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MULTIPLE EQUILIBRIA IN COMPLEX CHEMICAL REACTION NETWORKS: SEMIOPEN MASS ACTION SYSTEMS*

GHEORGHE CRACIUN† AND MARTIN FEINBERG‡

Abstract. In two earlier articles, we provided sufficient conditions on (mass action) reaction network structure for the preclusion of multiple positive steady states in the context of what chemical engineers call the continuous flow stirred tank reactor. In such reactors, all species are deemed to be present in the effluent stream, a fact which played a strong role in the proofs. When certain species are deemed to be entrapped within the reactor, the questions that must be asked are more subtle, and the mathematics becomes substantially more difficult. Here we extend results of the earlier papers to semiopen reactors and show that very similar results obtain, provided that the network of chemical reactions satisfies certain weak structural conditions; weak reversibility is sufficient but not necessary.

Key words. reaction network, bistability, multistability, enzyme

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1. Introduction.

In two earlier papers [1], [3], we developed means to determine whether a given (mass action) chemical reaction network has the capacity to exhibit multiple positive steady states in the context of what chemical engineers call the (isothermal) *continuous flow stirred tank reactor (CFSTR)*. Some of those results are reviewed in [4] with special focus on biochemistry. When we say that a network has the capacity to admit multiple positive steady states, we mean that there are certain combinations of parameter values (e.g., kinetic rate constants, reactant supply rates) such that, for the network, the corresponding isothermal CFSTR mass action differential equations admit at least two distinct rest points at which all species concentrations are positive. (Among mass action networks generally, this is far less common than might be supposed.) In the absence of an overarching theory, determination of a network's capacity for multiple steady states is difficult, for one is confronted with a large system of polynomial equations in the species concentrations, in which many parameters appear.

Nevertheless, the aforementioned articles provide means to assert for quite broad classes of reaction networks—including highly complex ones—that multiple positive steady states are impossible, regardless of parameter values. The test provided in the first article is largely computational, while the test provided in the second is tied to subtle aspects of a reaction network's structure as revealed in its *species-reaction graph*. In fact, a theorem in [3] ensures that *multiple positive steady states are impossible for a particular network unless the species-reaction graph for the network*

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†Departments of Mathematics and Biomolecular Chemistry, 405 Van Vleck Hall, University of Wisconsin Madison, Madison, WI 54706 (cracium@wise.edu). The work of this author was supported by US National Institutes of Health grant 1R01GM086881-01.

‡William G. Lowrie Department of Chemical & Biomolecular Engineering and Department of Mathematics, The Ohio State University, 125 Koffolt Laboratories, 140 W. 19th Avenue, Columbus, OH 43210 (feinberg@chbmeng.ohio-state.edu). The work of this author was supported by US National Science Foundation grant BES-042549 and US National Institutes of Health grant 1R01GM086881-01.

meets very stringent conditions. (It is striking that certain classical mechanisms for enzyme catalysis do indeed meet those conditions [4]. In this sense, biology finds ways to circumvent strictures against bistable dynamics that biochemical switches might require.)

In the classical chemical engineering picture of a CFSTR, only certain species might be fed to the reactor (usually at fixed rates), but *all* species leave the reactor at rates proportional to their concentrations within the reactor. That is, the molar effluent rate (per unit reactor volume) of each species s is given by $\xi_s c_s$, where ξ_s is a time-invariant positive number and c_s is the molar concentration of species s within the reactor. (In the classical picture, all the ξ_s are equal to a species-independent constant, but, for reasons discussed in [1], we impose no such restriction in what follows.)

The fact that *all* species are in the CFSTR effluent played a strong role in the mathematics underlying results in [1] and [3]. In some instances, however, it is useful to consider models in which only certain species are free to enter or exit the reactor, while certain other species remain entrapped within it. In [4], for example, we considered instances in which small-molecule metabolites were free to enter or leave the reactor vessel, while larger enzymes and enzyme-metabolite complexes were denied passage either inward or outward.

At first glance it might appear that models of the classical CFSTR variety are highly similar to semiopen models of the entrapped species type. Although these models are indeed similar in a physical sense, there are distinctions between the two that make them mathematically different in important ways. As we shall try to make clear in section 3, certain stoichiometric considerations that arise naturally in the entrapped-species case actually change the nature of the questions that one should ask. When asking, for example, about the capacity for multiple steady states in the entrapped-species case, one needs to ask whether there can exist two distinct steady states *that are stoichiometrically compatible.*

Before proceeding further, we call to the reader's attention reference [9], which differs in its objectives from this article but which has points in common with it.

2. A question and an answer. A question arises, then, about the extent to which results presented in [1] and [3] also give information about models of the entrapped-species variety: *If we know that, for a certain chemical reaction network, there is no combination of parameter values that gives rise to multiple positive steady states in the classical fully open CFSTR context, can we also assert that the same is true in an entrapped-species context?*

This question was addressed in [2], where a partial answer was provided. *Without invoking mass action kinetics*, we showed that if a reaction network does not have the capacity for multiple steady states in the classical CFSTR setting (with all species permitted passage outward), then, for the same chemistry in the entrapped-species setting, multiple positive steady states can arise *only if all but perhaps one of the steady states is degenerate* (in a sense we shall make precise in section 3).

It is the purpose of this article to show that when, for a reaction network, results in [1], [3], and [4] preclude multiple positive steady states in the classical CFSTR context, then those same results preclude *any* degenerate positive steady state—and therefore multiple positive steady states—in the entrapped species context, provided that the kinetics is mass action and provided that the network of chemical reactions satisfies certain weak structural conditions. Although it is beyond the scope of this introduction to describe those conditions here, we can say that *they will be satisfied*

when the underlying chemical reaction network is weakly reversible [5], [6], [7], [10] *and, in particular, when every reaction is reversible.* (Weak reversibility is defined formally in the next section, but for now it will suffice to say that a reaction network is weakly reversible if every reaction arrow is in a directed cycle.)

In fact, we can go even further. Suppose that the chemistry in the reactor is given by network (2.1), which represents one classical mechanism for the enzyme-promoted combination of two substrates, $S1$ and $S2$, to form a product, P, and suppose also that the kinetics is mass action. The first step indicates the reversible binding of $S1$ to an enzyme E to form $ES1$. The second step represents reversible binding of $S2$ to $ES1$ to form $ES1S2$. Once both substrates are bound to the enzyme, they combine to form P, which is then released from the enzyme. These last events are represented by the third reaction step, which is deemed to be irreversible. (That is, the reverse reaction is deemed to proceed at negligible rate.)

$$\begin{array}{c} E + S1 \rightleftarrows ES1, \\ ES1 + S2 \rightleftarrows ES1S2 \to P + E. \end{array} \tag{2.1}$$

Results in [3] and [4] immediately give the information, via the species-reaction graph, that network (2.1) does not have the capacity for multiple positive equilibria in the classical CFSTR context—that is, when all species are permitted passage out of the reactor.

Suppose, however, that we wish to consider a reactor in which the (mass action) chemistry is again as in (2.1) but in which $E, ES1$, and $ES1S2$ are deemed to be entrapped within the reactor, while $S1, S2$, and P are permitted passage outward. Can we once again assert—based on the same species-reaction graph—that multiple positive *stoichiometrically compatible* equilibria are impossible, regardless of parameter values?

In this case, network (2.1) is not weakly reversible because the reaction $ES1S2 \to P$ is not in a directed cycle, but we will nevertheless argue that *information given about the fully open CFSTR in* [1], [3], *and* [4] *also applies to an entrapped species reactor, provided that the kinetics is mass action and provided that the* entrapped-species projection *of the original chemical reaction network is weakly reversible.* By the *entrapped-species projection* of a reaction network we mean the reaction network formed by stripping away all species that are not entrapped, leaving behind only the entrapped species. Thus, when $E, ES1$, and $ES1S2$ are entrapped, (2.2) is the entrapped-species projection of network (2.1):

$$\begin{array}{ccc} E & \rightleftarrows & ES1 \\ & \nwarrow \quad \swarrow & \\ & ES1S2 & \end{array} \tag{2.2}$$

Although network (2.1) is not weakly reversible, its entrapped-species projection (2.2) *is* weakly reversible, so we can assert that for the entrapped-species reactor multiple positive stoichiometrically compatible equilibria are again impossible, regardless of parameter values.

A specific aim of this article, then, is to prove that when, for a particular (mass action) network of chemical reactions, results in [1], [3], *and* [4] *deny the capacity for multiple positive equilibria in the fully open CFSTR context, then the capacity for multiple (stoichiometrically compatible) positive equilibria will* also *be denied in an entrapped-species context, provided that the entrapped-species projection of the original*

network is weakly reversible. Along the way, the capacity for a degenerate positive steady state will be denied as well. (As we shall see, similar conclusions obtain under even broader circumstances—whenever the entrapped-species projection of the original network is what we call a *normal network*. Normal networks are defined in section 7. All weakly reversible networks are normal.)

