the task of finding the solutions is daunting. We develop an algebraic framework and procedure for linear elimination of variables. The procedure reduces the variables in the system to a set of "core" variables by eliminating variables corresponding to a set of noninteracting species. The steady states are parameterized algebraically by the core variables, and a graphical condition is given that ensures that a steady state with positive core variables necessarily takes positive values for all variables. Further, we characterize graphically the sets of eliminated variables that are constrained by a conservation law and show that this conservation law takes a specific form.

Key words. semiflow, species graph, noninteracting, spanning tree, polynomial equations

AMS subject classifications. 92C42, 80A30

DOI. 10.1137/110847305

1. Introduction.

The goal of this work is to discuss linear elimination of variables at steady state in chemical reaction networks taken with mass-action kinetics. We use the formalism of chemical reaction network theory (CRNT), which puts chemical reaction networks into a mathematical, particularly algebraic, framework. CRNT was developed around 40 years ago, mainly by Horn, Jackson, and Feinberg [15, 16, 20, 27]. Its usefulness for analysis of chemical reaction networks has continuously been supported [9, 11, 33].

We introduce the elimination procedure by going through a specific example. Consider five chemical species A, B, C, D, E that interact according to the reactions

$$r_1 : A + 2B \longrightarrow D\ , \quad r_2 : D \longrightarrow A + C\ , \quad r_3 : C + D \longrightarrow E\ , \quad r_4 : E \longrightarrow A + B.$$

The molar concentration of a species S at time t is denoted by $c_S = c_S(t)$. By employing the common assumption that reaction rates are of *mass-action* type, the concentrations change with time according to a system of ordinary differential equations (ODEs):

$$\dot{c_A} = -k_1 c_A c_B^2 + k_2 c_D + k_4 c_E, \quad \dot{c_B} = -2k_1 c_A c_B^2 + k_4 c_E, \quad \dot{c_C} = k_2 c_D - k_3 c_C c_D,$$
$$\dot{c_D} = k_1 c_A c_B^2 - k_2 c_D - k_3 c_C c_D, \quad \dot{c_E} = k_3 c_C c_D - k_4 c_E,$$

where k_i denotes the *positive* rate constant of reaction r_i. Observe that $\dot{c_A}+\dot{c_D}+\dot{c_E} = 0$, which implies that $c_A + c_D + c_E$ is constant over time and fixed by the sum of initial concentrations $c_0 = c_A(0) + c_D(0) + c_E(0)$. The equation $c_0 = c_A + c_D + c_E$ is called a *conservation law*.

*Received by the editors September 8, 2011; accepted for publication (in revised form) April 4, 2012; published electronically July 12, 2012.

http://www.siam.org/journals/siap/72-4/84730.html

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We are interested in the steady-state solutions of this system, in particular, the *positive* steady-state solutions, that is, the solutions for which all concentrations are positive. The steady-state solutions are found by setting the ODEs to zero. Consider the equations $\dot{c_A} = 0$, $\dot{c_D} = 0$, and $\dot{c_E} = 0$:

$$0 = -k_1 c_A c_B^2 + k_2 c_D + k_4 c_E, \qquad 0 = k_1 c_A c_B^2 - k_2 c_D - k_3 c_C c_D, \tag{1.1}$$
$$0 = k_3 c_C c_D - k_4 c_E.$$

The equations form a system of polynomial equations in c_A, c_B, c_C, c_D, c_E with real coefficients. None of the equations contain a monomial with more than one of the variables c_A, c_D, c_E. Further, the degree of c_A, c_D, c_E is one in all equations. In other words, if we let $\mathrm{Con} = \{k_1, k_2, k_3, k_4\}$, then (1.1) is a linear system of equations in the variables c_A, c_D, c_E with coefficients in the field $\mathbb{R}(\mathrm{Con} \cup \{c_B, c_C\})$:

$$\begin{pmatrix} -k_1 c_B^2 & k_2 & k_4 \\ k_1 c_B^2 & -k_2 - k_3 c_C & 0 \\ 0 & k_3 c_C & -k_4 \end{pmatrix} \begin{pmatrix} c_A \\ c_D \\ c_E \end{pmatrix} = 0.$$

The column sums of the 3×3 matrix are zero because of the conserved amount c_0. In fact, the matrix has rank 2 in $\mathbb{R}(\mathrm{Con} \cup \{c_B, c_C\})$, and the solutions of the system form a line parameterized, for example, by c_D:

$$c_A = \frac{k_1 c_B^2}{k_2 + k_3} c_D, \qquad c_E = \frac{k_4}{k_3 c_C} c_D.$$

These solutions are well defined in the field $\mathbb{R}(\mathrm{Con} \cup \{c_B, c_C\})$. If positive values of c_B, c_C, c_D are given, then the steady-state values of c_A, c_E are positive and completely determined. Further, since $c_A + c_D + c_E = c_0$, we find that

$$c_D = c_0 \left(1 + \frac{k_1 c_B^2}{k_2 + k_3} + \frac{k_4}{k_3 c_C}\right)^{-1}$$

and conclude that the positive steady states are fully determined by the positive steady-state solutions of c_B, c_C. These solutions are found from the equations $\dot{c_B} = 0$ and $\dot{c_C} = 0$ in c_B, c_C by substituting the values of c_A, c_D, c_E. The resulting equations can always be rewritten in polynomial form.

Let us now start from the equations $\dot{c_C} = 0$ and $\dot{c_E} = 0$: $k_2 c_D - k_3 c_C c_D = 0$ and $k_3 c_C c_D - k_4 c_E = 0$. This system is linear in the variables c_C, c_E with coefficients in the field $\mathbb{R}(\mathrm{Con} \cup \{c_D\})$. Further, the system has maximal rank in $\mathbb{R}(\mathrm{Con} \cup \{c_D\})$ and thus has a unique solution $c_C = k_2/k_3$, $c_E = k_2 c_D/k_4$ in $\mathbb{R}(\mathrm{Con} \cup \{c_D\})$. As above, the variables c_C, c_E can be eliminated and recovered from any positive steady-state solution c_A, c_B, c_D of the remaining equations.

The approaches that are used to eliminate the variables in $\{c_A, c_D, c_E\}$ and $\{c_C, c_E\}$ differ: In the former case the system is homogeneous and does not have maximal rank, and the conservation law is required for full elimination. In the latter the system has maximal rank but is not homogeneous. At this point we might ask: What are the similarities between the two sets of variables that enable their elimination from the steady-state equations? What are the differences that lead to different approaches? The species in both sets do not interact with each other; that is, they do not appear on the same side of a reaction, and further all concentrations have degree one in all the equations in which they appear. Such sets are called *noninteracting*.

However, in the first case the sum of concentrations is conserved, and the set is what we call a *cut*, while in the second case it is not. Importantly, the eliminated variables are nonnegative whenever the noneliminated variables (the *core* variables) are positive. Furthermore, the cases in which zero concentrations of the eliminated variables can occur can be completely characterized. The concentration c_B cannot be linearly eliminated because it has degree 2 in some equations.

This reaction system is small compared to real biochemical systems and can be manipulated manually. For an arbitrary network, most noninteracting sets can be eliminated using one of the approaches outlined above, depending on the presence or absence of a conserved amount. After reduction, the (positive) steady states are the solutions to polynomial equations depending on the core variables only. Thus the steady states form an algebraic variety in the core variables.

In this manuscript we discuss a general procedure for linear elimination of variables, embracing the two approaches described above. The design of the procedure relies on (a specific version of) the *species graph*. Subsets of species that can be eliminated are noninteracting. These sets correspond to a specific type of subgraph, and in any such set, the corresponding subgraph encodes the presence or absence of a conservation law relating the concentrations in the set. We study the interplay between noninteracting sets, subgraphs, and conservation laws and relate subgraph connectedness to minimality of conservation laws and the existence of conservation laws to the so-called *full* subgraphs. Thus, the results obtained here are of interest in their own right. The Matrix-Tree theorem [36] is key to a study of the positivity of solutions [35].

The elimination procedure has interesting potential applications. First, essential information about the system at steady state is contained in the equations for the core variables. Thus, experimental knowledge about the concentrations of the core variables is sufficient to explore the system at steady state. Further, the species graph can be used in experimental planning by choosing (if possible) a subgraph that optimizes the information in the experiment.

Second, two different reaction systems involving the same chemical species can be discriminated based on the core variables alone and thus used for model selection. This is possible, irrespective of whether the rate constants are known or not [23, 29], if the two algebraic varieties described by the core variables take different forms. A series of measurements with different initial concentrations can determine which variety the measurements belong to. Although exciting, this approach for model discrimination is statistically challenged by limitations in real data collection [25].

As discussed in [24], linear elimination is a common procedure underlying many model simplifications in biochemistry and systems biology. The main example is perhaps the so-called Quasi–Steady-State Approximation (QSSA), which is used in many important enzymatic models such as the Michaelis–Menten model (see, for instance, [8]). The QSSA is applied to reaction networks in which some reactions are assumed to occur at a much faster rate than others, such that a steady state effectively has been reached for the fast reactions. Mathematically, the approximation consists of eliminating some of the species by assuming that they are at steady state with respect to the others.

Finally, another potential application concerns the emergence of multiple positive steady states in a specific system. Many mathematical tools for detecting whether a system has at most one positive steady state exist [3, 9, 17]. However, when these fail, it is not straightforward to conclude that the system admits more than one positive

steady state, and rate constants need to be found for which this is true. The CRNT Toolbox software [14] and other software [5, 6, 32] provide algorithms to determine the existence of multiple steady states, but their applicability is limited for complex and big networks. Elimination of variables might reduce the computational burden substantially and decrease the likelihood of numerical errors.

This work provides a mathematical unifying framework for linear elimination of variables in chemical reaction networks and underlies most of the elimination examples presented in [24]. Previous work on variable elimination has been concerned with the so-called *posttranslational modification* (PTM) systems [21]. PTM systems form a special type of biochemical reaction network that is particularly abundant in cell signaling, and they have been the focus of much theoretical research [26, 28, 30]. A subclass of PTM systems was initially studied by Thomson and Gunawardena [35]. In [21], we analyzed an extended class of PTM systems including signaling cascades. In both papers, a two-step procedure based on the existence of *intermediate species* was essential for the elimination procedure. The general framework presented here shows that this particularity of the PTM systems is irrelevant for the purpose of elimination. Additionally, the existence of conservation laws is always guaranteed in PTM systems, and this is not the case for arbitrary networks.

The outline of the paper is the following. We introduce the notation, some preliminaries, and networks together with their associated mass-action ODEs. We proceed to discuss conservation laws arising from so-called semiflows, with special attention to minimal semiflows. Next, the species graph and its relevant subgraphs (full and noninteracting) are defined, and we proceed to discuss relations between the subgraphs and semiflows. We then present the variable elimination procedure and the reduction of the steady-state equations to a polynomial system in the core variables. Using the graphical representation, we show that positive solutions of the core variables in the reduced system correspond to nonnegative steady states of the network, in which only the eliminated variables can possibly be zero.

2. Notation. Let $\mathbb{R}_+$ denote the set of positive real numbers (without zero) and $\overline{\mathbb{R}}_+$ the set of nonnegative real numbers (with zero). Given a finite set $\mathcal{E}$, let $\mathbb{R}^{\mathcal{E}}$ be the real vector space of formal sums $v = \sum_{E\in\mathcal{E}} \lambda_E E$ with $\lambda_E \in \mathbb{R}$. If $\lambda_E \in \mathbb{R}_+$ (resp., $\overline{\mathbb{R}}_+$) for all $E \in \mathcal{E}$, then we write $v \in \mathbb{R}_+^{\mathcal{E}}$ (resp., $\overline{\mathbb{R}}_+^{\mathcal{E}}$).

S-positivity. Let $\mathbb{R}[\mathcal{E}]$ denote the ring of real polynomials in $\mathcal{E}$. A monomial is a polynomial of the form $\lambda \prod_{E\in\mathcal{E}} E^{n_E}$ for some $\lambda \in \mathbb{R} \setminus \{0\}$ and $n_E \in \mathbb{N}_0$ (the natural numbers including zero). A nonzero polynomial in $\mathbb{R}[\mathcal{E}]$ with nonnegative coefficients is called *S-positive*. Any assignment $a\colon \mathcal{E} \to \mathbb{R}_+$ induces an evaluation map $e_a\colon \mathbb{R}[\mathcal{E}] \to \mathbb{R}$. If $p \in \mathbb{R}[\mathcal{E}]$ is S-positive, then $e_a(p) > 0$.

A rational function f in $\mathcal{E}$ is called S-positive if it is a quotient of two S-positive polynomials in $\mathcal{E}$. Then $e_a(f)$ is well defined and positive for any assignment $a\colon \mathcal{E} \to \mathbb{R}_+$. In general, a rational function $f = p/q$ in $z_1, \dots, z_s$ and coefficients in $\mathbb{R}(\mathcal{E})$ are called S-positive if the coefficients of p and q are S-positive rational functions in $\mathcal{E}$. Then $e_a(f)$ is an S-positive rational function in $\mathbb{R}(z_1, \dots, z_s)$ for any assignment $a : \mathcal{E} \to \mathbb{R}_+$. Assume that $E = g(\widehat{\mathcal{E}})$ for some rational function g in $\widehat{\mathcal{E}} = \mathcal{E} \setminus \{E\}$ and $E \in \mathcal{E}$. Then, if f is a rational function in $\mathcal{E}$, substituting g into f gives f as a rational function in $\widehat{\mathcal{E}}$.

