SIAM J. Appl. Math.
Vol. 69, No. 4, pp. 977–998

SENSITIVITY AND ROBUSTNESS IN CHEMICAL REACTION NETWORKS*

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Abstract. For a wide class of chemical reaction networks, including all those governed by detailed balanced mass-action kinetics, we examine the robustness of equilibrium species concentrations against fluctuations in the overall reactant supply. In particular, we present lower bounds on the individual species-concentration sensitivities that derive from reaction network structure alone, independent of kinetic parameters or even of the particular equilibrium state at which sensitivities are calculated. These bounds suggest that, in the class of reaction networks considered here, very high robustness (i.e., very low sensitivities) should be expected only when the various molecules are constructed from a large number of distinct elemental building blocks that appear in high multiplicity or that combine gregariously. This situation is often encountered in biology.

Key words. equilibrium points, chemical reaction networks, sensitivity, robustness, systems biology

AMS subject classifications. 80A30, 37C05, 37C25, 92C45

DOI. 10.1137/080719820

1. Introduction.

1.1. Motivation.

Our interest is in understanding the relationship between the general features of a chemical reaction network and the sensitivity of its equilibria to changes in the overall supply of reactants.

Although our concerns are with reaction networks in general, we are motivated by questions that arise naturally in biology. The cell is highly dynamic. Nevertheless, it is reasonable to suppose that in the cytoplasm certain biochemical modules (reaction networks) act on a timescale that is fast relative to other cellular processes (e.g., relative to the production of large proteins or relative to the transport of small molecules across membranes). Thus, the concentrations of species participating in such a "fast" reaction network might be regarded as instantaneously at or near equilibrium.

At the same time, the "fast" biochemical module under consideration might be perturbed within the cell by slower changes in the overall reactant supply, as fresh proteins are produced or as smaller molecules enter the cell from its exterior. In response, the "fast" equilibrium composition would shift accordingly. For some purposes it might be advantageous for certain species concentrations within the module to be insensitive (robust) against such disturbances, while for other species great sensitivity might be highly desirable. Thus, as has been suggested by Veitia [25], it becomes important to understand how reaction network structure affects the responsiveness of its equilibrated species concentrations to changes in the ambient cellular environment.

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*Received by the editors March 31, 2008; accepted for publication (in revised form) September 5, 2008; published electronically January 14, 2009.
http://www.siam.org/journals/siap/69-4/71982.html

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The present inquiry is not dissimilar in nature to other lines of research. One is biochemical systems theory pioneered by Savageau [23]. Another is metabolic control analysis developed from work of Higgins [15] by Kacser and Burns [19] and Heinrich and Rapoport [13, 14]. Here we provide general results along similar lines, but results that depend only on network structure. These results apply, within a large and relevant class of chemical reaction systems, to networks of arbitrarily large size and complexity.

For our purposes, it will suffice to presume that the reaction networks we study are governed by mass-action kinetics conforming to what chemists call *detailed balance* [26, 20, 10]. In fact, even these presumptions are overly strong, for we shall require only that the system considered have what Horn and Jackson called the *quasi-thermostatic* property [16]. (Detailed balancing in mass-action systems is sufficient but not necessary to ensure quasi-thermostatic behavior.)

The remainder of this introduction is organized as follows: In section 1.2 we define, in the context of an informal example, what we mean by *species sensitivities.* In section 1.3, we indicate the type of theory we seek by stating one principal result: lower bounds for species sensitivities *that derive from network structure alone.* In very rough terms, these bounds suggest that *strong robustness (i.e., very low sensitivities) of equilibrium concentrations against variations in reactant supply can be expected only when the species are built from highly gregarious or multiplicitous building blocks—that is, from certain* elements *that associate indiscriminately with each other or that appear in high multiplicity within the compound species.* In section 1.4 we discuss the organization of the remainder of the article.

1.2. Sensitivities. To illustrate more concretely the problems that are of interest, it will be useful to consider, informally, the relatively simple reaction network

$$2A \rightleftharpoons A_2, \qquad A_2 + B \rightleftharpoons A_2B, \qquad A_2B + C \rightleftharpoons A_2BC. \tag{1.1}$$

Note. Network (1.1) is inspired by a regulated recruitment [22] model for a gene transcription control mechanism: Species A corresponds to a protein monomer that can dimerize to form A_2, an active transcription factor. The dimer A_2 (but not the monomer A) can bind the DNA promoter B to form the compound A_2B. The compound A_2B can subsequently recruit the enzyme RNA polymerase, denoted C, to form the active compound A_2BC. It is the concentration of this last compound that determines the system's activity—the rate of transcription of the gene downstream from B.

Although we shall eventually consider a far wider class of reaction networks, our example here is an instance of what we shall later call a *constructive network*: Network (1.1) contains, explicitly, certain elemental species A, B, and C, from which all other species are ultimately constructed. (It needn't be the case in a constructive network that, as with our example, all reactions merely add a monomer of one of the elements. Thus, for example, a reaction such as $A_2B + A_2BC \rightleftharpoons A_4B_2C$ might also be included.) Certainly not all reaction networks are of this kind, but here and in section 1.3 consideration of constructive networks will facilitate both an introduction to the questions of interest and a description of some results.

As indicated earlier, we suppose that the individual reactions in network (1.1) are governed by mass-action kinetics, with fixed positive rate constants. (In the case of network (1.1), the detailed balance requirements are automatically satisfied, with no constraints imposed on rate constant values [10].) For the purposes of our discussion, we suppose also that the reactions take place in the context of a closed vessel, the contents of which are maintained at a fixed volume and temperature. Corresponding to a certain initial supply of various species, the mixture composition will evolve in time and ultimately approach equilibrium.

Note that the reactions conserve the elements A, B, and C, although these elements may reside latently within the compound species A_2, A_2B, and A_2BC. In fact, if $c_s(t)$ indicates the molar concentration of species s at time t, we expect that, for all time, the conservation conditions

$$\begin{aligned} &c_A(t) + 2c_{A_2}(t) + 2c_{A_2B}(t) + 2c_{A_2BC}(t) = T_A, \\ &c_{A_2B}(t) + c_B(t) + c_{A_2BC}(t) = T_B, \\ &c_C(t) + c_{A_2BC}(t) = T_C \end{aligned} \tag{1.2}$$

would be respected. In (1.2) we have denoted by T_A, T_B, and T_C the time-invariant *total* molar concentrations of the elements A, B, and C, regardless of whether they appear overtly or latently.

For specified positive values of the total element concentrations, say T_A^*, T_B^*, and T_C^*, the (polynomial) mass-action differential equations for network (1.1), formulated in the usual way [7], will give rise to precisely one equilibrium composition $c_A^*, c_B^*, c_C^*, c_{A_2}^*, c_{A_2B}^*, c_{A_2BC}^*$, consistent with that specification. On the other hand, if the vessel is temporarily opened and additional amounts of A, B, and C are added (either directly or by the addition of A_2, A_2B, and A_2BC), the resealed vessel will eventually come to a new and different equilibrium $c_A^{**}, c_B^{**}, c_C^{**}, c_{A_2}^{**}, c_{A_2B}^{**}, c_{A_2BC}^{**}$, which is the equilibrium consistent with the, now different, total concentrations T_A^{**}, T_B^{**}, and T_C^{**} of the elements. As we shall see later in the article, the equilibrium concentrations of the six species are given by smooth functions of T_A, T_B, and T_C.

Our interest is in the way that equilibrium concentrations of the various species are affected by small variations in the supply of the elements A, B, and C. By the *sensitivity matrix* for the system we mean the array whose entries are given by

$$\left(\frac{\partial \ln \bar{c}_s}{\partial \ln T_e}\right), \tag{1.3}$$

where $s \in \{A, B, C, A_2, A_2B, A_2BC\}$, $e \in \{A, B, C\}$, and $\bar{c}_s(\cdot,\cdot,\cdot)$ is the function that gives, for each specification of T_A, T_B, and T_C, the equilibrium concentration of species s. Values of the entries in the sensitivity matrix will depend, of course, on the equilibrium composition at which they are evaluated (in particular, on the values of T_A, T_B, and T_C at that equilibrium). The entry $\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_B}$, for example, indicates the magnitude, at a particular equilibrium, of the fractional change in the equilibrium concentration of A_2BC in response to a small fractional change in the total concentration of element B. (The use of logarithms to reflect *fractional* changes, which is common in biology [23], is especially compelling when the various species concentrations can be of very different magnitudes.)

By the *sensitivity of species s* at equilibrium composition c^*, denoted $\Lambda^s(c^*)$, we mean the largest of the absolute values of the entries in the sensitivity matrix row corresponding to species s. Thus, for example,

$$\Lambda^{A_2BC}(c^*) = \max\left\{\left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_A}\right|, \left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_B}\right|, \left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_C}\right|\right\}_{c^*}. \tag{1.4}$$

In this instance, $\Lambda^{A_2BC}(c^*)$ gives an indication, at equilibrium c^*, of the most pronounced of the variations in the equilibrium concentration of A_2BC, as the mixture is perturbed, respectively, by variations in the supply of A, B, and C.

Our interest is in providing means to calculate the sensitivity matrix at a particular equilibrium composition and, even more, in coming to a qualitative understanding of how reaction network character imposes *intrinsic* lower bounds on its species sensitivities, *bounds that derive from the reaction network alone, independent of rate constant values or even of the equilibrium composition at which they are calculated.* Bounds of this kind are described in the next section.

