

**Multiple Equilibria in Complex Reaction Networks:
III. Extensions to Entrapped Species Models**

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Abstract: In two earlier papers, means were provided to decide the capacity of complex chemical reaction networks, taken with mass action kinetics, to admit multiple equilibria in the context of the isothermal homogeneous continuous flow stirred tank reactor (CFSTR). In such a reactor, all species are deemed to be in the outflow, a fact which has an important bearing on the nature of the governing equations. On the other hand, one can imagine CFSTR-like models of the cell in which certain large molecules (e.g., enzymes) remain entrapped within the cell while smaller ones (e.g., metabolites) are free to diffuse through the cell boundary. Although such models bear a strong physical resemblance to the classical CFSTR picture, there are substantive differences in the corresponding mathematics. Without a presumption of mass action kinetics, this paper is intended to indicate a general way in which results about uniqueness of equilibria in the classical CFSTR context extend to entrapped species models.

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1. Introduction. In two earlier papers^{1,2} we developed means to determine whether a given complex reaction network, taken with mass action kinetics, has the capacity to exhibit more than one steady state. That is, our interest was in whether, for the network, there exist parameter values such that the corresponding isothermal mass action differential equations admit two distinct rest points. (This is surprisingly uncommon.) In the first paper, the theory led to a test that lends itself to computational implementation, while, in the second paper, the capacity for multiple steady states was tied more directly to subtle aspects of reaction network structure (as revealed in the network's species-reaction graph).

In both papers, it was understood that the reactions were taking place in the context of what chemical engineers call a continuous flow stirred tank reactor (CFSTR). In particular, the reacting mixture was presumed to be an incompressible liquid, filling a perfectly-stirred vessel maintained at constant temperature and volume. (We assume isothermal incompressible mixtures throughout.) Moreover, it was supposed that at least certain reactants were fed to the vessel at constant rate and that all species were removed from the vessel at rates proportional to their molar concentrations within the vessel. (In the normal chemical engineering context, mixture is withdrawn from the vessel at a constant volumetric flow rate, which is identical to the volumetric flow rate of the feed stream. The molar removal rate of a particular species, therefore, is just the volumetric flow rate of the outflow stream multiplied by the molar per-unit-volume concentration of that species within the vessel.)

Our aim here is to extend results in the earlier papers to settings that are only slightly different physically from the classical CFSTR but that are substantively different mathematically. In particular, we want to consider variants of the continuous flow stirred tank reactor in which only certain species are in the outflow. We are motivated by consideration of CFSTR-like models for the cell in which biochemical reactions are driven by enzyme-catalysis. The presumption is that the enzyme(s) remain within the cell, neither entering it nor leaving it, while playing their catalytic role in the cell's interior repeatedly. On the other hand, small metabolites are free to cross the cell boundary, playing the role of substrates and products of the various enzyme-catalyzed reactions. We presume, as in earlier work, that the

mixture within the cell remains spatially homogeneous. Of course, this model for the cell is only a metaphor, but, given the complexities of most real biochemical networks, it is a metaphor that at least serves to isolate sources of dynamical behavior that have their origins in the chemistry itself. (Although we shall have in mind the image of the CFSTR-like cell, the same mathematics serves to describe intracellular biochemical modules [3,4] in which certain species are synthesized at constant rate while certain species are degraded at rates proportional to their concentrations. In such cases, constant-rate species synthesis plays the role of transport to the cell while species degradation plays the role of transport from the cell.)

To understand the substantive technical differences between the "entrapped enzyme" picture and the situation in which all species are free to leave the cell, it will be useful to consider the very simplest (mass-action) model of enzyme catalysis, depicted in eqn. (1). The substrate S binds to an enzyme E to form an enzyme-substrate complex SE, from which the product P is released while the enzyme returns to its original state. Suppose that these reactions occur within a spatially homogeneous cell immersed in a time-invariant ambient medium and that, in the medium, concentrations of the species are maintained at fixed values c_S^0 , c_E^0 , c_{SE}^0 and c_P^0 . We denote by c_S , c_E , c_{SE} and c_P the concentrations of the species in the cell's interior.



