HOMOTOPY METHODS FOR COUNTING REACTION NETWORK EQUILIBRIA

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ABSTRACT. Dynamical system models of complex biochemical reaction networks are usually high-dimensional, nonlinear, and contain many unknown parameters. In some cases the reaction network structure dictates that positive equilibria must be unique for all values of the parameters in the model. In other cases multiple equilibria exist if and only if special relationships between these parameters are satisfied. We describe methods based on homotopy invariance of degree which allow us to determine the number of equilibria for complex biochemical reaction networks and how this number depends on parameters in the model.

Contents

1. Introduction

Dynamical system models of complex biochemical reaction networks are usually high-dimensional, nonlinear, and contain many unknown parameters. As was shown recently in [CTF06], based on the assumption of mass-action kinetics, graph-theoretical properties of some biochemical reaction networks can guarantee the uniqueness of positive equilibrium points for any values of the reaction rate parameters in the model. On the other hand, relatively simple reaction networks do admit multiple positive equilibria for some values of the parameters as shown in [CF05, CF06, CTF06].

The aforementioned results do not address the dependence of the number of equilibria on the parameter values unless there is a unique equilibrium for every set of parameters. Also they do not address the general problem of *existence* of positive equilibria. Here we describe methods using degree theory to analyze general biochemical dynamics (not only mass-action kinetics). These methods allow us to determine how the number of positive equilibria for a complex biochemical reaction network depends on the parameters of the model. They will often also imply the existence of positive equilibria. Also we obtain uniqueness of positive equilibria in various situations under significantly weaker assumptions than in [CF05, CF06, CTF06].

1.1. **Overview.** We are interested in equilibria for high-dimensional, nonlinear dynamical systems that originate from chemical dynamics. These dynamical systems are systems of ordinary differential equations of the form

$$(1.1) \qquad \frac{dc}{dt} = f(c)$$

where f is a smooth function defined on a subset of the orthant $\mathbb{R}^n_{\geq 0}$ of vectors c in $\mathbb{R}^n$ having nonnegative components. Such dynamical systems usually have a large number of state variables, i.e., n is large. In addition, the parameters defining f are often not well known. The focus of this paper is on equilibria for dynamical systems of the form (1.1), that is on c^* for which $f(c^*) = 0$.

We consider the dynamical system (1.1) on a subset $\overline{\Omega}$ of $\mathbb{R}^n_{\geq 0}$ which is the closure of a domain Ω in $\mathbb{R}^n_{>0}$. We give conditions for the number of equilibria of (1.1) to remain constant as we "continuously deform" (homotopy) the function f through a family of functions. A key assumption is that the following condition holds for all members of the family:

> **(DetSign)** *The determinant of the Jacobian matrix $\frac{\partial f}{\partial c}(\cdot)$ of f is either strictly positive or strictly negative on Ω.*

(Recall that the Jacobian $\frac{\partial f}{\partial c}(c)$ at c is the matrix $\{\frac{\partial f_j}{\partial c_i}(c), \ i, j = 1, \ldots, n\}$.)

What we observe is that when the condition (DetSign) holds for all f in the family and Ω is bounded, then the number of equilibria for the dynamical system (1.1) is a constant for all f in the family, provided there are no equilibria on the boundary of Ω for any f in the family (see Theorem 1.1). We further indicate how this result extends to unbounded domains such as $\Omega = \mathbb{R}^n_{>0}$ under suitable conditions (see e.g., Theorem 4.1), including those associated with a mass-conserving reaction network operating in a chemical reactor with inflows and outflows.

This paper extends previous findings in several ways.

The (DetSign) condition was introduced by Craciun and Feinberg [CF05, CF06] in the context of chemistry with $\Omega = \mathbb{R}^n_{>0}$ and they observed that many chemical reaction networks have the property (DetSign). They gave many examples and many tests for this condition to hold in the case where f is a system of polynomials and Ω is $\mathbb{R}^n_{>0}$. Then they [CF05, CF06] proved that if the components of f are polynomials corresponding to mass-action kinetics (operating in a continuous flow stirred tank reactor), and if (DetSign) holds on $\mathbb{R}^n_{>0}$ for *all positive* "rate constants", then for each particular choice of rate constant, when an equilibrium exists, it is unique. Here we obtain stronger conclusions with weaker assumptions. In particular the following are features of our approach.

   (1) Rather than all positive "rate constants" we can select a (vector valued) rate constant k_0 of interest at which (DetSign) holds. Then one merely needs a continuous curve $k(\lambda)$ of "rate constants" joining k_0 to another k_1 at which (DetSign) holds and at which the dynamical system has a unique positive equilibrium.
   (2) For a mass-conserving reaction network operating in a chemical reactor with inflows and outflows, under the (DetSign) assumption in (1), we prove existence and uniqueness of a positive equilibrium, see Theorem 5.8.
   (3) In (1) and (2), the function f need not be polynomial, but is required only to be continuously differentiable. Of practical importance are rational f as one finds in Michaelis-Menten or Hill type chemical models, see §5, §6.
   (4) We give methods, see §6, combining the items above to describe large regions of rate constants where a chemical reaction network has a unique positive equilibrium.

We also point out in this paper that the biochemical reaction network models introduced and analyzed by Arcak and Sontag [AS06, AS08] satisfy (DetSign) and we can also rule out boundary equilibria (where they give enough data). Consequently, under extremely weak hypotheses, we obtain that each of these models has a unique positive equilibrium, see §2.2. The findings of Arcak and Sontag are impressive in that under strong hypotheses they prove global asymptotic stability of equilibria, a topic that this paper does not address.

1.2. **More detail.** Now we give some formal definitions. Let Ω be a **domain** in $\mathbb{R}^n$, i.e., an open, connected set in $\mathbb{R}^n$. We denote the closure of Ω by $\overline{\Omega}$ and the boundary of Ω by $\partial\Omega$. A function $f : \overline{\Omega} \to \mathbb{R}^n$ is **smooth** if it is once continuously differentiable on $\overline{\Omega}$. If Ω

is bounded, for such a smooth function f, the following norm is finite:

$$\|f\|_\Omega := \sup_{c \in \Omega} \|f(c)\|$$

Here $\|\cdot\|$ denotes the Euclidean norm on $\mathbb{R}^n$. When Ω is bounded, a family $f_\lambda : \overline{\Omega} \to \mathbb{R}^n$ for $\lambda \in [0, 1]$, is a **continuously varying family of functions** provided each f_λ is smooth and the mapping $\lambda \to f_\lambda$ is continuous on $[0, 1]$ with the norm $\|\cdot\|_\Omega$ on the functions f_λ. A **zero** of $f : \overline{\Omega} \to \mathbb{R}^n$ is a value $c \in \overline{\Omega}$ such that $f(c) = 0$, where 0 is the zero vector in $\mathbb{R}^n$. A zero of f is an equilibrium point for the dynamical system (1.1).

The following is an immediate consequence of Theorem 3.2 which will be proved in §3. This theorem and examples given in §2 are designed to illustrate our approach; then more targeted theorems are given in §4 and §5, followed by more examples in §6.

Theorem 1.1. *Suppose $\Omega \subset \mathbb{R}^n$ is a bounded domain and $f_\lambda : \overline{\Omega} \to \mathbb{R}^n$ for $\lambda \in [0, 1]$, is a continuously varying family of smooth functions such that f_λ does not have any zeros on the boundary of Ω for all $\lambda \in [0, 1]$. If $\det\left(\frac{\partial f_\lambda}{\partial c}(c)\right) \neq 0$ for all $c \in \Omega$ whenever $\lambda = 0$ and whenever $\lambda = 1$, then the number of zeros of f_0 in Ω equals the number of zeros of f_1 in Ω.*

The domain of interest for chemical dynamics (cf. (1.1)) is typically the orthant $\mathbb{R}^n_{>0}$, but this is not bounded, so it violates the hypothesis "Ω is bounded". Thus in applying Theorem 1.1 we must approximate $\mathbb{R}^n_{>0}$ by a large bounded domain Ω and check for the absence of boundary equilibria. One can think of the boundary $\partial\Omega$ in two pieces: that which intersects the boundary of $\mathbb{R}^n_{>0}$, called the **sides of Ω**, and the **outer boundary**, $\partial\Omega \cap \mathbb{R}^n_{>0}$. We show that if we assume conservation of mass (e.g., by atomic balance) in our model and augment with suitable "outflows", then natural bounded domains Ω can be chosen which have no equilibria on the outer boundary. An example of such a natural bounded domain in $\mathbb{R}^2_{>0}$ is shown in Figure 1. Also under assumptions of positive invariance and augmentation with "inflows", there are no equilibria on the sides. In these cases we conclude that there is exactly one nonnegative equilibrium c^* for (1.1) and that it is actually a positive equilibrium, i.e., it lies in $\mathbb{R}^n_{>0}$. This result is described in detail in §4 and §5.