1.3. Network-imposed sensitivity bounds: Gregarious or multiplicitous elements are necessary for highly robust equilibria. Our goal in this section is to describe, informally and in the context of constructive networks, ways in which the network itself places a lower bound on species sensitivities. For a constructive network, we denote by $\mathscr{S}$ the set of species and by $\mathscr{E}$ the set of elements. In the case of network (1.1), $\mathscr{S} = \{A, B, C, A_2, A_2B, A_2BC\}$ and $\mathscr{E} = \{A, B, C\}$. For each element $e \in \mathscr{E}$ and each species $s \in \mathscr{S}$ we denote by M_e^s the *e-content* of species s—that is, the content of element e in species s. Thus, in our example, $M_A^A = 1$, $M_A^B = 0$, $M_A^C = 0$, $M_A^{A_2} = 2$, $M_A^{A_2B} = 2$, $M_A^{A_2BC} = 2$, and so on. (Later on, beginning in section 3, the notion of “element” will have meaning for reaction networks broadly, not just for those that are constructive. Even in this broader context it will make sense to speak of “the e-content of species s.” Results described in this section will then extend to reaction networks in general.)

For each pair of elements $e, e' \in \mathscr{E}$ we denote by $M_e^{max}(e')$ the maximal e-content that can be found as we search over all species that contain the element e' (i.e., over all species that have positive e'-content). In network (1.1), the species that contain A are A, A_2, A_2B, and A_2BC. Thus, for example,

$$M_B^{max}(A) = \max\left\{M_B^A, M_B^{A_2}, M_B^{A_2B}, M_B^{A_2BC}\right\} = \max\{0, 0, 1, 1\} = 1.$$

Evaluating $M_e^{max}(e')$ for all combinations of $e, e' \in \mathscr{E}$ in network (1.1), we obtain

$$\begin{aligned} &M_A^{max}(A) = 2, \qquad M_A^{max}(B) = 2, \qquad M_A^{max}(C) = 2,\\ &M_B^{max}(A) = 1, \qquad M_B^{max}(B) = 1, \qquad M_B^{max}(C) = 1,\\ &M_C^{max}(A) = 1, \qquad M_C^{max}(B) = 1, \qquad M_C^{max}(C) = 1. \end{aligned} \tag{1.5}$$

Note that in general $M_e^{max}(e')$ will be high when there is an e'-containing species in which e appears with high multiplicity.

The *degree* of element e is given by

$$\deg(e) = \sum_{e' \in \mathscr{E}} M_e^{max}(e'). \tag{1.6}$$

Thus, for network (1.1),

$$\deg(A) = 6, \qquad \deg(B) = 3, \qquad \deg(C) = 3. \tag{1.7}$$

Note that a high degree for a particular element will result if it is free to combine with many other element partners (*resulting in many nonzero terms in* (1.6)) *or if it is multiplicitous in at least one instance of those various liaisons* (*resulting in a high* *value for at least one of the terms in* (1.6)). Indeed, a very high degree for a particular element *requires* such gregarious or multiplicitous couplings (or a combination of both).

All this is relevant to the description of a result we shall present as Theorem 7.3, a theorem that provides a *network-imposed* lower bound on the species sensitivities: *At every equilibrium composition* c^* *and for each species* $s \in \mathscr{S}$,

$$\Lambda^s(c^*) \geq \max_{e \in \mathscr{E}} \left\{ \frac{M_e^s}{\deg(e)} \right\}. \tag{1.8}$$

It should be noted that the lower bound afforded by (1.8) *is an attribute of the network alone. It is independent of rate constant values and of the particular equilibrium compositions at which sensitivities are of interest.* For network (1.1) we have at every equilibrium composition c^*,

$$\begin{aligned} &\Lambda^A(c^*) \geq \frac{1}{6}, \qquad \Lambda^B(c^*) \geq \frac{1}{3}, \qquad \Lambda^C(c^*) \geq \frac{1}{3}, \\ &\Lambda^{A_2}(c^*) \geq \frac{1}{3}, \qquad \Lambda^{A_2B}(c^*) \geq \frac{1}{3}, \qquad \Lambda^{A_2BC}(c^*) \geq \frac{1}{3}. \end{aligned} \tag{1.9}$$

The bound given by (1.8) *tells us that for an equilibrium concentration of a particular species s to be very robust against fluctuations in the overall supply of elements (that is, if s is to have very low sensitivity), the elements of which s is composed should have very high degree (so they should be gregarious or multiplicitous).* It helps too if species s itself is composed of very *few* copies of the various elements—that is, if M_e^s is low for the various $e \in \mathscr{E}$, especially those elements of low degree. In the setting of constructive systems, if we consider the sensitivities of the elements themselves (that is, for $s = e$), we note that $M_{e'}^e = 1$ when $e' = e$ and 0 otherwise. Thus, for each $e \in \mathscr{E}$, (1.8) reduces to

$$\Lambda^e(c^*) \geq \frac{1}{\deg(e)}. \tag{1.10}$$

As indicated earlier, we have stated here just one principal result, carried by (1.8), and then only in the context of what we have called constructive networks. That restriction was solely for the purpose of this introduction. Our concerns extend to networks in general, and our interests are not limited to the establishment of bounds. We seek, for example, to provide means to determine how entries in the sensitivity matrix depend on the current equilibrium state.

1.4. Organization. In section 2 we provide an introduction to the rudimentary aspects of chemical reaction network theory, in particular to properties of *quasi-thermostatic* systems.

In section 3 we introduce the idea of *elemented reaction networks.* These constitute a broad generalization of what we call constructive reaction networks in this section: The fact is that the differential equations that derive from reaction networks typically reflect certain "conservation conditions" (integrals of motion), although what is conserved cannot always be clearly associated with total concentrations of species appearing overtly in the network (A, B, and C in our example). Nevertheless, one can generally associate with the network certain "elements," not necessarily overt species, whose total concentrations are conserved along solutions of the differential equations that the network induces. (An element might, for example, be identified with a moiety that manifests itself latently within the various species of the network while not itself appearing overtly.) For a given network there can be many such choices of (independently) conserved elements, and a particular application might favor one choice over another. By an elemented reaction network we mean a reaction network taken with one such choice of elements.

In section 4 we begin to examine properties of elemented quasi-thermostatic systems. This will set the stage for a discussion of sensitivities in section 5. Extended to elemented systems generally, our interest (as in this introductory section) will be in the variations of the equilibrium concentrations of the species in response to variations in the total concentrations of its elements. In section 6 we provide computational means to determine the sensitivity matrix—that is, to determine *through explicit formulas* how its entries depend on the particular equilibrium state at which they are calculated.

In section 7 we deduce lower bounds on the species sensitivities, bounds that are induced by the network alone, independent of rate constants and even of the equilibrium state at which the sensitivities are calculated. As in this introductory section, these bounds will be related to the *degrees* of the various elements. For elemented networks generally, the degree of an element is again influenced by the extent to which it combines in a gregarious and multiplicitous fashion. In section 8, we define formally what we mean by a constructive system, and we state a result that is particular to them. In section 9 we offer some brief concluding remarks.

2. Some ideas from chemical reaction network theory.

In this section we provide a brief review of rudimentary material from chemical reaction network theory [10, 16, 7, 5, 6, 8, 9, 2, 3, 4, 17, 11]. (An introduction for mathematicians, with more motivational discussion, can be found in [7].) Although chemical reaction network theory addresses a wide variety of dynamical issues, our focus here is exclusively on what Horn and Jackson [16] called *quasi-thermostatic* behavior.

2.1. Notation.

We denote the real numbers by $\mathbb{R}$, the strictly positive real numbers by $\mathbb{R}_+$, and the nonnegative real numbers by $\bar{\mathbb{R}}_+$. For an arbitrary finite set I (usually the set of species in a reaction network) we denote by $\mathbb{R}^I$ the real vector space of all formal sums $\sum_{i\in I} u_i i$ in which all u_i are real. By $\mathbb{R}^I_+$ (respectively, $\bar{\mathbb{R}}^I_+$) we mean the set of all $u \in \mathbb{R}^I$ for which all u_i are positive (respectively, nonnegative). By the *support* of an element $u \in \mathbb{R}^I$ we mean the subset of I defined by $\operatorname{supp}(u) = \{i \in I : u_i \neq 0\}$.

We use the symbol "$\circ$" to indicate componentwise multiplication. That is, for every u and v in $\mathbb{R}^I$, $u \circ v$ is the element of $\mathbb{R}^I$ such that $(u \circ v)_i = u_i v_i$. We denote by "$\cdot$" the (standard) scalar product in $\mathbb{R}^I : u \cdot v = \sum_{i\in I} u_i v_i$.

The function $\ln(\cdot) : \mathbb{R}^I_+ \to \mathbb{R}^I$ is the componentwise logarithm. That is, for each $u \in \mathbb{R}^I_+$, $(\ln u)_i = \ln(u_i)$. The function $\exp(\cdot) : \mathbb{R}^I \to \mathbb{R}^I_+$ is defined similarly: For each $u \in \mathbb{R}^I$, $(\exp u)_i = \exp(u_i)$.

If I is an arbitrary finite set, then by $\#(I)$ we mean the number of distinct elements in I.

2.2. Chemical reaction networks and their differential equations.

We begin with a definition of a chemical reaction network. By the *complexes* of a reaction network we shall mean the linear combinations of the species that appear before and after the reaction arrows. In network (1.1) there are six complexes: $2A, A_2, A_2 + B, A_2B, A_2B + C, A_2BC$. If $\mathscr{S}$ is the set of species of the network, we view the complexes of the network to be members of $\bar{\mathbb{R}}^{\mathscr{S}}_+$. We take a reaction network to be a specification of its species, a specification of its complexes, and, finally, a specification of a "reacts to" relation among the complexes.