Graphs and the Matrix-Tree theorem. Let G be a directed graph with node set $\mathcal{N}$. A *spanning tree* τ of G is a directed subgraph with node set $\mathcal{N}$ and such that the corresponding undirected graph is connected and acyclic. Self-loops are by definition excluded from a spanning tree. There is a (unique) undirected path

between any two nodes in a spanning tree [13]. We say that the spanning tree τ is *rooted* at a node v if the undirected path between any node w and v is directed from w to v. As a consequence, v is the only node in τ with no edges of the form $v \to w$ (called out-edges). Further, there is no node in τ with two out-edges. The graph G is *strongly connected* if there is a directed path from v to w for any pair of nodes v, w. Any directed path from v to w in a strongly connected graph can be extended to a spanning tree rooted at w. Some general references for graph theory are [13] and [22].

If G is labeled, then τ inherits a labeling from G, and we define

$$\pi(\tau) = \prod_{x \xrightarrow{a} y \in \tau} a.$$

Assume that G has no self-loops. We order the node set $\{v_1, \dots, v_n\}$ of G and let $a_{i,j}$ be the label of the edge $v_i \to v_j$. Further, we set $a_{i,j} = 0$ for $i \neq j$ if there is no edge from v_i to v_j and $a_{i,i} = 0$. Let $\mathcal{L}(G) = \{\alpha_{i,j}\}$ be the *Laplacian* of G, that is, the matrix with $\alpha_{i,j} = a_{j,i}$ if $i \neq j$ and $\alpha_{i,i} = -\sum_{k=1}^{n} a_{i,k}$, such that the column sums are zero. Any matrix whose column sums are zero can be realized as the Laplacian of a directed labeled graph with no self-loops.

For each node v_j, let $\Theta(v_j)$ be the set of spanning trees of G rooted at v_j. Let $\mathcal{L}(G)_{(ij)}$ denote the determinant of the minor of $\mathcal{L}(G)$ obtained by removing the ith row and the jth column of $\mathcal{L}(G)$. Then, by the Matrix-Tree theorem [36],

$$\mathcal{L}(G)_{(ij)} = (-1)^{n-1+i+j} \sum_{\tau \in \Theta(v_j)} \pi(\tau).$$

Note that for notational simplicity we have defined the Laplacian as the transpose of how it is usually defined, and the Matrix-Tree theorem has been adapted consequently.

3. Chemical reaction networks. We introduce the definition of a chemical reaction network and some related concepts. See, for instance, [16, 18] for extended discussions.

DEFINITION 3.1. *A* chemical reaction network *(called* network*) consists of three finite sets:*

(1) *A set* $\mathcal{S}$ *of* species*.*
(2) *A set* $\mathcal{C} \subset \overline{\mathbb{R}}_+^{\mathcal{S}}$ *of* complexes*.*
(3) *A set* $\mathcal{R} \subset \mathcal{C} \times \mathcal{C}$ *of* reactions*, such that* $(y, y) \notin \mathcal{R}$ *for all* $y \in \mathcal{C}$*, and if* $y \in \mathcal{C}$*, then there exists* $y' \in \mathcal{C}$ *such that either* $(y, y') \in \mathcal{R}$ *or* $(y', y) \in \mathcal{R}$*.*

Inflow and outflow of species are accommodated in this setting by incorporating the complex $0 \in \overline{\mathbb{R}}_+^{\mathcal{S}}$ and reactions $0 \to A$, $A \to 0$, respectively [19].

Following the usual convention, an element $r = (y, y') \in \mathcal{R}$ is denoted by $r\colon y \to y'$. For a reaction $r\colon y \to y'$, the reactant and product (complexes) are denoted by $y(r) := y$ and $y'(r) := y'$, respectively. By definition, any complex is either the reactant or product of some reaction.

Let s be the cardinality of $\mathcal{S}$. We fix an order in $\mathcal{S}$ so that $\mathcal{S} = \{S_1, \dots, S_s\}$ and identify $\mathbb{R}^{\mathcal{S}}$ with $\mathbb{R}^s$. The species S_i is identified with the ith canonical vector of $\mathbb{R}^s$ with 1 in the ith position and zeros elsewhere. An element in $\mathbb{R}^s$ is then given as $\sum_{i=1}^{s} \lambda_i S_i$. In particular, a complex $y \in \mathcal{C}$ is given as $y = \sum_{i=1}^{s} y_i S_i$ or $(y_1, \dots, y_s)$. If r is a reaction, $y_i(r), y_i'(r)$ denote the ith entries of $y(r), y'(r)$, respectively.

DEFINITION 3.2. *We say the following:*

(i) y_i *is the* stoichiometric coefficient *of* S_i *in* y*.*

(ii) *If $y_i \neq 0$ for some i and $y \in \mathcal{C}$, then S_i* is part of *y, y* involves *S_i, and r* involves *S_i for any reaction r such that $y(r) = y$ or $y'(r) = y$.*

(iii) *$S_i, S_j \in \mathcal{S}$* interact *if $y_i, y_j \neq 0$, $i \neq j$ for some complex y.*

(iv) *$y \in \mathcal{C}$* reacts to *$y' \in \mathcal{C}$ if there is a reaction $y \to y'$.*

(v) *$y \in \mathcal{C}$* ultimately reacts to *$y' \in \mathcal{C}$ (denoted $y \Rightarrow y'$) if either y reacts to y' or there exists a sequence of reactions $y \to y^1 \to \cdots \to y^r \to y'$ with $y^m \in \mathcal{C}$ for all $m = 1, \ldots, r$.*

(vi) *$S_i \in \mathcal{S}$* produces *$S_j \in \mathcal{S}$ if there exist two complexes y, y' with $y_i \neq 0$, $y'_j \neq 0$ and a reaction $y \to y'$.*

(vii) *$S_i \in \mathcal{S}$* ultimately produces *$S_j \in \mathcal{S}$ if there exist $S_{i_1}, \ldots, S_{i_r}$ with $S_{i_1} = S_i$, $S_{i_r} = S_j$ and such that $S_{i_{k-1}}$ produces S_{i_k} for $k = 2, \ldots, r$. If each S_{i_k} belongs to a subset $\mathcal{S}_\alpha \subseteq \mathcal{S}$ for $k = 2, \ldots, r-1$, then S_i* ultimately produces *S_j* via *$\mathcal{S}_\alpha$.*

If y ultimately reacts to y', then y and y' are *linked*. Being linked generates an equivalence relation among the complexes, and the classes are called *linkage classes*. Two complexes y, y' are *strongly linked* if both $y \Rightarrow y'$ and $y' \Rightarrow y$. Being strongly linked also defines an equivalence relation, and the classes are called *strong linkage classes*. A strong linkage class is called *terminal* if none of its complexes react to a complex outside the class.

Example 3.3. We introduce an example that we use to illustrate the definitions and constructions below. Consider the network with set of species $\mathcal{S} = \{S_1, \ldots, S_9\}$ and set of complexes $\mathcal{C} = \{S_1 + S_2, S_4, S_1 + S_3, S_5, S_3 + S_4, S_6, S_2 + S_5, S_1 + S_7, S_7 + S_8, S_9, S_2 + S_3 + S_8\}$, reacting according to

$$S_1 + S_2 \rightleftarrows S_4, \qquad S_1 + S_3 \rightleftarrows S_5, \qquad S_3 + S_4 \rightleftarrows S_6 \rightleftarrows S_2 + S_5,$$

$$S_6 \downarrow S_1 + S_7$$

$$S_7 + S_8 \rightleftarrows S_9 \longrightarrow S_2 + S_3 + S_8.$$

That is, the set of reactions $\mathcal{R}$ consists of

$$r_1\colon S_1 + S_2 \to S_4, \quad r_2\colon S_4 \to S_1 + S_2, \quad r_3\colon S_1 + S_3 \to S_5, \quad r_4\colon S_5 \to S_1 + S_3,$$
$$r_5\colon S_3 + S_4 \to S_6, \quad r_6\colon S_6 \to S_3 + S_4, \quad r_7\colon S_6 \to S_2 + S_5, \quad r_8\colon S_2 + S_5 \to S_6,$$
$$r_9\colon S_6 \to S_1 + S_7, \quad r_{10}\colon S_7 + S_8 \to S_9, \quad r_{11}\colon S_9 \to S_7 + S_8, \quad r_{12}\colon S_9 \to S_2 + S_3 + S_8.$$

This system represents a two substrate enzyme catalysis with unordered substrate binding [12] in which S_1 is an enzyme, S_2, S_3 are substrates, S_4, S_5, S_6 are intermediate enzyme-substrate complexes, and S_7 is considered the product of the reaction system. The product dissociates via catalysis by an enzyme S_8 and the formation of an intermediate complex S_9. The stoichiometric coefficients of all species that are part of a complex are one. The complex $S_2 + S_3 + S_8$ involves S_2, S_3, S_8, and its vector expression is $(0, 1, 1, 0, 0, 0, 0, 1, 0) \in \overline{\mathbb{R}}^9_+$. The species S_3 and S_4 interact. The complex $S_1 + S_3$ reacts to the complex S_5, implying that species S_1 produces species S_5. Also, the complex $S_3 + S_4$ ultimately reacts to $S_1 + S_7$, and $S_7 + S_8$ ultimately reacts to $S_2 + S_3 + S_8$. It follows that S_3 ultimately produces S_7 and S_8.

4. Mass-action kinetics. The molar concentration of species S_i at time t is denoted by $c_i = c_i(t)$. To any complex y we associate a monomial $c^y = \prod_{i=1}^s c_i^{y_i}$. For example, if $y = (2, 1, 0, 1) \in \overline{\mathbb{R}}^4_+$, then the associated monomial is $c^y = c_1^2 c_2 c_4$.

We assume that each reaction $r : y \to y'$ has an associated positive *rate constant* $k_{y \to y'} \in \mathbb{R}_+$ (also denoted k_r). The set of reactions together with their associated rate

constants gives rise to a polynomial system of ODEs taken with *mass-action kinetics*:

$$\dot{c_i} = \sum_{y \to y' \in \mathcal{R}} k_{y \to y'} c^y (y'_i - y_i), \qquad S_i \in \mathcal{S}. \tag{4.1}$$

These ODEs describe the dynamics of the concentrations c_i in time. The steady states of the system are the solutions to the following system of polynomial equations in $c_1, \ldots, c_s$ obtained by setting the derivatives of the concentrations to zero:

$$0 = \sum_{y \to y' \in \mathcal{R}} k_{y \to y'} c^y (y'_i - y_i) \qquad \text{for all } i. \tag{4.2}$$

These polynomial equations can be written as

$$0 = \sum_{r \in \mathcal{R}} k_r c^{y(r)} y'_i(r) - \sum_{r \in \mathcal{R}} k_r c^{y(r)} y_i(r) \qquad \text{for all } i. \tag{4.3}$$

It is convenient to treat the rate constants as parameters with unspecified values, that is, as symbols (as we did in the example in the introduction). For that, let

$$\mathrm{Con} = \{k_{y \to y'} | y \to y' \in \mathcal{R}\}$$

be the set of the symbols. Then, the equations (4.2) form a system of polynomial equations in $c_1, \ldots, c_s$ with coefficients in the field $\mathbb{R}(\mathrm{Con})$.

Only *nonnegative solutions* of the steady-state equations are biologically or chemically meaningful, and we focus on these only. The concept of S-positivity introduced above will be key in what follows. Consider Example 3.3 and denote by k_i the rate constant of reaction r_i. The mass-action ODEs are

$$\begin{aligned}
\dot{c_1} &= -k_1 c_1 c_2 + k_2 c_4 - k_3 c_1 c_3 + k_4 c_5 + k_9 c_6, & \dot{c_5} &= k_3 c_1 c_3 - k_4 c_5 + k_7 c_6 - k_8 c_2 c_5,\\
\dot{c_2} &= -k_1 c_1 c_2 + k_2 c_4 + k_7 c_6 - k_8 c_2 c_5 + k_{12} c_9, & \dot{c_7} &= k_9 c_6 - k_{10} c_7 c_8 + k_{11} c_9,\\
\dot{c_3} &= -k_3 c_1 c_3 + k_4 c_5 - k_5 c_3 c_4 + k_6 c_6 + k_{12} c_9, & \dot{c_8} &= -k_{10} c_7 c_8 + k_{11} c_9 + k_{12} c_9,\\
\dot{c_4} &= k_1 c_1 c_2 - k_2 c_4 - k_5 c_3 c_4 + k_6 c_6, & \dot{c_9} &= k_{10} c_7 c_8 - k_{11} c_9 - k_{12} c_9.\\
\dot{c_6} &= k_5 c_3 c_4 - k_6 c_6 - k_7 c_6 + k_8 c_2 c_5 - k_9 c_6,
\end{aligned}$$

Take, for instance, species S_1. The only reactions that involve S_1 are r_1, r_2, r_3, r_4, r_9. The reactions r_1, r_3 involve S_1 in the reactant complex, and thus the monomials contain c_1 and have negative coefficients. Similarly, r_2, r_4, r_9 involve S_1 only in the product complex, and thus the monomials do not include c_1 and have positive coefficients.