1.3. **Organization of the paper.** In this paper we give many examples of widely varying types to illustrate our contention that our method applies broadly and is easy to use. Section 2 gives several examples illustrating Theorem 1.1. Section 3 summarizes degree theory since our proof is based on this and relies on the observation that when (DetSign) is true, and there are no boundary equilibria, then "the degree of f with respect to 0" equals $\pm$ the number of equilibria in a bounded domain. Then in §3 we prove Theorem 1.1 and more. Section 4 describes the mathematical benefits of mass dissipation (including mass conservation) and "inflows" and "outflows". Section 5 describes a chemical reaction network framework which contains in addition to mass-action kinetics, Michaelis-Menten and Hill dynamics. We conclude in §6 with more examples and new methods presented in the context of these examples. For many of our examples, the determinant of the Jacobian of f was computed symbolically using Mathematica. As a complement to this paper, we
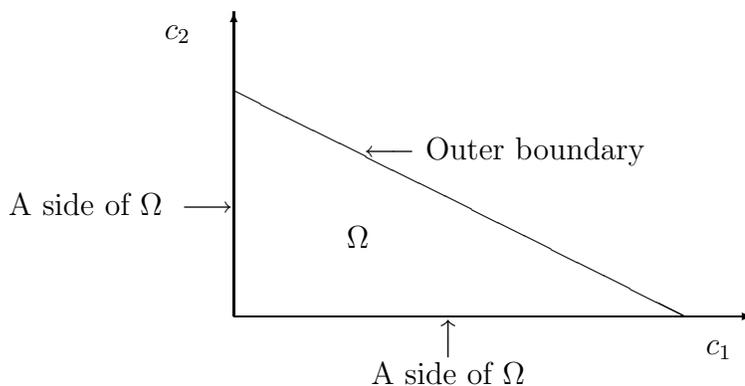
Figure 1: An example of a natural bounded domain in $\mathbb{R}^2_{>0}$ with the outer boundary and sides indicated.

have established a webpage at http://www.math.ucsd.edu/~helton/chemjac.html containing Mathematica notebooks for many examples in this paper and a demonstration notebook that readers may edit to run their own examples.

2. Examples

Our goal in this section is to present some examples showing how to use Theorem 1.1. In the process we mention that all chemical reaction examples of Arcak and Sontag [AS06, AS08] satisfy (DetSign) and fit well into our approach here. Later in §6 we give broader categories of examples.

2.1. **Two examples on treating boundary equilibria.** We start with two examples, the study of which goes back to a class of examples studied by Thron [TO78, T91]. Here, c satisfies (1.1) and the Jacobian for all c has the form:

$$(2.1) \qquad \frac{\partial f}{\partial c} = \begin{bmatrix} -a_1 & 0 & \cdots & 0 & -b_n \\ b_1 & -a_2 & \ddots & & 0 \\ 0 & b_2 & -a_3 & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & 0 \\ 0 & \cdots & 0 & b_{n-1} & -a_n \end{bmatrix}$$

where $a_i \geq 0$, $b_i \geq 0$, $i = 1, \ldots, n$, may depend on c. This cyclic feedback structure is common in gene regulation networks, cellular signaling pathways, and metabolic pathways [AS08]. Thron showed that all eigenvalues of $\frac{\partial f}{\partial c}$ have nonnegative real part (local stability)

if $\frac{b_1 \cdots b_n}{a_1 \cdots a_n} < (\sec(\pi/n))^n$. Arcak and Sontag showed that an equilibrium of such a dynamical system is unique and globally stable under strong global restrictions.

Here we observe that our key assumption, the determinant of the Jacobian never changes sign, is met, which is a major step toward checking when a unique positive equilibrium exists for this class of problem.

Lemma 2.1. *When the Jacobian has the form (2.1), we have*

$$(2.2) \qquad \det\left(\frac{\partial f}{\partial c}\right) = (-1)^n \left[\Pi_{j=1}^n a_j + \Pi_{j=1}^n b_j\right]$$

which if not zero has sign independent of $a_i, b_i \geq 0$.

Proof: Direct computation. ∎

We shall now consider several examples from papers of Arcak and Sontag primarily to illustrate that checking for absence of boundary equilibria is straightforward; subsequently we obtain existence and uniqueness of an equilibrium. In this section we assume that all parameters in the reactions are strictly positive. In the sequel, we shall use $\dot{c}$ as an abbreviation for the time derivative of c.

Example 2.2. We start with an example from §6 of [AS06] which they took from Thron [T91]. For this,

$$(2.3) \qquad \dot{c}_1 = \frac{p_1 c_0}{p_2 + c_3} - p_3 c_1$$

$$(2.4) \qquad \dot{c}_2 = p_3 c_1 - p_4 c_2$$

$$(2.5) \qquad \dot{c}_3 = p_4 c_2 - \frac{p_5 c_3}{p_6 + c_3}.$$

Now we apply Theorem 1.1 to obtain the conclusion:

> *For each set of parameters $p_j > 0, j = 1, 2, \ldots, 6, c_0 > 0$, there is a unique equilibrium point c^* in $\mathbb{R}^3_{>0}$ for the chemical reaction network with dynamics given by (2.3)–(2.5), and there is no equilibrium point on the boundary of $\mathbb{R}^3_{\geq 0}$.*

However, we have made no comment on stability (even local), while [AS06] gives certain conditions ensuring global stability. (In fact, one can algebraically solve for the two equilibria of (2.3)–(2.5) as functions of the parameters. Inspection reveals that exactly one of these is in $\mathbb{R}^3_{>0}$ and neither is on the boundary of $\mathbb{R}^3_{\geq 0}$. The point of us treating this example is to show in a simple context how our method works.)

Proof: We shall apply Theorem 1.1 to prove that there is a unique equilibrium, inside any sufficiently large box; hence there is a unique equilibrium in the orthant. The right hand

side $f_{p,c_0}(c)$ of the differential equations (2.3)–(2.5), while a function of c, also depends on positive parameters (p, c_0). One can check that the Jacobian for any of these parameters has the form in (2.1) for all $c \in \mathbb{R}^3_{>0}$, and since $n = 3$ and all parameters are strictly positive, the Jacobian determinant is strictly negative. Note that for any two values of the positive parameters, (p^*, c_0^*) and $(p^\dagger, c_0^\dagger)$, $f_{\lambda(p^*,c_0^*)+(1-\lambda)(p^\dagger,c_0^\dagger)}, \lambda \in [0, 1]$, defines a continuously varying family of smooth functions on any bounded subset of $\mathbb{R}^n_{\geq 0}$. We check below that the no equilibria (i.e., no zeros of $f_{(p,c_0)}$) on the boundary hypothesis holds on any sufficiently large box, for all positive parameters (p, c_0), and thereby conclude using Theorem 1.1 that the number of equilibria of (2.3)–(2.5) in $\mathbb{R}^n_{>0}$ does not depend on (p, c_0) provided the parameters are all strictly positive. Computing the equilibria at one simple "initial" value of (p^*, c_0^*) then finishes the proof.

No equilibria on the boundary of the orthant: Suppose an equilibrium has $c_2 = 0$. Then equation (2.4) implies $c_1 = 0$ which contradicts (2.3). Likewise if we start by assuming $c_1 = 0$ we get $c_1 > 0$ and a contradiction. On the other hand if $c_3 = 0$, then (2.5) implies $c_2 = 0$, which reverts to the case considered first. Thus there are no equilibria on the boundary of $\mathbb{R}^3_{\geq 0}$.

No equilibria on the outer boundary of some big box: Suppose $0 < \delta < \frac{1}{2}$ and $\frac{1}{\delta} > p_j > \delta$ for all j and $c_0 < 1$. Pick Ω to be a box $\Omega := \{c \in \mathbb{R}^3_{>0} : c_j < (\frac{1}{\delta})^4$ for $j = 1, 2, 3\}$. An equilibrium on the outer boundary of the box satisfies

(1) $c_1 = (\frac{1}{\delta})^4$ which by (2.3) implies $(\frac{1}{\delta})^2 > \frac{p_1 c_0}{p_2 + c_3} = p_3 c_1 > (\frac{1}{\delta})^3$. A contradiction.
   OR
(2) $c_2 = (\frac{1}{\delta})^4$ which by (2.5) implies $\frac{1}{\delta} > \frac{p_5 c_3}{p_6 + c_3} = p_4 c_2 > (\frac{1}{\delta})^3$. A contradiction.
   OR
(3) $c_3 = (\frac{1}{\delta})^4$ which by adding (2.3), (2.4), (2.5) implies that

$$\delta^3 = \frac{\frac{1}{\delta}}{\frac{1}{\delta^4}} \geq \frac{p_1 c_0}{p_2 + c_3} = \frac{p_5 c_3}{p_6 + c_3} \geq \frac{\delta \cdot \frac{1}{\delta^4}}{\frac{1}{\delta} + \frac{1}{\delta^4}} = \frac{\delta}{\delta^3 + 1}.$$

A contradiction.