We note that chemists generally suppose that all chemical reactions are reversible, at least to some extent. For chemical systems that are modeled with this stricture in mind, we can assert that entrapped-species projected networks will inherit reversibility (and weak reversibility) from the original network of chemical reactions. Thus, it is only for models in which the putative network of chemical reactions does *not* conform to the reversibility orthodoxy that results in [1], [3], and [4] might fail to extend to the entrapped-species context.

3. Chemical reaction networks and mass action systems. In this section we provide a framework for discussion of chemical reaction networks. Much of this material is also available, with more motivation, in [5], [6], [7]. The simple reaction network (2.1), discussed in the preceding section, will provide a useful vehicle for introducing some language. The *species* of the network are, of course, $S1, S2, E, ES1, ES2, ES1S2$, and P. By the *complexes* of the network we mean the entities that appear before and after the reaction arrows. Thus, the complexes of network (2.1) are $E + S1, ES1, ES1 + S2, ES1S2$, and $P + E$. The *reactions* in the network are regarded to be a specification of a "reacts to" relation (indicated by "$\rightarrow$") in the set of complexes. After introduction of some notation, we posit a formal definition of a chemical reaction network.

Notation. We denote by $\mathbb{R}$ the set of real numbers, by $\mathbb{R}_+$ the positive real numbers, and by $\overline{\mathbb{R}}_+$ the nonnegative real numbers.

So that we can speak, for example, of a "vector of species concentrations" or a "vector of reaction rate constants," we will want to associate a real vector space with the set of species, with the set of reactions, and also with the set of complexes. At the same time, it will prove awkward to number these various objects just so that we can work in the familiar $\mathbb{R}^N$. With this in mind, we proceed as follows.

If $\mathscr{I}$ is a finite index set (for example, the set of species), we denote by $\mathbb{R}^{\mathscr{I}}$ the vector space of real-valued functions with domain $\mathscr{I}$. By ω_i we mean the element of $\mathbb{R}^{\mathscr{I}}$ that assigns 1 to $i \in \mathscr{I}$ and 0 to all other members of $\mathscr{I}$. It is easy to see that $\{\omega_i : i \in \mathscr{I}\}$ is a basis for $\mathbb{R}^{\mathscr{I}}$, and each $x \in \mathbb{R}^{\mathscr{I}}$ has a representation $x = \sum_{i\in\mathscr{I}} x_i\omega_i$, where x_i is the value of x associated with i. In effect, then, $\mathbb{R}^{\mathscr{I}}$ is a real vector space with a distinguished basis labeled by the elements of $\mathscr{I}$. By $\mathbb{R}^{\mathscr{I}}_+$ ($\overline{\mathbb{R}}^{\mathscr{I}}_+$) we mean the set of all $x \in \mathbb{R}^{\mathscr{I}}$ such that x_i is positive (nonnegative) for all $i \in \mathscr{I}$. If x is a member of $\mathbb{R}^{\mathscr{S}}$, the *support* of x is defined by $\mathrm{supp}(x) := \{i \in \mathscr{I} : x_i \neq 0\}$.

By the *standard scalar product* in $\mathbb{R}^{\mathscr{I}}$, we mean the scalar product that makes the basis $\{\omega_i : i \in \mathscr{I}\}$ orthonormal. When we have in mind the standard scalar product we will use "$\cdot$". Thus, for x and y in $\mathbb{R}^{\mathscr{I}}$ we have $x \cdot y = \sum_{i\in\mathscr{I}} x_i y_i$.

Only in the special case of $\mathbb{R}^{\mathscr{S}}$, where $\mathscr{S}$ is the set of species in a network, will we choose to replace symbols for the canonical basis vectors $\{\omega_s : s \in \mathscr{S}\}$ with the names of the species themselves. Thus, in the context of network (2.1) we would regard the sum $ES1 + S2$ as a surrogate for $\omega_{ES1} + \omega_{S2} \in \mathbb{R}^{\mathscr{S}}$. In this way, the complexes of a reaction network can be identified with elements of $\overline{\mathbb{R}}^{\mathscr{S}}_+$.

Definition 3.1 (see [5], [7]). *A* chemical reaction network *consists of three finite sets:*

(i) *a set* $\mathscr{S}$ *of* species *of the network;*
(ii) *a set* $\mathscr{C} \subset \bar{\mathbb{R}}_+^{\mathscr{S}}$ *of* complexes *of the network;*
(iii) *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* $(y', y) \in \mathscr{R}$.

When (y, y') is a member of $\mathscr{R}$ we say that the complex y *reacts to* complex y', and we write $y \to y' \in \mathscr{R}$ or simply $y \to y'$ to indicate that y reacts to y'. We say that y and y' are, respectively, the *reactant complex* and the *product complex* of the reaction $y \to y'$.

For use later, we also record the following definitions.

DEFINITION 3.2. *A reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *is* reversible *if, whenever* $y \to y'$ *is a member of* $\mathscr{R}$, $y' \to y$ *is also a member of* $\mathscr{R}$. *The reaction network is* weakly reversible *if, whenever* $y \to y'$ *is a member of* $\mathscr{R}$, *either* $y' \to y$ *is also a member of* $\mathscr{R}$ *or there exists a finite sequence of complexes* $\{y_1, y_2, \ldots, y_k\}$ *such that* $\mathscr{R}$ *contains the reactions* $y' \to y_1 \to y_2 \to \cdots \to y_k \to y$.

Definition 3.2 asserts that a reaction network is weakly reversible if every reaction is contained within a directed reaction cycle. Clearly, every reversible network is also weakly reversible.

DEFINITION 3.3. *For a reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, *the* reaction vector *corresponding to reaction* $y \to y' \in \mathscr{R}$ *is* $y' - y \in \mathbb{R}^{\mathscr{S}}$. *The* stoichiometric subspace *for the network, generally denoted by S, is the span of its reaction vectors:*

$$S := \operatorname{span}\{y' - y \in \mathbb{R}^{\mathscr{S}} : y \to y' \in \mathscr{R}\}. \tag{3.1}$$

Thus, for example, the reaction vector corresponding to $E + S1 \to ES1$ in network (2.1) is $ES1 - E - S1$. Note that for species $s \in \mathscr{S}$ the s-component, $y'_s - y_s$, of reaction vector $y' - y$ is the net number of molecules of s produced with each occurrence of the reaction $y \to y'$.

For a network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ the occurrence of the reactions will generally lead to a change in the population of the various species. Our interest will be in differential equations that govern the mixture composition. We identify the *mixture composition* with a vector $c \in \bar{\mathbb{R}}_+^{\mathscr{S}}$, where, for each $s \in \mathscr{S}$, c_s is the molar concentration of species s. The occurrence rates of the various reactions generally depend upon the instantaneous composition.

A *mass action kinetics* for a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ amounts to a prescription of a reaction-rate function for each reaction, one that relates occurrence rate to composition in a classical way: Associated with each reaction $y \to y'$ is a positive *rate constant* $k_{y \to y'}$ such that the molar occurrence rate per unit volume of $y \to y'$ is given by $k_{y \to y'} c^y$, where

$$c^y := \prod_{s \in \mathscr{S}} c_s^{y_s}. \tag{3.2}$$

DEFINITION 3.4. *A* mass action system *is a reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *taken with a* rate constant specification $k \in \mathbb{R}_+^{\mathscr{R}}$. *The positive number* $k_{y \to y'}$ *is the* rate constant *assigned to reaction* $y \to y' \in \mathscr{R}$.

Once a mass action kinetics is associated with a reaction network, we can calculate the net molar production rate (per unit volume) of each species due to the simultaneous occurrence of the various reactions.

DEFINITION 3.5. *For a mass action system* $\{\mathscr{S},\mathscr{C},\mathscr{R},k\}$*, the* species-formation-rate function $r:\overline{\mathbb{R}}_+^{\mathscr{S}}\to\mathbb{R}^{\mathscr{S}}$ *is given by*

$$r(c)\equiv\sum_{y\to y'\in\mathscr{R}}k_{y\to y'}c^y(y'-y). \tag{3.3}$$

In particular, the species-formation-rate at composition c for species $s\in\mathscr{S}$ is given by

$$r_s(c)\equiv\sum_{y\to y'\in\mathscr{R}}k_{y\to y'}c^y(y'_s-y_s). \tag{3.4}$$

Thus, the overall net production rate of s is taken to be the sum of all the various reaction rates, each multiplied by the net molecular gain of s associated with the corresponding reaction.