5. Conservation laws and P-semiflows. The dynamics of a network might preserve quantities that remain constant over time. If this is the case, the dynamics takes place in a proper invariant subspace of $\mathbb{R}^s$. Let $x \cdot x'$ denote the Euclidian scalar product of two vectors x, x'. If A is a set of vectors in some vector space, then $\langle A \rangle$ denotes the linear span of A.

DEFINITION 5.1. *The* stoichiometric subspace *of a network,* $(\mathcal{S}, \mathcal{C}, \mathcal{R})$*, is the following subspace of* $\mathbb{R}^s$*:*

$$\Gamma = \langle y' - y | \, y \to y' \in \mathcal{R} \rangle.$$

A semiflow *is a nonzero vector* $\omega = (\lambda_1, \ldots, \lambda_s) \in \Gamma^\perp$*. If* $\lambda_i \geq 0$ *for all* i*, then* ω *is a* P-semiflow.

By the definition of the mass-action ODEs, the vector $\dot{c}$ points along the stoichiometric subspace Γ. If $\omega = (\lambda_1, \dots, \lambda_s)$ is a semiflow, then $\sum_{i=1}^{s} \lambda_i \dot{c}_i = 0$. Thus, $\omega \cdot c = \sum_{i=1}^{s} \lambda_i c_i$ is independent of time and determined by the initial concentrations of the system. In particular, any steady-state solution of the system preserves the total initial amounts and lies in a particular coset of Γ. In general, a linear combination $\sum_{i=1}^{s} \lambda_i c_i$ that is independent of time gives rise to an equation, called a *conservation law*, with a fixed *total amount* $\overline{\omega} \in \mathbb{R}_+$:

$$\overline{\omega} = \sum_{i=1}^{s} \lambda_i c_i. \tag{5.1}$$

The *stoichiometric class* of a concentration vector $c \in \overline{\mathbb{R}}^s_+$ is $\mathcal{C}_c = \{c + \Gamma\} \cap \overline{\mathbb{R}}^s_+$. Two steady states c, c' are called *stoichiometrically compatible* if $c - c' \in \Gamma$ or, equivalently, $\omega \cdot c = \omega \cdot c'$ for all $\omega \in \Gamma^\perp$. Therefore, a stoichiometric class is characterized by the value of $\omega \cdot c$ for all semiflows ω. In particular, if $\{\omega^1, \dots, \omega^d\}$ is a basis of $\Gamma^\perp$, then each stoichiometric class corresponds to a set of total amounts $\overline{\omega}^1, \dots, \overline{\omega}^d \in \mathbb{R}_+$ and c belongs to the class if and only if $\overline{\omega}^l = \omega^l \cdot c$ for all l.

In Example 3.3, the dimension of Γ is 5:

$$\Gamma = \langle S_1 + S_2 - S_4, S_1 + S_3 - S_5, S_3 + S_4 - S_6, S_1 + S_7 - S_6, S_7 + S_8 - S_9 \rangle.$$

Thus the space $\Gamma^\perp$ has dimension 4, and a basis is

$$\begin{aligned} &\omega^1 = S_1 + S_4 + S_5 + S_6, && \omega^2 = S_8 + S_9, \\ &\omega^3 = S_2 + S_4 + S_6 + S_7 + S_9, && \omega^4 = S_2 + S_3 + S_4 + S_5 + 2S_6 + 2S_7 + 2S_9. \end{aligned} \tag{5.2}$$

The conservation law corresponding to ω^1 is $\overline{\omega}^1 = c_1 + c_4 + c_5 + c_6$ for a given $\overline{\omega}^1 \in \mathbb{R}_+$.

Remark. Questions like "How many steady states does a system possess?" refer to the number of stoichiometrically compatible steady states, that is, the steady states that fulfill the conservation laws obtained from semiflows with the same total amounts. If this restriction is not imposed and conservation laws exist, then the steady-state solutions form an algebraic variety of dimension at least 1 (over the complex numbers).

Remark. Not all networks have semiflows. Consider, for instance, the network with $s = 6$ and reactions $S_1 \to S_2$, $S_2 \to S_3$, $S_1 + S_2 + S_3 \to S_6$, $S_4 + S_5 \to S_6$, $S_4 \to S_5$, and $S_5 \to S_1$. The stoichiometric subspace is $\mathbb{R}^6$, and thus $\Gamma^\perp = 0$. If the last reaction is removed, then the stoichiometric subspace has dimension 1, and there is, up to scaling, one P-semiflow: $\omega = 2S_1 + 2S_2 + 2S_3 + 3S_4 + 3S_5 + 6S_6$. In general, a basis for $\Gamma^\perp$ consisting of P-semiflows is not guaranteed. Consider the following network with $s = 3$ and one reaction, $A + B + C \to A$. There is no basis of $\Gamma^\perp = \langle A, B - C \rangle$ consisting of P-semiflows alone.

LEMMA 5.2. *The following statements are equivalent:*

(i) *The stoichiometric class* $\mathcal{C}_c = \{c + \Gamma\} \cap \overline{\mathbb{R}}^s_+$, $c \in \overline{\mathbb{R}}^s_+$, *is compact.*

(ii) $\Gamma \cap \overline{\mathbb{R}}^s_+ = \{0\}$.

(iii) $\Gamma^\perp$ *has a basis* $\{\omega^1, \dots, \omega^d\}$ *of P-semiflows with* $\lambda^j_i > 0$ *if* $\omega^j = (\lambda^j_1, \dots, \lambda^j_s)$.

(iv) *There is an element* $\omega = (\lambda_1, \dots, \lambda_s)$ *of* $\Gamma^\perp$ *with* $\lambda_i > 0$ *for all* i.

Proof. We will prove (i)⇒(ii)⇒(iii)⇒(iv)⇒(i). Assume that $\mathcal{C}_c$ is compact, and consider $V := \Gamma \cap \overline{\mathbb{R}}^s_+$. If $V \neq \{0\}$, then for any nonzero $v \in V$, the set $c + \langle v \rangle$ is unbounded in $\overline{\mathbb{R}}^s_+$. Hence $\mathcal{C}_c$ cannot be compact, and thus (ii) must be the case. Let $\mathrm{bd}(A)$ denote the boundary of a set A. If (ii), then $\Gamma^\perp \cap \overline{\mathbb{R}}^s_+ \neq \{0\}$ and further

$\Gamma^\perp \cap \overline{\mathbb{R}}^s_+ \not\subseteq \mathrm{bd}(\overline{\mathbb{R}}^s_+)$. If the latter were not the case, then also $\Gamma \cap \mathrm{bd}(\overline{\mathbb{R}}^s_+) \neq \{0\}$, contradicting (ii). Hence, there exists an open set $\Omega \subseteq \Gamma^\perp \cap \mathbb{R}^s_+$ in $\Gamma^\perp$, and we can choose a basis $\{\omega^1, \ldots, \omega^d\}$ of P-semiflows with $\omega^j \in \Omega$, that is, $\lambda^j_i > 0$. Thus (iii) is fulfilled. (iii) gives (iv) directly. Assume (iv). For $x = (x_i)_i \in \mathcal{C}_c$, $\omega \cdot x = \omega \cdot c$ is independent of x. Since $\lambda_i > 0$ and $x_i \geq 0$, $x_i \leq (\omega \cdot c)/\lambda_i$ for all i, and thus $\mathcal{C}_c$ is bounded. Since it is a closed set, $\mathcal{C}_c$ is compact and (i) is proven. □

Lemma 5.2 is well known in dynamical systems theory and Petri Net theory. In the latter semiflows are known as P-invariants (place invariants) [31]. The equivalence between (i) and (iv) in the context of chemical reaction networks was shown in [27, App. 1].

Remark. All conservation laws might not be obtained from semiflows [20], that is, the semiflows in $\Gamma^\perp$ might not give the minimal affine space in which the dynamics of the system takes place. There can be additional conservation laws depending on the rate constants and not merely on the stoichiometric coefficients. The next lemma is proven in [20] and stated here for future reference.

LEMMA 5.3 (see [20, section 6]). *If each linkage class contains exactly one terminal strong linkage class, then all conservation laws correspond to semiflows.*

As shown in [20], any weakly reversible network fulfills the condition of the lemma. Also, Example 3.3 fulfills the criterion. The network with reactions $r_1\colon S_1 \to S_2$, $r_2\colon S_1 \to S_3$, and $r_3\colon S_2 + S_3 \to 2S_1$ does not fulfill it [10]. Here, $\Gamma^\perp = \langle S_1 + S_2 + S_3 \rangle$, providing the conservation law $c_1 + c_2 + c_3 = \overline{\omega}$. However, when $k_1 = k_2 = k_3$, then $c_1 + 2c_2$ is also conserved, because $\dot{c}_2 = \dot{c}_3$.

Minimal and terminal semiflows.

DEFINITION 5.4. *The* support *of a semiflow $\omega = (\lambda_1, \ldots, \lambda_s)$ is the set $\mathcal{S}(\omega) = \{S_i |\ \lambda_i \neq 0\}$. We say that ω is*

(i) minimal *if, for any semiflow $\widetilde{\omega}$ with $\mathcal{S}(\widetilde{\omega}) \subseteq \mathcal{S}(\omega)$, there is $a \in \mathbb{R}$ such that $a\widetilde{\omega} = \omega$.*

(ii) terminal *if any semiflow $\widetilde{\omega}$ with $\mathcal{S}(\widetilde{\omega}) \subseteq \mathcal{S}(\omega)$ satisfies $\mathcal{S}(\widetilde{\omega}) = \mathcal{S}(\omega)$.*

That is, a semiflow ω is minimal if any semiflow given by a linear combination of the species in its support is a multiple of ω and terminal if there is no semiflow with smaller support.

LEMMA 5.5.

(i) *A semiflow is minimal if and only if it is terminal.*

(ii) *If ω is a P-semiflow that is not minimal, then there is a P-semiflow $\widetilde{\omega}$ such that $\mathcal{S}(\widetilde{\omega}) \subsetneq \mathcal{S}(\omega)$.*

Proof. (i) If ω is a minimal semiflow, then by definition any semiflow $\widetilde{\omega}$ with $\mathcal{S}(\widetilde{\omega}) \subseteq \mathcal{S}(\omega)$ satisfies $\widetilde{\omega} = a\omega$ for some $a \in \mathbb{R}$. Thus, $\mathcal{S}(\widetilde{\omega}) = \mathcal{S}(\omega)$, which implies that ω is terminal. To prove the reverse, assume that ω is terminal but not minimal; that is, there exists $\widetilde{\omega}$ such that $\mathcal{S}(\widetilde{\omega}) = \mathcal{S}(\omega)$ and $\widetilde{\omega} \neq a\omega$ for all $a \in \mathbb{R}$. Let $\mathcal{I} = \{i | S_i \in \mathcal{S}(\widetilde{\omega})\}$, $\omega = (\lambda_1, \ldots, \lambda_s)$, and $\widetilde{\omega} = (\widetilde{\lambda}_1, \ldots, \widetilde{\lambda}_s)$. Choose $u \in \mathcal{I}$ such that $|\widetilde{\lambda}_u/\lambda_u| \geq |\widetilde{\lambda}_i/\lambda_i|$ for all $i \in \mathcal{I}$, and define $\gamma = \widetilde{\lambda}_u$ and $\widetilde{\gamma} = \lambda_u$. Then

$$\widehat{\omega} := \gamma\omega - \widetilde{\gamma}\widetilde{\omega} = \sum_{i=1}^{s} (\widetilde{\lambda}_u \lambda_i - \lambda_u \widetilde{\lambda}_i) S_i = \sum_{i=1}^{s} \mu_i S_i$$

is a semiflow, since $\widehat{\omega} \neq 0$ (otherwise $\widetilde{\omega} = a\omega$ for some a). Since $\mu_u = 0$, $\mathcal{S}(\widehat{\omega}) \subsetneq \mathcal{S}(\omega)$, which contradicts that ω is terminal.

(ii) If ω is a P-semiflow that is not minimal, then there exists a semiflow $\widetilde{\omega}$ such that $\mathcal{S}(\widetilde{\omega}) \subsetneq \mathcal{S}(\omega)$. The construction above provides a new semiflow $\widehat{\omega}$. Since $\lambda_i > 0$

for all $i \in \mathcal{I}$, we have $|\widetilde{\lambda}_u|\lambda_i - \lambda_u|\widetilde{\lambda}_i| \geq 0$ and either $\widetilde{\lambda}_u > 0$ and $\mu_i \geq 0$ for all i or $\widetilde{\lambda}_u < 0$ and $\mu_i \leq 0$ for all i. Hence, either $\widehat{\omega}$ or $-\widehat{\omega}$ is a P-semiflow fulfilling (ii). ∎

Therefore, there cannot exist two linearly independent minimal P-semiflows with the same support. For example, if $S_1 + S_2 + S_3$ is conserved and minimal, then $\lambda_1 S_1 + \lambda_2 S_2 + \lambda_3 S_3$ with $\lambda_1 \neq \lambda_2$ cannot be a semiflow. We will see below that the P-semiflows $\omega^1, \omega^2, \omega^3$ in (5.2) of Example 3.3 are minimal. However, ω^4 is not minimal since $\mathcal{S}(\omega^3) \subsetneq \mathcal{S}(\omega^4)$.