Initializing: Up to this point, Theorem 1.1 tells us that each choice of parameters yields the same number of equilibria! It is easy to compute for oneself that there is a simple choice of parameters which yields a unique positive equilibrium, for example $p_j = 1$ for all j yields the unique equilibrium, $c_1 = c_2 = \frac{c_0}{1+c_0}, c_3 = c_0$. Thus there is one and only one equilibrium in $\mathbb{R}^3_{>0}$ for each value of the positive parameters (p, c_0). ■

Example 2.3.   In Example 1 of §4 in [AS08], the authors describe a simplified model of mitogen activated protein kinase (MAPK) cascades with inhibitory feedback, proposed

in [K00, SHYDWL01]. For this,

$$(2.6) \qquad \dot{c}_1 = -\frac{b_1 c_1}{c_1 + a_1} + \frac{d_1(1 - c_1)}{e_1 + (1 - c_1)} \frac{\mu}{1 + k c_3}$$

$$(2.7) \qquad \dot{c}_2 = -\frac{b_2 c_2}{c_2 + a_2} + \frac{d_2(1 - c_2)}{e_2 + (1 - c_2)} c_1$$

$$(2.8) \qquad \dot{c}_3 = -\frac{b_3 c_3}{c_3 + a_3} + \frac{d_3(1 - c_3)}{e_3 + (1 - c_3)} c_2.$$

The variables $c_j \in [0, 1]$, $j = 1, 2, 3$ denote the concentrations of the active forms of the proteins, and the terms $1 - c_j$, $j = 1, 2, 3$, indicate the inactive forms (after non-dimensionalization and assuming that the total concentration of each of the proteins is 1). Here the parameters $a_1, a_2, a_3, b_1, d_1, e_1, b_2, d_2, e_2, b_3, d_3, e_3, \mu, k$ are strictly positive.

Let $\Omega := \{c \in \mathbb{R}^3 : 0 < c_j < 1, \ j = 1, 2, 3\}$ denote the open unit cube, the domain where this model holds. Now we show how to apply Theorem 1.1 on Ω to conclude that:

> There is a unique equilibrium in Ω for any choice of the strictly positive parameters, $a_1, a_2, a_3, b_1, d_1, e_1, b_2, d_2, e_2, b_3, d_3, e_3, \mu, k$.

Proof: First the Jacobian has the form (2.1). Thus (2.2) implies that the determinant is strictly negative for all strictly positive parameters and concentrations $c \in \Omega$. The proof follows the same outline as Example 2.2. Now we check the required items:

No equilibria on the boundary of the unit cube: Suppose there is an equilibrium c on the boundary of Ω. Then the equilibrium equations imply that

(1) If $c_1 = 0$ then (2.6) forces $c_1 = 1$. Contradiction.
(2) If $c_1 = 1$ then (2.6) forces $c_1 = 0$. Contradiction.
(3) If $c_2 = 0$ then (2.7) forces $c_1 = 0$. Contradiction as above.
(4) If $c_2 = 1$ then (2.7) forces $c_2 = 0$. Contradiction.
(5) If $c_3 = 0$ then (2.8) forces $c_2 = 0$. Contradiction as above.
(6) If $c_3 = 1$ then (2.8) forces $c_3 = 0$. Contradiction.

Initializing: [AS08] proves that there are choices of parameters compatible with this model for which there is a unique stable equilibrium point in Ω. Alternatively, one can compute for a simple choice of parameters that there is a unique positive equilibrium.

The discussion exactly as before implies that there is a unique equilibrium for all fixed strictly positive values of the parameters. ∎

We mention here that the question of how one finds good intializations for the rate constants might be a topic for further research. The goal would be to find methods for systematically selecting rate constants that produce systems whose equilibria can be determined by analytic means. We have not explored this topic at all.

2.2. **The theory of Arcak and Sontag.** Now we shall make some general comments on [AS06, AS08]. There were four chemical reaction examples presented in the two papers [AS06, AS08]. So far we have treated two of the four here in this section. The third example, Example 2 in §4 of [AS08], is a small variant of Example 2.3 above and it can be treated in a similar manner to that example. In particular, it has a Jacobian of the form (2.1). We now turn to the fourth example of Arcak and Sontag.

Example 2.4. This is Example 3 in §4 of [AS08] which we do not describe in detail, since it requires about a page. While its Jacobian does not have the form (2.1), it is easy to analyze (using Mathematica) and what we found is that the determinant of the Jacobian of f is positive at all strictly positive c. Thus the theory described here applies provided suitable boundary behavior holds. Boundary behavior was not possible to determine since the example was a rather general class whose boundary behavior was not specified. In a particular case where more information is specified one might expect that this could be done. ∎

Arcak and Sontag [AS08] present a general theory which contains the examples considered in this section and which does not match up simply with ours. Their theory assumes an equilibrium exists (we do not). It places global restrictions on the equilibrium which guarantee that it is a unique globally stable equilibrium (we address uniqueness but not stability). However, while the Arcak-Sontag theory is different than ours, we do point out in this section that all four of their chemical examples have Jacobians whose determinant sign does not depend on chemical concentration, so our approach applies directly, and with a bit of attention to boundary behavior, gives existence of a unique positive equilibrium. However, we do not obtain the very impressive global stability in [AS08].

3. Degree and homotopy of maps

The proof of Theorem 1.1 and other results in this paper is based on classical degree theory. The degree of a function is invariant if we continuously deform (homotopy) the function and we use that to advantage in this paper.

Now we give the setup. If $\Omega \subset \mathbb{R}^n$ is a bounded domain, and if a smooth (once continuously differentiable) function $f : \overline{\Omega} \to \mathbb{R}^n$ has no degenerate zeros, and has no zeros on the boundary of Ω, then the *topological degree with respect to zero of f* (or simply the *degree*

of f) equals

$$(3.1) \qquad \deg(f) = \deg(f, \Omega) = \sum_{c \in Z_f} \text{sgn}\ \left(\det \left(\frac{\partial f}{\partial c}(c) \right) \right),$$

where $\text{sgn} : \mathbb{R} \to \{-1, 0, 1\}$ is the sign function, Z_f is the set of zeros of f in Ω, and c^* is a degenerate point means $\det \left(\frac{\partial f}{\partial c}(c^*) \right) = 0$. The degree of a map naturally extends from nondegenerate smooth functions to continuous functions $f : \overline{\Omega} \to \mathbb{R}^n$. For this, one can approximate f uniformly with smooth functions F_k that have no degenerate zeros and no zeros on the boundary of Ω, and then define the degree of f as the limit of the degrees of F_k. The key fact is: this construction of the degree of f is independent of the approximates F_k. Fortunately, we shall only need to compute $\deg(f)$ on smooth nondegenerate f. For a quick account of this theorem and the main properties of degree, see Ch 1.6A of [B77].

Homotopy invariance of the degree is the following well known property:

Theorem 3.1. *Consider some bounded domain $\Omega \subset \mathbb{R}^n$ and a continuously varying family of smooth functions $f_\lambda : \overline{\Omega} \to \mathbb{R}^n$ for $\lambda \in [0, 1]$, such that f_λ does not have any zeros on the boundary of Ω for all $\lambda \in [0, 1]$. Then $\deg(f_\lambda)$ is constant for all $\lambda \in [0, 1]$.*

Now we give a slightly more general theorem than Theorem 1.1 stated in the introduction.

Theorem 3.2. *Suppose Ω and f_λ, $\lambda \in [0, 1]$, are as in Theorem 3.1. Then for any $\lambda \in [0, 1]$ such that $\det \left(\frac{\partial f_\lambda}{\partial c}(c) \right) \neq 0$ for all $c \in \Omega$, the number of zeros of f_λ in Ω must equal the absolute value of the degree of f_λ in Ω, which equals the absolute value of the degree of $f_{\lambda'}$ for any $\lambda' \in [0, 1]$.*

Proof: If $\lambda \in [0, 1]$ is such that the determinant $\det(\partial f_\lambda / \partial c)$ does not vanish in Ω, then $\text{sgn}\,(\det(\partial f_\lambda / \partial c))$ is independent of c. This implies that the zeros of f_λ are nondegenerate and, by the formula for the degree of f_λ, that $|\deg(f_\lambda)|$ equals the number of zeros of f_λ in Ω. The fact that the degree does not vary with λ is immediate from Theorem 3.1. ∎

Remark 3.3. For $|\deg(f_\lambda)|$ to count the number of zeros of f_λ in Ω, $\text{sgn}\left(\det \left(\frac{\partial f_\lambda}{\partial c}(c^*) \right) \right)$ need only be the same for all zeros c^* in Ω, not for all $c \in \Omega$. Sadly this weakening of hypotheses is hard to take advantage of in practice.

Remark 3.4. From the viewpoint of numerical calculation, Theorem 3.2 strongly suggests that if the no boundary zeros hypothesis holds, and (DetSign) holds for $f = f_\lambda$ at one value of $\lambda = \lambda_1$, and if one can calculate all zeros of f_λ at some other value of $\lambda = \lambda_2$ where (DetSign) also holds, then we can determine the number of zeros at $\lambda = \lambda_1$. Indeed, often we can find a λ_2 for which f_{λ_2} is "simple" in the sense that all zeros for λ_2 are non-degenerate and the zeros can be readily computed along with the Jacobians there, and consequently $\deg(f_{\lambda_2})$ can be computed. The import for numerical calculation is that finding a single equilibrium is often not so onerous. After finding one equilibrium one typically makes a new initial guess and tries to find another. Knowing if one has found

all of the equilibria is the truly daunting task, since it is nearly impossible to ensure this by experiment. Thus theoretical results (hopefully those here) help with this very difficult computational question.