DEFINITION 3.6. *The* differential equation associated with a mass action system $\{\mathscr{S},\mathscr{C},\mathscr{R},k\}$ *is*

$$\dot{c}=r(c), \tag{3.5}$$

where the overdot indicates time-differentiation and $r(\cdot)$ *is the corresponding species-formation-rate function. An* equilibrium *of the mass action system is a composition* $a\in\overline{\mathbb{R}}_+^{\mathscr{S}}$ *such that* $r(a)=0$*. A* positive equilibrium *is an equilibrium in* $\mathbb{R}_+^{\mathscr{S}}$*.*

With rate constants for network (2.1) as indicated in (3.6),

$$\begin{gathered}E+S1\underset{k_2}{\overset{k_1}{\rightleftarrows}}ES1,\\ ES1+S2\underset{k_4}{\overset{k_3}{\rightleftarrows}}ES1S2\overset{k_5}{\to}P+E,\end{gathered} \tag{3.6}$$

the component form of the corresponding mass action differential equations is

$$\begin{aligned}\dot{c}_{S1}&=k_2c_{ES1}-k_1c_{S1}c_E,\\ \dot{c}_{S2}&=k_4c_{ES1S2}-k_3c_{S2}c_{ES1},\\ \dot{c}_E&=k_5c_{ES1S2}+k_2c_{ES1}-k_1c_{S1}c_E,\\ \dot{c}_{ES1}&=k_4c_{ES1S2}-k_3c_{S2}c_{ES1}-k_2c_{ES1}+k_1c_{S1}c_E,\\ \dot{c}_{ES1S2}&=-\left(k_5+k_4\right)c_{ES1S2}+k_3c_{S2}c_{ES1},\\ \dot{c}_P&=k_5c_{ES1S2}.\end{aligned} \tag{3.7}$$

Note that the species-formation-rate function of a mass action system takes values in S, the stoichiometric subspace for the underlying reaction network. For reasons rooted in mass-conservation conditions it is often (but, as we shall see, not always) the case that the stoichiometric subspace is a *proper* linear subspace of $\mathbb{R}^{\mathscr{S}}$. Because $\dot{c}$ invariably points along S, it is not difficult to see that composition trajectories will reside entirely within parallels of S. In particular, a trajectory passing through c^0 will lie in the set $(c^0+S)\cap\overline{\mathbb{R}}_+^{\mathscr{S}}$.

With this in mind we say that two compositions c and c' are *stoichiometrically compatible* if $c'-c$ is a member of S. In fact, stoichiometric compatibility is an equivalence relation that serves to partition $\overline{\mathbb{R}}_+^{\mathscr{S}}$ into *stoichiometric compatibility classes.* In

rough terms, one is interested in dynamics *within stoichiometric compatibility classes*. Thus, when we consider the capacity of a network for multiple positive equilibria, *we shall always mean its capacity for more than one positive equilibrium within a stoichiometric compatibility class.* (Of course, if $S = \mathbb{R}^{\mathscr{S}}$, then this qualification becomes moot.)

DEFINITION 3.7. *A reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ has the capacity to admit multiple positive equilibria *if there exists* $k \in \mathbb{R}_+^{\mathscr{R}}$ *such that the mass action system* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, k\}$ *admits (at least) two distinct positive equilibria that are stoichiometrically compatible.*

Remark 3.1. For a mass action system, we shall be interested in the derivative of the species-formation-function evaluated at a positive equilibrium point. Because our interest is in dynamics within stoichiometric compatibility classes, it is natural to restrict the derivative, viewed as a linear transformation, to the stoichiometric subspace. In fact, if $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, k\}$ is a mass action system and $a \in \mathbb{R}_+^{\mathscr{S}}$ is an equilibrium, then the derivative $\mathrm{d}r(a) : S \to S$ is given by [7]

$$\mathrm{d}r(a)\sigma \equiv \sum_{y \to y' \in \mathscr{R}} \kappa_{y \to y'}(y * \sigma)(y' - y), \tag{3.8}$$

where

$$\kappa_{y \to y'} := k_{y \to y'} a^y = k_{y \to y'} \prod_{s \in \mathscr{S}} a_s^{y_s} \tag{3.9}$$

and "$*$" is a scalar product in $\mathbb{R}^{\mathscr{S}}$ defined by

$$u * w := \sum_{s \in \mathscr{S}} \frac{u_s w_s}{a_s}. \tag{3.10}$$

DEFINITION 3.8. *For a mass action system* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, k\}$, *an equilibrium* $a \in \mathbb{R}_+^{\mathscr{S}}$ *is* degenerate *if* $\mathrm{d}r(a)$ *is singular—that is, if there exists a nonzero* $\sigma \in S$ *such that* $\mathrm{d}r(a)\sigma = 0$.

4. Mass action models of open systems. In the example of the preceding section it was supposed tacitly that the mixture under consideration was enclosed in a well-mixed vessel impervious to the transport of any of the species either inward or outward. Suppose, however, that the nonenzymatic species $S1$ and $S2$ are fed to the vessel at constant rates (per unit vessel volume) of F_{S1} and F_{S2}, while $S1$, $S2$, and P are removed from the vessel at (per unit volume) rates proportional to their concentrations within the vessel, with proportionality constants ξ_{S1}, ξ_{S2}, and ξ_P. In this case, the governing differential equations would be

$$\begin{aligned}
\dot{c}_{S1} &= k_2 c_{ES1} - k_1 c_{S1} c_E + F_{S1} - \xi_{S1} c_{S1}, \\
\dot{c}_{S2} &= k_4 c_{ES1S2} - k_3 c_{S2} c_{ES1} + F_{S2} - \xi_{S2} c_{S2}, \\
\dot{c}_E &= k_5 c_{ES1S2} + k_2 c_{ES1} - k_1 c_{S1} c_E, \\
\dot{c}_{ES1} &= k_4 c_{ES1S2} - k_3 c_{S2} c_{ES1} - k_2 c_{ES1} + k_1 c_{S1} c_E, \\
\dot{c}_{ES1S2} &= -(k_5 + k_4)\, c_{ES1S2} + k_3 c_{S2} c_{ES1}, \\
\dot{c}_P &= k_5 c_{ES1S2} - \xi_P c_P.
\end{aligned} \tag{4.1}$$

These are not the differential equations corresponding to mass action system (3.6), but they are the differential equations corresponding to the mass action system

shown in (4.2). There, a "pseudoreaction" of the form $s \to 0$ is appended to the network of true reactions to indicate that species s is in the effluent stream, while $0 \to s$ is appended to indicate that s is in the feed stream. (Here 0 denotes the "zero complex," identified with the zero vector of $\mathbb{R}^{\mathscr{S}}$.) In examining connections between reaction network structure and properties of the corresponding differential equations, it is network (4.2), rather than (3.6), that is relevant to the study of (4.1):

$$
\begin{array}{ll}
E + S1 \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES1, & S1 \underset{F_{S1}}{\overset{\xi_{S1}}{\rightleftarrows}} 0 \underset{F_{S2}}{\overset{\xi_{S2}}{\leftrightarrows}} S2 \\
ES1 + S2 \underset{k_4}{\overset{k_3}{\rightleftarrows}} ES1S2 \overset{k_5}{\to} P + E, & \xi_P \uparrow \\
 & P.
\end{array}
\tag{4.2}
$$

More generally, in considering reaction network models of open systems, the species set $\mathscr{S}$ will be partitioned into two subsets: $\mathscr{M}$, called the *mobile species*, and $\mathscr{E}$, called the *entrapped species*. The mobile species are precisely those permitted passage outward in the effluent stream, while the entrapped species are those denied such passage. We denote by $\mathscr{F} \subset \mathscr{S}$ the set of species deemed to be present in the feed stream. (As in our example, it is typically the case that $\mathscr{F}$ is contained in $\mathscr{M}$, and we shall suppose that this is the case hereafter. Results similar to those reported here will obtain even when $\mathscr{F}$ is not contained in $\mathscr{M}$, but at the expense of a somewhat more fussy development.)

An *open system reaction network* amounts, in effect, to specification of a network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ of true chemical reactions taken together with specifications of the set $\mathscr{M}$ of mobile species and the set $\mathscr{F}$ of species deemed present in the feed stream. The resulting open system reaction network will be $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, where $\mathscr{C} = \mathscr{C}_t \cup \mathscr{M} \cup \{0\}$ and $\mathscr{R} = \mathscr{R}_t \cup \{m \to 0 : m \in \mathscr{M}\} \cup \{0 \to m : m \in \mathscr{F}\}$. (For the special case in which $\mathscr{M}$ and $\mathscr{F}$ are empty, we take $\mathscr{C} = \mathscr{C}_t$. It will be convenient to admit this extreme case as an instance of an open system reaction network.) It is worth mentioning that when we speak of the "true chemical reactions," we simply mean those reactions deemed to model chemical transformations, exclusive of pseudoreactions such as $m \to 0$ or $0 \to m$ added to model the effect of species addition and removal.

If $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is an open system reaction network, then the differential equation corresponding to the open system mass action model $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, k\}$ is

$$
\dot{c} = \sum_{y \to y' \in \mathscr{R}_t} k_{y \to y'} c^y (y' - y) + \sum_{m \in \mathscr{F}} k_{0 \to m} m + \sum_{m \in \mathscr{M}} k_{m \to 0} c_m (-m). \tag{4.3}
$$

Remark 4.1 (degradation and synthesis models). The mass action description of open systems described above can be adapted to model still other situations. For example, we might imagine a closed system in which certain species participate in the true chemistry but also decompose to inerts at rates proportional to their current concentrations. The effect of the degradation could be modeled *as if* those species were being removed from the reactor, with reactions such as $m \to 0$ employed to account for the degradation effect. Similarly, a reaction such as $0 \to m$ could be used to account for the constant-rate supply of species m by means of a synthesis otherwise divorced from the chemistry under study.