6. The species graph. Given a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$, we define the *species graph* $G_{\mathcal{S}}$ as the labeled directed graph with node set $\mathcal{S}$ and a directed edge from S_i to S_j with label $r\colon y \to y'$ whenever $y_i \neq 0$ and $y'_j \neq 0$ for some reaction r in $\mathcal{R}$. That is, there is a directed edge from S_i to S_j if and only if S_i produces S_j. There can be multiple edges with different labels between a pair of nodes. In addition, if S_i is involved both in the reactant and product of a reaction, then there is a self-edge $S_i \to S_i$. The species graph of Example 3.3 is depicted in Figure 6.1.

The species graph does not characterize a network uniquely, since information coming from the stoichiometric coefficients is ignored. For instance, the following two networks have the same species graph:

$$R_1 = \{A + B \to 2C, C \to A\}, \qquad R_2 = \{A + B \to C, C \to A\}. \tag{6.1}$$

Remark. A reaction $r\colon y \to y'$ is called *reversible* if the reaction $y' \to y$ also belongs to the network. In Example 3.3, all reactions but r_9, r_{12} are reversible. In contrast to other papers [3, 35], we consider reversible reactions as two (independent) irreversible reactions. Thus, reversible reactions provide two edges with opposite directions and different labels in the species graph. This is required when we consider spanning trees in section 8. Changing a reaction from reversible to irreversible does not change the stoichiometric subspace, and a system with all reactions considered irreversible has the same (P-)semiflows as a system with some (all) reactions considered reversible. However, the number of steady states and their values may depend on whether reactions are reversible or not.

DEFINITION 6.1. *A graph G with node set $\mathcal{S}_\alpha$ is a* subgraph *of $G_{\mathcal{S}}$ if $\mathcal{S}_\alpha \subseteq \mathcal{S}$ and the labeled directed edges of G are inherited from $G_{\mathcal{S}}$. We denote $G = G_{\mathcal{S}_\alpha}$. Further:*

(i) *$\mathcal{S}_\alpha$ is* full *if any reaction involving some $S_i \in \mathcal{S}_\alpha$ appears at least once as a label of an edge in $G_{\mathcal{S}_\alpha}$. If this is the case, then $G_{\mathcal{S}_\alpha}$ is said to be* full.

(ii) *$\mathcal{S}_\alpha$ is* noninteracting *if it contains no pair of interacting species and all stoichiometric coefficients are either 0 or 1, that is, $y_i = 0, 1$ for all $S_i \in \mathcal{S}_\alpha$ and*

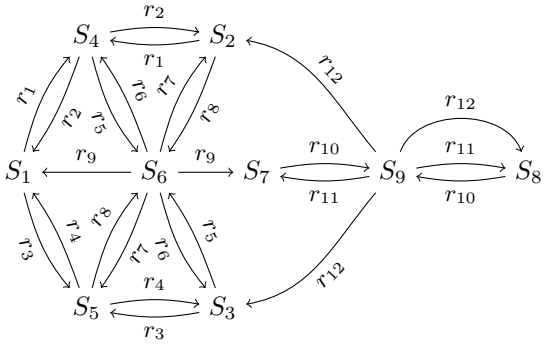


FIG. 6.1. *Species graph of Example* 3.3.

$y \in \mathcal{C}$. *If this is the case, then* $G_{\mathcal{S}_\alpha}$ *is said to be* noninteracting.

(iii) *If* $\mathcal{S}_\alpha$ *is full and noninteracting, then* $\mathcal{S}_\alpha$ *is a* cut *of* $\mathcal{S}$.

Remark. The notion of a full set is related to the concepts of a siphon and a trap from the Petri Net literature [4, 7]. These concepts have also appeared in recent papers on chemical reaction networks [1, 2, 34]. A *siphon*, also called a *semilocking set*, is a set $\mathcal{S}'$ such that if the support of the product y' of a reaction $y \to y'$ intersects $\mathcal{S}'$, then the support of the reactant y also intersects $\mathcal{S}'$. A *trap* is a set $\mathcal{S}'$ such that if the support of y of a reaction $y \to y'$ intersects $\mathcal{S}'$, then the support of y' also intersects $\mathcal{S}'$. It is clear from the definition that a full set is exactly a set that is both a siphon and a trap. If all reactions are reversible, then full, siphon, and trap are identical concepts.

The definition of subgraphs of $G_{\mathcal{S}}$ extends to subgraphs of $G_{\mathcal{S}_\alpha}$. We depict in Figure 6.2 four different subgraphs of the species graph of Example 3.3, corresponding to four different subsets $\mathcal{S}_\alpha \subseteq \mathcal{S}$.

The proof of the following lemma is left to the reader.

LEMMA 6.2. *Let* $\mathcal{S}_1, \mathcal{S}_2 \subseteq \mathcal{S}$. *Then the following hold:*

(i) *If* $\mathcal{S}_1$ *and* $\mathcal{S}_2$ *are full, then so is* $\mathcal{S}_1 \cup \mathcal{S}_2$.

(ii) *If* $\mathcal{S}_1 \cup \mathcal{S}_2$ *is noninteracting, then so are* $\mathcal{S}_1$ *and* $\mathcal{S}_2$.

If $\mathcal{S}_1$ *and* $\mathcal{S}_2$ *are disjoint, and* $G_{\mathcal{S}_1 \cup \mathcal{S}_2} = G_{\mathcal{S}_1} \cup G_{\mathcal{S}_2}$, *then the reverse statements are also true.*

It follows that if $\mathcal{S}_\alpha$ is a cut, then the node set of any connected component of $G_{\mathcal{S}_\alpha}$ is also a cut (as illustrated in Figure 6.2(a)). The next lemma connects some properties of full and noninteracting graphs that will be used in what follows. A nonempty subgraph $G_{\mathcal{S}'_\alpha}$ of $G_{\mathcal{S}_\alpha}$ is *proper* if $G_{\mathcal{S}'_\alpha} \neq G_{\mathcal{S}_\alpha}$.

LEMMA 6.3. *Let* $\mathcal{S}_\alpha \subseteq \mathcal{S}$ *be a subset.*

(i) *If* $\mathcal{S}_\alpha$ *is noninteracting, then any reaction label* r *appears at most once in* $G_{\mathcal{S}_\alpha}$.

(ii) *If* $\mathcal{S}_\alpha$ *is noninteracting and* $S_i \in \mathcal{S}_\alpha$ *is involved in a reaction* r *that is a label of an edge of* $G_{\mathcal{S}_\alpha}$, *then the edge is to/from* S_i.

(iii) *If* $\mathcal{S}_\alpha$ *is full and* $S_i \in \mathcal{S}_\alpha$ *is involved in a reaction* r, *then there is an edge to/from* S_i *labeled* r.

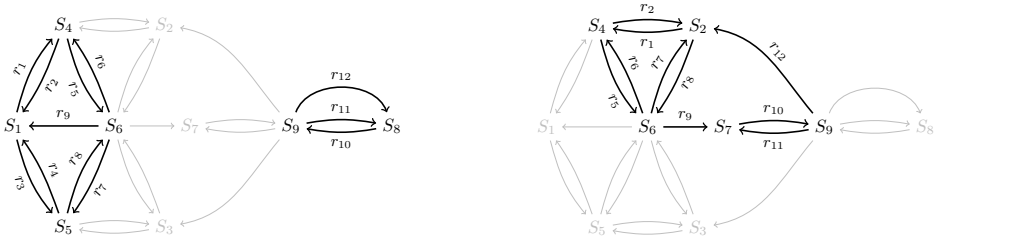
(iv) *If* $\mathcal{S}_\alpha$ *is full and has no repeated reaction labels and the stoichiometric coefficients of its nodes in all complexes are either* 0 *or* 1, *then* $\mathcal{S}_\alpha$ *is a cut.*

(v) *If* $G_{\mathcal{S}_\alpha}$ *has no repeated reaction labels and* $G_{\mathcal{S}_\alpha}$ *is connected, then* $G_{\mathcal{S}_\alpha}$ *has no proper full subgraphs.*

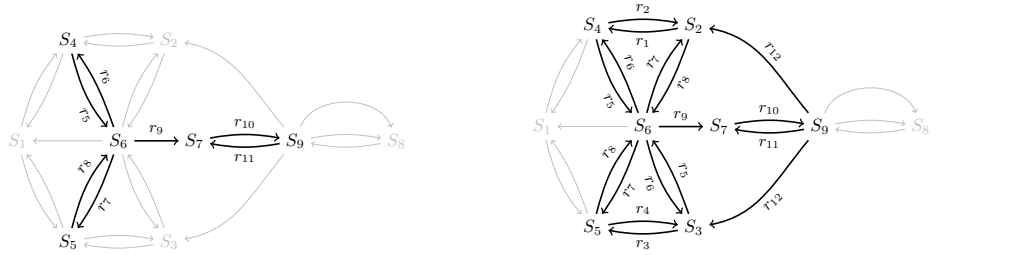
Proof. (i) Assume that a reaction r appears in two different edges $S_i \xrightarrow{r} S_j$ and $S_u \xrightarrow{r} S_v$ of $G_{\mathcal{S}_\alpha}$. If $S_i \neq S_u$ or $S_j \neq S_v$, then either S_i and S_u or S_j and S_v interact, and thus $G_{\mathcal{S}_\alpha}$ is not noninteracting.

(ii)–(iii) In both cases there is an edge in $G_{\mathcal{S}_\alpha}$ labeled r—in (ii) by assumption and in (iii) because $\mathcal{S}_\alpha$ is full. Assume that the edge with label r is not from/to S_i but between $S_j, S_u \in \mathcal{S}_\alpha$, $j, u \neq i$. Then, S_i and S_j (or S_u) interact reaching a contradiction in case (ii) and implying that there is an edge with label r between S_i and S_u (or S_j) in case (iii).

(iv) Assume that there are two (different) interacting nodes $S_i, S_j \in \mathcal{S}_\alpha$. Then there exists a reaction $r\colon y \to y'$ such that $y_i = y_j = 1$ or $y'_i = y'_j = 1$. Let us assume that $y_i = y_j = 1$, and the other case follows by symmetry. Since $\mathcal{S}_\alpha$ is full, r is the label of an edge in $G_{\mathcal{S}_\alpha}$. Let S_u (potentially equal to S_i or S_j) be the end node of the edge. Since $S_i, S_j, S_u \in \mathcal{S}_\alpha$, the edges $S_i \xrightarrow{r} S_u$ and $S_j \xrightarrow{r} S_u$ are in $G_{\mathcal{S}_\alpha}$, contradicting that there are no repeated labels. Hence, $\mathcal{S}_\alpha$ is noninteracting and hence a cut.



(a) $\mathcal{S}_\alpha^1 = \{S_1, S_4, S_5, S_6, S_8, S_9\}$ (b) $\mathcal{S}_\alpha^2 = \{S_2, S_4, S_6, S_7, S_9\}$



(c) $\mathcal{S}_\alpha^3 = \{S_4, S_5, S_6, S_7, S_9\}$ (d) $\mathcal{S}_\alpha^4 = \{S_2, S_3, S_4, S_5, S_6, S_7, S_9\}$

FIG. 6.2. *Species subgraphs of Example* 3.3. *The node sets in* (a) *and* (b) *are cuts, but the graph in* (a) *has two connected components, and the graph in* (b) *one;* (c) *the node set is noninteracting but not full and cannot be extended to a cut;* (d) *is a full graph but two of its nodes,* S_2, S_3*, interact.*

(v) Assume that there is a proper subgraph G. Since $G_{\mathcal{S}_\alpha}$ is connected, one can find a node S_i in $G_{\mathcal{S}_\alpha}$ that is not in G and a node S_j in G for which there is an edge $S_i \xrightarrow{r} S_j$ or $S_j \xrightarrow{r} S_i$ in $G_{\mathcal{S}_\alpha}$. By assumption a label appears at most once in $G_{\mathcal{S}_\alpha}$. Thus G is not full since r is not a label of an edge in G, but r involves $S_j \in G$. ∎

It follows from Lemma 6.3(i), (iii) that if $\mathcal{S}_\alpha$ is a cut, then all reactions involving $S_i \in \mathcal{S}_\alpha$ label edges of $G_{\mathcal{S}_\alpha}$ to/from S_i, and they appear exactly once. From (i), (v) we find that noninteracting connected graphs have no proper full subgraphs. In particular, if $\mathcal{S}_\alpha$ is a cut such that $G_{\mathcal{S}_\alpha}$ is connected, then $G_{\mathcal{S}_\alpha}$ has no proper full subgraphs.

7. Semiflows and the species graph. In this section we explore the relationship between semiflows, P-semiflows, and full subgraphs of $G_{\mathcal{S}}$. We come to the main results on semiflows in relation to variable elimination: (1) if the support of a semiflow is a cut and the associated species graph is connected, then all nonzero coefficients of the semiflow are equal; (2) the support of a semiflow is noninteracting if and only if it is a cut.