DEFINITION 2.1. *A* chemical reaction network *consists of three finite sets:*

1. *A set* $\mathscr{S}$ *of distinct* species *of the network;*
2. *a set* $\mathscr{C} \subset \bar{\mathbb{R}}_+^{\mathscr{S}}$ *of* complexes *of the network;*
3. *a set* $\mathscr{R} \subset \mathscr{C} \times \mathscr{C}$ *of* reactions, *with the following properties:*
   (a) $(y, y) \notin \mathscr{R}$ *for any* $y \in \mathscr{C}$*;*
   (b) *for each* $y \in \mathscr{C}$ *there exists* $y' \in \mathscr{C}$ *such that* $(y, y') \in \mathscr{R}$ *or such that* $(y', y) \in \mathscr{R}$*.*

Following the usual notation in chemistry we write $y \to y'$ to indicate the reaction whereby complex y reacts to complex y'. With each reaction $y \to y'$ we associate the *reaction vector* $y' - y \in \mathbb{R}^{\mathscr{S}}$. In the context of our example, the reaction vector corresponding to $A_2B + C \to A_2BC$ is $A_2BC - A_2B - C$. For reasons that will become apparent, the span of a network's reaction vectors has special significance. This serves as motivation for the following definition.

DEFINITION 2.2. *The* stoichiometric subspace *of a reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *is the set* $\mathrm{S} \subset \mathbb{R}^{\mathscr{S}}$ *defined by*

$$\mathrm{S} = \operatorname{span}\left\{y' - y \in \mathbb{R}^{\mathscr{S}} : y \to y' \in \mathscr{R}\right\}.$$

When a particular network is under discussion, it will be understood that the symbol S is reserved to denote its stoichiometric subspace. We denote by $\mathrm{S}^{\perp} \subset \mathbb{R}^{\mathscr{S}}$ the orthogonal complement of S relative to the standard scalar product in $\mathbb{R}^{\mathscr{S}}$. We reserve the symbol $p\ (= \#(\mathscr{S}) - \dim \mathrm{S})$ for the dimension of $\mathrm{S}^{\perp}$.

Note that if $M \in \mathbb{R}_+^{\mathscr{S}}$ is the vector of molecular weights of the species in a network and if $y \to y'$ is a reaction, then $y \cdot M$ is the total mass of molecules on the reactant side of the reaction, while $y' \cdot M$ is the total mass of molecules on the product side. If the reaction respects conservation of mass, then we expect that $y \cdot M = y' \cdot M$, or equivalently, that $(y' - y) \cdot M = 0$. If all the reactions in the network respect conservation of mass, then M should be orthogonal to each of the reaction vectors, which is to say that M should be a member of $\mathrm{S}^{\perp}$. Following Horn and Jackson [16], we say that a reaction network is conservative if there exists for it at least one positive member of $\mathrm{S}^{\perp}$ that might play the role of a vector of molecular weights, relative to which all reactions are mass conserving.

DEFINITION 2.3. *A chemical reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *is* conservative *if* $\mathrm{S}^{\perp} \cap \mathbb{R}_+^{\mathscr{S}} \neq \emptyset$.

In order to write the differential equations governing the species concentrations in a reaction network, it is first necessary to specify, at each mixture composition, the rate at which the various reactions occur. For each reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ we generally denote by $c \in \bar{\mathbb{R}}_+^{\mathscr{S}}$ the vector of molar concentrations of the species. That is, for each $s \in \mathscr{S}$, c_s is the molar concentration of species s. By a *rate function* for the reaction $y \to y'$ we mean a function $\mathscr{K}_{y \to y'}(\cdot) : \bar{\mathbb{R}}_+^{\mathscr{S}} \to \bar{\mathbb{R}}_+$ that gives information about the dependence of occurrence rate on mixture composition. In particular, $\mathscr{K}_{y \to y'}(c)$ is the molar occurrence rate per unit volume at composition c. It is natural to expect that, at a particular mixture composition c, $\mathscr{K}_{y \to y'}(c)$ will be positive if and only if all species appearing with nonzero stoichiometric coefficients in the *reactant complex* y are present at composition c—that is, if and only if $\operatorname{supp}(y) \subset \operatorname{supp}(c)$. Very often, rate functions are taken to be of *mass-action* type—monomials in the species concentrations reflecting the probability of an encounter between molecules appearing in the reactant complex.

Definition 2.4. *A* kinetics $\mathscr{K}$ *for a reaction network* $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ *is an assignment to each reaction* $y \to y' \in \mathscr{R}$ *of a continuous rate function* $\mathscr{K}_{y\to y'}(\cdot) : \bar{\mathbb{R}}_+^{\mathscr{S}} \to \bar{\mathbb{R}}_+$ *such that* $\mathscr{K}_{y\to y'}(c) > 0$ *if and only if* $\operatorname{supp}(y) \subset \operatorname{supp}(c)$. *A* mass-action kinetics *for the network is a kinetics of the following kind: For each reaction* $y \to y' \in \mathscr{R}$ *there is a positive* rate constant $k_{y\to y'}$ *such that*

$$\mathscr{K}_{y\to y'}(c) \equiv k_{y\to y'} \prod_{s\in\mathscr{S}} (c_s)^{y_s}. \tag{2.1}$$

A kinetic system *is a reaction network endowed with a kinetics. A* mass-action system *is a reaction network endowed with a mass-action kinetics.*

Definition 2.5. *The* species-formation-rate function *for a kinetic system* $\{\mathscr{S}, \mathscr{C},\mathscr{R},\mathscr{K}\}$ *is the function* $r(\cdot) : \bar{\mathbb{R}}_+^{\mathscr{S}} \to \mathbb{R}^{\mathscr{S}}$ *defined by*

$$r(c) = \sum_{y\to y'\in\mathscr{R}} \mathscr{K}_{y\to y'}(c)(y' - y), \tag{2.2}$$

and the associated differential equation *is*

$$\dot{c} = r(c). \tag{2.3}$$

We say that $c \in \bar{\mathbb{R}}_+^{\mathscr{S}}$ *is an* equilibrium composition *if* $r(c) = 0$. *By the* positive equilibrium set *for the kinetic system we mean the set*

$$E = \left\{c \in \mathbb{R}_+^{\mathscr{S}} : r(c) = 0\right\}. \tag{2.4}$$

Example 2.6. Network (1.1), taken with mass action kinetics, gives rise to the species-formation-rate function (2.5), which is constructed according to (2.1) and (2.2):

$$\begin{aligned}
r_A(c) &= 2k_{A_2\to 2A}(c_{A_2}) - 2k_{2A\to A_2}(c_A)^2,\\
r_B(c) &= k_{A_2B\to A_2+B}(c_{A_2B}) - k_{A_2+B\to A_2B}(c_{A_2})(c_B),\\
r_C(c) &= k_{A_2BC\to A_2B+C}(c_{A_2BC}) - k_{A_2B+C\to A_2BC}(c_{A_2B})(c_C),\\
r_{A_2}(c) &= k_{2A\to A_2}(c_A)^2 - k_{A_2\to 2A}(c_{A_2})\\
&\qquad + k_{A_2B\to A_2+B}(c_{A_2B}) - k_{A_2+B\to A_2B}(c_{A_2})(c_B),\\
r_{A_2B}(c) &= k_{A_2+B\to A_2B}(c_{A_2})(c_B) - k_{A_2B\to A_2+B}(c_{A_2B})\\
&\qquad + k_{A_2BC\to A_2B+C}(c_{A_2BC}) - k_{A_2B+C\to A_2BC}(c_{A_2B})(c_C),\\
r_{A_2BC}(c) &= k_{A_2B+C\to A_2BC}(c_{A_2B})(c_C) - k_{A_2BC\to A_2B+C}(c_{A_2BC}).
\end{aligned} \tag{2.5}$$

Remark 2.7. Note that the species-formation-rate function takes values in the stoichiometric subspace S, which, for a conservative system, will be a *proper* linear subspace of $\mathbb{R}^{\mathscr{S}}$. Because in (2.3) the "velocity vector" $\dot{c}$ is restricted to point along S, it is not difficult to see that a composition trajectory that begins at composition c' can pass through composition c'' only if $c'' - c'$ lies in S.

With this in mind we say that two compositions are *stoichiometrically compatible* if $c'' - c'$ lies in S. Stoichiometric compatibility is an equivalence relation that serves to partition the set $\bar{\mathbb{R}}_+^{\mathscr{S}}$ of all compositions into *stoichiometric compatibility classes* (and partition the set $\mathbb{R}_+^{\mathscr{S}}$ of strictly positive compositions into *positive stoichiometric compatibility classes*). Stoichiometric compatibility classes are those subsets of parallels of S that lie in $\bar{\mathbb{R}}^{\mathscr{S}}_{+}$. In particular, the stoichiometric compatibility class containing a composition c^0 is the intersection of $\bar{\mathbb{R}}^{\mathscr{S}}_{+}$ with the parallel $c^0 + \mathrm{S} = \{c^0 + \sigma \in \mathbb{R}^{\mathscr{S}} : \sigma \in \mathrm{S}\}$.

Because two compositions along a trajectory governed by (2.3) must be stoichiometrically compatible, it is evident that each composition trajectory must lie entirely within a stoichiometric compatibility class.

2.3. Quasi-thermostatic kinetic systems. It is remarkable that a wide variety of mass-action systems fall into a class called by Horn and Jackson [16] *quasi-thermostatic*. Quasi-thermostatic systems are characterized by a simply described positive equilibrium set.