4. Mass-dissipating dynamical systems

In this section we consider a general dynamical system model which includes the more specific dynamics of conservative chemical reaction networks, augmented with inflows and outflows, as described in the next section. In chemical engineering, the latter is commonly refered to as dynamics that goes with a continuous flow stirred tank reactor (CFSTR). In biochemistry, one may view this as a model for intracellular behavior with production and degradation, or with inflow and outflow across the cell boundary. Here all species components are subject to inflow and outflow, however, to approximate the conservation of some species such as enzymes, one may take the associated inflow rate value in c_{in} and degradation factor in Λ_o to be arbitrarily small, if desired.

In preparation for defining a dynamical system on the orthant $\mathbb{R}^n_{\geq 0}$, we consider a smooth function $g : \mathbb{R}^n_{\geq 0} \to \mathbb{R}^n$, where g has the property that for each $j \in \{1, ..., n\}$, the jth coordinate of $g(c)$ is nonnegative whenever the jth coordinate of $c \in \mathbb{R}^n_{\geq 0}$ is zero. Consider the dynamical system associated with this function given by

$$(4.1) \qquad\qquad \dot{c} = g(c) \quad \text{ for } c \in \mathbb{R}^n_{\geq 0}.$$

This dynamical system (4.1) is called *positive-invariant* because of the condition on g. This guarantees that the dynamics leaves the orthant $\mathbb{R}^n_{\geq 0}$ invariant. Given $m \in \mathbb{R}^n_{>0}$, the dynamical system (4.1) is called *mass-dissipating with respect to m* if

$$(4.2) \qquad\qquad m \cdot g(c) \leq 0$$

for all $c \in \mathbb{R}^n_{\geq 0}$; it is called *mass-dissipating* if it is mass-dissipating with respect to m for some $m \in \mathbb{R}^n_{>0}$. In this case, on $\mathbb{R}^n_{\geq 0}$,

$$(4.3) \qquad\qquad \frac{d(m \cdot c)}{dt} = m \cdot g(c) \leq 0.$$

Now we consider the dynamical system (4.1) augmented with inflows and outflows:

$$(4.4) \qquad\qquad \dot{c} = c_{in} - \Lambda_o c + g(c),$$

where Λ_o is an $n \times n$ diagonal matrix with strictly positive entries on the diagonal. We interpret the term $c_{in} \in \mathbb{R}^n_{>0}$ as a constant *inflow rate*, and the term $\Lambda_o c$ as an *outflow rate* which for each component is proportional to the concentration of that component. It is easy to check that with this augmentation, the dynamics still leaves the orthant $\mathbb{R}^n_{\geq 0}$ invariant. However, the mass-dissipating property is only inherited at large values of the concentration c.

We are now ready to state our main theorem in this context.

Theorem 4.1. *Let $c_{in}, m \in \mathbb{R}_{>0}^n$, and Λ_o be an $n \times n$ diagonal matrix with strictly positive diagonal entries. Consider a smooth function $g : \mathbb{R}_{\geq 0}^n \to \mathbb{R}^n$ such that the dynamical system (4.1) is positive-invariant and mass-dissipating with respect to m. Define*

$$f(c) := c_{in} - \Lambda_o c + g(c) \quad for \ c \in \mathbb{R}_{\geq 0}^n.$$

Then the augmented system (4.4), with inflows and outflows, has no equilibria on the boundary of $\mathbb{R}_{\geq 0}^n$, and if $\det \left(\frac{\partial f}{\partial c} \right) \neq 0$ on $\mathbb{R}_{>0}^n$, then there is exactly one equilibrium point for this system in $\mathbb{R}_{>0}^n$.

Proof. It suffices to prove that f has no zeros on the boundary of $\mathbb{R}_{\geq 0}^n$ and if $\det \left(\frac{\partial f}{\partial c} \right) \neq 0$ on $\mathbb{R}_{>0}^n$, then f has exactly one zero in $\mathbb{R}_{>0}^n$.

Define

$$f_\lambda(c) := c_{in} - \Lambda_o c + \lambda g(c), \quad for \ c \in \mathbb{R}_{\geq 0}^n, \ \lambda \in [0, 1].$$

Fix $M > m \cdot c_{in}$ and let

$$\Omega_M = \{ c \in \mathbb{R}_{>0}^n : m \cdot (\Lambda_o c) < M \}.$$

Then Ω_M is a bounded domain and $\{ f_\lambda : \lambda \in [0, 1] \}$ is a continuously varying family of smooth functions on $\overline{\Omega}_M$. For $j = 1, \ldots, n$, consider $c^j \in \overline{\Omega}_M$ such that the jth coordinate of c^j is zero. Then the jth coordinate of $f_\lambda(c^j)$ must be strictly positive, because the jth coordinate of c_{in} is strictly positive, and the jth coordinate of $g(c^j)$ is nonnegative, by the positive-invariance assumption. Therefore f_λ has no zeros on the sides of Ω_M, i.e., on $\overline{\Omega}_M \cap \partial \mathbb{R}_{\geq 0}^n$. Also, we have

$$(4.5) \qquad m \cdot f_\lambda(c) = m \cdot c_{in} - m \cdot (\Lambda_o c) + \lambda m \cdot g(c) \leq m \cdot c_{in} - m \cdot (\Lambda_o c) < 0$$

for all $c \in \overline{\Omega}_M$ such that $m \cdot (\Lambda_o c) = M$. Here we have used the mass-dissipating property of m for the first inequality and the fact that $M > m \cdot c_{in}$ for the second inequality. It follows that f_λ has no zeros on the outer boundary of Ω_M, i.e., on $\{ c \in \mathbb{R}_{>0}^n : m \cdot (\Lambda_o c) = M \}$. Thus, f_λ has no zeros on the boundary of Ω_M for all $\lambda \in [0, 1]$. Then, by Theorem 3.1, the degree of f_λ on Ω_M, $\deg(f_\lambda, \Omega_M)$, is constant for all $\lambda \in [0, 1]$. Next we observe that $c^* = (\Lambda_o)^{-1} c_{in}$ is the unique zero of f_0 and is inside Ω_M, and $\frac{\partial f}{\partial c} = -\Lambda_o$, and so by (3.1), we obtain $\deg(f_0, \Omega_M) = \text{sgn}(\det(-\Lambda_o)) = (-1)^n$. Hence, by Theorem 3.2, if $\det \left(\frac{\partial f}{\partial c} \right) = \det \left(\frac{\partial f_1}{\partial c} \right) \neq 0$ on Ω_M, then $f = f_1$ has exactly one zero in Ω_M.

Since $M > m \cdot c_{in}$ was arbitrary and the sets $\overline{\Omega}_M : M > m \cdot c_{in}$ fill out $\mathbb{R}_{\geq 0}^n$, it follows that f has no zeros on the boundary of $\mathbb{R}_{\geq 0}^n$. If furthermore, $\det \left(\frac{\partial f}{\partial c} \right) \neq 0$ on all of $\mathbb{R}_{>0}^n$, then it follows that f has exactly one zero in $\mathbb{R}_{>0}^n$. ∎

Remark 4.2. A special case of mass-dissipating is *mass-conserving*, namely $m \cdot g(c) = 0$. For dynamical systems associated to chemical reaction networks this has a natural interpretation. Indeed, the dynamics of chemical concentrations resulting from chemical interactions among several types of molecules will be mass-conserving whenever there exists a mass

assignment for each chemical species which is conserved by each reaction, or whenever each chemical species (or molecule) is made up of atoms that are also conserved by each reaction. More generally, the dynamics will be mass-dissipating whenever no reaction produces more mass than it consumes, respectively, produces more atoms than it consumes. Mass conservation implies $\det(\frac{\partial g}{\partial c}) = 0$, since $m \cdot \frac{\partial g}{\partial c} = 0$ when $m \cdot g = 0$. Thus augmenting with outflows is required to make the hypothesis on the sign of $\det(\frac{\partial f}{\partial c})$ in our theorems meaningful. The paper [HKG08], which builds on the current one, introduces a more general determinant that applies when there are no outflows (or only some outflows). This then helps one count equilibria in a manner generalizing what we have done here.

Remark 4.3. Theorem 4.1 still holds with a much less restrictive definition of mass-dissipating, e.g., by replacing "m" with the gradient "∇L" for an appropriate class of functions $L : \mathbb{R}_{\geq 0}^n \to \mathbb{R}_{\geq 0}$. Here mass or atom count is behaving like what is called *storage function* in engineering systems theory, see [K02]. Indeed the inequality $m \cdot f_\lambda(c) \leq m \cdot c_{in} - m \cdot (\Lambda_o c)$ derived in (4.5) is what is called a dissipation inequality on the storage function $c \to m \cdot c$, which in fact also plays the role of a "running cost".