LEMMA 7.1. *Let* ω *be a semiflow.*

(i) *If* ω *is minimal, then* $G_{\mathcal{S}(\omega)}$ *is connected.*
(ii) *If* ω *is a P-semiflow or* $\mathcal{S}(\omega)$ *is noninteracting, then* $G_{\mathcal{S}(\omega)}$ *is full.*
(iii) *If* ω *is a P-semiflow or* $\mathcal{S}(\omega)$ *is noninteracting, and* $G_{\mathcal{S}(\omega)}$ *has no proper full subgraphs, then* ω *is minimal.*

Proof. (i) The idea is that if $G_{\mathcal{S}(\omega)}$ is not connected, then the semiflow splits into two, contradicting minimality. If $G_{\mathcal{S}(\omega)}$ is not connected, then $G_{\mathcal{S}(\omega)} = G_1 \cup G_2$ with G_1, G_2 being two nonempty disjoint subgraphs of $G_{\mathcal{S}(\omega)}$. Let $\mathcal{S}_1$, $\mathcal{S}_2$ be the node sets of G_1, G_2, respectively. If $\omega = \sum_{i=1}^s \lambda_i S_i$, let $\omega_k = \sum_{i|S_i \in \mathcal{S}_k} \lambda_i S_i \neq 0$, $k = 1, 2$. Since $\mathcal{S}_1 \cap \mathcal{S}_2 = \emptyset$, $\omega = \omega_1 + \omega_2$. By hypothesis, $\omega \cdot (y' - y) = 0$ for all $y \to y' \in \mathcal{R}$, and thus $0 = \omega_1 \cdot (y' - y) + \omega_2 \cdot (y' - y)$. Since G_1, G_2 are disjoint, there is no edge between a

node in $\mathcal{S}_1$ and a node in $\mathcal{S}_2$. If $y \to y'$ is a reaction with $y_i' - y_i \neq 0$ for some $S_i \in \mathcal{S}_1$, then $y_j = y_j' = 0$ for all $S_j \in \mathcal{S}_2$ and trivially $\omega_2 \cdot (y' - y) = 0$. Thus $\omega_1 \cdot (y' - y) = 0$. By symmetry, we have that $\omega_1 \cdot (y' - y) = \omega_2 \cdot (y' - y) = 0$ for all reactions $y \to y'$, and hence ω_k is a semiflow for $k = 1, 2$.

(ii) Let $S_i \in \mathcal{S}(\omega)$ and $r\colon y \to y'$ be a reaction with either y_i or $y_i' \neq 0$. We assume that $y_i \neq 0$, and the other case follows by symmetry. We want to show that r is a label in $G_{\mathcal{S}(\omega)}$, that is, $y_j' \neq 0$ for some $S_j \in \mathcal{S}(\omega)$. By hypothesis, $\omega \cdot (y' - y) = 0$. If $\omega = (\lambda_1, \ldots, \lambda_s)$ is a P-semiflow (that is, $\lambda_i \geq 0$), we have $\omega \cdot y' = \omega \cdot y \geq \lambda_i y_i > 0$. If $\mathcal{S}(\omega)$ is noninteracting, then $y_j = 0$ for any $i \neq j$ such that $S_j \in \mathcal{S}(\omega)$, and hence $\omega \cdot y' = \omega \cdot y = \lambda_i y_i \neq 0$. In either case $\lambda_j y_j' \neq 0$ for some j, and hence $y_j' \neq 0$ for some $S_j \in \mathcal{S}(\omega)$. Note that the case $j = i$ is accepted.

(iii) Assume that ω is not minimal. Then by Lemma 5.5(i) there exists a semiflow $\widetilde{\omega}$ such that $\mathcal{S}(\widetilde{\omega}) \subsetneq \mathcal{S}(\omega)$. If ω is a P-semiflow, then by Lemma 5.5(ii) we can assume that $\widetilde{\omega}$ is a P-semiflow. If $\mathcal{S}(\omega)$ is noninteracting, then so is $\mathcal{S}(\widetilde{\omega})$. Using (ii), $G_{\mathcal{S}(\widetilde{\omega})}$ is full and thus a proper full subgraph of $G_{\mathcal{S}(\omega)}$, which is a contradiction. ∎

Consider the P-semiflows ω^i (5.2) of Example 3.3 and the subsets $\mathcal{S}_\alpha^i$ in Figure 6.2. Here, $\mathcal{S}(\omega^1) \cup \mathcal{S}(\omega^2) = \mathcal{S}_\alpha^1$, $\mathcal{S}(\omega^3) = \mathcal{S}_\alpha^2$ and $\mathcal{S}(\omega^4) = \mathcal{S}_\alpha^4$, giving full subgraphs. The two components of $G_{\mathcal{S}_\alpha^1}$ and the graph $G_{\mathcal{S}_\alpha^2}$ have no proper full subgraphs, while $G_{\mathcal{S}_\alpha^2}$ is a proper full subgraph of $G_{\mathcal{S}_\alpha^4}$. Thus, it follows from Lemma 7.1(iii) that $\omega^1, \omega^2, \omega^3$ are minimal.

Lemma 7.1(iii) cannot be reversed. Consider, for example, the network R_2 in (6.1). The vector $\omega = A + B + C$ is a minimal P-semiflow. The associated species graph $G_{\mathcal{S}(\omega)}$ is $A \underset{r_2}{\overset{r_1}{\rightleftarrows}} C \xleftarrow{r_1} B$ and has a proper full subgraph $A \underset{r_2}{\overset{r_1}{\rightleftarrows}} C$. Further, $\omega_2 = A + C$ is a P-semiflow for the network R_1 in (6.1), but there is not a P-semiflow involving all species. This implies that if $\widetilde{G}$ is a full connected subgraph of G and G corresponds to a P-semiflow, then $\widetilde{G}$ does not necessarily correspond to a P-semiflow. Similarly, if $\widetilde{G}$ corresponds to a P-semiflow, then G does not necessarily correspond to one.

PROPOSITION 7.2. *Let $\mathcal{S}_\alpha \subseteq \mathcal{S}$ be a subset. If $\mathcal{S}_\alpha$ is a cut, then $\omega = \sum_{i|S_i \in \mathcal{S}_\alpha} S_i$ is a P-semiflow. In this case, ω is minimal if and only if $G_{\mathcal{S}_\alpha}$ is connected.*

Proof. The stoichiometric coefficients of $S_i \in \mathcal{S}_\alpha$ are either 0 or 1 since $\mathcal{S}_\alpha$ is a cut. Consider $r\colon y \to y' \in \mathcal{R}$. If $y_i, y_i' = 0$ for all i such that $S_i \in \mathcal{S}_\alpha$, then clearly $\omega \cdot (y' - y) = 0$. Since $\mathcal{S}_\alpha$ is noninteracting, if $y_i \neq 0$ for some $S_i \in \mathcal{S}_\alpha$, then $y_j = 0$ for all $S_j \in \mathcal{S}_\alpha$ such that $i \neq j$. From Lemma 6.3(ii), (iii), r is the label of exactly one edge of $G_{\mathcal{S}_\alpha}$ connected to S_i, and there exists exactly one species $S_j \in \mathcal{S}_\alpha$ such that $y_j' \neq 0$. Thus, $\omega \cdot (y' - y) = y_j' - y_i = 1 - 1 = 0$. This shows that ω is a P-semiflow.

For the second part of the statement, Lemma 7.1(i) implies that if ω is minimal, then $G_{\mathcal{S}_\alpha}$ is connected. On the other hand, if $G_{\mathcal{S}_\alpha}$ is connected, then by Lemma 6.3(i), (v), $G_{\mathcal{S}_\alpha}$ has no proper full subgraphs, and by Lemma 7.1(iii), ω is minimal. ∎

The reverse is not true: In the reaction system $S_1 + S_2 \to Y_1 + Y_2$, $S_3 \to S_1$, $Y_1 \to S_3$, $Y_2 \to S_3$, the vector $\omega = S_1 + S_3 + Y_1 + Y_2$ is a P-semiflow, but the set $\{S_1, S_3, Y_1, Y_2\}$ is not a cut. Further, ω is minimal. Thus, the noninteracting property cannot be read from the coefficients of the P-semiflow.

Remark. If $\mathcal{S}_\alpha$ is a cut, we denote the P-semiflow given in Proposition 7.2 by $\omega(\mathcal{S}_\alpha) = \sum_{i|S_i \in \mathcal{S}_\alpha} S_i$. The operation $\omega(\cdot)$ defines a map between the set of cuts and the set of P-semiflows such that sets with connected associated species graphs are mapped to minimal P-semiflows. The operation $\mathcal{S}(\cdot)$ defines a map between the set of P-semiflows and the subsets of $\mathcal{S}$ with full associated species graphs such that

minimal P-semiflows are mapped to subsets with connected associated species graphs. The map $\mathcal{S}(\omega(\cdot))$ is the identity.

From Lemma 7.1(ii) and Proposition 7.2 we derive the following corollary.

COROLLARY 7.3.

(i) *Let ω be a semiflow such that $\mathcal{S}(\omega)$ is noninteracting. Then $\mathcal{S}(\omega)$ is a cut.*

(ii) *If $\mathcal{S}_\alpha \subseteq \mathcal{S}$ is a noninteracting subset and $\omega(\mathcal{S}_\alpha)$ is not a P-semiflow, then $\mathcal{S}_\alpha$ is not a cut, and there is no semiflow with support $\mathcal{S}_\alpha$.*

Therefore, there is a one-to-one correspondence between cuts and P-semiflows with noninteracting support and nonzero entries equal to one. The results of this section give rise to the following corollary.

COROLLARY 7.4. *Let $\mathcal{S}_\alpha$ be a noninteracting set such that $G_{\mathcal{S}_\alpha}$ is connected.*

(i) *If $\mathcal{S}_\alpha$ is a cut, then any semiflow with support included in $\mathcal{S}_\alpha$ is a multiple of $\omega(\mathcal{S}_\alpha)$.*

(ii) *If $\mathcal{S}_\alpha$ is not a cut, then there are no semiflows with support included in $\mathcal{S}_\alpha$.*

Proof. Part (i) follows from Proposition 7.2 and Lemma 5.5. Part (ii) follows from Lemma 6.3(i), (v), Lemma 7.1(ii), and the fact that $G_{\mathcal{S}_\alpha}$ is not full. ∎

Consider the noninteracting subset $\mathcal{S}^3_\alpha$ of Example 3.3. The vector $\omega = S_4 + S_5 + S_6 + S_7 + S_9$ is not a P-semiflow since $\omega \cdot (S_1 + S_2 - S_4) = -1 \neq 0$. It follows that $\mathcal{S}^3_\alpha$ is not a cut and there is no semiflow with support included in $\mathcal{S}^3_\alpha$. Reciprocally, consider the P-semiflow ω^1. Since $\mathcal{S}^1_\alpha = \mathcal{S}(\omega^1)$ is noninteracting, it is a cut. Further, since the graph $G_{\mathcal{S}^2_\alpha}$ is connected and $\mathcal{S}^2_\alpha$ is a cut, any semiflow involving the species in $\mathcal{S}^2_\alpha$ is a multiple of ω^3. Checking if a set is noninteracting is easy from the set of complexes. However, checking that the associated species subgraph is full is in general not straightforward. We have shown that the relationship between semiflows and cuts gives simple conditions for determining if a noninteracting set is a cut.

Remark. Using Lemma 6.2, we find that if $\mathcal{S}_\alpha$ is a cut but $G_{\mathcal{S}_\alpha}$ is not connected, then $\mathcal{S}_\alpha$ decomposes into a disjoint union of cuts, $\mathcal{S}_\alpha = \mathcal{S}^1_\alpha \cup \cdots \cup \mathcal{S}^r_\alpha$, such that $G_{\mathcal{S}^i_\alpha}$ is connected for all i. Thus, $\omega(\mathcal{S}_\alpha) = \omega(\mathcal{S}^1_\alpha) + \cdots + \omega(\mathcal{S}^r_\alpha)$, and the P-semiflow $\omega(\mathcal{S}_\alpha)$ decomposes into a sum of minimal P-semiflows. Further, if $G_{\mathcal{S}_\alpha}$ is not connected, e.g., has two connected components $G_{\mathcal{S}^1_\alpha}$ and $G_{\mathcal{S}^2_\alpha}$, then $\omega(\mathcal{S}^1_\alpha) - \omega(\mathcal{S}^2_\alpha)$ is a semiflow with support in $\mathcal{S}_\alpha = \mathcal{S}^1_\alpha \cup \mathcal{S}^2_\alpha$, but it is not a multiple of $\omega(\mathcal{S}_\alpha)$. It follows that requiring $G_{\mathcal{S}_\alpha}$ to be connected is a necessary condition in Corollary 7.4.

Remark. Note that Lemma 7.1(ii) implies that the support of a P-semiflow is always a full set (that is, a set that is both a siphon and a trap). We have shown that if a full set is noninteracting, then it is the support of a P-semiflow. The existence of P-semiflows with support in a given full set can be used to prove results about the persistence of a network [2].