To begin, imagine that every species is free to diffuse through the boundary of the cell and that the net molar transfer rate of each species to the cell from the ambient medium is proportional to the concentration difference of that species across the boundary. Thus, for example, the net molar transfer rate (per unit cell volume) of species S to the cell from the ambient medium is $\alpha_S(c_S^0 - c_S)$, where α_S is a mass transfer coefficient for species S. Taking account of the contributions of mass transfer and chemical reactions, we write the differential equations governing the species concentrations within the cell as in eqns. (2), where $k_{S+E \rightarrow SE}$, $k_{SE \rightarrow S+E}$ and $k_{SE \rightarrow P+E}$ are the mass action rate constants for the corresponding reactions. (Note that eqns.(2) become those of the classical continuous flow stirred tank reactor if all the mass transfer coefficients are set to g/V , where V is the volume of the reacting mixture and g is the volumetric flow rate of the outflow and feed streams.)

$$\begin{aligned}
\dot{c}_S &= \alpha_S(c_S^0 - c_S) - k_{S+E \rightarrow SE}c_Sc_E + k_{SE \rightarrow S+E}c_{SE} \\
\dot{c}_E &= \alpha_E(c_E^0 - c_E) - k_{S+E \rightarrow SE}c_Sc_E + (k_{SE \rightarrow S+E} + k_{SE \rightarrow P+E})c_{SE} \\
\dot{c}_{SE} &= \alpha_{SE}(c_{SE}^0 - c_{SE}) + k_{S+E \rightarrow SE}c_Sc_E - (k_{SE \rightarrow S+E} + k_{SE \rightarrow P+E})c_{SE} \\
\dot{c}_P &= \alpha_P(c_P^0 - c_P) + k_{SE \rightarrow P+E}c_{SE}
\end{aligned} \tag{2}$$

To ask whether, in the context described, network (1) has the capacity for multiple positive equilibria is to ask whether there are positive values for the rate constants, mass transfer coefficients, and ambient concentrations such that the system of polynomial equations, obtained by setting the time derivatives in eqns. (2) to zero, admit two or more distinct positive solutions for c_S , c_E , c_{SE} , and c_P . In fact, theory in references [1] and [2] indicates very quickly that eqns. (2) do not have the capacity for multiple positive equilibria. (The species-reaction graph has a single cycle, and it is an s-cycle[2].)

Now consider the "entrapped enzyme" picture. That is, suppose that the cell boundary is impermeable to species E and ES. In this case, the governing differential equations become those shown in eqns. (3). Note that the equations for equilibria, obtained by setting the time derivatives to zero, become redundant; in particular, the second and third (equilibrium) equations are identical up to a change in sign. Thus, there are essentially three equilibrium equations to determine the four equilibrium concentrations, c_S , c_E , c_{SE} and c_P . In contrast to the situation for eqns. (2) there are now an uncountable number of positive equilibria, no matter what positive values the parameters take.

$$\begin{aligned}
\dot{c}_S &= \alpha_S(c_S^0 - c_S) - k_{S+E \rightarrow SE}c_Sc_E + k_{SE \rightarrow S+E}c_{SE} \\
\dot{c}_E &= - k_{S+E \rightarrow SE}c_Sc_E + (k_{SE \rightarrow S+E} + k_{SE \rightarrow P+E})c_{SE} \\
\dot{c}_{SE} &= k_{S+E \rightarrow SE}c_Sc_E - (k_{SE \rightarrow S+E} + k_{SE \rightarrow P+E})c_{SE} \\
\dot{c}_P &= \alpha_P(c_P^0 - c_P) + k_{SE \rightarrow P+E}c_{SE}
\end{aligned} \tag{3}$$

This, however, is to be expected on physical grounds. Note that from eqns. (3) we have $\dot{c}_E + \dot{c}_{SE} = 0$ so, for all t , $c_E(t) + c_{SE}(t) = c_E(0) + c_{SE}(0)$. That is, the total amount of enzyme (either with or without S bound to it) remains equal to its initial supply in the cell. Thus, two different initial supplies of enzyme in the cell cannot possibly result in the same equilibrium. The appropriate uniqueness question, then, becomes this: For a given

supply of enzyme (i.e., for a given value of $c_E(0) + c_{SE}(0)$), can there be more than one positive equilibrium? In assessing network (1)'s capacity for multiple positive equilibria in the entrapped enzyme context, we would now ask: Are there positive values of the mass transfer coefficients, the rate constants, c_S^0 , c_P^0 , and a value of the total enzyme concentration such that two or more distinct equilibria corresponding to this enzyme supply are admitted by eqs.(3)?

It should be clear, then, that there is a difference in questions appropriate to the "entrapped enzyme" picture and the picture in which all species are permitted to diffuse through the cell boundary. Some care is required in the passage from results about one situation to assertions about the other.

Some of the most important results in [1] and [2] are of the kind that assert that certain highly complex mass action networks do not have the capacity for multiple positive equilibria when all species are permitted to cross the cell boundary. That is, no matter what the parameters values are, the corresponding differential equations (exemplified by eqns.(2)) cannot admit more than one positive equilibrium. Note that these are assertions about the reaction network that gives rise to the corresponding equations, for when the kinetics is mass action the network itself determines the shape of the equations up to parameter values.