5. Dynamics of chemical reaction networks

We now introduce the standard terminology of Chemical Reaction Network Theory (see [HJ72, F72, F95, CF05]). A chemical reaction network is usually specified by a finite set of reactions $\mathscr{R}$ involving a finite set of chemical species $\mathscr{S}$.

For example, a chemical reaction network with two chemical species A_1 and A_2 is schematically given in the diagram

$$(5.1) \qquad 2A_1 \rightleftharpoons A_1 + A_2 \rightleftharpoons 2A_2$$
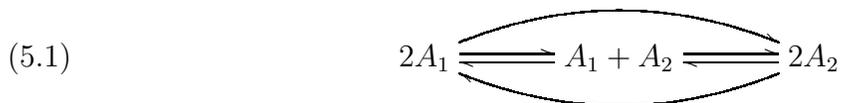
The dynamics of the state of this chemical system is defined in terms of functions $c_{A_1}(t)$ and $c_{A_2}(t)$ which represent the concentrations of the species A_1 and A_2 at time t. The occurrence of a chemical reaction causes changes in concentrations; for instance, whenever the reaction $A_1 + A_2 \to 2A_1$ occurs, the net gain is a molecule of A_1, whereas one molecule of A_2 is lost. Similarly, the reaction $2A_2 \to 2A_1$ results in the creation of two molecules of A_1 and the loss of two molecules of A_2.

A common assumption is that the rate of change of the concentration of each species is governed by *mass-action kinetics* [HJ72, F72, F79, F87, F95, S01, CF05, CF06, CTF06], i.e., that each reaction takes place at a rate that is proportional to the product of the concentrations of the species being consumed in that reaction. For example, under the mass-action kinetics assumption, the contribution of the reaction $A_1 + A_2 \to 2A_1$ to the rate of change of c_{A_1} has the form $k_{A_1+A_2 \to 2A_1} c_{A_1} c_{A_2}$, where $k_{A_1+A_2 \to 2A_1}$ is a positive number

called the *reaction rate constant.* In the same way, the reaction $2A_2 \to 2A_1$ contributes the negative value $-2k_{2A_2 \to 2A_1} c_{A_2}^2$ to the rate of change of c_{A_2}. Collecting the contributions of all the reactions, we obtain the following dynamical system associated to the chemical reaction network depicted in (5.1):

$$
\begin{aligned}
(5.2) \qquad \dot{c}_{A_1} = \;& -k_{2A_1 \to A_1 + A_2} c_{A_1}^2 + k_{A_1 + A_2 \to 2A_1} c_{A_1} c_{A_2} - k_{A_1 + A_2 \to 2A_2} c_{A_1} c_{A_2} \\
& + k_{2A_2 \to A_1 + A_2} c_{A_2}^2 - 2k_{2A_1 \to 2A_2} c_{A_1}^2 + 2k_{2A_2 \to 2A_1} c_{A_2}^2 \\
\dot{c}_{A_2} = \;& k_{2A_1 \to A_1 + A_2} c_{A_1}^2 - k_{A_1 + A_2 \to 2A_1} c_{A_1} c_{A_2} + k_{A_1 + A_2 \to 2A_2} c_{A_1} c_{A_2} \\
& - k_{2A_2 \to A_1 + A_2} c_{A_2}^2 + 2k_{2A_1 \to 2A_2} c_{A_1}^2 - 2k_{2A_2 \to 2A_1} c_{A_2}^2
\end{aligned}
$$

The objects on both sides of the reaction arrows (i.e., $2A_1$, $A_1 + A_2$, and $2A_2$) are called *complexes* of the reaction network. Note that the complexes are non-negative integer combinations of the species. On the other hand, we will see later that it is very useful to think of the complexes as (column) vectors in $\mathbb{R}^n$, where n is the number of elements of $\mathscr{S}$, via an identification of the set of species with the standard basis of $\mathbb{R}^n$, given by a fixed ordering of the species. For example, via this identification, the complexes above become $2A_1 = \begin{bmatrix} 2 \\ 0 \end{bmatrix}$, $A_1 + A_2 = \begin{bmatrix} 1 \\ 1 \end{bmatrix}$, and $2A_2 = \begin{bmatrix} 0 \\ 2 \end{bmatrix}$. We can now formulate a general setup which includes many situations, certainly those above.

5.1. **The general setup.** Now we present basic definitions and illustrate them.

Definition 5.1. A *chemical reaction network* is a triple $(\mathscr{S}, \mathscr{C}, \mathscr{R})$, where $\mathscr{S}$ is a set of n chemical *species*, $\mathscr{C}$ is a finite set of vectors in $\mathbb{R}^n_{\geq 0}$ with nonnegative integer entries called the set of *complexes*, and $\mathscr{R} \subset \mathscr{C} \times \mathscr{C}$ is a finite set of relations between elements of $\mathscr{C}$, denoted $y \to y'$ which represents the set of *reactions* in the network. Moreover, the set $\mathscr{R}$ cannot contain elements of the form $y \to y$; for any $y \in \mathscr{C}$ there exists some $y' \in \mathscr{C}$ such that either $y \to y'$ or $y' \to y$; and the union of the supports of all $y \in \mathscr{C}$ is $\mathscr{S}$, where the *support* of an element $\alpha \in \mathbb{R}^n$ is $\mathrm{supp}(\alpha) = \{j : \alpha_j \neq 0\}$. To each reaction $y \to y' \in \mathscr{R}$, we associate a reaction vector given by $y' - y$.

The last two constraints of the definition amount to requiring that each complex appears in at least one reaction, and each species appears in at least one complex. For the system (5.1), the set of species is $\mathscr{S} = \{A_1, A_2\}$, the set of complexes is $\mathscr{C} = \{2A_1, A_1 + A_2, 2A_2\}$ and the set of reactions is $\mathscr{R} = \{2A_1 \rightleftharpoons A_1 + A_2, A_1 + A_2 \rightleftharpoons 2A_2, 2A_2 \rightleftharpoons 2A_1\}$, and consists of 6 reactions, represented as three reversible reactions.

In examples we will often refer to a chemical reaction network by specifying $\mathscr{R}$ only, since $\mathscr{R}$ encompasses all of the information about the network. In the sequel we shall sometimes simply say reaction network in place of chemical reaction network.

Definition 5.2. A *kinetics* for a reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ is an assignment to each reaction $y \to y' \in \mathscr{R}$ of a *reaction rate function*

$$K_{y \to y'} : \mathbb{R}^n_{\geq 0} \to \mathbb{R}^n_{\geq 0}.$$

By a *kinetic system*, which we denote by $(\mathscr{S}, \mathscr{C}, \mathscr{R}, K)$, we mean a reaction network taken together with a kinetics.

For each *concentration* $c \in \mathbb{R}^n_{\geq 0}$, the nonnegative number $K_{y \to y'}(c)$ is interpreted as the occurrence rate of the reaction $y \to y'$ when the chemical mixture has concentration c. Hereafter, we suppose that reaction rate functions are smooth on $\mathbb{R}^n_{\geq 0}$, and that $K_{y \to y'}(c) = 0$ whenever $\mathrm{supp}(y) \not\subset \mathrm{supp}(c)$. Although it will not be important in this article, it is natural to also require that, for each $y \to y' \in \mathscr{R}$ the function $K_{y \to y'}$ is strictly positive precisely when $\mathrm{supp}(y) \subset \mathrm{supp}(c)$, i.e., precisely when the concentration c contains at non-zero concentrations those species that appear in the reactant complex y.

Definition 5.3. The *species formation rate function* for a kinetic system $(\mathscr{S}, \mathscr{C}, \mathscr{R}, K)$ is defined by $r : \mathbb{R}^n_{\geq 0} \to \mathbb{R}^n$ where

$$r(c) = \sum_{y \to y' \in \mathscr{R}} K_{y \to y'}(c)(y' - y) \quad \text{for} \quad c \in \mathbb{R}^n_{\geq 0}.$$

The *associated dynamical system* for the kinetic system $(\mathscr{S}, \mathscr{C}, \mathscr{R}, K)$ is

$$(5.3) \qquad \dot{c} = r(c) = \sum_{y \to y' \in \mathscr{R}} K_{y \to y'}(c)(y' - y),$$

where $c \in \mathbb{R}^n_{\geq 0}$ is the nonnegative vector of species concentrations.

The interpretation of $r(\cdot)$ is as follows: if the chemical concentration is $c \in \mathbb{R}^n_{\geq 0}$, then $r_j(c)$ is the production rate of species j due to the occurrence of all chemical reactions. To see this, note that

$$r_j(c) = \sum_{y \to y' \in \mathscr{R}} K_{y \to y'}(c)(y'_j - y_j),$$

and that $y'_j - y_j$ is the net number of molecules of species j produced with each occurrence of reaction $y \to y'$. Thus, the right hand side of the equation above is the sum of all reaction occurrence rates, each weighted by the net gain in molecules of species j with each occurrence of the corresponding reaction. Note that $r_j(c)$ could be less than zero, in which case $|r_j(c)|$ represents the overall rate of consumption of species j.