8. Elimination of variables. Let $\mathcal{S}_\alpha \subseteq \mathcal{S}$ be a noninteracting subset. Let $G_{\mathcal{S}_\alpha}$ be the species graph associated to $\mathcal{S}_\alpha$, and assume that it is connected. By Corollary 7.4, either $\mathcal{S}_\alpha$ is a cut and $\omega(\mathcal{S}_\alpha)$ is minimal, or $\mathcal{S}_\alpha$ is not a cut and there is no semiflow with support included in $\mathcal{S}_\alpha$. In what follows we discuss conditions such that the concentrations of the species in $\mathcal{S}_\alpha$ can be fully eliminated from the steady-state equations. The discussion depends on whether $\mathcal{S}_\alpha$ is a cut or not.

For simplicity, we assume that $\mathcal{S}_\alpha = \{S_1, \ldots, S_m\}$. Let $\mathcal{R}^c_\alpha$ be the set of reactions not appearing as labels in $G_{\mathcal{S}_\alpha}$ but involving some $S_i \in \mathcal{S}_\alpha$, and $\mathcal{R}^c_{\alpha,out}(i)$, $\mathcal{R}^c_{\alpha,in}(i)$ the sets of reactions in $\mathcal{R}^c_\alpha$ involving $S_i \in \mathcal{S}_\alpha$ in the reactants and products, respectively. Clearly, $\mathcal{R}^c_\alpha = \bigcup_{i=1}^m \mathcal{R}^c_{\alpha,out}(i) \cup \mathcal{R}^c_{\alpha,in}(i)$. Note that $\mathcal{R}^c_\alpha = \emptyset$ if and only if $\mathcal{S}_\alpha$ is a cut.

We restrict our attention to the steady-state equations (4.2) for a fixed $S_i \in \mathcal{S}_\alpha$. Using the expression in (4.3) and the fact that the stoichiometric coefficients are 1,

we can write this equation as $\dot{S}_i = X_i - Y_i$ with

$$X_i = \sum_{\substack{j=1\\ j\neq i}}^{m} \sum_{S_j \xrightarrow{r} S_i} k_r c^{y(r)} + \sum_{r\in\mathcal{R}^c_{\alpha,in}(i)} k_r c^{y(r)}, \quad Y_i = \sum_{\substack{j=1\\ j\neq i}}^{m} \sum_{S_i \xrightarrow{r} S_j} k_r c^{y(r)} + \sum_{r\in\mathcal{R}^c_{\alpha,out}(i)} k_r c^{y(r)},$$

where the first summand in each term is taken over the edges in $G_{\mathcal{S}_\alpha}$. Recall that there can be more than one edge between two species. Further, since the stoichiometric coefficient of S_i is 0 or 1, any edge $S_i \to S_i$ provides no summand in the steady-state equations (4.2).

Let $C_\alpha = C(\mathcal{S}_\alpha) = \{c_i | S_i \in \mathcal{S}_\alpha\} = \{c_1, \dots, c_m\}$. Each of the monomials $c^{y(r)}$ in Y_i involves c_i, and if another c_k is involved, then S_k interacts with S_i (and, in particular, $k \notin \{1, \dots, m\}$). Similarly, $c^{y(r)}$ in X_i involves the variable c_k if and only if S_k produces S_i. Further, for $r \in \mathcal{R}^c_{\alpha,in}(i)$, $c^{y(r)}$ does not involve any $c_k \in C_\alpha$, while if r is the label of $S_j \xrightarrow{r} S_i$ with $S_j \in \mathcal{S}_\alpha$, then the only variable from C_α involved in $c^{y(r)}$ is c_j. It follows that the system is linear in C_α with coefficients in $\mathbb{R}[\mathrm{Con} \cup C^c(\mathcal{S}_\alpha)]$, where

$$C^c(\mathcal{S}_\alpha) = \{c_i | S_i \in \mathcal{S} \setminus \mathcal{S}_\alpha \text{ interacts with or produces some } S_j \in \mathcal{S}_\alpha\}. \tag{8.1}$$

We write $C^c_\alpha = C^c(\mathcal{S}_\alpha)$ for short and note that $C_\alpha \cap C^c_\alpha = \emptyset$. Let $\widehat{y}_j(r)$ denote the vector in $\mathbb{R}^{s-1}$ obtained from $y(r)$ by removing the jth coordinate. We have shown that X_i, Y_i can be written so that (4.2) for $S_i \in \mathcal{S}_\alpha$ becomes

$$0 = \sum_{j=1}^{m} a_{i,j} c_j + z_i, \tag{8.2}$$

where $a_{i,i} = e_i + d_i$ and

$$e_i = -\sum_{\substack{j=1\\ j\neq i}}^{m} \sum_{S_i \xrightarrow{r} S_j} k_r c^{\widehat{y}_i(r)}, \qquad a_{i,j} = \sum_{S_j \xrightarrow{r} S_i} k_r c^{\widehat{y}_j(r)},$$

$$d_i = -\sum_{r\in\mathcal{R}^c_{\alpha,out}(i)} k_r c^{\widehat{y}_i(r)}, \qquad z_i = \sum_{r\in\mathcal{R}^c_{\alpha,in}(i)} k_r c^{y(r)}.$$

Let $A = \{a_{i,j}\}$ be the $m \times m$ matrix with $a_{i,j}$ defined as above, $d = (d_1, \dots, d_m)$, and $z = (z_1, \dots, z_m)$. Note that $\mathcal{S}_\alpha$ is a cut if and only if $z = d = 0$. The discussion above provides a proof of the following lemma.

LEMMA 8.1. *Let $\mathcal{S}_\alpha \subseteq \mathcal{S}$ be a noninteracting set. The steady-state equations (4.2) for $S_i \in \mathcal{S}_\alpha$ form an $m \times m$ linear system of equations in C_α, $Ax + z = 0$, where the entries of the matrix A and the independent term z are either zero or S-positive in $\mathbb{R}[\mathrm{Con} \cup C^c_\alpha]$. Further, $\mathcal{S}_\alpha$ is a cut if and only if $z = d = 0$, in which case the system is homogeneous.*

If A has maximal rank m, then the system has a unique solution in $\mathbb{R}(\mathrm{Con} \cup C^c_\alpha)$. By Corollary 7.4, if $\mathcal{S}_\alpha$ is a cut, then the column sums of A are all zero, and the system cannot have maximal rank. If $\mathcal{S}_\alpha$ is not a cut, then there are no semiflows with support in $\mathcal{S}_\alpha$. The column sums of the matrix A are (for column i)

$$\sum_{k=1}^{m} a_{k,i} = \sum_{i\neq k} \sum_{S_i \xrightarrow{r} S_k} k_r c^{\widehat{y}_i(r)} - \sum_{\substack{j=1\\ j\neq i}}^{m} \sum_{S_i \xrightarrow{r} S_j} k_r c^{\widehat{y}_i(r)} + d_i = d_i.$$

These are zero as polynomials in $\mathbb{R}[\mathrm{Con} \cup C_\alpha^c]$ if and only if $\mathcal{R}^c_{\alpha,out}(i) = \emptyset$ for all i, and the condition $d = 0$ is equivalent to the column sums being zero. If $\mathcal{S}_\alpha$ is not a cut, but $d = 0$, then $z \neq 0$ as a tuple with entries in $\mathbb{R}[\mathrm{Con} \cup C_\alpha^c]$. It follows that the system is incompatible in $\mathbb{R}(\mathrm{Con} \cup C_\alpha^c)$, because $\sum_i z_i \neq 0$. The only possible nonnegative steady-state solutions must satisfy $z_i = 0$ for some i and hence $c_j = 0$ for some $c_j \in C_\alpha^c$ such that S_j produces $S_i \in \mathcal{S}_\alpha$.

We proceed now to discuss the case in which $\mathcal{S}_\alpha$ is a cut and the case in which it is not a cut. Both cases could be merged into a single approach, but the discussion of the first situation becomes more transparent when it is treated separately.

Elimination of variables in a cut. Let $\mathcal{S}_\alpha \subseteq \mathcal{S}$ be a cut such that $G_{\mathcal{S}_\alpha}$ is connected. For $S_i \in \mathcal{S}_\alpha$ the equations (4.2) form an $m \times m$ homogeneous linear system of equations with variables C_α and coefficients in $\mathbb{R}[\mathrm{Con} \cup C_\alpha^c]$. Using (8.2), the steady-state equations (4.2) become

$$0 = \sum_{j=1}^{m} a_{i,j} c_j, \qquad i = 1, \dots, m. \tag{8.3}$$

Because the column sums of A are zero, A is the Laplacian of a labeled directed graph $\widehat{G}_{\mathcal{S}_\alpha}$ with node set $\mathcal{S}_\alpha$ and a labeled edge $S_j \xrightarrow{a_{i,j}} S_i$ whenever $a_{i,j} \neq 0$, $i \neq j$. Note that $a_{i,j} \in \mathbb{R}[\mathrm{Con} \cup C_\alpha^c]$ is S-positive. We have that $G_{\mathcal{S}_\alpha}$ is (strongly) connected if and only if $\widehat{G}_{\mathcal{S}_\alpha}$ is. The two graphs differ in the labels and in that multiple directed edges from S_i to S_j in $G_{\mathcal{S}_\alpha}$ are collapsed to a single directed edge in $\widehat{G}_{\mathcal{S}_\alpha}$. Further, the graph $\widehat{G}_{\mathcal{S}_\alpha}$ has no self-loops (that is, those of $G_{\mathcal{S}_\alpha}$ are removed by construction).

By the Matrix-Tree theorem, the minors $A_{(i,j)}$ of $A = \mathcal{L}(\widehat{G}_{\mathcal{S}_\alpha})$ are

$$A_{(i,j)} = (-1)^{m-1+i+j} \sum_{\tau \in \Theta(S_j)} \pi(\tau).$$

Thus, A has rank $m-1$ if and only if there exists at least one spanning tree in $\widehat{G}_{\mathcal{S}_\alpha}$ rooted at some S_j, $j = 1, \dots, m$. The next proposition follows from the discussion above. In particular, the proposition holds if $\widehat{G}_{\mathcal{S}_\alpha}$ (or, equivalently, $G_{\mathcal{S}_\alpha}$) is strongly connected.

PROPOSITION 8.2. *Assume that $\mathcal{S}_\alpha$ is a cut such that $G_{\mathcal{S}_\alpha}$ is connected, and let $\overline{\omega} = \sum_{i=1}^m c_i$ be the conservation law obtained from the P-semiflow $\omega(\mathcal{S}_\alpha)$. The following statements are equivalent:*

(i) *$\overline{\omega} = \sum_{i=1}^m c_i$ is, up to scaling, the only conservation law with variables in C_α.*
(ii) *$\widehat{G}_{\mathcal{S}_\alpha}$ has at least one rooted spanning tree.*
(iii) *The rank of A is $m-1$.*

Since $G_{\mathcal{S}_\alpha}$ is connected and $\mathcal{S}_\alpha$ is a cut, any semiflow with support in $\mathcal{S}_\alpha$ is a multiple of $\omega(\mathcal{S}_\alpha)$. The proposition says that $\widehat{G}_{\mathcal{S}_\alpha}$ has a rooted spanning tree if and only if there are no other conservation laws with concentrations only in C_α.

Remark. Let $\mathcal{S}_\alpha$, $G_{\mathcal{S}_\alpha}$ be as in the proposition above. Let $\mathcal{C}_\alpha$ be the set of complexes involving at least one species in $\mathcal{S}_\alpha$. Consider the linkage classes in $\mathcal{C}_\alpha$ given by the relation "ultimately reacts to" (Definition 3.2). If $G_{\mathcal{S}_\alpha}$ has a rooted spanning tree, then the root must be in a terminal strong linkage class, because the elements in such a class cannot react to complexes outside the class. Further, using the same reasoning, there cannot be two terminal strong linkage classes. Consequently, if $G_{\mathcal{S}_\alpha}$ has a rooted spanning tree, there is only one terminal strong linkage class. This remark is closely related to Lemma 5.3.

For simplicity we assume that there exists a spanning tree rooted at S_1. Then, the variables $c_2, \dots, c_m$ can be solved in the coefficient field $\mathbb{R}(\mathrm{Con} \cup C_\alpha^c \cup \{c_1\})$. In particular, using Cramer's rule and the Matrix-Tree theorem, we obtain

$$c_j = \frac{(-1)^{j+1} A_{(1,j)}}{A_{(1,1)}} = \frac{\sigma_j(C_\alpha^c)}{\sigma_1(C_\alpha^c)} c_1 = \varphi_j(C_\alpha^c) c_1, \text{ where } \sigma_j(C_\alpha^c) = \sum_{\tau \in \Theta(S_j)} \pi(\tau) \tag{8.4}$$

and $j = 1, \dots, m$. Since there is a spanning tree rooted at S_1, it follows that $\sigma_1(C_\alpha^c)$ is S-positive and $\sigma_j(C_\alpha^c)$ is either zero or S-positive in $\mathbb{R}[\mathrm{Con} \cup C_\alpha^c]$. If the graph $\widehat{G}_{\mathcal{S}_\alpha}$ is strongly connected, then $\sigma_j(C_\alpha^c) \neq 0$ for all j and any choice of root S_j could be used instead of S_1. The arguments given above and the definition of $a_{i,j}$ provide a proof of the following lemma.