Definition 2.8. *A kinetic system* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, \mathscr{K}\}$ *is called* quasi-thermostatic *if it admits a positive equilibrium* $c^* \in \mathbb{R}^{\mathscr{S}}_{+}$ *and if its positive equilibrium set is given by*

$$E = \left\{ c \in \mathbb{R}^{\mathscr{S}}_{+} : \ln c - \ln c^* \in \mathrm{S}^{\perp} \right\}. \tag{2.6}$$

Remark 2.9. Given the stoichiometric subspace for a quasi-thermostatic system, specification of its entire positive equilibrium set amounts to the specification of a single member, c^*. In fact, if c^{**} is any other member, then the positive equilibrium set has the alternative characterization

$$E = \left\{ c \in \mathbb{R}^{\mathscr{S}}_{+} : \ln c - \ln c^{**} \in \mathrm{S}^{\perp} \right\}.$$

Remark 2.10. Not all mass-action systems are quasi thermostatic [16, 9, 2, 3, 4], but very important categories of them are. In particular, *detailed balanced* mass-action systems are quasi thermostatic [16]. In the terminology of chemistry, a kinetic system $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, \mathscr{K}\}$ is detailed balanced if all of its reactions are reversible (i.e., $y \to y'$ implies $y' \to y$) and if for each $y \to y' \in \mathscr{R}$ and at each equilibrium $c^* \in \mathbb{R}^{\mathscr{S}}_{+}$,

$$\mathscr{K}_{y \to y'}(c^*) = \mathscr{K}_{y' \to y}(c^*).$$

Not all reversible mass-action systems are detailed balanced: In some instances, reaction network structure alone will suffice to ensure detailed balancing, regardless of rate-constant values, but in other instances detailed balancing obtains only if the rate constants are suitably orchestrated [10]. It is a commonly held belief among chemists that closed mass-action systems occurring in nature *should* be detailed balanced (and, therefore, quasi thermostatic). This belief seems to go back to Wegscheider [26] and Lewis [20].

Horn and Jackson [16] showed that mass-action systems satisfying the far weaker condition of *complex balancing* are also quasi thermostatic. (Detailed balancing implies complex balancing, but the converse is not true.) In turn, Feinberg [5] and Horn [17] showed that for a large class of mass-action systems (those that derive from *weakly reversible deficiency zero* networks), complex balancing, and therefore quasi-thermostatic behavior, is a consequence of reaction network structure alone, independent of rate-constant values. Subsequently, Feinberg [7, 8, 11] showed that, regardless of rate-constant values, quasi-thermostatic behavior (but not necessarily complex balancing) is a property of mass-action systems that derive from a still wider class of networks, those satisfying the requirements of the *deficiency one theorem.*

The following proposition tells us that, for a quasi-thermostatic kinetic system, each positive stoichiometric compatibility class (Remark 2.7) contains exactly one member of the positive equilibrium set. A proof can be found in [16], and a somewhat different proof is given in Appendix B of [11].

Proposition 2.11. *Let* $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ *be a reaction network with stoichiometric subspace* S, *and let* c^*, c^0 *be elements of* $\mathbb{R}_+^{\mathscr{S}}$. *Then the sets*

$$c^0 + \mathrm{S} = \left\{c^0 + \sigma \in \mathbb{R}^{\mathscr{S}} : \sigma \in \mathrm{S}\right\}$$

and

$$E = \left\{c \in \mathbb{R}_+^{\mathscr{S}} : \ln c - \ln c^* \in \mathrm{S}^{\perp}\right\}$$

meet at exactly one point.

Remark 2.12. If, for a quasi-thermostatic system, we write the equilibrium set in the form

$$E = \left\{c \in \mathbb{R}_+^{\mathscr{S}} : c = c^* \circ \exp(\gamma),\ \gamma \in \mathrm{S}^{\perp}\right\}, \tag{2.7}$$

it becomes clear that E is a p-dimensional smooth manifold ($p = \dim \mathrm{S}^{\perp}$) parametrized globally by the map $\tilde{c}(\cdot) : \mathrm{S}^{\perp} \to \mathbb{R}^{\mathscr{S}}$, where $\tilde{c}(\gamma) \equiv c^* \circ \exp(\gamma)$. (After choosing a basis for $\mathrm{S}^{\perp}$, one can of course identify $\mathrm{S}^{\perp}$ with $\mathbb{R}^p$.) Clearly, $\tilde{c}(0) = c^*$. For future reference we note that the derivative of $\tilde{c}(\cdot)$, evaluated at 0, and denoted $\mathrm{d}\,\tilde{c}[0] : \mathrm{S}^{\perp} \to \mathbb{R}^{\mathscr{S}}$, acts on each $\gamma \in \mathrm{S}^{\perp}$ in the following way: $\mathrm{d}\,\tilde{c}[0]\gamma = c^* \circ \gamma$. From this it is apparent that the linear subspace tangent to E at c^* is

$$c^* \circ \mathrm{S}^{\perp} = \left\{c^* \circ \gamma : \gamma \in \mathrm{S}^{\perp}\right\}. \tag{2.8}$$

3. Elemented reaction networks.

By an *elemented reaction network* we mean a reaction network taken together with a distinguished choice of nonnegative basis for $\mathrm{S}^{\perp}$. As will be readily apparent, each basis vector gives rise to a linear combination of the species concentrations that is conserved along solutions of the differential equations associated with any kinetic system deriving from the network. Loosely speaking, then, each basis vector can be associated with an "indestructible element" whose total concentration is conserved by the reactions. In some instances, as in the case of network (1.1), these conserved elements (A, B, and C in our example) have natural interpretations and correspond to a natural choice of basis for $\mathrm{S}^{\perp}$. For a different network, the choice of elements might be more arbitrary and may, in fact, vary from one application to another.

As we shall see later, whatever the choice of elements, specification of their total (time-invariant) concentrations will serve to specify, bijectively, a particular stoichiometric compatibility class (Remark 2.7). For a quasi-thermostatic kinetic system, each positive stoichiometric compatibility class contains precisely one equilibrium (Proposition 2.11). In effect, then, the positive equilibrium set E can be parametrized by the total concentrations of the elements. As suggested in the introduction, our interest is in how the equilibria change in response to a change in the element concentrations, which, in fact, corresponds to a change in stoichiometric compatibility class.

Definition 3.1. *An* elemented reaction network *consists of the following:*

1. *A reaction network* $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$;
2. *a set* $\mathscr{E}$ *of* p ($= \dim \mathrm{S}^{\perp}$) *distinct members called the* elements *of the network;*
3. *a basis* $\mathscr{M} = \{M_e\}_{e\in\mathscr{E}} \subset \bar{\mathbb{R}}_+^{\mathscr{S}}$ *for* $\mathrm{S}^{\perp}$.

For each $e \in \mathscr{E}$ *and* $s \in \mathscr{S}$, *the component* M_e^s *is called the* e-content of species s.

Remark 3.2. Although we have not built such a requirement into the definition, we shall, in this article, suppose that all networks under consideration are conservative in the sense of Definition 2.3. (There are instances, not considered here, in which one might want to study the more general situation.) It is easy to see that, for a conservative network, it is always possible to choose a basis for $S^\perp$ that lies in $\bar{\mathbb{R}}_+^{\mathscr{S}}$. Moreover, it is not difficult to see that, for an elemented conservative network, there exists for each species s at least one element e such that the e-content of s is not zero.

Example 3.3. One elemented network derived from (1.1) results from the choice $\mathscr{E} = \{A, B, C\}$ and the basis for $S^\perp$ given by

$$\begin{aligned} M_A &= A + 2A_2 + 2A_2B + 2A_2BC, \\ M_B &= B + A_2B + A_2BC, \\ M_C &= C + A_2BC. \end{aligned} \tag{3.1}$$

In this case all of the elements are associated with certain species that appear explicitly in the network.

Example 3.4. It is instructive to consider a simple network of the general form

$$W + X \rightleftharpoons Y + Z, \tag{3.2}$$

for which $\dim S^\perp = 3$. The network might be "elemented" in different ways, depending on the context. If, for example, $W = AQ_2$, $Y = AQ$, and $Z = XQ$, then (3.2) represents a transfer of a molecular component Q from species AQ_2 to species X. In this case, it is natural to take $\mathscr{E} = \{A, Q, X\}$ and

$$\begin{aligned} M_A &= AQ_2 + AQ = W + Y, \\ M_Q &= 2AQ_2 + AQ + XQ = 2W + Y + Z, \\ M_X &= X + XQ = X + Z. \end{aligned}$$

Note that the element X is an overt species, whereas the elements A and Q are not.

If, on the other hand, $W = AB$, $X = CD$, $Y = AC$, and $Z = BD$, then (3.2) represents a component exchange. One natural choice of elements is $\mathscr{E} = \{A, B, C\}$ and the basis for $S^\perp$ given by

$$\begin{aligned} M_A &= AB + AC = W + Y, \\ M_B &= AB + BD = W + Z, \\ M_C &= CD + AC = X + Y. \end{aligned}$$

Still another choice is $\mathscr{E} = \{A, B, D\}$ and

$$\begin{aligned} M_A &= AB + AC = W + Y, \\ M_B &= AB + BD = W + Z, \\ M_D &= CD + BD = X + Z. \end{aligned}$$

With neither choice does any element appear explicitly as a species.

Recall that for the network discussed in the introduction we considered the *total concentrations* of the three elements. For each element e, the total concentration was calculated from the current mixture composition c by a sum of the following kind:

$$\sum_{s \in \mathscr{S}} M_e^s c_s,$$

where M_e^s is the e-content of species s. We now extend this idea using the following definition.

DEFINITION 3.5. *Let* $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{E},\mathscr{M}\}$ *be an elemented reaction network. The* element concentration functions $\bar{T}_e(\cdot):\mathbb{R}^{\mathscr{S}}\to\mathbb{R}$ *are defined for each* $e\in\mathscr{E}$ *by*

$$\bar{T}_e(x) := M_e\cdot x. \tag{3.3}$$

For use later, we define the function $\bar{T}(\cdot):\mathbb{R}^{\mathscr{S}}\to\mathbb{R}^{\mathscr{E}}$ by

$$\bar{T}(x) := \sum_{e\in\mathscr{E}}\bar{T}_e(x)e. \tag{3.4}$$

It is not difficult to see that the kernel of $\bar{T}(\cdot)$ is the stoichiometric subspace S.

Remark 3.6. Two compositions c' and c'' are associated with precisely the same element concentrations (i.e., $\bar{T}(c')=\bar{T}(c'')$) if and only if $c'-c''$ is a member of the stoichiometric subspace, which is to say that c' and c'' reside in the same stoichiometric compatibility class. In this way each specification of positive element concentrations (i.e., each member of $\bar{T}(\mathbb{R}_+^{\mathscr{S}})\subset\mathbb{R}_+^{\mathscr{E}}$) can be identified bijectively with a positive stoichiometric compatibility class.