Remark 4.2. Suppose that $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, k\}$ is a mass action description of an open system, with $\mathscr{S} = \mathscr{M} \sqcup \mathscr{E}$. As before, we denote by S the stoichiometric subspace

for the underlying reaction network (including pseudoreactions). If $a \in \mathbb{R}^{\mathscr{S}}_+$ is an equilibrium, then, in this case, the derivative $\mathrm{d}r(a) : S \to S$ is given by

$$\mathrm{d}r(a)\sigma \equiv \sum_{y \to y' \in \mathscr{R}_t} \kappa_{y\to y'}(y * \sigma)(y' - y) + \sum_{m \in \mathscr{M}} \kappa_{m\to 0}(m * \sigma)(-m), \tag{4.4}$$

where the $\kappa_{y\to y'}$ and "$*$" are as in Remark 3.1 and $\mathscr{R}_t$ refers to the set of true reactions.

5. Entrapped-species versus fully open systems. In a mass action model of an open system, the set of mobile species might coincide with the full set of species (i.e., $\mathscr{M} = \mathscr{S}$). In this case all species are in the effluent stream, and we say that the model (and the underlying network) are *fully open*. Otherwise, we say that the model (and the underlying network) are of the *entrapped-species* kind. If $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is a fully open reaction network, then the reaction set will contain as a subset $\{s \to 0 : s \in \mathscr{S}\}$. From this it follows that the stoichiometric subspace coincides with $\mathbb{R}^{\mathscr{S}}$ and that all compositions are stoichiometrically compatible. In this respect, fully open models are somewhat easier to study than semiopen models. In particular, for fully open models questions about the existence of multiple equilibria reduce simply to questions about the number of equilibria, unqualified by questions about whether those equilibria are stoichiometrically compatible.

With more formal definitions now in place, it will be useful to revisit some ideas described informally in the preceding sections: In [1] and [3] we examined, for *fully open* mass action models, connections between the capacity for multiple positive equilibria and the structure of the network of true chemical reactions. Among other things, those papers contain broad theorems asserting that, if the network of true chemical reactions satisfies certain weak conditions, then multiple positive equilibria cannot result from a corresponding *fully open* mass action model, *regardless of parameter values*. In [2] we argued that a theorem that denies the capacity for multiple positive equilibria in a particular fully open system will *also* deny the capacity for multiple positive equilibria in an entrapped-species context, but in a restricted sense: There can be multiple stoichiometrically compatible positive equilibria in the entrapped-species model *only if all but perhaps one of those equilibria are degenerate in the sense of Definition* 3.8. In fact, results in [2] were not restricted to mass action kinetics.

Our aim now is to show that, when results in [1] and [3] preclude multiple positive steady states in the fully open context, then, regardless of parameter values, *those same results preclude* any *degenerate positive equilibrium (and therefore multiple stoichiometrically compatible positive equilibria) in the entrapped-species context, provided that the kinetics is mass action and provided that the true reaction network satisfies, in addition, very weak structural conditions.* (It is sufficient that the entrapped-species projection of the true chemical reaction network fall into a class of networks we call *normal*. Every weakly reversible network is normal.)

All results in [1] and [3] were based on the idea of network *injectivity*. In section 6, we define what we mean by an injective reaction network, and we discuss the relationship between injectivity and the capacity for multiple positive equilibria. In section 7 we define normal networks, and then in section 8 we state the main theorem of this article.

Remark 5.1 (the "$*$"-scalar product). The scalar product defined in (3.10) will appear frequently in the remainder of this article. It depends, of course, on the choice of $a \in \mathbb{R}^{\mathscr{S}}_+$. When a certain construct—a linear transformation, for example—depends upon that choice, we will attach a subscripted "$*$" to the name of the construct as

a reminder of the a-dependence. When we say that a certain property obtains "for every choice of $*$-scalar product," we mean for every choice of $a \in \mathbb{R}^{\mathscr{S}}_+$.

We note that the scalar product in $\mathbb{R}^{\mathscr{S}}$ given by (3.10) is of a special form, motivated by the particular reaction network considerations that we have in mind. It will be evident, however, that this special form is inconsequential to the proof of several assertions made along the way, in which case "for every choice of $*$-scalar product" can be taken in its more general sense.

6. Injective reaction networks. In [1] we defined the notion of an *injective reaction network*, but only in consideration of fully open reactors. Here we generalize that definition with an eye toward study of entrapped-species reactors, as follows.

DEFINITION 6.1. *Let $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ be a reaction network with stoichiometric subspace S. The network is* injective *if, for every choice of $\eta \in \mathbb{R}^{\mathscr{R}}_+$ and for every choice of $*$-scalar product, the map $T_{*\eta} : S \to S$ defined by*

$$T_{*\eta}\sigma \equiv \sum_{y \to y' \in \mathscr{R}} \eta_{y \to y'}(y * \sigma)(y' - y) \tag{6.1}$$

is nonsingular.

Remark 6.1 (injectivity for open system reaction networks). Let $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ be an open system reaction network, let $\mathscr{M}$ be the set of mobile species, and let S be the stoichiometric subspace for the network. In this case, the map $T_{*\eta} : S \to S$ in Definition 6.1 takes the form

$$T_{*\eta}\sigma \equiv \sum_{y \to y' \in \mathscr{R}_t} \eta_{y \to y'}(y * \sigma)(y' - y) + \sum_{m \in \mathscr{M}} \eta_{m \to 0}(m * \sigma)(-m). \tag{6.2}$$

Remark 6.2 (notation). Note that for an open system reaction network, the set $\mathscr{F}$ of species deemed present in the feed stream plays no role in the determination of injectivity. The presence or absence of injectivity is determined solely by the network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ of true chemical reactions and the set $\mathscr{M}$ of mobile species. Because our concerns will be largely with injectivity, we shall find it convenient hereafter to refer to the "open system network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$," where $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ is the network of true chemical reactions and $\mathscr{M}$ is the set of mobile species. When we refer to the "entrapped species network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$," we mean that $\mathscr{M}$ is smaller than $\mathscr{S}$; the set of entrapped species $\mathscr{E}$ is then just $\mathscr{S} \setminus \mathscr{M}$, possibly $\mathscr{S}$ itself. Against this background, the fully open network with true chemistry $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ is indicated by $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{S}\}$.

Remark 6.3 (the path forward, in terms of injectivity). In [1] we showed that if a *fully open* reaction network is injective, then for any choice of rate constants the resulting fully open mass action model cannot admit multiple positive equilibria. Nevertheless, it is not a simple matter to determine whether, for a complex reaction network, injectivity obtains. In [1] and [3], however, we provided means to determine—via computation or via inspection of the *species-reaction graph*—that a particular *fully open* reaction network is injective. It is our objective here to examine the extent to which those same methods extend to entrapped-species networks.

In more specific terms, if $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{S}\}$ is a fully open network that is known to be injective, and if $\mathscr{M} \subset \mathscr{S}$ is a specification of the mobile species, *we want to know when it can be said that the entrapped-species network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ is also injective.* If $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ *is* injective, it follows directly from Remark 3.1, Definition 3.8, and Definition 6.1 that *no mass action system deriving from it can*

give rise to a degenerate positive equilibrium. In light of the discussion at the close of section 5, *it also follows that the entrapped species network* $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ *does not have the capacity for multiple positive equilibria.*

Remark 6.4. There is another path, different from the one described in the preceding remark, that connects injectivity in the sense of Definition 6.1 to the preclusion of multiple positive stoichiometrically compatible equilibria. For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with stoichiometric subspace S, it can be shown that the network is *not* injective if and only if there are $k \in \mathbb{R}^{\mathscr{S}}_+$ and distinct $c^*, c^{**} \in \mathbb{R}^{\mathscr{S}}_+$, with $c^* - c^{**} \in S$, such that

$$\sum_{y \to y' \in \mathscr{R}} k_{y \to y'} (c^*)^y (y' - y) = \sum_{y \to y' \in \mathscr{R}} k_{y \to y'} (c^{**})^y (y' - y). \tag{6.3}$$

That is, noninjectivity in the sense of Definition 6.1 is equivalent to the existence of positive rate constants such that there are two distinct stoichiometrically compatible positive compositions at which the species-formation-rate function takes the same value. Clearly, then, if the network *is* injective in the sense of Definition 6.1, then for no assignment of rate constants can there exist two distinct stoichiometrically compatible positive equilibria—that is, two distinct stoichiometrically compatible positive compositions at which the species-formation-rate function takes the value *zero.* Although this connection between Definition 6.1 and the preclusion of equilibrium multiplicity is compelling, our preference is to proceed through the logical route described in the preceding remark, for then the capacity for a degenerate equilibrium is also precluded. Moreover, the connection of Definition 6.1 to the preclusion of a degenerate equilibrium is direct.

7. Normal reaction networks. Here we say what it means for a reaction network to be *normal.*[1] Normality is closely related to injectivity.