5.1.1. *Special case: Mass-action kinetics.*

Definition 5.4. A *mass-action system* is a quadruple $(\mathscr{S}, \mathscr{C}, \mathscr{R}, k)$, where $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ is a chemical reaction network and $k = (k_{y \to y'})$ is a vector of *reaction rate constants*, so that the reaction rate function $K_{y \to y'} : \mathbb{R}^n_{\geq 0} \to \mathbb{R}^n_{\geq 0}$, for each reaction $y \to y' \in \mathscr{R}$, is given by *mass-action kinetics*:

$$K_{y \to y'}(c) = k_{y \to y'} c^y \qquad where \quad c^y = \prod_{i=1}^{n} c_i^{y_i}.$$

(Here we adopt the convention that $0^0 = 1$.) The *associated mass-action dynamical system* is

(5.4) $$\dot{c} = \sum_{y \to y' \in \mathscr{R}} k_{y \to y'} c^y (y' - y).$$

In the vector equation (5.4), the total rate of change of the vector of concentrations c is computed by summing the contributions of all the reactions in $\mathscr{R}$. Each reaction $y \to y'$ contributes proportionally to the product of the concentrations of the species in its *source* y, that is, c^y, and also proportional to the number of molecules gained or lost in this reaction. Finally, the proportionality factor is $k_{y \to y'}$. For example, we can rewrite (5.2) in the vector form (5.4) as

(5.5)
$$\begin{bmatrix} \dot{c}_1 \\ \dot{c}_2 \end{bmatrix} = k_{2A_1 \to A_1 + A_2} c_1^2 \begin{bmatrix} -1 \\ 1 \end{bmatrix} + k_{A_1 + A_2 \to 2A_1} c_1 c_2 \begin{bmatrix} 1 \\ -1 \end{bmatrix} + k_{A_1 + A_2 \to 2A_2} c_1 c_2 \begin{bmatrix} -1 \\ 1 \end{bmatrix}$$
$$+ k_{2A_2 \to A_1 + A_2} c_2^2 \begin{bmatrix} 1 \\ -1 \end{bmatrix} + k_{2A_1 \to 2A_2} c_1^2 \begin{bmatrix} -2 \\ 2 \end{bmatrix} + k_{2A_2 \to 2A_1} c_2^2 \begin{bmatrix} 2 \\ -2 \end{bmatrix}.$$

5.2. **Mass conservation.** Now we see in terms of this setup how one obtains mass conservation as defined in §4.

Definition 5.5. The *stoichiometric subspace* $S \subset \mathbb{R}^n$ of a reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ is the linear subspace of $\mathbb{R}^n$ spanned by the *reaction vectors* $y' - y$, for all reactions $y \to y' \in \mathscr{R}$.

Note that, according to (5.3), for a given value of c, the vector $\dot{c}$ is a linear combination of the reaction vectors. This implies that each *stoichiometric compatibility class* $(c_0 + S) \cap \mathbb{R}^n_{\geq 0}$ is an invariant set for the dynamical system (5.3) with initial condition $c_0 \in \mathbb{R}^n_{\geq 0}$.

Definition 5.6. A reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ is called *conservative* if there exists some positive vector $m \in \mathbb{R}^n_{> 0}$ which is orthogonal to all its reaction vectors, i.e.,

$$m \cdot (y' - y) = 0$$

for all reactions $y \to y'$ in $\mathscr{R}$. Then m is called a *conserved mass vector*.

Remark 5.7. Each trajectory of a conservative reaction network is bounded. A conservative reaction network can have no inflows or outflows (see the definition of inflow and outflow in the next section).

5.3. **Main results.** We now consider conservative reaction networks augmented with inflow and outflow reactions (for each of the species). An inflow reaction is a reaction of the form $0 \to A$ and an outflow reaction is one of the form $A \to 0$, where A is a species. The reaction vector $y' - y$ associated with an inflow reaction for species j is the vector containing all zeros, except that it has a one in the jth position. The reaction vector associated with an outflow reaction for species j is the negative of the vector associated with an inflow reaction for that species. Here, for the kinetics associated with the inflows and outflows, we assume that the reaction rate function for each inflow reaction is a positive constant and the value of the reaction rate function for each outflow reaction is a positive constant times the concentration of the species flowing out. The latter corresponds to degradation of each species at a rate proportional to its concentration. The following theorem may be used to determine the number of equilibria for conservative reaction networks augmented by such inflows and outflows. It requires a positive determinant condition and is the analog of Theorem 4.1 in this context.

Theorem 5.8. *Consider some conservative reaction network* $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ *with conserved mass vector m. Let K be a kinetics for this network with associated species formation rate function g. Consider an augmented kinetic system* $(\mathscr{S}, \tilde{\mathscr{C}}, \tilde{\mathscr{R}}, \check{\mathscr{K}})$ *obtained by adding inflow and outflow reactions for all species so that the associated dynamical system is:*

$$(5.6) \qquad\qquad \dot{c} = r(c) := c_{in} - \Lambda_o c + g(c),$$

where $c_{in} \in \mathbb{R}^n_{>0}$ and Λ_o is an $n \times n$ diagonal matrix with strictly positive diagonal entries. Suppose that

$$\det\left(\frac{\partial r}{\partial c}(c)\right) \neq 0,$$

for all $c \in \mathbb{R}^n_{>0}$. Then the dynamical system (5.6) has exactly one equilibrium c^* in $\mathbb{R}^n_{>0}$ and no equilibria on the boundary of $\mathbb{R}^n_{\geq 0}$.

Proof. We want to apply Theorem 4.1. The function g is given by the right member of (5.3), where the functions $K_{y \to y'}$ are all smooth and have the property that $K_{y \to y'}(c) = 0$ whenever $\mathrm{supp}(y) \not\subset \mathrm{supp}(c)$. Consequently, g is smooth and, whenever $c \in \mathbb{R}^n_{\geq 0}$ is such that $c_j = 0$ for some j, then we have

$$g_j(c) \geq \sum_{y \to y' \in \mathscr{R}} K_{y \to y'}(c)(-y_j) = 0,$$

because $K_{y \to y'}(c) = 0$ whenever $y_j > 0$ and $c_j = 0$, by the support property of K. It follows that the dynamical system (5.6) is positive-invariant. Furthermore, the system is mass-dissipating, since

$$m \cdot g(c) = \sum_{y \to y' \in \mathscr{R}} K_{y \to y'}(c)\, m \cdot (y - y') = 0,$$

by the assumption that the reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ is conservative. The conclusion then follows immediately from Theorem 4.1. ∎

Remark 5.9. The results described above use the assumption that all species have inflows, in order to conclude that there are no equilibria on the boundary of $\mathbb{R}^n_{>0}$. On the other hand, for very large classes of chemical reaction networks described in [ADS07], this assumption is actually not needed in order to rule out the existence of such boundary equilibria (an observation by David Anderson University of Wisconsin [A]).

Remark 5.10. The paper [BDB07], for the case of "nonautocatalytic reactions", gives a condition on the "stoichiometric matrix" (in our terminology the matrix whose columns are the vectors $y' - y$ for $y \rightarrow y' \in \mathscr{R}$) which is necessary and sufficient for the determinant of the Jacobian of r to be of one sign for all concentrations and all $K_{y \rightarrow y'}$ which are monotone increasing in each variable. Our theory is less restrictive as illustrated by Example 6.1.

6. APPLICATIONS

In practice, most dynamical system models of biochemical reaction networks contain a large number of unknown parameters. These parameters correspond to reaction rates and other chemical properties of the reacting species. In this section we treat a variety of examples of such models and illustrate the use of Theorems 4.1 and 5.8. In some of these examples, the determinant of the Jacobian $\det\left(\frac{\partial r}{\partial c}\right)$ is of one sign everywhere on the open orthant for all parameters and in some it is not. Even in the latter cases we describe ways to find classes of rate functions for which there exists a unique positive equilibrium.

The first subsection assumes mass-action kinetics and defines (reminds) the reader of the Craciun-Feinberg "determinant expansion" via an example. Critical is the sign of each term in the expansion and whether all terms have the same sign or miss this by "a little", namely, only one or two terms in the determinant expansion has a coefficient with an anomalous sign. Here we point out that all examples in [CF05, CF06, CTF06] have at most one or two anomalous signs. When there are no anomalous signs, these papers show that any positive equilibrium is unique for all parameter values, and they develop and use graph theoretical methods for determining when there are no anomalous signs. Here, for cases of few anomalous signs, we propose and illustrate a technique for identifying parameter values for which a positive equilibrium exists and is unique. The paper, [HKG08], subsequent to this one, gives ways of counting the number of anomalous signs in terms of graphs associated to a chemical reaction network.

In the second subsection, we continue with the general framework of §5, and move beyond mass-action kinetics to rate functions satisfying certain monotonicity conditions (see Definitions 6.3 and 6.4). The weaker condition, Definition 6.4, holds for many biochemical reactions and allows one to make sense of the signs which occur in the determinant expansion. Hence one can apply the methods in this paper.