DEFINITION 3.7. *An* elemented kinetic system *is an elemented reaction network endowed with kinetics.*

Remark 3.8. It is apparent from Remark 3.6 that the element concentrations remain invariant along solutions of the differential equations associated with an elemented kinetic system: Composition trajectories reside entirely within stoichiometric compatibility classes (Remark 2.7), and, within each stoichiometric compatibility class, all compositions give rise to the same element concentrations. The constancy of the element concentrations can, of course, be seen more directly: If $c(\cdot)$ is a solution of the differential equation for a particular elemented kinetic system, and if e is an element, then, at each t,

$$\frac{d}{dt}\bar{T}_e(c(t)) = M_e\cdot\frac{dc(t)}{dt} = M_e\cdot r(c(t)) = 0. \tag{3.5}$$

Here, of course, $M_e\in \mathrm{S}^\perp$ is the basis vector associated with element e, and $r(\cdot)$ is the species-formation-rate function for the kinetic system. The last equality in (3.5) follows from the fact that $r(\cdot)$ takes values in S.

4. Elemented quasi-thermostatic systems.

We asserted in Remark 3.6 that, for an elemented network, there is a bijective correspondence between positive stoichiometric compatibility classes and distinct positive specifications of element concentrations. On the other hand, for a quasi-thermostatic system, each positive stoichiometric compatibility class contains precisely one equilibrium (Proposition 2.11). Thus, for a quasi-thermostatic elemented system, there is a bijective correspondence between the positive equilibrium set E and $\bar{T}(\mathbb{R}_+^{\mathscr{S}})$, the set of all (realizable) positive specifications of the element concentrations. Our goal in this section is to show that this correspondence is, in fact, a diffeomorphism. Then we will be able to speak of the smooth dependence of equilibrium species concentrations on the element concentrations, and the way will be paved for discussion and analysis of sensitivities, which were described only informally in the introduction.

We consider, therefore, a quasi-thermostatic elemented kinetic system $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{K},\mathscr{E},\mathscr{M}\}$ with positive equilibrium set $E\in\mathbb{R}_+^{\mathscr{S}}$. We denote by $\bar{T}_E(\cdot):E\to\bar{T}(\mathbb{R}_+^{\mathscr{S}})$ the restriction of $\bar{T}(\cdot)$ to E, taken with the indicated choice of codomain. (It is the function $\bar{T}_E(\cdot)$ that we want to show is a diffeomorphism.) Note that $\bar{T}_E(\cdot)$ is a map from the p-dimensional smooth manifold E to the p-dimensional smooth manifold $\bar{T}(\mathbb{R}_+^{\mathscr{S}})$ (an open set in $\mathbb{R}^{\mathscr{E}}$ by the open mapping theorem [1]). Recall from Remark 2.12 that if c^* is a member of E, then at c^* the space tangent to E is

$$c^* \circ \mathrm{S}^{\perp} = \left\{c^* \circ \gamma : \gamma \in \mathrm{S}^{\perp}\right\}.$$

NOTATION 4.1. *Occasionally we will focus on a fixed but arbitrary positive equilibrium $c^* \in E$. It will be useful to have available a scalar product "$*$" in $\mathbb{R}^{\mathscr{S}}$ defined by*

$$x * y = x \cdot (c^* \circ y) \equiv \sum_{s \in \mathscr{S}} x_s c_s^* y_s.$$

LEMMA 4.2. *The derivative of $\bar{T}_E(\cdot)$ at an equilibrium $c^* \in E$ is the nonsingular map $\mathrm{d}\,\bar{T}_E\,[c^*]\,(\cdot) : c^* \circ \mathrm{S}^{\perp} \to \mathbb{R}^{\mathscr{E}}$ given for each $\gamma \in \mathrm{S}^{\perp}$ by*

$$\mathrm{d}\,\bar{T}_E\,[c^*]\,(c^* \circ \gamma) = \sum_{e \in \mathscr{E}} (M_e * \gamma) e. \tag{4.1}$$

Proof. Equation (4.1) derives from straightforward calculation. To see that the map $\mathrm{d}\,\bar{T}_E\,[c^*]\,(\cdot)$ is nonsingular, suppose that $\gamma^{\#} \in \mathrm{S}^{\perp}$ is such that $\mathrm{d}\,\bar{T}_E\,[c^*]\,(c^* \circ \gamma^{\#}) = 0$. This requires that $M_e * \gamma^{\#} = 0$ for each $e \in \mathscr{E}$. Thus, $\gamma^{\#} \in \mathrm{S}^{\perp}$ is orthogonal, with respect to the "$*$" scalar product, to each member of a basis for $\mathrm{S}^{\perp}$. Hence, $\gamma^{\#}$ and therefore $c^* \circ \gamma^{\#}$ are each the zero vector in $\mathbb{R}^{\mathscr{S}}$. ☐

PROPOSITION 4.3. *$\bar{T}_E(\cdot) : E \to \bar{T}(\mathbb{R}_+^{\mathscr{S}})$ is a diffeomorphism.*

Proof. To show that $\bar{T}_E(\cdot)$ is bijective, suppose that T^0 is some fixed but arbitrary member of $\bar{T}(\mathbb{R}_+^{\mathscr{S}})$. We will argue that there is precisely one member of E mapped to the point T^0 by $\bar{T}_E(\cdot)$. Because T^0 is a member of $\bar{T}(\mathbb{R}_+^{\mathscr{S}})$, there is a $c^0 \in \mathbb{R}_+^{\mathscr{S}}$ such that $\bar{T}(c^0) = T^0$. That is,

$$\bar{T}(c^0) = \sum_{e \in \mathscr{E}} (M_e \cdot c^0) e = T^0.$$

In fact, the full set of vectors in $\mathbb{R}^{\mathscr{S}}$ mapped by $\bar{T}(\cdot)$ to T^0 is $c^0 + \ker \bar{T}(\cdot) = c^0 + \mathrm{S}$. Our interest, then, is in the members of $c^0 + \mathrm{S}$ that are also members of E. If c^* is a member of E, then, for the quasi-thermostatic system under study, the equilibrium set is given by

$$E = \left\{c \in \mathbb{R}_+^{\mathscr{S}} : \ln c - \ln c^* \in \mathrm{S}^{\perp}\right\}.$$

From Proposition 2.11, however, E meets $c^0 + \mathrm{S}$ in *exactly* one point. Thus, $\bar{T}_E(\cdot)$ is bijective.

Because from Lemma 4.2 we have that $\mathrm{d}\,\bar{T}_E\,[c^*]\,(\cdot)$ is nonsingular for each $c^* \in E$, the inverse function theorem [12] ensures that, at each point in $\bar{T}(\mathbb{R}_+^{\mathscr{S}})$, there is a local inverse of the smooth function $\bar{T}_E(\cdot)$ that is also smooth. Since any such local inverse must be a restriction of the (unique) global inverse whose existence was established above, it follows that the inverse of $\bar{T}(\cdot)$ is smooth. ☐

5. Sensitivities.

Hereafter we consider an elemented quasi-thermostatic kinetic system $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, \mathscr{K}, \mathscr{E}, \mathscr{M}\}$, and we denote by c^* a fixed but arbitrary member of its positive equilibrium set E. We denote by $T^* \in \mathbb{R}^{\mathscr{E}}$ the value $\bar{T}(c^*)$. That is, T^* is the vector of element concentrations associated with the equilibrium composition c^*.

Proposition 4.3 tells us that the map $\bar{T}_E(\cdot) : E \to \bar{T}(\mathbb{R}^{\mathscr{S}}_+)$ gives a global coordinate system on E. That is, each equilibrium in E is characterized uniquely by a specification of the concentrations of the elements. We will choose to introduce a change in coordinates so that we can work instead with logarithms of the element concentrations. For this purpose we introduce the map $\ln \bar{T}_E(\cdot) : E \to \ln \bar{T}(\mathbb{R}^{\mathscr{S}}_+)$ defined in the obvious way: For each $c \in E$, $\ln \bar{T}_E(c) = \ln(\bar{T}_E(c))$. As a variant of (4.1), it is not difficult to see that $\mathrm{d} \ln \bar{T}_E[c^*](\cdot) : c^* \circ \mathrm{S}^\perp \to \mathbb{R}^{\mathscr{E}}$ is given by

$$\mathrm{d} \ln \bar{T}_E[c^*](c^* \circ \gamma) = \sum_{e \in \mathscr{E}} \frac{1}{T^*_e}(M_e \cdot c^* \circ \gamma) e = \sum_{e \in \mathscr{E}} \frac{1}{T^*_e}(M_e * \gamma) e \tag{5.1}$$

for all $\gamma \in \mathrm{S}^\perp$.

Because $\ln \bar{T}_E(\cdot)$ amounts to the composition of the diffeomorphisms $\bar{T}_E(\cdot)$ and $\ln(\cdot) : \bar{T}(\mathbb{R}^{\mathscr{S}}_+) \to \ln \bar{T}(\mathbb{R}^{\mathscr{S}}_+)$, it too is a diffeomorphism. Hereafter, we denote by $\bar{c}(\cdot) : \ln \bar{T}(\mathbb{R}^{\mathscr{S}}_+) \to E$ the inverse of $\ln \bar{T}_E(\cdot)$. Thus, if $\ln T$ is a member of $\ln \bar{T}(\mathbb{R}^{\mathscr{S}}_+)$ it makes sense to speak of "the equilibrium composition $\bar{c}(\ln T)$." Note that $\ln \bar{T}_E(c^*) = \ln \bar{T}(c^*) = \ln T^*$ and $\bar{c}(\ln T^*) = c^*$. Note too that $\mathrm{d}\, \bar{c}[\ln T^*](\cdot) : \mathbb{R}^{\mathscr{E}} \to c^* \circ \mathrm{S}^\perp$ is just the inverse of $\mathrm{d} \ln \bar{T}_E[c^*]$, which is given by (5.1). Thus we have

$$\mathrm{d}\, \bar{c}[\ln T^*] \left(\sum_{e \in \mathscr{E}} \frac{1}{T^*_e}(M_e * \gamma) e \right) = c^* \circ \gamma \tag{5.2}$$

for all $\gamma \in \mathrm{S}^\perp$.