DEFINITION 7.1. *Let* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *be a reaction network with stoichiometric subspace* S*. The network is* normal *if, for each* $*$*-scalar product in* $\mathbb{R}^{\mathscr{S}}$*, there exists* $\eta \in \mathbb{R}^{\mathscr{R}}$ *such that the linear transformation* $T_{*\eta} : S \to S$ *defined by*

$$T_{*\eta}\sigma \equiv \sum_{y \to y' \in \mathscr{R}} \eta_{y \to y'} (y * \sigma)(y' - y) \tag{7.1}$$

is nonsingular.

It is apparent that a network can be injective only if it is normal. The following proposition describes a wide class of normal networks.

PROPOSITION 7.2. *Every weakly reversible network is normal.*

Proof. Let $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ be a weakly reversible reaction network with stoichiometric subspace S, and let "$*$" denote a fixed but arbitrary scalar product in $\mathbb{R}^{\mathscr{S}}$. Choose $\eta \in \mathbb{R}^{\mathscr{S}}_+$ to satisfy the condition

$$\sum_{y \to y' \in \mathscr{R}} \eta_{y \to y'} (\omega_{y'} - \omega_y) = 0. \tag{7.2}$$

(Recall that $\{\omega_y\}_{y \in \mathscr{C}}$ is the standard basis for $\mathbb{R}^{\mathscr{C}}$.) That such an η exists for a weakly reversible network is proved in [5]. With η chosen this way, we want to show that the map $T_{*\eta}$ defined by (7.1) is nonsingular.

[1] In a recently published article [8], Gnacadja also uses the term "normal network," but his usage is different from ours.

In fact, we can show that $T_{*\eta}$ is $*$-negative-definite—i.e., that $\sigma * T_{*\eta}\sigma < 0$ for all nonzero $\sigma \in S$:

$$\sigma * T_{*\eta}\sigma = \sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(y*\sigma)(y'*\sigma - y*\sigma) \tag{7.3}$$

$$\leq \frac{1}{2}\sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}((y'*\sigma)^2 - (y*\sigma)^2) \tag{7.4}$$

$$= \frac{1}{2}\left[\sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(\omega_{y'} - \omega_y)\right]\cdot\left[\sum_{\tilde{y}\in\mathscr{C}} (\tilde{y}*\sigma)^2\omega_{\tilde{y}}\right] \tag{7.5}$$

$$= 0. \tag{7.6}$$

In (7.5), "$\cdot$" indicates the standard scalar product in $\mathbb{R}^{\mathscr{C}}$. Note that equality holds in (7.4) if and only if $(y' - y) * \sigma = 0$ for all $y \to y' \in \mathscr{R}$—that is, if and only if σ is orthogonal to S relative to the $*$-scalar product. Because σ is presumed to be a member of S, this can be the case only if $\sigma = 0$.

To complete the proof we note that if $T_{*\eta}\bar{\sigma} = 0$, in which case $\bar{\sigma} * T_{*\eta}\bar{\sigma} = 0$, we must have $\bar{\sigma} = 0$. Thus, $T_{*\eta}$ is nonsingular. □

The simple network $A \to B$ suffices to demonstrate that a network can be normal without being weakly reversible.

8. The main theorem and its consequences. In this section we state the main theorem of this article, and we review its consequences. The theorem describes circumstances under which injectivity of a fully open network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{S}\}$ implies injectivity of an entrapped-species network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ sharing the same true chemistry. (To see that an entrapped-species network does not always inherit injectivity from the fully open network, it is enough to consider the simple true chemistry $A \to B$. The corresponding fully open network containing reactions $\{A \to B,\ A \to 0,\ B \to 0\}$ is injective. On the other hand, if B is entrapped, the entrapped species network containing the reactions $\{A \to B, A \to 0\}$ is not injective.)

Here, then, we consider the entrapped-species network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$. The set $\mathscr{E}$ of entrapped species is $\mathscr{S} \setminus \mathscr{M}$. We denote by $\Gamma_{\mathscr{M}}$ and $\Gamma_{\mathscr{E}}$ the sets of vectors in $\mathbb{R}^{\mathscr{S}}$ having supports in $\mathscr{M}$ and $\mathscr{E}$, respectively. Note that $\mathbb{R}^{\mathscr{S}} = \Gamma_{\mathscr{M}} \oplus \Gamma_{\mathscr{E}}$. We denote by M: $\mathbb{R}^{\mathscr{S}} \to \Gamma_{\mathscr{M}}$ and E: $\mathbb{R}^{\mathscr{S}} \to \Gamma_{\mathscr{E}}$ the obvious projections.

We come now to the *entrapped-species projection* of the true network of chemical reactions. This was described informally, with an example, in section 2. Recall that the entrapped-species projection of the true network is obtained by stripping away all of the species in $\mathscr{M}$, leaving behind only the species of $\mathscr{E}$. Although the resulting network contains no species of $\mathscr{M}$, we shall nevertheless find it convenient to continue viewing its species set as $\mathscr{S}$.

DEFINITION 8.1. *For an entrapped-species reaction network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$, the* entrapped-species projection *of the true chemical reaction network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ is the reaction network $\{\mathscr{S}, \mathscr{C}_{\mathscr{E}}, \mathscr{R}_{\mathscr{E}}\}$ with complex and reaction sets defined as follows:*

$$\mathscr{C}_{\mathscr{E}} := \{\tilde{y} \in \Gamma_{\mathscr{E}} : \exists\, y \in \mathscr{C}_t \text{ with } \tilde{y} = Ey\}, \tag{8.1}$$

$$\mathscr{R}_{\mathscr{E}} := \{(\tilde{y}, \tilde{y}') \in \mathscr{C}_{\mathscr{E}} \times \mathscr{C}_{\mathscr{E}} : \exists\, y \to y' \in \mathscr{R}_t \text{ with } \tilde{y} = Ey,\ \tilde{y}' = Ey', \tilde{y} \neq \tilde{y}'\}. \tag{8.2}$$

We write $\tilde{y} \to \tilde{y}'$ (or $Ey \to Ey'$) to indicate the reaction whereby complex $\tilde{y}$ reacts to complex $\tilde{y}'$.

We are now in a position to state the central theorem of this article, as follows.

THEOREM 8.2. *Suppose that* $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ *is an entrapped-species network and that the fully open network* $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{S}\}$ *is injective. If the entrapped-species projection of* $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ *is a normal reaction network, then* $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ *is also injective. In particular,* $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ *is injective if the entrapped-species projection of* $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ *is weakly reversible.*

Proof of the theorem will begin in the next section. Here we survey the theorem's consequences.

It should be kept in mind that in [1], [3], [4] we presented easily applied means to determine whether *fully open* reaction networks are injective. (Tests are applied to the network of true chemical reactions.) For an entrapped-species network sharing the same chemistry, it will often happen that the entrapped-species projection of the true chemical reaction network is weakly reversible—in particular, if the true network is itself weakly reversible. In such instances, the theorem tells us immediately that the entrapped species network inherits injectivity from its fully open counterpart. Then, as we have argued in section 5 and Remark 6.3, a mass action system derived from the entrapped-species network cannot admit a degenerate positive equilibrium, nor can it admit multiple positive stoichiometrically compatible equilibria. This we record as the following corollary of the theorem.

COROLLARY 8.3. *For an entrapped-species network satisfying the conditions of Theorem* 8.2*, no choice of rate constants can result in a mass action system that admits a degenerate positive equilibrium or two distinct positive equilibria that are stoichiometrically compatible.*

9. A useful proposition. Here we begin our proof of Theorem 8.2. In consideration of injectivity and normality, both for fully open networks and for entrapped species networks, we shall have occasion to calculate determinants of maps such as those shown in (4.4), (6.1), and (7.1). With this in mind, we shall find it useful to have on record a proposition that describes such calculations in terms that are sufficiently abstract that it can be employed in a variety of situations.

We suppose that $\mathscr{S}$ is the set of species, that "$*$" is a scalar product in $\mathbb{R}^{\mathscr{S}}$, and that U is a linear subspace in $\mathbb{R}^{\mathscr{S}}$ of dimension p. Moreover, we suppose that $\det_{\mathrm{U}}[\cdot, \cdot, \ldots, \cdot]$ is a determinant function on U. That is, $\det_{\mathrm{U}}$ is a skew-symmetric p-linear real-valued function on $U \times U \times \cdots \times U$ (p times). We presume that $\det_{\mathrm{U}}$ is normalized such that for some $*$-orthonormal basis for U, say $\{u_1, , u_2, \ldots, u_p\}$, $\det_{\mathrm{U}}[u_1, , u_2, \ldots, u_p] = 1$. If $L : U \to U$ is a linear transformation, then by det L we mean the number $\det_{\mathrm{U}}[Lu_1, Lu_2 \ldots, Lu_p]$.