LEMMA 8.3. *If $c_k \in C_\alpha^c$ is a variable of the function $\sigma_j(C_\alpha^c)$ for some j, then there exists $S_i \in \mathcal{S}_\alpha$ that interacts with S_k and S_i ultimately produces S_j via $\mathcal{S}_\alpha$. Specifically, there is a complex y^1 involving S_i and S_k, and a complex y^2 involving some species $S_u \in \mathcal{S}_\alpha$, such that y^1 reacts to y^2 and S_u ultimately produces S_j via $\mathcal{S}_\alpha$. If $\widehat{G}_{\mathcal{S}_\alpha}$ is strongly connected, then the reverse is true.*

The sum of the concentrations in C_α is conserved. Using the equation $\overline{\omega} = \sum_{i=1}^m c_i$, we obtain

$$\overline{\omega} = (1 + \varphi_2(C_\alpha^c) + \cdots + \varphi_m(C_\alpha^c)) c_1,$$

where the coefficient of c_1 is S-positive in $\mathbb{R}(\mathrm{Con} \cup C_\alpha^c)$. Thus,

$$c_1 = \overline{\varphi}_1(C_\alpha^c) = \frac{\overline{\omega}}{1 + \varphi_2(C_\alpha^c) + \cdots + \varphi_m(C_\alpha^c)} = \frac{\overline{\omega}\sigma_1(C_\alpha^c)}{\sigma_1(C_\alpha^c) + \sigma_2(C_\alpha^c) + \cdots + \sigma_m(C_\alpha^c)},$$

with $\overline{\varphi}_1$ being an S-positive rational function in C_α^c with coefficients in $\mathbb{R}(\mathrm{Con} \cup \{\overline{\omega}\})$. Observe that $\overline{\omega}$ becomes an extra parameter and can be treated as a symbol as well. Further, if $\overline{\omega}$ is assigned a positive value, then $c_1 > 0$ at steady state for positive values of C_α^c. By substitution of c_1 by $\overline{\varphi}_1$, we obtain

$$c_j = \overline{\varphi}_j(C_\alpha^c) := \varphi_j(C_\alpha^c)\overline{\varphi}_1(C_\alpha^c), \qquad j = 2, \dots, m, \tag{8.5}$$

with $\overline{\varphi}_j$ being either zero or an S-positive rational function in C_α^c with coefficients in $\mathbb{R}(\mathrm{Con} \cup \{\overline{\omega}\})$.

If, in the discussion above, we replace the fixed species S_1 by any other species S_i in $\mathcal{S}_\alpha$, we obtain the following proposition.

PROPOSITION 8.4. *Let $\mathcal{S}_\alpha \subseteq \mathcal{S}$ be a cut such that $G_{\mathcal{S}_\alpha}$ is connected. Assume that there is a spanning tree of $\widehat{G}_{\mathcal{S}_\alpha}$ rooted at some species S_i for some i. Then, there exists a zero or S-positive rational function φ_j in C_α^c with coefficients in $\mathbb{R}(\mathrm{Con})$, such that the steady-state equation (4.2) for $c_j \in C_\alpha$ is satisfied in $\mathbb{R}(\mathrm{Con} \cup C_\alpha^c)$ if and only if*

$$c_j = \varphi_j(C_\alpha^c) c_i, \qquad c_j \in C_\alpha.$$

Further, there exists an S-positive rational function $\overline{\varphi}_i$ in C_α^c with coefficients in $\mathbb{R}(\mathrm{Con} \cup \{\overline{\omega}\})$, such that the conservation law $\overline{\omega} = \sum_{k=1}^m c_k$ is fulfilled if and only if $c_i = \overline{\varphi}_i(C_\alpha^c)$.

Consider Example 3.3 and the cut $\mathcal{S}_\alpha = \{S_1, S_4, S_5, S_6\}$ corresponding to a connected component of the graph $G_{\mathcal{S}^1_\alpha}$ in Figure 6.2(a). System (8.3) becomes

$$\begin{pmatrix} -k_1c_2 - k_3c_3 & k_2 & k_4 & k_9 \\ k_1c_2 & -k_2 - k_5c_3 & 0 & k_6 \\ k_3c_3 & 0 & -k_4 - k_8c_2 & k_7 \\ 0 & k_5c_3 & k_8c_2 & -k_6 - k_7 - k_9 \end{pmatrix} \begin{pmatrix} c_1 \\ c_4 \\ c_5 \\ c_6 \end{pmatrix} = 0.$$

The column sums are zero because of the conservation law $\overline{\omega}^1 = c_1 + c_4 + c_5 + c_6$. The graph $\widehat{G}_{\mathcal{S}_\alpha}$ is strongly connected (Figure 8.1(a)), as is observed in many real (bio)chemical systems. Thus rooted spanning trees exist, and the system has rank 3. This also follows from Proposition 8.2 and Lemma 5.3, since each linkage class of the network has exactly one terminal strong linkage class.

The polynomials σ_j are

$$\begin{aligned}
\sigma_1 &= k_2k_4(k_6 + k_7 + k_9) + k_2k_8(k_6 + k_9)c_2 + k_4k_5(k_7 + k_9)c_3 + k_5k_8k_9c_2c_3, \\
\sigma_4 &= k_1k_4(k_6 + k_7 + k_9)c_2 + k_1k_8(k_6 + k_9)c_2^2 + k_3k_6k_8c_2c_3, \\
\sigma_5 &= k_2k_3(k_6 + k_7 + k_9)c_3 + k_3k_5(k_7 + k_9)c_3^2 + k_1k_5k_7c_2c_3, \\
\sigma_6 &= (k_1k_4k_5 + k_2k_3k_8)c_2c_3 + k_1k_5k_8c_2^2c_3 + k_3k_5k_8c_2c_3^2.
\end{aligned}$$

Each monomial in σ_j corresponds to a spanning tree rooted at S_j. The species S_2, S_3 are the only species interacting with a species in $\mathcal{S}_\alpha$, and thus only c_2, c_3 appear in the expressions. Using (8.4) and (8.5) we find the steady-state expressions of c_1, c_4, c_5, c_6 in terms of the rate constants, the total amount $\overline{\omega}^1$, and the concentrations c_2, c_3.

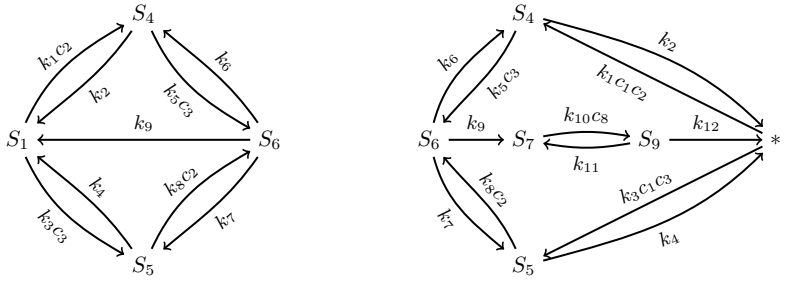
Remark. It is straightforward to find σ_j by computing the minors of A using any computer algebra software. The advantage of the Matrix-Tree description in the theoretical discussion is that S-positivity of the solutions is easily obtained.

Elimination of variables in a subset that is not a cut. Let $\mathcal{S}_\alpha \subseteq \mathcal{S}$ be a noninteracting subset that is not a cut, and assume that $G_{\mathcal{S}_\alpha}$ is connected. As discussed above, if the column sums of the matrix A are zero, then there are no positive steady-state solutions. If the column sums of A are not all zero, then A is not a Laplacian. However, A can be extended such that its determinant is a minor of a Laplacian.

Consider the labeled directed graph $\widehat{G}_{\mathcal{S}_\alpha}$ with node set $\mathcal{S}_\alpha \cup \{*\}$. We order the nodes such that S_i is the ith node and $*$ the $(m+1)$th node. The graph $\widehat{G}_{\mathcal{S}_\alpha}$ has the following labeled directed edges: $S_j \xrightarrow{a_{i,j}} S_i$ if $a_{i,j} \neq 0$ and $i \neq j$, $S_i \xrightarrow{-d_i} *$ if $d_i \neq 0$, and $* \xrightarrow{z_i} S_i$ if $z_i \neq 0$. All labels are S-positive in $\mathbb{R}[\mathrm{Con} \cup C_\alpha^c]$. Let $\mathcal{L} = \{\lambda_{i,j}\}$ be the Laplacian of $\widehat{G}_{\mathcal{S}_\alpha}$. If $i, j \leq m$, then $\lambda_{i,j} = a_{i,j}$. The entries of the last row are $\lambda_{m+1,i} = -d_i$ for $i \leq m$, and the entries of the last column are $\lambda_{i,m+1} = z_i$ for $i \leq m$. We conclude that the $(m+1, m+1)$ minor of $\mathcal{L}$ is exactly A, and thus, by the Matrix-Tree theorem, we have

$$\sigma(C_\alpha^c) := (-1)^m \det(A) = (-1)^m \mathcal{L}_{(m+1,m+1)} = \sum_{\tau \in \Theta(*)} \pi(\tau).$$

If there exists at least one spanning tree rooted at $*$, then $(-1)^m \det(A)$ is S-positive in $\mathbb{R}[\mathrm{Con} \cup C_\alpha^c]$. In this case the system $Ax + z = 0$ has a unique solution in $\mathbb{R}(\mathrm{Con} \cup C_\alpha^c)$. A spanning tree rooted at $*$ exists if and only if for all species $S_i \in \mathcal{S}_\alpha$ there exists a reaction $y \to y'$ such that y' does not involve any species in $\mathcal{S}_\alpha$, y involves some



(a) $\mathcal{S}_\alpha = \{S_1, S_4, S_5, S_6\}$ (b) $\mathcal{S}_\alpha^2 = \{S_4, S_5, S_6, S_7, S_9\}$

FIG. 8.1. (a) *The graph* $\widehat{G}_{\mathcal{S}_\alpha}$ *in Example* 3.3 *for the cut* $\mathcal{S}_\alpha$. (b) *The graph* $\widehat{G}^2_{\mathcal{S}_\alpha}$ *for the noninteracting set* $\mathcal{S}^2_\alpha$*, which is not a cut.*

$S_u \in \mathcal{S}_\alpha$, and S_i ultimately produces S_u. The existence of such a spanning tree ensures that $\mathcal{R}^c_{\alpha,out}(i) \neq \emptyset$ and thus $d_i \neq 0$ for some i.

Since A is noninteracting but not a cut, there are no semiflows with support in $\mathcal{S}_\alpha$ by Corollary 7.4(ii). Similarly to Proposition 8.2, we obtain the following proposition.

PROPOSITION 8.5. *Assume that* $\mathcal{S}_\alpha$ *is a noninteracting set that is not a cut and such that* $G_{\mathcal{S}_\alpha}$ *is connected. Then* A *has maximal rank if and only if there exists a spanning tree rooted at* $*$*. Further, if* A *has maximal rank, then there are no conservation laws in the concentrations in* C_α*.*

If A does not have maximal rank, then there is a vanishing linear combination of the rows of A, $0 = \sum_{k=1}^m \lambda_k a_{k,j}$ for all j. If there are no conservation laws in the concentrations in C_α, then $0 \neq \sum_{k=1}^m \lambda_k \dot{c}_k = \sum_{k=1}^m \sum_{j=1}^m \lambda_k (a_{k,j} c_j + z_k)$, and it follows that $\sum_{k=1}^m \lambda_k z_k \neq 0$. If $\lambda_k \geq 0$ for all k, then we conclude that the system (8.2) is incompatible in $\mathbb{R}(\mathrm{Con} \cup C^c_\alpha)$ and there are no positive steady states.

Assume that a spanning tree rooted at $*$ exists. For $i = 1, \ldots, m$, let σ_i be the following polynomial in C^c_α:

$$\sigma_i(C^c_\alpha) = (-1)^{i+1} \mathcal{L}_{(m+1,i)} = \sum_{\tau \in \Theta(S_i)} \pi(\tau),$$

which is either zero or S-positive in $\mathbb{R}[\mathrm{Con} \cup C^c_\alpha]$. By Cramer's rule, we have

$$c_i = \varphi_i(C^c_\alpha) = \frac{(-1)^{m+1-i} \mathcal{L}_{(m+1,i)}}{(-1)^m \mathcal{L}_{(m+1,m+1)}} = \frac{\sigma_i(C^c_\alpha)}{\sigma(C^c_\alpha)},$$

which is either zero or S-positive in $\mathbb{R}(\mathrm{Con} \cup C^c_\alpha)$. If there exists at least one spanning tree rooted at S_i, then $\sigma_i \neq 0$ as a polynomial in $\mathbb{R}[\mathrm{Con} \cup C^c_\alpha]$. A necessary condition for $\sigma_i \neq 0$ is the existence of a directed path from $*$ to S_i, which implies that S_i is ultimately produced from some species $S_k \in \mathcal{S} \setminus \mathcal{S}_\alpha$. In particular, if $\widehat{G}_{\mathcal{S}_\alpha}$ is strongly connected, then all concentrations are nonzero as elements in $\mathbb{R}(\mathrm{Con} \cup C^c_\alpha)$.