We will also be interested in the map $\ln \bar{c}(\cdot) : \ln \bar{T}(\mathbb{R}^{\mathscr{S}}_+) \to \ln E$ $(= \ln c^* + \mathrm{S}^\perp)$ defined in the obvious way. (Note that the tangent spaces of the manifolds $\ln \bar{T}(\mathbb{R}^{\mathscr{S}}_+)$ and $\ln c^* + \mathrm{S}^\perp$ are, respectively, everywhere $\mathbb{R}^{\mathscr{E}}$ and $\mathrm{S}^\perp$.) Because $\mathrm{d} \ln \bar{c}[\ln T^*](\cdot) : \mathbb{R}^{\mathscr{E}} \to \mathrm{S}^\perp$ is given by $\frac{1}{c^*} \circ \mathrm{d}\, \bar{c}[\ln T^*]$, it follows from (5.2) that

$$\mathrm{d} \ln \bar{c}[\ln T^*] \left(\sum_{e \in \mathscr{E}} \frac{1}{T^*_e}(M_e * \gamma) e \right) = \gamma \tag{5.3}$$

for all $\gamma \in \mathrm{S}^\perp$.

We are now in position to make precise definitions of the sensitivities discussed informally in the introduction.

Definition 5.1. *By the* sensitivity vector for element e at equilibrium composition c^* *we mean the vector in* $c^* \circ \mathrm{S}^\perp \subset \mathbb{R}^{\mathscr{S}}$ *given by*

$$\frac{\partial \ln \bar{c}}{\partial \ln T_e}(c^*) := \mathrm{d} \ln \bar{c}\, [\ln T^*]\, e. \tag{5.4}$$

By the sensitivity matrix at c^* *we mean the array whose elements are given by*

$$\left(\frac{\partial \ln \bar{c}_s}{\partial \ln T_e}(c^*) \right)_{s \in \mathscr{S},\, e \in \mathscr{E}}. \tag{5.5}$$

6. Means to calculate the sensitivity matrix.

Here we provide means to calculate the sensitivity matrix at the equilibrium $c^* \in E$.[1] For the specified equilibrium c^*, we denote by $\{M^e\}_{e \in \mathscr{E}} \subset \mathrm{S}^\perp$ the basis for $\mathrm{S}^\perp$ that is reciprocal, relative

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[1] We note that these calculations have points of contact with issues raised some years ago in two Ph.D. theses, by K. Israel [18] and J. Nailor [21], supervised by Michael Reed. Stated in very rough and somewhat inaccurate terms, their interest was in signs of entries in what we call the sensitivity matrix—signs that might be determined by experiment. The motivation was that such experimentally determined signs might provide clues about the underlying reaction network when the network is unknown.

to the "$*$" scalar product in $\mathbb{R}^{\mathscr{S}}$, to the basis $\mathscr{M} = \{M_e\}_{e\in\mathscr{E}}$. (Recall Notation 4.1.) That is, $M^{e'} * M_e = 1$ if $e' = e$ and is 0 otherwise. Because the "$*$" scalar product depends upon c^*, so will the reciprocal basis it induces. It is not difficult to determine such reciprocal bases computationally within the context of readily available symbolic mathematics programs.

Theorem 6.1. *Let* $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{K},\mathscr{E},\mathscr{M}\}$ *be an elemented quasi-thermostatic kinetic system, let* $c^* \in E$ *be a positive equilibrium (corresponding to an element specification* $T^* \in \mathbb{R}^{\mathscr{E}}$*), and let* $\{M^e\}_{e\in\mathscr{E}}$ *be the basis for* $\mathrm{S}^\perp$ *reciprocal to* $\mathscr{M}$ *relative to the "$*$" scalar product in* $\mathbb{R}^{\mathscr{S}}$. *Then for each* $e \in \mathscr{E}$, *the sensitivity vector is given by*

$$\frac{\partial \ln \bar{c}}{\partial \ln T_e}(c^*) = T_e^* M^e. \tag{6.1}$$

Entries of the sensitivity matrix are given by

$$\frac{\partial \ln \bar{c}_s}{\partial \ln T_e}(c^*) = T_e^* M^{es} \tag{6.2}$$

for each $s \in \mathscr{S}$ *and* $e \in \mathscr{E}$.

Proof. Equation (6.1) results from a simple substitution in (5.3): To obtain the sensitivity vector for a particular element $e' \in \mathscr{E}$, set $\gamma = T_{e'}^* M^{e'}$. Equation (6.2) is just the component form of (6.1). ☐

Example 6.2. Consider network (6.3), which can serve to model a process whereby an active biomolecule A_2B_2 is assembled from elements A and B:

$$A + B \rightleftharpoons AB, \qquad 2AB \rightleftharpoons A_2B_2. \tag{6.3}$$

One elemented network derived from (6.3) is afforded by the choices $\mathscr{E} = \{A, B\}$ and $\mathscr{M} = \{M_A, M_B\}$, where

$$\begin{aligned} M_A &= A + AB + 2A_2B_2, \\ M_B &= B + AB + 2A_2B_2. \end{aligned} \tag{6.4}$$

If the kinetics for the network is mass action, then, for any choice of rate constants, detailed balance will obtain [10] and the system will be quasi thermostatic. Thus, Theorem 6.1 can be used to calculate all entries in the sensitivity matrix, evaluated at an arbitrary equilibrium c^*. In particular, the entries corresponding to the "active" species A_2B_2 are

$$\begin{aligned} \frac{\partial \ln \bar{c}_{A_2B_2}}{\partial \ln T_A}(c^*) &= \frac{2(c_A^* + c_{AB}^* + 2c_{A_2B_2}^*)c_B^*}{c_A^*c_B^* + (c_A^* + c_B^*)(c_{AB}^* + 4c_{A_2B_2}^*)}, \\ \frac{\partial \ln \bar{c}_{A_2B_2}}{\partial \ln T_B}(c^*) &= \frac{2(c_B^* + c_{AB}^* + 2c_{A_2B_2}^*)c_A^*}{c_A^*c_B^* + (c_A^* + c_B^*)(c_{AB}^* + 4c_{A_2B_2}^*)}. \end{aligned} \tag{6.5}$$

7. Degree, connectivity, and network-imposed sensitivity bounds.

As before, we consider an elemented quasi-thermostatic kinetic system $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{K},\mathscr{E},\mathscr{M}\}$, and we let c^* be a positive equilibrium, corresponding to an element concentration specification $T^* \in \mathbb{R}^{\mathscr{E}}$. Motivated by considerations discussed in the introduction, we have interest in understanding the extent to which the equilibrium concentration of a particular species $s \in \mathscr{S}$ might be robust against variations in the supply of elements. Our interest, then, is in the "worst case"—that is, in the most sensitive response in the concentration of s, taken against variation in each of the element concentrations.

DEFINITION 7.1. *The* sensitivity of species $s \in \mathscr{S}$ at c^*, *denoted* $\Lambda^s(c^*)$, *is defined by*

$$\Lambda^s(c^*) = \max_{e \in \mathscr{E}} \left\{ \left| \frac{\partial \ln \bar{c}_s}{\partial \ln T_e}(c^*) \right| \right\}. \tag{7.1}$$

By the system sensitivity at c^*, *denoted* $\Lambda(c^*)$, *we mean the least of the species sensitivities. That is,*

$$\Lambda(c^*) = \min_{s \in \mathscr{S}} \Lambda^s(c^*). \tag{7.2}$$

Remark 7.2. In general, the species sensitivities and the system sensitivity will depend on the choice of basis $\mathscr{M}$ for $\mathrm{S}^\perp$.

If the system sensitivity is high at c^*, then *all* species sensitivities at c^* are high, so it makes sense to say that the system itself is highly sensitive to element concentration variations.

Our goal in this section is to provide lower bounds on the species sensitivities and on the system sensitivity, bounds that derive *from network properties alone*, independent of kinetic parameters and even of the equilibrium at which the sensitivities are calculated. By way of preparation, we posit formally some ideas that were discussed informally in the introduction. All the following are attributes of the elemented *network* underlying the kinetic system we have been studying:

For each $e \in \mathscr{E}$ we let

$$M_e^{max} = \max_{s \in \mathscr{S}} \{M_e^s\}. \tag{7.3}$$

That is, M_e^{max} is the largest number of copies of element e that can be found in any species. It is a measure of the extent to which element e combines in a multiplicitous way.

For each $e, e' \in \mathscr{E}$ we let

$$M_e^{max}(e') = \max_{s \in \operatorname{supp} M_{e'}} \{M_e^s\}. \tag{7.4}$$

That is, $M_e^{max}(e')$ is the largest number of copies of element e that can be found in any species *that also contains* e'. Clearly we have

$$M_e^{max}(e') \leq M_e^{max} \tag{7.5}$$

for each $e, e' \in \mathscr{E}$.

By the *degree* of element e we mean

$$\deg(e) = \sum_{e' \in \mathscr{E}} M_e^{max}(e'). \tag{7.6}$$

As we indicated in the introduction, an element will have high degree if it combines gregariously with many different elements or if it combines in high multiplicity with at least one of the elements.