Finally, let $\mathscr{R}^{\#}$ denote a set of reactions, the number of reactions being at least p. When we write $C(\mathscr{R}^{\#}, p)$, we mean the set of all combinations of (distinct) reactions in $\mathscr{R}^{\#}$ taken p at a time. If χ is a member of $C(\mathscr{R}^{\#}, p)$, we indicate the p reactions of χ by symbols $\{\chi(1), \chi(2), \ldots, \chi(p)\}$. Moreover, we indicate the reactant and product complexes of reaction $\chi(i)$ by $y_{\chi(i)}$ and $y'_{\chi(i)}$, respectively. Thus, $\chi(i)$ and $y_{\chi(i)} \to y'_{\chi(i)}$ are alternative symbols for the same reaction. In particular, an alternative display of χ takes the form

$$\{y_{\chi(1)} \to y'_{\chi(1)},\ y_{\chi(2)} \to y'_{\chi(2)},\ \ldots,\ y_{\chi(p)} \to y'_{\chi(p)}\}.$$

The numbering of reactions imparts an artificial order to members of χ, but that order will have no significance in anything that follows.

PROPOSITION 9.1. *Let* $\{v_{y \to y'}\}_{y \to y' \in \mathscr{R}^{\#}}$ *and* $\{u_{y \to y'}\}_{y \to y' \in \mathscr{R}^{\#}}$ *be members of U,*

let $\{\alpha_{y\to y'}\}_{y\to y'\in\mathscr{R}^{\#}}$ *be real numbers, and let* $L : U \to U$ *be defined by*

$$Lx := \sum_{y\to y'\in\mathscr{R}^{\#}} \alpha_{y\to y'}(v_{y\to y'} * x)u_{y\to y'}. \tag{9.1}$$

Then

$$\det L = \sum_{\chi\,\in\, C(\mathscr{R}^{\#},\, p)} \left(\prod_{i=1}^{p} \alpha_{\chi(i)}\right) \det_{\mathrm{U}}\,[v_{\chi(1)},\,\ldots,\,v_{\chi(p)}]\,\det_{\mathrm{U}}[u_{\chi(1)},\,\ldots,\,u_{\chi(p)}]. \tag{9.2}$$

Proof. Apart from some minor variations, the proof is similar to the proof of Theorem 3.2 in [1]. ∎

10. Injectivity, normality, and critical subnetworks. Let $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ be a reaction network with stoichiometric subspace S, and suppose that $\mathbb{R}^{\mathscr{S}}$ is given a $*$-scalar product. As before, for each $\eta \in \mathbb{R}^{\mathscr{R}}$, we take the linear transformation $T_{*\eta} : S \to S$ to be defined by

$$T_{*\eta}\sigma \equiv \sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(y * \sigma)(y' - y). \tag{10.1}$$

Let $\pi_* : \mathbb{R}^{\mathscr{S}} \to S$ be the projection onto S along $S^{\perp^*}$, where it is understood that $\perp^*$ indicates orthogonality relative to the $*$-scalar product. With this in mind, we can rewrite (10.1) as

$$T_{*\eta}\sigma \equiv \sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(\pi_* y * \sigma)(y' - y). \tag{10.2}$$

With s denoting the dimension of S and with $\det_S[\cdot\,,\cdot,\ldots\,,\cdot]$ denoting a determinant function on S chosen as in section 9, it follows from Proposition 9.1 that

$$\det T_{*\eta} = \sum_{\chi\,\in\, C(\mathscr{R},\, s)} \left(\prod_{i=1}^{s} \eta_{\chi(i)}\right) \det_S\,[\pi_* y_{\chi(1)},\ldots,\pi_* y_{\chi(s)}]\,\det_S[y'_{\chi(1)} - y_{\chi(1)},\ldots,y'_{\chi(s)} - y_{\chi(s)}]. \tag{10.3}$$

From this it is clear that for $T_{*\eta}$ to be nonsingular for at least *some* choice of $\eta \in \mathbb{R}^{\mathscr{R}}$ it is necessary that for at least one combination of s reactions—that is, for at least one $\chi \in C(\mathscr{R}, s)$—it must be the case that

$$\det_S\,[\pi_* y_{\chi(1)},\,\ldots,\,\pi_* y_{\chi(s)}]\,\det_S[y'_{\chi(1)} - y_{\chi(1)},\,\ldots,\,y'_{\chi(s)} - y_{\chi(s)}] \neq 0, \tag{10.4}$$

which is to say that the sets

$$\{\pi_* y_{\chi(1)},\,\ldots,\,\pi_* y_{\chi(s)}\} \quad\text{and}\quad \{y'_{\chi(1)} - y_{\chi(1)},\,\ldots,\,y'_{\chi(s)} - y_{\chi(s)}\} \tag{10.5}$$

are *both* independent (and are *both* bases for S).

It should be understood that, in the preceding discussion, a fixed but arbitrary choice of the $*$-scalar product was made, and that choice in turn determined the projection π_*. In particular, the set $\{\pi_* y_{\chi(1)},\,\ldots,\,\pi_* y_{\chi(s)}\}$ might be independent of one choice of $*$-scalar product but dependent on another. Recall that a reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ is *normal* if for *each* choice of $*$-scalar product there exists $\eta \in$

$\mathbb{R}^{\mathscr{R}}$, depending perhaps on "$*$," such that $T_{*\eta}$ is nonsingular. *Thus, the network is normal only if for* each *choice of "$*$" there is a reaction combination* $\chi \in C(\mathscr{R}, s)$*, depending perhaps on "$*$," such that* (10.4) *holds.* (It is easy to see that the network will, in fact, be normal if this condition is satisfied.)

Note that each $\chi \in C(\mathscr{R}, s)$ can be associated in an obvious way with a subnetwork of $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ having s reactions. Hereafter, we let

$$D_*(\chi) := \det{}_S\,[\pi_* y_{\chi(1)}, \ldots, \pi_* y_{\chi(s)}]\,\det{}_S[y'_{\chi(1)} - y_{\chi(1)}, \ldots, y'_{\chi(s)} - y_{\chi(s)}]. \tag{10.6}$$

Again, the subscript "$*$" is intended to serve as reminder that the value of $D_*(\chi)$ depends on the choice of $*$-scalar product in $\mathbb{R}^{\mathscr{S}}$.

DEFINITION 10.1. *Let* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *be a reaction network with an* s*-dimensional stoichiometric subspace. Relative to a choice of* $*$*-scalar product in* $\mathbb{R}^{\mathscr{S}}$*, we say that* $\chi \in C(\mathscr{R}, s)$ *is a* $*$-critical subnetwork *of* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *if* $D_*(\chi) \neq 0$*; the subnetwork is* positive (*resp.,* negative) *if* $D_*(\chi)$ *is positive* (*negative*).

In language we now have available, we can summarize some of the preceding discussion in the following proposition.

PROPOSITION 10.2. *A reaction network is normal if and only if for each choice of* $*$*-scalar product there exists at least one* $*$*-critical subnetwork. In particular, a weakly reversible network will have at least one* $*$*-critical subnetwork for each choice of* $*$*-scalar product.*

The following proposition connects injectivity of a reaction network to the existence and properties of its $*$-critical subnetworks.

PROPOSITION 10.3. *A reaction network is injective if and only if, for each choice of* $*$*-scalar product, both of the following conditions are satisfied:* (i) *there exists at least one* $*$*-critical subnetwork, and* (ii) *all* $*$*-critical subnetworks are of the same sign. In particular, a normal reaction network can fail to be injective only if, for some* $*$*-scalar product, there is a positive and a negative* $*$*-critical subnetwork.*

Proof. For reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ having an s-dimensional stoichiometric subspace, consider a fixed but arbitrary choice of $*$-scalar product in $\mathbb{R}^{\mathscr{S}}$. Injectivity then is equivalent to the requirement that, for each $\eta \in \mathbb{R}^{\mathscr{R}}_+$, we have $\det T_{*\eta} \neq 0$. Combining (10.3) and (10.6), we can write

$$\det T_{*\eta} = \sum_{\chi \in C(\mathscr{R}, s)} \left(\prod_{i=1}^{s} \eta_{\chi(i)} \right) D_*(\chi). \tag{10.7}$$

If both conditions of the proposition statement are satisfied, it is clear that the sum on the right will be nonzero (and will have the common sign of the critical subnetworks). In this case the network is injective.

If, on the other hand, condition (i) is not satisfied, then the sum must be zero, and we will, in fact, have $\det T_{*\eta} = 0$ for *all* $\eta \in \mathbb{R}^{\mathscr{R}}_+$. Suppose, then, that condition (i) is satisfied but condition (ii) is not. Then there are critical subnetworks χ^+ and χ^- such that $D_*(\chi^+) > 0$ and $D_*(\chi^-) < 0$. Now choose $\eta^+ \in \mathbb{R}^{\mathscr{R}}_+$ as follows: $\eta^+_{y \to y'} := \theta$ for all $y \to y' \in \chi^+$ and $\eta^+_{y \to y'} := 1$ for all $y \to y' \notin \chi^+$. In this case, $\det T_{*\eta^+}$ becomes an s-order polynomial in θ, with leading coefficient $D_*(\chi^+) > 0$. Thus, by choosing θ sufficiently large, we can ensure that $\det T_{*\eta^+}$ is positive, and we suppose that θ has been chosen in this way. In a very similar manner (by replacing χ^+ with χ^-), we can construct $\eta^- \in \mathbb{R}^{\mathscr{R}}_+$ to ensure that $\det T_{*\eta^-}$ is negative. Then, along a line segment in $\mathbb{R}^{\mathscr{R}}_+$ connecting η^+ to η^-, there will exist η^0 such that $\det T_{*\eta^0} = 0$, in which case the network is not injective. ∎

In the next section we connect the normality of an entrapped-species network to the normality of the entrapped-species projection of its true chemical reaction network. Then we will be in a position to complete the proof of Theorem 8.2.