The number of anomalous signs can be determined for the examples in this section using: (a) the graph-theoretic methods of Craciun and Feinberg [CF05, CF06] when there are no

anomalous signs and the kinetics are of mass-action type, and (b) symbolic computation of the determinant of the Jacobian using Mathematica when there are some anomalous signs or the kinetics are general (not necessarily mass-action). The reader will find Mathematica notebooks at

$$\text{http://www.math.ucsd.edu/}\sim\text{helton/chemjac.html}$$

for many of the examples in this section (including all of those that fall under (b)), as well as a demonstration notebook that readers may edit to run their own examples. This software works well when the number of species is small; for larger numbers, the determinant expansion has too many terms to be handled readily.

We conclude this preamble with some intuition underlying the case when there are anomalous signs. In general, we expect that for very small values of the parameters appearing in the reaction rate functions for a conservative reaction network (and, in the limit, for vanishing parameter values), the dynamics of the system augmented by inflows and outflows will be dominated by the inflow and outflow terms, and $\det(\partial r/\partial c)$ will not vanish; moreover, if the inflow and (linear) outflow terms dominate the dynamics, then the equilibrium will be unique. Examples 6.1 and 6.6 illustrate how this observation can be made rigorous and can be used together with Theorem 5.8 and the proof of Theorem 4.1 to conclude the existence and uniqueness of an equilibrium for a subset of the parameter space, even if the result does not hold for the entire parameter space.

6.1. **The determinant expansion, its signs and uses.**

Example 6.1. Consider the mass-action kinetics system given by the chemical reaction network (6.1), which is an irreversible version of the network shown in Table 1.1(i) of [CF05] (see Table 1(i) below):

$$\begin{aligned} A + B &\rightarrow P \\ B + C &\rightarrow Q \\ C &\rightarrow 2A \end{aligned}$$

(6.1)

If we add inflow and outflow reactions for all species, the associated dynamical system model for (6.1) is

$$\begin{aligned} \dot{c}_A &= k_{0\rightarrow A} - k_{A\rightarrow 0}c_A - k_{A+B\rightarrow P}c_A c_B + 2k_{C\rightarrow 2A}c_C \\ \dot{c}_B &= k_{0\rightarrow B} - k_{B\rightarrow 0}c_B - k_{A+B\rightarrow P}c_A c_B - k_{B+C\rightarrow Q}c_B c_C \\ \dot{c}_C &= k_{0\rightarrow C} - k_{C\rightarrow 0}c_C - k_{B+C\rightarrow Q}c_B c_C - k_{C\rightarrow 2A}c_C \\ \dot{c}_P &= k_{0\rightarrow P} - k_{P\rightarrow 0}c_P + k_{A+B\rightarrow P}c_A c_B \\ \dot{c}_Q &= k_{0\rightarrow Q} - k_{Q\rightarrow 0}c_Q + k_{B+C\rightarrow Q}c_B c_C. \end{aligned}$$

(6.2)

According to Remark 4.3 in [CF05] the dynamical system above does have multiple positive equilibria for some values of the reaction rate parameters.

If we assume that all outflow rate constants $k_{A\to 0}, ..., k_{Q\to 0}$ are equal to 1, then the determinant of the Jacobian of the reaction rate function is:

$$
\begin{aligned}
\det(\partial r/\partial c) \;=\; & -1 \;-\; k_{A+B\to P}c_A \;-\; k_{B+C\to Q}c_C \;-\; k_{B+C\to Q}c_B \\
& -\; k_{B+C\to Q}k_{A+B\to P}c_A c_B \;-\; k_{C\to 2A} \;-\; k_{C\to 2A}k_{A+B\to P}c_A \\
& -\; k_{C\to 2A}k_{B+C\to Q}c_C \;-\; k_{A+B\to P}c_B \;-\; k_{A+B\to P}k_{C\to 2A}c_B \\
& -\; k_{A+B\to P}k_{B+C\to Q}c_B^2 \;-\; k_{A+B\to P}k_{B+C\to Q}c_B c_C \\
& +\; k_{A+B\to P}k_{B+C\to Q}k_{C\to 2A}c_B c_C .
\end{aligned}
$$

(6.3)

Note that there is only one positive monomial in the expansion in (6.3). The concentrations in it are $c_B c_C$, but there is also a negative monomial with concentrations $c_B c_C$, and the two combine to give

$$
[- k_{A+B\to P}k_{B+C\to Q} + k_{A+B\to P}k_{B+C\to Q}k_{C\to 2A}]c_B c_C .
$$

Thus if $k_{C\to 2A} \leq 1$, then the positive monomial will be dominated by a negative monomial. Therefore, if $k_{C\to 2A} \leq 1$, then $\det(\partial r/\partial c) \neq 0$ for this network, everywhere on $\mathbb{R}_{>0}^5$.

Note that $(m_A, m_B, m_C, m_P, m_Q) = (1, 1, 2, 2, 3)$ is a conserved mass vector for the reaction network (6.1). It follows from Theorem 5.8 that (6.2), the dynamical system for (6.1), augmented with inflows and outflows (with outflow rate constants equal to one), has a unique positive equilibrium for all positive values of the reaction rates such that $k_{C\to 2A} \leq 1$. Note that this uniqueness conclusion would not follow directly from the theory of [CF05, CF06] nor from that in [BDB07], since these works pertain only when the determinant has the same sign for *all* rate constants and species concentrations.

The same method can be applied to conclude that the reversible version of the reaction network (6.1), augmented with inflows and outflows (with outflow rate constants set equal to one), also has a unique positive equilibrium for all positive values of the reaction rates such that $k_{C\to 2A} \leq 1$; moreover, even if the (positive) outflow rate constants $k_{A\to 0}, ..., k_{Q\to 0}$ are not necessarily equal to 1, the same conclusion holds if we know that $k_{C\to 2A} \leq k_{C\to 0}$. ∎

Example 6.2. Here we summarize several examples with mass-action kinetics (in the next subsection we consider some of these reactions with more general kinetics). Of the eight examples in [CF05, CF06], which are chemical reaction networks augmented with inflows and outflows (with outflow rate constants equal to one), two have the property that the coefficients of the terms in their Jacobian determinant expansion all have the same sign, and the other six have all but one sign the same. The first observation is from [CF05] and the second observation, emphasizing that there is *only one* anomalous sign, is new here. An analysis as in Example 6.1 can be applied here. Table 1 is a list of the examples showing how many "anomalous" signs each determinant expansion has.

A similar accounting holds for examples of reaction networks in [CTF06], see Table 1, page 8699. These reactions involve enzymes which [CTF06] treat with mass-action kinetic models. They find reaction networks 1,2,3,5,7,9 in this table do not have any anomalous

	Reaction network		Num. of "anomalous" signed terms in det expansion
(i)	$A + B \rightleftharpoons P$ $\quad B + C \rightleftharpoons Q$ $C \rightleftharpoons 2A$		1
(ii)	$A + B \rightleftharpoons P$ $\quad B + C \rightleftharpoons Q$ $C + D \rightleftharpoons R$ $\quad D \rightleftharpoons 2A$		0
(iii)	$A + B \rightleftharpoons P$ $\quad B + C \rightleftharpoons Q$ $C + D \rightleftharpoons R$ $\quad D + E \rightleftharpoons S$ $E \rightleftharpoons 2A$		1
(iv)	$A + B \rightleftharpoons P$ $\quad B + C \rightleftharpoons Q$ $C \rightleftharpoons A$		0
(v)	$A + B \rightleftharpoons F$ $\quad A + C \rightleftharpoons G$ $C + D \rightleftharpoons B$ $\quad C + E \rightleftharpoons D$		1
(vi)	$A + B \rightleftharpoons 2A$		1
(vii)	$2A + B \rightleftharpoons 3A$		1
(viii)	$A + 2B \rightleftharpoons 3A$		1

Table 1: Some examples of reaction networks and the signs of coefficients in their Jacobian determinant expansion when augmented with inflows and outflows (with outflow rate constants equal to one).

signs. Here we point out that the remaining reaction networks, 4, 6 and 8 have very few anomalous signs. The reaction network 4 is

$$S + E \rightleftharpoons ES \rightarrow E + P, \quad I + E \rightleftharpoons EI, \quad I + ES \rightleftharpoons ESI \rightleftharpoons EI + S$$

and has only 1 "anomalous" sign, and the reaction network 6 is

$$S1 + E \rightleftharpoons ES1, \quad S2 + E \rightleftharpoons ES2, \quad S2 + ES1 \rightleftharpoons ES1S2 \rightleftharpoons S1 + ES2, \quad ES1S2 \rightarrow E + P$$

and has only 2 "anomalous" signs. Reaction network 8 has 4 anomalous signs out of a total of over 3000 terms. Here all reactions are augmented by outflows with outflow rate constants set to one. (For the cases of no anomalous signs these outflow rate constants can be taken to be arbitrarily small without changing the answer[1].) The theory of Sections 4 and 5 applies, if there are (arbitrarily small) inflows and outflows, to yield that there is a unique positive equilibrium, for reaction networks 1,2,3,5,7,9. It also leaves open the possibility that one can apply the technique in Example 6.1 to get a unique positive equilibrium for certain rate constants in reaction networks 4, 6, 8. These applications of our theory require finding a conserved "mass" for the system without inflow and outflow, which is easy to do in all cases.