Theorem 7.3. *Let $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{K},\mathscr{E},\mathscr{M}\}$ be an elemented quasi-thermostatic kinetic system with positive equilibrium set E. For each $c^* \in E$ and each $s \in \mathscr{S}$,*

$$\Lambda^s(c^*) \geq \max_{e\in\mathscr{E}} \left\{ \frac{M_e^s}{\deg(e)} \right\}. \tag{7.7}$$

Moreover,

$$\Lambda(c^*) \geq \min_{s\in\mathscr{S}} \max_{e\in\mathscr{E}} \left\{ \frac{M_e^s}{\deg(e)} \right\}. \tag{7.8}$$

Proof. We begin with the equation

$$M_e = \mathrm{d}\ln\bar{c}\,[\ln T^*] \left(\sum_{e'\in\mathscr{E}} \frac{1}{T^*_{e'}} (M_{e'} * M_e) e' \right), \tag{7.9}$$

which is an immediate consequence of (5.3). Written in terms of components, (7.9) gives for each $e \in \mathscr{E}$ and $s \in \mathscr{S}$,

$$M_e^s = \sum_{e'\in\mathscr{E}} \frac{\partial \ln \bar{c}_s}{\partial \ln T_{e'}}(c^*) \frac{1}{T^*_{e'}} (M_{e'} * M_e). \tag{7.10}$$

From this we can write the inequality

$$M_e^s \leq \sum_{e'\in\mathscr{E}} \left| \frac{\partial \ln \bar{c}_s}{\partial \ln T_{e'}}(c^*) \right| \frac{1}{T^*_{e'}} (M_{e'} * M_e). \tag{7.11}$$

Note that for each $e', e \in \mathscr{E}$ we have

$$\begin{aligned} M_{e'} * M_e &= \sum_{s\in\mathscr{S}} M^s_{e'} c^*_s M^s_e = \sum_{s\in \operatorname{supp} M_{e'}} M^s_{e'} c^*_s M^s_e \\ &\leq M_e^{max}(e') \sum_{s\in \operatorname{supp} M_{e'}} M^s_{e'} c^*_s = M_e^{max}(e') T^*_{e'}. \end{aligned} \tag{7.12}$$

Thus, from inequalities (7.11) and (7.12) and equations (7.1) and (7.6) we obtain for every $e \in \mathscr{E}$ and $s \in \mathscr{S}$ the inequality

$$M_e^s \leq \Lambda^s(c^*) \sum_{e'\in\mathscr{E}} M_e^{max}(e') = \Lambda^s(c^*) \deg(e), \tag{7.13}$$

or equivalently,

$$\Lambda^s(c^*) \geq \frac{M_e^s}{\deg(e)}. \tag{7.14}$$

Since (7.14) holds for every element $e \in \mathscr{E}$, we in fact have, for each $s \in \mathscr{S}$, the bound given in (7.7). The bound (7.8) is then just a consequence of the definition of system sensitivity. ▯

Example 7.4. In the case of the elemented kinetic system of Example 6.2, we have from (6.4), (7.4), and (7.6) that

$$\deg(A) = 4, \qquad \deg(B) = 4. \tag{7.15}$$

From (6.4), (7.7), and (7.15) we obtain

$$\begin{aligned} &\Lambda^{A}(c^*) \geq \frac{1}{4}, \qquad &&\Lambda^{B}(c^*) \geq \frac{1}{4}, \\ &\Lambda^{AB}(c^*) \geq \frac{1}{4}, \qquad &&\Lambda^{A_2B_2}(c^*) \geq \frac{1}{2}. \end{aligned} \tag{7.16}$$

Note that the lower bound on the "active" species A_2B_2 is sharp: If, for example, $c^* = \epsilon A + \epsilon B + \epsilon AB + A_2B_2$, then, from (6.5) and (7.1), we have that $\Lambda^{A_2B_2}(c^*) = \frac{1}{2}(1+\epsilon) + o(\epsilon^2)$. Thus, as ϵ tends to zero, $\Lambda^{A_2B_2}$ tends to the lower bound $\frac{1}{2}$ in (7.16). We note that for any choice of c^* there is an assignment of rate constants for network (6.2) consistent with the existence of the equilibrium c^* and at which detailed balance obtains. Thus, there are quasi-thermostatic systems for which, at certain equilibria, the sensitivity of A_2B_2 approaches the stipulated lower bound arbitrarily closely.

Remark 7.5. In a conservative elemented network, for each species $s \in \mathscr{S}$ there exists an element $e \in \mathscr{E}$ such that the e-content of s is positive (Remark 3.2). Thus, inequality (7.7) implies that a species (or system) sensitivity of zero is impossible in conservative quasi-thermostatic systems. It should be noted, however, that kinetic systems exist that *do* allow for zero sensitivity. One such system, proposed in the context of bacterial signaling, is treated in [24]. In this system the quasi-thermostatic condition is *not* satisfied.

Remark 7.6. Because the sensitivities themselves will depend on the choice of elements, so will the bounds given by Theorem 7.3. Consider, for example, the very simple network

$$2A + B \rightleftharpoons A_2B. \tag{7.17}$$

This network can be elemented in various ways: One choice is given by

$$\begin{aligned} M_A &= A + 2A_2B, \\ M_B &= B + A_2B; \end{aligned} \tag{7.18}$$

another equally valid choice is given by

$$\begin{aligned} M_Q &= A + \epsilon B + (2+\epsilon)A_2B, \\ M_R &= A + 2\epsilon B + (2+2\epsilon)A_2B, \end{aligned} \tag{7.19}$$

where ϵ is a positive number much smaller than 1. From Theorem 7.3 we have that, for choice (7.18), the species sensitivities are bounded from below according to the inequalities $\Lambda^{A}(c^*) \geq \frac{1}{4}$, $\Lambda^{B}(c^*) \geq \frac{1}{2}$, and $\Lambda^{A_2B}(c^*) \geq \frac{1}{2}$. For the system sensitivity we have that $\Lambda(c^*) \geq \frac{1}{4}$. On the other hand, for choice (7.19) the same theorem gives the bounds $\Lambda^{A}(c^*) \geq \frac{1}{4} + o(\epsilon)$, $\Lambda^{B}(c^*) \geq o(\epsilon)$, $\Lambda^{A_2B}(c^*) \geq \frac{1}{2}$, and $\Lambda(c^*) \geq o(\epsilon)$. Thus, different choices of basis for $\mathrm{S}^{\perp}$ can lead to markedly different lower bounds. Note that while choice (7.18) carries a natural interpretation for the elements A and B as building blocks that appear either overtly as species or latently in the compound A_2B, choice (7.19) offers no immediate physical interpretation for the elements Q and R.

Note also, that in the general case, if each vector in the basis for $\mathrm{S}^{\perp}$ is multiplied by a (potentially different) scalar, then the lower bounds corresponding to the new basis remain unchanged.

The bounds given in Theorem 7.3 give rise to different (weaker) bounds that are stated not in terms of the *degrees* of the elements, but, rather, in terms of what we call the element *connectivities.* For each $e \in \mathscr{E}$ we denote by $\Gamma(e)$ the set of elements that appear together with e in at least one species. More precisely,

$$\Gamma(e) = \{e' \in \mathscr{E} : \operatorname{supp} M_{e'} \cap \operatorname{supp} M_e \neq \emptyset\}\,. \tag{7.20}$$

By the *connectivity* of e we mean the number of such elements:

$$\operatorname{conn}(e) := \#(\Gamma(e)). \tag{7.21}$$

From (7.5) and (7.6) it is not difficult to see that

$$\deg(e) \leq \operatorname{conn}(e) M_e^{max}. \tag{7.22}$$

Thus from Theorem 7.3 we have the following.

Corollary 7.7. *Let $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{K},\mathscr{E},\mathscr{M}\}$ be an elemented quasi-thermostatic system with positive equilibrium set E. For each $c^* \in E$ and each $s \in \mathscr{S}$,*

$$\Lambda^s(c^*) \geq \max_{e \in \mathscr{E}} \left\{ \frac{M_e^s}{\operatorname{conn}(e) M_e^{max}} \right\} \tag{7.23}$$

and

$$\Lambda(c^*) \geq \min_{s \in \mathscr{S}} \max_{e \in \mathscr{E}} \left\{ \frac{M_e^s}{\operatorname{conn}(e) M_e^{max}} \right\}. \tag{7.24}$$

8. Constructive reaction networks.

By a *constructive reaction network* we mean an elemented network with special properties: Its elements can be identified with certain building blocks that appear explicitly as species of the network and from which all other species are constructed by means of the network's reactions. Example 3.3, which is based on network (1.1) in the introduction, is an example of a constructive reaction network.

Definition 8.1. *A* constructive reaction network *is an elemented reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{E},\mathscr{M}\}$ such that $\mathscr{E} \subset \mathscr{S}$ and such that, for each $e \in \mathscr{E}$,*

$$M_e = e + \sum_{q \in \mathscr{Q}} M_e^q q, \tag{8.1}$$

where $\mathscr{Q} = \mathscr{S} \setminus \mathscr{E}$, and where the M_e^q take nonnegative integer values. The members of $\mathscr{Q}$ are called the compounds *of the network.*

For constructive networks the following proposition indicates the sense in which the compounds are built from the elements in terms of reactions appearing in the network.

Proposition 8.2. *Let $\{\mathscr{S},\mathscr{C},\mathscr{R},\mathscr{E},\mathscr{M}\}$ be a constructive reaction network. Then the set*

$$\left\{ q - \sum_{e \in \mathscr{E}} M_e^q e : q \in \mathscr{Q} \right\} \tag{8.2}$$

is a basis for the stoichiometric subspace S.