11. An entrapped-species network is normal if its true chemistry entrapped-species projection is normal. Throughout this section, we consider an entrapped species network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$, and we denote by $\{\mathscr{S}, \mathscr{C}_\mathscr{E}, \mathscr{R}_\mathscr{E}\}$ the entrapped-species projection of the true network, $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$. Moreover, we suppose that $\{\mathscr{S}, \mathscr{C}_\mathscr{E}, \mathscr{R}_\mathscr{E}\}$ is normal. Our aim is to show that the original entrapped species network is also normal. We denote by S the stoichiometric subspace of the entrapped-species network, and by $S_\mathscr{E}$ the stoichiometric subspace of the entrapped-species projection. Recall that $\Gamma_\mathscr{M}$ and $\Gamma_\mathscr{E}$ are the sets of vectors in $\mathbb{R}^\mathscr{S}$ having supports in, respectively, $\mathscr{M}$ and $\mathscr{E}$. Thus, $\mathbb{R}^\mathscr{S} = \Gamma_\mathscr{M} \oplus \Gamma_\mathscr{E}$. Recall also that $M\colon \mathbb{R}^\mathscr{S} \to \Gamma_\mathscr{M}$ and $E\colon \mathbb{R}^\mathscr{S} \to \Gamma_\mathscr{E}$ are the corresponding projections.

LEMMA 11.1. *$S = S_\mathscr{E} \oplus \Gamma_\mathscr{M}$. In particular,* $\dim S = \dim S_\mathscr{E} + \#(\mathscr{M})$.

Proof. We first note that $S = \operatorname{span}\{y' - y \in \mathbb{R}^\mathscr{S}\colon\ y \to y' \in \mathscr{R}_t\} + \Gamma_\mathscr{M}$. On the other hand, for each $y \to y' \in \mathscr{R}_t$ we have $y' - y = E(y' - y) + M(y' - y)$, from which it follows that $S = \operatorname{span}\{E(y' - y) \in \mathbb{R}^\mathscr{S}\colon\ y \to y' \in \mathscr{R}_t\} \oplus \Gamma_\mathscr{M}$. But $S_\mathscr{E} = \operatorname{span}\{E(y' - y) \in \mathbb{R}^\mathscr{S}\colon\ y \to y' \in \mathscr{R}_t\}$. ∎

Remark 11.1. From Lemma 11.1 it follows that, when $\mathbb{R}^\mathscr{S}$ is given a scalar product "$*$" as in (3.10),

$$S^{\perp^*} = S_\mathscr{E}^{\perp^*} \cap \Gamma_\mathscr{M}^{\perp^*} = S_\mathscr{E}^{\perp^*} \cap \Gamma_\mathscr{E}. \tag{11.1}$$

Now consider a vector $x \in \Gamma_\mathscr{E}$. Viewed as a member of the vector space $\Gamma_\mathscr{E}$ (with scalar product inherited from $\mathbb{R}^\mathscr{S}$), x has a certain projection onto $S_\mathscr{E}$ along $S_\mathscr{E}^{\perp^*} \cap \Gamma_\mathscr{E}$. Viewed as a member of $\mathbb{R}^\mathscr{S}$, x has a certain projection onto S along $S^{\perp^*}$. Lemma 11.1 and (11.1) ensure that those two projections are identical.

LEMMA 11.2. *Let "$*$" be a scalar product in $\mathbb{R}^\mathscr{S}$ given by* (3.10)*, and let $\pi_*\colon \mathbb{R}^\mathscr{S} \to S$ be the projection onto S along $S^{\perp^*}$. Then, for each $x \in \mathbb{R}^\mathscr{S}$, $E\pi_* x = \pi_* E x$.*

Proof. First we note that $S^{\perp^*}$ is contained in $\Gamma_\mathscr{E}$. In fact, let v be a member of $S^{\perp^*}$, and let m be a member of $\mathscr{M}$. Because m is also a member of S, we must have $m * v = 0$. Then, from (3.10), it follows that $v_m = 0$. (That $S^{\perp^*}$ is contained in $\Gamma_\mathscr{E}$ also follows from (11.1).)

Now let x be a member of $\mathbb{R}^\mathscr{S}$. It has a representation as the sum of a vector $\sigma \in S$ and a vector $\nu \in S^{\perp^*}$. In fact, by virtue of Lemma 11.1, we can write $\sigma = \sigma_\mathscr{E} + \sigma_\mathscr{M}$, with $\sigma_\mathscr{E} \in S_\mathscr{E}$. Note that $\sigma_\mathscr{E}$ and $\sigma_\mathscr{M}$ are both members of S. Thus, we have

$$x = \sigma_\mathscr{E} + \sigma_\mathscr{M} + \nu, \tag{11.2}$$

whereupon

$$\pi_* x = \sigma_\mathscr{E} + \sigma_\mathscr{M} \quad \text{and} \quad E x = \sigma_\mathscr{E} + \nu. \tag{11.3}$$

From this it follows that $E\pi_* x = \pi_* E x = \sigma_\mathscr{E}$. ∎

PROPOSITION 11.3. *An entrapped species network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ is normal if the entrapped species projection of $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$ is normal.*

Proof. We suppose that $\{\mathscr{S}, \mathscr{C}_\mathscr{E}, \mathscr{R}_\mathscr{E}\}$, the entrapped species projection of $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t\}$, is normal. As usual, S is the stoichiometric subspace for the entrapped species network, and $s = \dim S$. We denote by p the number of species in $\mathscr{M}$, and by $\bar{s}$ the dimension of $S_\mathscr{E} \subset \Gamma_\mathscr{E}$, the stoichiometric subspace of $\{\mathscr{S}, \mathscr{C}_\mathscr{E}, \mathscr{R}_\mathscr{E}\}$. Thus, from Lemma 11.1, $s = \bar{s} + p$.

Now let "$*$" be a scalar product in $\mathbb{R}^{\mathscr{S}}$ as in (3.10). Because $\{\mathscr{S}, \mathscr{C}_{\mathscr{E}}, \mathscr{R}_{\mathscr{E}}\}$ is normal there exists for it a $*$-critical subnetwork. In particular, there are reactions $\{Ey_i \to Ey_i'\}_{i=1,\dots,\bar{s}} \subset \mathscr{R}_{\mathscr{E}}$ (with $\{y_i \to y_i'\}_{i=1,\dots,\bar{s}} \subset \mathscr{R}_t$) such that the sets

$$\{\pi_* Ey_1, \pi_* Ey_2, \dots, \pi_* Ey_{\bar{s}}\} \quad \text{and} \quad \{Ey_1' - Ey_1, Ey_2' - Ey_2, \dots, Ey_{\bar{s}}' - Ey_{\bar{s}}\}$$

are both independent. Here π_* indicates the projection of $\Gamma_{\mathscr{E}}$ onto $S_{\mathscr{E}}$ along $S_{\mathscr{E}}^{\perp^*} \cap \Gamma_{\mathscr{E}}$. If $\{m_1, m_2, \dots, m_p\}$ are the elements of $\mathscr{M}$, it is clear that the sets

$$\{\pi_* Ey_1, \dots, \pi_* Ey_{\bar{s}}, m_1, \dots, m_p\} \quad \text{and} \quad \{Ey_1' - Ey_1, \dots, Ey_{\bar{s}}' - Ey_{\bar{s}}, m_1, \dots, m_p\}$$

are both independent and are both bases for S. (Here we view π_* as the projection of $\mathbb{R}^{\mathscr{S}}$ onto S along $S^{\perp^*}$; see Remark 11.1.) Thus, with $\det_S[\cdot, \cdot, \dots, \cdot]$ denoting a determinant function on S chosen as in section 9, we have

$$\begin{aligned} \det_S[\pi_* Ey_1, \dots, \pi_* Ey_{\bar{s}}, m_1, \dots, m_p] &= \det_S[E\pi_* y_1, \dots, E\pi_* y_{\bar{s}}, m_1, \dots, m_p] \\ &= \det_S[\pi_* y_1, \dots, \pi_* y_{\bar{s}}, m_1, \dots, m_p] \\ &\neq 0 \end{aligned} \tag{11.4}$$

and

$$\begin{aligned} \det_S[Ey_1' - Ey_1, \dots, Ey_{\bar{s}}' - Ey_{\bar{s}}, m_1, \dots, m_p] &= \det_S[y_1' - y_1, \dots, y_{\bar{s}}' - y_{\bar{s}}, m_1, \dots, m_p] \\ &\neq 0. \end{aligned} \tag{11.5}$$

From this it follows that the reactions $\{y_i \to y_i'\}_{i=1,\dots,\bar{s}} \subset \mathscr{R}_t$ taken together with reactions $\{m_1 \to 0, \dots, m_p \to 0\}$ constitute a $*$-critical subnetwork of the entrapped-species network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$, whereupon by Proposition 10.2 the entrapped-species network is normal. □

12. Completion of the proof of Theorem 8.2. Proof of Theorem 8.2 will be almost complete once we have available the following proposition.