6.2. **General reaction rate functions.** In this subsection, we follow the setup in §5 and move beyond mass-action kinetics to a very general classes of rate functions.

---

[1]See [CF06iee] for how one can eliminate outflows for the enzymes.

Definition 6.3. We say that a reaction rate function $K_{y \to y'}$ is *consumptively increasing*, if for each species i belonging to the support of the vector y, the partial derivative of the reaction rate function, $\partial K_{y \to y'} / \partial c_i$, is strictly positive on the open orthant.

It is very common to assume that the reaction rate functions $K_{y \to y'}$ are *consumptively increasing*, since this simply means that the rate of a reaction increases whenever the concentration of a consumed species is increased unilaterally. In particular, the consumptively increasing property is true for many common chemical reaction rate laws, such as many Michaelis-Menten and Hill laws, as well as for all mass-action kinetics [KS98]. All examples in this section entail consumptively increasing reaction rates.

The consumptively increasing property can fail to hold for some classes of reactions including those involving inhibitory enzymes and for those in which a Michaelis-Menten rate depends on the products of the reaction [Rec81]. However, the next more lenient assumption handles these and many additional biochemical situations.

Definition 6.4. We say that a reaction rate function $K_{y \to y'}$ is *strictly monotone*, if for each species i on which the function $K_{y \to y'}$ actually depends, the partial derivative of the reaction rate function, $\partial K_{y \to y'} / \partial c_i$, has one strict sign on the open orthant.

More generally, the main technique used in this section is to compute the determinant expansion of the Jacobian as a sum of terms, each of which is a product of partial derivatives $\partial K_{y \to y'} / \partial c_i$ where i belongs to the support of y. For strictly monotone rate functions we can assign a $\pm$ to each term according to whether that term is everywhere positive or negative on the domain $\mathbb{R}^n_{>0}$. That is, strict monotonicity guarantees the technique of tracking anomalous signs in the determinant expansion applies.

Examples 2.2, 2.3 and 2.4 which involve inhibitory feedback can be written in the form (5.3) with strictly monotone rate functions. As we observed in §2 the determinant of the Jacobian, $(\frac{\partial r}{\partial c})$, is positive for all of these situations. However, at this point the terminology is in place so that we can mention the more refined property that each of these examples has no anomalous signs.

Example 6.5. Consider the chemical reaction network (6.4), which is the reversible network shown in (ii) in Table 1 but, unlike in [CF05], in this example we don't assume that the kinetics is mass-action.

$$
\begin{aligned}
A + B &\rightleftharpoons P \\
B + C &\rightleftharpoons Q \\
C + D &\rightleftharpoons R \\
D &\rightleftharpoons 2A
\end{aligned}
$$
(6.4)

We augment this reaction with inflows and outflows where the outflow matrix Λ_o is normalized to be the identity. We suppose that each of the reaction rate functions $K_{y \to y'}$ is

consumptively increasing as in Definition 6.3. We compute the expansion of $\det(\partial r/\partial c)$ in terms of the partial derivatives $\partial K_{y\rightarrow y'}(c)/\partial c_i$, for i belonging to the support of y. It is a sum of coefficients times monomials in these partial derivatives; the set of coefficients is shown in (6.5).

$$
\begin{aligned}
\{&-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,\\
&-1,-1,-1,-1,-1,-1,-1,-2,-2,-2,-2,-2,-2,-2,\\
&-2,-2,-2,-2,-2,-2,-3,-1,-1,-1,-1,-1,-1,-1,\\
&-3,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,\\
&-2,-2,-2,-2,-2,-1,-1,-1,-1,-1,-1,-1,-1,-1,\\
&-1,-1,-1,-1,-1,-1,-1,-1,-2,-2,-2,-2,-2,-2,\\
&-1,-1,-1,-1,-1,-1,-1,-1,-2,-2,-1,-1,-1,-1,\\
&-1,-1,-1,-1,-1,-1,-1,-2,-2,-2,-2,-2,-2,-2,\\
&-2,-1,-1,-1,-1,-1,-1,-1,-1,-2,-2,-2,-1,-1,\\
&-1,-1,-1,-1,-2,-2,-2,-1,-1,-1,-2,-1\}
\end{aligned}
$$

(6.5)

To summarize this list there are 138 terms in the expansion of $\det(\partial r/\partial c)$ and the set of coefficients of these terms contains exactly: 96 minus ones, 40 minus twos, 2 minus threes and no positive terms. This is more information than we need, since the point is that all these numbers are negative. This implies that $\det(\partial r/\partial c) \neq 0$ for this network, for all values of $c \in \mathbb{R}^7_{>0}$ and all "consumptively increasing" rate laws. Also, note that $(m_A, m_B, m_C, m_D, m_P, m_Q, m_R) = (1,1,1,2,2,2,3)$ is a conserved mass vector for the reaction network (6.4). Therefore, we can apply Theorem 5.8 to conclude that for the reaction network (6.4) with rate laws which are all consumptively increasing, and with inflows and outflows (with rate constants equal to one), has a unique positive equilibrium. ∎

Example 6.6. This example is exactly parallel to Example 6.5 except that now we consider the chemical reaction network which is the network shown in (v) in Table 1. Unlike in [CF05], in this example we don't assume that the kinetics is mass-action. We assume that the reaction rate functions are consumptively increasing. Also we augment with inflows and outflows where the outflow matrix Λ_o is normalized to be the identity matrix. We find that there are 167 terms in the expansion of $\det(\partial r/\partial c)$ involving the partial derivatives of $\partial K_{y\rightarrow y'}/\partial c_i$ for i belonging to the support of y and the set of coefficients of these terms contains exactly: 146 minus ones, 20 minus twos and one positive term. The positive term is

$$
K'_{B\rightarrow C+D}(c_B)\, K'_{D\rightarrow C+E}(c_D)\, K^{(0,1)}_{A+C\rightarrow G}(c_A, c_C)\, K^{(1,0)}_{A+B\rightarrow F}(c_A, c_B)
$$

Here $F^{(1,0)}$ (resp. $F^{(0,1)}$) denotes the partial derivative of F with respect to its first (respectively second) variable.

One can think of many conditions on the reaction rate functions that make the determinant have one sign on the open orthant. Typically, the more complicated they look, the more

lenient is the assumption. Here are some examples. We first note that the reaction network (v) of Table 1 is mass-conserving with mass vector $m = (1, 3, 1, 2, 1, 4, 2)$.

(1) We can collect all terms containing $K'_{D \to C+E}(c_D) \, K^{(0,1)}_{A+C \to G}(c_A, c_C) \, K^{(1,0)}_{A+B \to F}(c_A, c_B)$, this yields $(-1 + K'_{B \to C+D}(c_B)) \, K'_{D \to C+E}(c_D) \, K^{(0,1)}_{A+C \to G}(c_A, c_C) \, K^{(1,0)}_{A+B \to F}(c_A, c_B)$. Thus if we assume that $1 \geq K'_{B \to C+D}(c_B)$ for all $c_B > 0$, then the determinant is negative on the open orthant.

(2) Alternatively, we can collect all terms containing $K^{(0,1)}_{A+C \to G}(c_A, c_C) \, K^{(1,0)}_{A+B \to F}(c_A, c_B)$ and extract its coefficient which yields

$$(6.6) \quad -[1 - K'_{B \to C+D}(c_B)] \, K'_{D \to C+E}(c_D) - [1 + K^{(0,1)}_{C+D \to B}(c_C, c_D)] \, [1 + K^{(0,1)}_{C+D \to B}(c_C, c_D)].$$

Thus assuming this is negative for all positive c_B, c_C, c_D makes the determinant negative on the open orthant. We see that the second requirement is less stringent than the first.

(3) For any $M > m \cdot c_{in}$ the boundary of the set $\Omega_M := \{c \in \mathbb{R}^n_{>0} : m \cdot c < M\}$ contains no equilibria, as in the proof of Theorem 4.1. A yet weaker assumption than (1) and (2) is that the function (6.6) is negative on Ω_M for a particular $M > m \cdot c_{in}$.

In cases (1) and (2), we can apply Theorem 5.8 to conclude that for the reaction network in Table 1 (v) with rate laws which are all consumptively increasing, after augmentation with inflows and outflows (where Λ_o is the identity matrix), there exists a unique positive equilibrium. For case (3) we can apply the proof of Theorem 4.1 to conclude under the same conditions that there is a unique equilibrium in Ω_M . ∎

Remark 6.7. We emphasize that the homotopy-based methods described in this paper not only imply uniqueness, but also *existence* of a positive equilibrium for many dynamical systems derived from chemical reaction networks, while the methods in [CF05, CF06] only imply uniqueness of an equilibrium. Also, as we saw, our methods may be used for models containing very general chemical kinetics laws (not necessarily mass-action kinetics).

7. ACKNOWLEDGEMENTS

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