Consider the set $\mathcal{S}_\alpha = \mathcal{S}^3_\alpha = \{S_4, S_5, S_6, S_7, S_9\}$ in Example 3.3. It is noninteracting and not a cut, and $G_{\mathcal{S}_\alpha}$ is connected. Further, all species ultimately produce $S_9 \in \mathcal{S}_\alpha$, and S_9 reacts to $S_2 + S_3 + S_8$, which does not involve species in $\mathcal{S}_\alpha$. Hence a spanning tree rooted at $*$ exists. The graph $\widehat{G}_{\mathcal{S}_\alpha}$ is depicted in Figure 8.1(b) and is strongly connected. We have that $z = (k_1 c_1 c_2, k_3 c_1 c_3, 0, 0, 0)$, $C^c_\alpha = \{c_1, c_2, c_3, c_8\}$, and

$$\sigma = k_{10} k_{12} (k_2 k_4 (k_6 + k_7 + k_9) + k_2 k_6 k_8 c_2$$

$$
\begin{aligned}
&+ k_4k_5k_7c_3 + k_9(k_4k_5c_3 + k_2k_8c_2 + k_5k_8c_2c_3))c_8,\\
\sigma_4 =&k_{10}k_{12}(k_1k_4(k_6 + k_7 + k_9) + k_1k_8(k_6 + k_9)c_2 + k_3k_6k_8c_3)c_1c_2c_8,\\
\sigma_5 =&k_{10}k_{12}(k_2k_3(k_6 + k_7 + k_9) + k_1k_5k_7c_2 + k_3k_5(k_7 + k_9)c_3)c_1c_3c_8,\\
\sigma_6 =&k_{10}k_{12}(k_1k_4k_5 + k_2k_3k_8 + k_1k_5k_8c_2 + k_3k_5k_8c_3)c_1c_2c_3c_8,\\
\sigma_7 =&k_9(k_{11} + k_{12})(k_1k_4k_5 + k_2k_3k_8 + k_1k_5k_8c_2 + k_3k_5k_8c_3)c_1c_2c_3,\\
\sigma_9 =&k_9k_{10}(k_1k_4k_5 + k_2k_3k_8 + k_1k_5k_8c_2 + k_3k_5k_8c_3)c_1c_2c_3c_8.
\end{aligned}
$$

The concentration c_1 is only in the label of out-edges from $*$, and thus c_1 is not in σ.

PROPOSITION 8.6. *Assume that there is a spanning tree of $\widehat{G}_{\mathcal{S}_\alpha}$ rooted at $*$. Then, there exists a zero or S-positive rational function φ_i in C_α^c with coefficients in $\mathbb{R}(\mathrm{Con})$, such that the steady-state equations* (4.2) *for $c_i \in C_\alpha$ are satisfied in $\mathbb{R}(\mathrm{Con} \cup C_\alpha^c)$ if and only if $c_i = \varphi_i(C_\alpha^c)$.*

Remark. The procedure outlined here can be stated in full generality: Consider a square linear system of equations $Ax + z = 0$, such that the entries of z and the off-diagonal entries of A are positive and the column sums of A are zero or negative. If A has maximal rank, then the unique solution of the system is nonnegative.

Remark. If the matrix A does not have maximal rank, then we can always select a subset of $\mathcal{S}_\alpha$ such that the corresponding matrix has maximal rank and proceed with elimination of the variables in the subset.

Remarks. Let $\mathcal{S}_\alpha \subseteq \mathcal{S}$ be any noninteracting subset such that $G_{\mathcal{S}_\alpha}$ is connected. We have proven that for all $c_i \in C_\alpha$ there exists a rational function φ_i such that $c_i = \varphi_i(C_\alpha^c)$ at steady state, provided some spanning trees exist. When $\mathcal{S}_\alpha$ is a cut, the P-semiflow $\omega(\mathcal{S}_\alpha)$ is required in the elimination, while when $\mathcal{S}_\alpha$ is not a cut, variables are eliminated using only the steady-state equations.

If $G_{\mathcal{S}_\alpha}$ is not connected, then the results above apply to the connected components separately, since the underlying node set of each connected component is noninteracting. Further, let $\mathcal{S}_\alpha^1, \mathcal{S}_\alpha^2$ be two noninteracting sets such that $G_{\mathcal{S}_\alpha^1}$ and $G_{\mathcal{S}_\alpha^2}$ are disjoint and connected. One easily sees that $C(\mathcal{S}_\alpha^2) \cap C^c(\mathcal{S}_\alpha^1) = \emptyset$; that is, both sets of variables $C(\mathcal{S}_\alpha^1)$ and $C(\mathcal{S}_\alpha^2)$ can be simultaneously eliminated. Additionally, if we let $\mathcal{S}_\alpha = \mathcal{S}_\alpha^1 \cup \mathcal{S}_\alpha^2$, then $C^c(\mathcal{S}_\alpha) = C^c(\mathcal{S}_\alpha^1) \cup C^c(\mathcal{S}_\alpha^2)$. For instance, consider $\mathcal{S}_\alpha = \{S_1, S_4, S_5, S_6, S_8, S_9\}$ in Figure 6.2(a). The associated species graph has two connected components, which are strongly connected. The concentrations $c_i \in C_\alpha$ can be expressed as S-positive rational functions in $C_\alpha^c = \{c_2, c_3, c_7\}$.

The conditions to apply the variable elimination procedure are summarized in Table 8.1. The procedure guarantees that if positive values are assigned to all $c_j \in C_\alpha^c$, then c_i is nonnegative. For c_i to be positive, that is, $\sigma_i \neq 0$, the existence of at least one in-edge to S_i is necessary. Otherwise the concentration at steady state of S_i is zero, which is expected if S_i is only consumed and never produced. Further, we have the following proposition.

TABLE 8.1

Summary of the conditions required for the variable elimination procedure for a noninteracting set $\mathcal{S}_\alpha$. Here "only" indicates up to multiplication by a constant.

	$\mathcal{S}_\alpha$ cut	$\mathcal{S}_\alpha$ not a cut
Characterization	$\omega(\mathcal{S}_\alpha)$ semiflow or $z = d = 0$	$\nexists$ semiflow or $z - d \neq 0$
Elimination of C_α works if in $\widehat{G}_{\mathcal{S}_\alpha}$ ...	$\exists$ rooted spanning tree (equivalent to $\overline{\omega} = \sum_{c_i \in C_\alpha} c_i$ being the "only" conservation law in C_α)	$\exists$ spanning tree rooted at $*$ (implies $\nexists$ conservation law in C_α and $d \neq 0$)

PROPOSITION 8.7. *Let $\mathcal{S}_\alpha$ be a noninteracting subset of $\mathcal{S}$ such that the concentrations C_α can be eliminated from the steady-state equations using the procedure described in this section. Each component of the graph $\widehat{G}_{\mathcal{S}_\alpha}$ is strongly connected if and only if any steady-state solution satisfies $c_j > 0$ for all $c_j \in C_\alpha$ (and for any positive total amount if appropriate) whenever the variables in C_α^c take positive values.*

9. Steady-state equations. Let $\mathcal{S}_\alpha$ be any noninteracting subset such that $G_{\mathcal{S}_\alpha}$ is connected and such that the variables in C_α can be eliminated by the procedure above. Let $\Phi_u(C_\alpha^c) = 0$ be the equation obtained from $\dot{c}_u = 0$, $u = m+1, \dots, s$, after elimination of variables in C_α and removal of denominators. The denominators can be chosen to be S-positive. With this choice, the denominators have no positive zeros, and therefore multiplication by the denominators does not change the solutions or the positivity of them. Fix a maximal set of n independent combinations $\xi^l(c_1, \dots, c_s) = \sum_{i=1}^s \lambda_i^l c_i$ providing conservation laws that includes those corresponding to the full connected components of $G_{\mathcal{S}_\alpha}$ (that is, to cuts). For given total amounts, the steady-state equations are complemented with the equations $\overline{\omega}^l = \xi^l(c_1, \dots, c_s)$, $l = 1, \dots, n$. If the conservation law corresponds to a cut, then the elimination procedure ensures that $\xi^l(\varphi_1(C_\alpha^c), \dots, \varphi_m(C_\alpha^c), c_{m+1}, \dots, c_s) = \overline{\omega}^l$, and the equation becomes redundant.

THEOREM 9.1. *Consider a network with a noninteracting set $\mathcal{S}_\alpha$. Assume that $\mathcal{S}_\alpha = \mathcal{S}_\alpha^1 \cup \cdots \cup \mathcal{S}_\alpha^r$ is a partition of $\mathcal{S}_\alpha$ into disjoint sets such that $G_{\mathcal{S}_\alpha^j}$ is connected and $\widehat{G}_{\mathcal{S}_\alpha^j}$ admits a spanning tree for all j. If $\mathcal{S}_\alpha^j$ is a cut, assume that the spanning tree is rooted at some $S_i \in \mathcal{S}_\alpha$, and otherwise assume that it is rooted at $*$. Let total amounts $\overline{\omega}^l$ be given for the n conservation laws.*

The nonnegative steady states with positive values in C_α^c are in one-to-one correspondence with the positive solutions to

$$\Phi_u(C_\alpha^c) = 0, \qquad \overline{\omega}^l = \xi^l(C_\alpha^c) := \xi^l(\varphi_1(C_\alpha^c), \dots, \varphi_m(C_\alpha^c), c_{m+1}, \dots, c_s)$$

for $u = m+1, \dots, s$ and $l = 1, \dots, n$.

Proof. We have shown that any nonnegative steady-state solution with positive values for $c_i \in C_\alpha^c$ must satisfy these equations. For the reverse, we apply the following to each connected component of $G_{\mathcal{S}_\alpha}$. Consider a positive solution $c = (c_{m+1}, \dots, c_s)$ to the equations $\Phi_u(C_\alpha^c) = 0$ and $\overline{\omega}^l = \xi^l(C_\alpha^c)$. For $i = 1, \dots, m$, define c_i through Proposition 8.4 or 8.6, depending on whether S_i belongs to a cut $\mathcal{S}_\alpha^j$ or not. For positive rate constants and positive total amounts, c_i is nonnegative (because the rational functions defining it are S-positive). By construction this procedure automatically ensures that conservation laws corresponding to cuts are fulfilled. Using Propositions 8.4 and 8.6 the values c_i satisfy (4.2). Since $\Phi_u(C_\alpha^c) = 0$ is the steady-state equation $\dot{c_u} = 0$ after substitution of the eliminated variables, this equation is also satisfied, and the same reasoning applies to the equation $\overline{\omega}^l = \xi^l(c_1, \dots, c_m)$. Thus, $(c_1, \dots, c_m)$ is a solution to the steady-state equations and satisfies the conservation laws corresponding to the total amounts $\overline{\omega}^l$. ∎

This theorem and Proposition 8.7 together give the following corollary.

COROLLARY 9.2. *With the conditions of Theorem 9.1, assume further that each graph $\widehat{G}_{\mathcal{S}_\alpha^j}$ is strongly connected. Then, the positive steady states of the system are in one-to-one correspondence with the positive solutions to $\Phi_u(C_\alpha^c) = 0$ and $\overline{\omega}^l = \xi^l(C_\alpha^c)$ for $u = m+1, \dots, s$ and $l = 1, \dots, n$. Further, if a steady-state solution satisfies $c_i = 0$ for $c_i \in C_\alpha$, then there exists some $c_j \in C_\alpha^c$ such that $c_j = 0$.*

In Example 3.3, the set $\mathcal{S}_\alpha = \{S_1, S_4, S_5, S_6, S_8, S_9\}$ is the largest noninteracting subset of $\mathcal{S}$ and thus provides the maximal number of linearly eliminated

concentrations. The initial system of steady-state equations is reduced to three equations—for instance, the one corresponding to $\dot{c_7} = 0$, and the two conservation laws $\overline{\omega}^3 = c_2 + c_4 + c_6 + c_7 + c_9$ and $\overline{\omega} = c_3 + c_5 + c_6 + c_7 + c_9$ (which corresponds to $\omega^4 - \omega^3$). Because of the conservation laws, the equations $\dot{c_2} = 0$ and $\dot{c_3} = 0$ are redundant. The elimination from cuts provides $c_k = \sigma_k/\sigma_1$ for $k = 4, 5, 6$, $c_1 = \overline{\omega}^1\sigma_1/(\sigma_1+\sigma_4+\sigma_5+\sigma_6)$, $c_9 = \sigma_9/\sigma_8$, and $c_8 = \overline{\omega}^2\sigma_8/(\sigma_8+\sigma_9)$ with σ_i S-positive polynomials in c_2, c_3, c_7 and coefficients in $\mathbb{R}(\mathrm{Con} \cup \{\overline{\omega}^1, \overline{\omega}^2\})$ for all i. The steady-state equations are thus reduced to $0 = k_9\sigma_6(\sigma_8+\sigma_9)\sigma_8 - k_{10}\overline{\omega}^2\sigma_8^2\sigma_1c_7 + k_{11}\sigma_1\sigma_9(\sigma_8+\sigma_9)$, $\overline{\omega}^3\sigma_1\sigma_8 = \sigma_1\sigma_8c_2 + \sigma_4\sigma_8 + \sigma_6\sigma_8 + \sigma_1\sigma_8c_7 + \sigma_1\sigma_9$, and $\overline{\omega}\sigma_1\sigma_8 = \sigma_1\sigma_8c_3 + \sigma_5\sigma_8 + \sigma_6\sigma_8 + \sigma_1\sigma_8c_7 + \sigma_1\sigma_9$.

Acknowledgment. Part of this work was done while the authors were visiting Imperial College London in the fall of 2011.

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