PROPOSITION 12.1. *Suppose that $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{M}\}$ is a normal entrapped-species network that is not injective. Then the fully open network $\{\mathscr{S}, \mathscr{C}_t, \mathscr{R}_t, \mathscr{S}\}$ is not injective.*

Proof. We let $\tilde{\mathscr{R}}$ denote the reaction set $\mathscr{R}_t \cup (\mathscr{M} \to 0)$, where $\mathscr{M} \to 0 := \{m \to 0 : m \in \mathscr{M}\}$. Note that $\tilde{\mathscr{R}}$ is the set of all reactions for the entrapped-species network, apart from "feed reactions" of the form $0 \to m$, which play no role in considerations of normality or injectivity (Remarks 6.1 and 6.2). Similarly, we let $\mathscr{R}^{\#} := \tilde{\mathscr{R}} \cup (\mathscr{E} \to 0)$, where $\mathscr{E} \to 0 := \{e \to 0 : e \in \mathscr{E}\}$. Then $\mathscr{R}^{\#}$ is just the set of all reactions in the fully open network, apart from feed reactions. We denote the stoichiometric subspace for the entrapped-species network by S (= span $\{y' - y : y \to y' \in \tilde{\mathscr{R}}\}$), and we denote by s its dimension.

Because the entrapped-species network is normal but not injective, there is a $*$-scalar product relative to which there is a positive $*$-critical subnetwork $\chi^+ \in C(\tilde{\mathscr{R}}, s)$ and a negative $*$-critical subnetwork $\chi^- \in C(\tilde{\mathscr{R}}, s)$. That is, $D_*(\chi^+) > 0$ and $D_*(\chi^-) < 0$, where $D_*(\cdot)$ is given by (10.6). We denote by a the particular vector of $\mathbb{R}_+^{\mathscr{S}}$ with respect to which the $*$-scalar product is defined via (3.10).

Our aim is to show that the fully open network is not injective. With "$*$" denoting the same scalar product as indicated immediately above, we will argue that there is

some $\eta^0 \in \mathbb{R}_+^{\mathscr{R}^\#}$ such that there is a nonzero $\sigma \in \mathbb{R}^{\mathscr{S}}$ satisfying the equation

$$\sum_{y\to y'\in\mathscr{R}^\#} \eta^0_{y\to y'}(y*\sigma)(y'-y) = \sum_{y\to y'\in\tilde{\mathscr{R}}} \eta^0_{y\to y'}(y*\sigma)(y'-y) + \sum_{e\to 0\in\mathscr{E}\to 0} \eta^0_{e\to 0}(e*\sigma)(-e) = 0. \tag{12.1}$$

In fact, hereafter we make the particular choice $\eta^0_{e\to 0} = a_e$ for all $e \in \mathscr{E}$, in which case it follows from (3.10) that the second term on the right-hand side of (12.1) reduces to $-E\sigma$, the projection of $-\sigma$ onto $\Gamma_{\mathscr{E}}$.

With the $\eta^0_{e\to 0}$ chosen in this way, we want to show that it is possible to choose $\{\eta^0_{y\to y'}\}_{y\to y'\in\tilde{\mathscr{R}}}$, all positive, in such a way as to ensure the existence of nonzero $\sigma \in S$ satisfying (12.1). (Note the special focus on σ *in* S, the stoichiometric subspace for the entrapped-species network.) With this in mind, we consider only $\eta \in \mathbb{R}_+^{\mathscr{R}^\#}$ satisfying the requirement that, for each $e \in \mathscr{E}$, $\eta_{e\to 0} = \eta^0_{e\to 0} = a_e$. For each such η we define the map $\bar{T}_{*\eta} : S \to S$ by

$$\bar{T}_{*\eta}\,\sigma := \sum_{y\to y'\in\tilde{\mathscr{R}}} \eta_{y\to y'}(y*\sigma)(y'-y) - E\sigma \tag{12.2}$$

$$= \sum_{y\to y'\in\tilde{\mathscr{R}}} \eta_{y\to y'}(y*\sigma)(y'-y) + \sum_{e\to 0\,\in\,\mathscr{E}\to 0} \eta^0_{e\to 0}(e*\sigma)(-e) \tag{12.3}$$

$$= \sum_{y\to y'\in\tilde{\mathscr{R}}} \eta_{y\to y'}(\pi_* y*\sigma)(y'-y) + \sum_{e\to 0\,\in\,\mathscr{E}\to 0} \eta^0_{e\to 0}(\pi_* e*\sigma)(-\pi_* e). \tag{12.4}$$

Here, as before, $\pi_*{:}\mathbb{R}^{\mathscr{S}} \to S$ is the projection onto S along $S^{\perp^*}$. (To see that $\bar{T}_{*\eta}$ does indeed take values in S, note that the first term in (12.2) clearly takes values in S. Note also that $\sigma = E\sigma + M\sigma$. Because $M\sigma$ is a member of S, it follows that if σ is a member of S, so is $E\sigma$. In particular, for $\sigma \in S$, we have $E\sigma = \pi_* E\sigma$, which, in part, gives rise to the last term in (12.4).)

We seek to establish that, for some choice of η, the map $\bar{T}_{*\eta} : S \to S$ becomes singular. For this purpose, we will calculate $\det \bar{T}_{*\eta}$ with the help of Proposition 9.1, whereby we make these identifications: First, we take $U = S$ and $p = s$. Then, for $y \to y' \in \mathscr{R}^\# = \tilde{\mathscr{R}} \cup (\mathscr{E} \to 0)$ we choose $v_{y\to y'}$ and $u_{y\to y'}$ as follows: For $y \to y' \in \tilde{\mathscr{R}}$ we take $v_{y\to y'} = \pi_* y$ and $u_{y\to y'} = y' - y$. For $e \to 0 \in \mathscr{E} \to 0$ we take $v_{e\to 0} = \pi_* e$ and $u_{e\to 0} = -\pi_* e$. It remains understood that, for each $e \in \mathscr{E}$, $\eta_{e\to 0}$ is taken to be $\eta^0_{e\to 0} = a_e$. With $\det_S[\cdot,\cdot,\ldots,\cdot]$ denoting a determinant function on S chosen as in section 9, it follows from Proposition 9.1 that

$$\det \bar{T}_{*\eta} = \sum_{\chi\,\in\,C(\tilde{\mathscr{R}},\,s)} \left(\prod_{i=1}^{s} \eta_{\chi(i)}\right) D_*(\chi) + \sum_{\chi\,\in\,C(\mathscr{R}^\#,\,s)\,\smallsetminus\,C(\tilde{\mathscr{R}},\,s)} \left(\prod_{i=1}^{s} \eta_{\chi(i)}\right) \det_{\mathrm{S}}[v_{\chi(1)},\ldots,v_{\chi(s)}]\,\det_{\mathrm{S}}[u_{\chi(1)},\ldots,u_{\chi(s)}], \tag{12.5}$$

where again $D_*(\cdot)$ is given by (10.6).

Hereafter, the proof resembles that of Proposition 10.3: Choose $\eta^+ \in \mathbb{R}_+^{\mathscr{R}^\#}$ as follows: $\eta^+_{y\to y'} := \theta$ for all $y \to y' \in \chi^+$; $\eta^+_{y\to y'} := 1$ for all $y \to y' \in \tilde{\mathscr{R}} \smallsetminus \chi^+$; and

$\eta^+_{e\to 0} := \eta^0_{e\to 0} = a_e$ for all $e \to 0 \in \mathscr{E} \to 0$. Then, $\det \bar{T}_{*\eta^+}$ becomes an s-order polynomial in θ, with leading coefficient $D_*(\chi^+) > 0$. By choosing θ sufficiently large, we can ensure that $\det \bar{T}_{*\eta^+}$ is positive. Similarly, by exploiting the existence of χ^-, we can construct $\eta^- \in \mathbb{R}^{\mathscr{R}^\#}_+$ to ensure that $\det \bar{T}_{*\eta^-}$ is negative. Then, along the line segment in $\mathbb{R}^{\mathscr{R}^\#}_+$ connecting η^+ to η^- there will exist η^0 such that $\det \bar{T}_{*\eta^0} = 0$, in which case the network is not injective. In fact, with η^0 chosen in this way, there is a nonzero $\sigma \in S$ that satisfies (12.1). □

Theorem 8.2 is then a consequence of Propositions 11.3 and 12.1 taken together. □

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