Proof. Clearly, the vectors of (8.2) are linearly independent and their number is equal to dim S. To see that they are in fact members of S, it is enough to show that each of the vectors in (8.2) is orthogonal to all the vectors of (8.1). Toward this end, note that for each $q' \in \mathscr{Q}$ and each $e' \in \mathscr{E}$,

$$\left(q' - \sum_{e \in \mathscr{E}} M_e^{q'} e\right) \cdot \left(e' + \sum_{q \in \mathscr{Q}} M_{e'}^{q} q\right) = M_{e'}^{q'} - M_{e'}^{q'} = 0. \qquad \square$$

Remark 8.3. The vectors of (8.2) can be viewed as "reaction vectors" that derive from the "reactions"

$$\left\{ \sum_{e \in \mathscr{E}} M_e^q e \to q : q \in \mathscr{Q} \right\}. \tag{8.3}$$

Each of these "reactions" can be regarded as representing the production of a particular compound *solely* from the elements. Of course, these "construction" reactions need not all be "true" reactions of the original network, but, at least in terms of stoichiometry, each of the "true" reactions can be regarded as a linear combination of the "construction" reactions. Similarly, each member of (8.3) can be viewed as an overall reaction—one for each compound—that derives from a linear combination of the "true" reactions. Hence, the "true" reactions can be viewed as machinery for constructing the compounds from the elements.

Remark 8.4. Proposition 8.2 provides a way to distinguish a reaction network that might be constructive (for a suitable choice of elements) from one that cannot be constructive. For example, network (3.2) has a one-dimensional stoichiometric subspace spanned by $Y + Z - X - W$. Thus, there is no partition of the species set into elements and compounds that is consistent with a basis for the stoichiometric subspace of the form (8.2).

We note that for a *constructive* kinetic system the elements themselves are species, and so it makes sense to speak of their sensitivities. Recall that for a constructive system we have, for each pair $e, e' \in \mathscr{E}$, that $M_e^{e'}$ is 0 if $e' \neq e$ and is 1 otherwise. This observation gives rise to the following corollary to Theorem 7.3.

COROLLARY 8.5. *Let* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, \mathscr{K}, \mathscr{E}, \mathscr{M}\}$ *be a constructive quasi-thermostatic system with positive equilibrium set* E. *For each* $c^* \in E$ *and each* $e \in \mathscr{E}$,

$$\Lambda^e(c^*) \geq \frac{1}{\deg(e)} \geq \frac{1}{\mathrm{conn}(e) M_e^{max}}. \tag{8.4}$$

Moreover,

$$\Lambda(c^*) \geq \min_{e \in \mathscr{E}} \left\{ \frac{1}{\deg(e)} \right\} \geq \min_{e \in \mathscr{E}} \left\{ \frac{1}{\mathrm{conn}(e) M_e^{max}} \right\}. \tag{8.5}$$

Remark 8.6. For any elemented network, it is apparent that, for each element $e \in \mathscr{E}$, $\mathrm{conn}(e) \leq \#(\mathscr{E})$. If we let

$$M^{max} := \max_{e \in \mathscr{E}} \{ M_e^{max} \}, \tag{8.6}$$

then we have

$$\deg(e) \leq \mathrm{conn}(e) M_e^{max} \leq \#(\mathscr{E}) M^{max}. \tag{8.7}$$

For a constructive kinetic system in particular we get a crude bound on the system sensitivity:

$$\Lambda(c^*) \geq \frac{1}{\#(\mathscr{E})M^{max}}. \tag{8.8}$$

Although this bound is far less nuanced than (8.5), it already tells us that high system robustness (low sensitivity) requires that the system have a large number of elements or that it contain at least one species having high element content (or a combination of both).

9. Concluding remarks.

Remark 9.1. We emphasized both in the introduction and in section 7 that the species sensitivity bounds given in Theorem 7.3 depend on network structure alone, independent of kinetics or even of the equilibrium state at which they are evaluated. In fact, even the fine details of the reaction network are of limited consequence:

For a quasi-thermostatic kinetic system the entire equilibrium set is determined by specification of just one equilibrium state and the stoichiometric subspace for the underlying network of chemical reactions. This is to say that two elemented quasi-thermostatic systems that share a common equilibrium state and the same stoichiometric subspace have precisely the same positive equilibrium sets and, therefore, *the same species sensitivities (relative to the same choice of elements).* To the extent that the network influences the equilibrium set, then, that influence is felt through the stoichiometric subspace. In turn, the stoichiometric subspace is merely the span of the network's reaction vectors, but the same span might derive from two rather different reaction networks.

Certainly, though, there is *some* network information carried by the stoichiometric subspace S, and therefore, by its orthogonal complement $\mathrm{S}^{\perp}$. Indeed, the sensitivity formulas given by Theorem 7.3 derive squarely from the nature of $\mathrm{S}^{\perp}$ and the particular elemental basis chosen for it.

Remark 9.2. As we indicated earlier, the sensitivity bounds derived for elemented systems suggest that strong robustness (i.e., very low sensitivity) against fluctuations in the element concentrations requires that the elements manifest themselves within the species in high multiplicity or that the species combine with each other gregariously. There is a certain intuitive sense to these requirements, for when they are met, the effect of changes in an element's total concentration can be attenuated through propagation across many species or buffered within species containing multiple instances of that element. It is striking that the requirements for high robustness are highly suggestive of biochemistry, in which large molecules are often built from multiple copies of many distinct elements that readily combine with each other by means of relatively large reaction networks.

Acknowledgments. We thank Noam Berger, Erez Dekel, Yuval Hart, Yakar Kannai, and Ron Milo for their help.

REFERENCES

[1] R. Abraham, J. E. Marsden, and T. Ratiu, *Manifolds and Tensor Analysis*, 2nd ed., Springer-Verlag, New York, 1983.

[2] G. Craciun and M. Feinberg, *Multiple equilibria in complex chemical reaction networks:* I. *The injectivity property*, SIAM J. Appl. Math., 65 (2005), pp. 1526–1546.

[3] G. Craciun and M. Feinberg, *Multiple equilibria in complex chemical reaction networks:* II. *The species-reaction graph*, SIAM J. Appl. Math., 66 (2006), pp. 1321–1338.

[4] G. Craciun, Y. Tang, and M. Feinberg, *Understanding bistability in complex enzyme-driven reaction networks*, Proc. Nat. Acad. Sci. USA, 103 (2006), pp. 8697–702.

[5] M. Feinberg, *Complex balancing in general kinetic systems*, Arch. Ration. Mech. Anal., 49 (1972), pp. 187–194.

[6] M. Feinberg, *Mathematical aspects of mass action kinetics*, in Chemical Reactor Theory: A Review, N. Amundsen and L. Lapidus, eds., Prentice–Hall, Englewood Cliffs, NJ, 1977, pp. 1–78.

[7] M. Feinberg, *Lectures on Chemical Reaction Networks*, written version of lectures given at the Mathematical Research Center, University of Wisconsin, Madison, WI, 1979. Available online at http://www.chbmeng.ohio-state.edu/~feinberg/research/.

[8] M. Feinberg, *Chemical reaction network structure and the stability of complex isothermal reactors*–I. *The deficiency zero and deficiency one theorems*, Chem. Engrg. Sci., 42 (1987), pp. 2229–2268.

[9] M. Feinberg, *Chemical reaction network structure and the stability of complex isothermal reactors*–II. *Multiple steady states for networks of deficiency one*, Chem. Engrg. Sci., 43 (1988), pp. 1–25.

[10] M. Feinberg, *Necessary and sufficient conditions for detailed balancing in mass action systems of arbitrary complexity*, Chem. Engrg. Sci., 44 (1989), pp. 1819–1827.

[11] M. Feinberg, *The existence and uniqueness of steady states for a class of chemical reaction networks*, Arch. Ration. Mech. Anal., 132 (1995), pp. 311–370.

[12] V. Guillemin and A. Pollack, *Differential Topology*, Prentice–Hall, Englewood Cliffs, NJ, 1974.

[13] R. Heinrich and T. A. Rapoport, *A linear steady-state treatment of enzymatic chains. Critique of the crossover theorem and a general procedure to identify interaction sites with an effector*, European J. Biochem./FEBS, 42 (1974), pp. 97–105.

[14] R. Heinrich and T. A. Rapoport, *A linear steady-state treatment of enzymatic chains. General properties, control and effector strength*, European J. Biochem./FEBS, 42 (1974), pp. 89–95.

[15] J. Higgins, *Analysis of sequential reactions*, Ann. New York Acad. Sci., 108 (1963), pp. 305–321.

[16] F. Horn and R. Jackson, *General mass action kinetics*, Arch. Ration. Mech. Anal., 47 (1972), pp. 81–116.

[17] F. Horn, *Necessary and sufficient conditions for complex balancing in chemical kinetics*, Arch. Ration. Mech. Anal., 49 (1972), pp. 172–186.

[18] K. Israel, *Monotone Behavior for Equilibria of Dynamical Systems*, Ph.D. thesis, Duke University, Durham, NC, 1984.

[19] H. Kacser and J. A. Burns, *The control of flux*, Symposia of the Society for Experimental Biology, 27 (1973), pp. 65–104.

[20] G. Lewis, *A new principle of equilibrium*, Proc. Nat. Acad. Sci. USA, 11 (1925), pp. 179–183.

[21] J. Nailor, *Behavior of Equilibria in Quasi-Thermodynamic Chemical Reaction Networks with Mass-Action Kinetics*, Ph.D. thesis, Duke University, Durham, NC, 1991.

[22] M. Ptashne and A. Gann, *Genes and Signals*, 1st ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.

[23] M. A. Savageau, *Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology*, Addison-Wesley, New York, 1976.

[24] G. Shinar, R. Milo, M. Rodríguez Martínez, and U. Alon, *Input-output robustness in simple bacterial signaling systems*, Proc. Nat. Acad. Sci. USA, 104 (2007), pp. 19931–19935.

[25] R. Veitia, *Nonlinear effects in macromolecular assembly and dosage sensitivity*, J. Theoret. Biol., 220 (2003), pp. 19–25.

[26] R. Wegscheider, *Über simultane Gleichgewichte und die Beziehungen zwischen Thermodynamik und Reaktionskinetik homogener Systeme*, Z. Phys. Chem., 39 (1902), pp. 257–303.