We would like to assert that, with very slight modification in their statements, these results also serve to preclude multiple positive equilibria for a wide variety of intricate enzymatic networks in the context of the "entrapped enzyme" picture, provided that uniqueness of equilibria is construed in the sense described above. That is, we consider uniqueness of equilibria consistent with fixed supplies of enzyme(s). Our aim in this article is to show that, under the very same hypotheses, results in [1] and [2] do indeed carry over unchanged to preclude multiple positive equilibria in the entrapped enzyme context, except perhaps for multiple equilibria of a highly degenerate nature. In this way, broad theory about highly complex reaction networks becomes extensible to settings more suited for cell biology.

2. Reaction Networks and Kinetic Systems. We follow the general scheme in [1],[2],[5] and [6] for describing chemical reaction networks and their kinetics. The real numbers are denoted by \mathbb{R} , the positive real numbers by \mathbb{R}_+ and the non-negative real numbers by $\overline{\mathbb{R}}_+$. If I is a finite index set, we de-

note by \mathbb{R}^I the vector space of all formal (real) linear combinations of I . Thus, an element $x \in \mathbb{R}^I$ has a representation of the form

$$x = \sum_{i \in I} x_i i ,$$

with $x_i \in \mathbb{R}$. The support of an element $x \in \mathbb{R}^I$ (denoted $\text{supp } x$) is the set of all $i \in I$ such that $x_i \neq 0$. By \mathbb{R}_+^I [resp. $\overline{\mathbb{R}}_+^I$] we mean the set of $x \in \mathbb{R}^I$ for which $x_i > 0$ [resp. $x_i \geq 0$], for all $i \in I$. We give \mathbb{R}^I the scalar product (and resulting norm topology) defined by

$$x \cdot y := \sum_{i \in I} x_i y_i .$$

By the complexes in a reaction network we mean the formal linear combinations of the species that appear at the heads and tails of the reaction arrows - for example, $S + E$, SE and $P + E$ in network (1). Thus, if \mathcal{S} is the set of species in a network (e.g., $\{S, E, SE, P\}$ in (1)), then the complexes of the network are elements of $\overline{\mathbb{R}}_+^{\mathcal{S}}$. The reactions of a network are then specified by a "reacts to" relation in the set of complexes. With this as background, we define a reaction network as follows:

Definition 2.1. A reaction network is specified by a triplet $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, where

- (i) \mathcal{S} is a finite set of species
- (ii) $\mathcal{C} \subset \overline{\mathbb{R}}_+^{\mathcal{S}}$ is a finite set of complexes
- (iii) $\mathcal{R} \subset \mathcal{C} \times \mathcal{C}$ is a "reacts to" relation in \mathcal{C} such that
 - (a) $(y, y) \notin \mathcal{R}, \forall y \in \mathcal{C}$
 - (b) for each $y \in \mathcal{C}, \exists y' \in \mathcal{C}$ such that $(y, y') \in \mathcal{R}$ or $(y', y) \in \mathcal{R}$

Elements of \mathcal{R} are the reactions of the network. We write the more suggestive $y \rightarrow y'$ in place of (y, y') if and only if $(y, y') \in \mathcal{R}$.

A composition for a mixture with species set \mathcal{S} is a specification of a molar concentration $c_{\mathcal{V}}$ for each $\mathcal{V} \in \mathcal{S}$. Thus, we can identify a composition with an element $c \in \overline{\mathbb{R}}_+^{\mathcal{S}}$. A kinetics for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is an assignment to each reaction $y \rightarrow y' \in \mathcal{R}$ of a non-negative-real-valued rate function $\mathcal{K}_{y \rightarrow y'}(\cdot)$ with domain $\overline{\mathbb{R}}_+^{\mathcal{S}}$. For each composition $c \in \overline{\mathbb{R}}_+^{\mathcal{S}}$, $\mathcal{K}_{y \rightarrow y'}(c)$ is interpreted as the molar occurrence rate per unit volume of reaction $y \rightarrow y'$ when the mixture has composition c . Hereafter, we suppose that rate functions are continuously differentiable on $\overline{\mathbb{R}}_+^{\mathcal{S}}$. (Although it will not be important to this article, it is natural to require that, for each $y \rightarrow y' \in \mathcal{R}$, $\mathcal{K}_{y \rightarrow y'}(c)$ be strictly positive precisely when $\text{supp } y \subset \text{supp } c$ - that is, precisely when the composition c contains at nonzero concentrations those species that appear in the reactant complex y .) By a kinetic system, which we indicate symbolically as $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, we mean a reaction network taken together with a kinetics.

Example. A mass action kinetics for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is a kinetics having the following property: For each $y \rightarrow y' \in \mathcal{R}$, there is a positive rate constant $k_{y \rightarrow y'}$ such that

$$\mathcal{K}_{y \rightarrow y'}(c) \equiv k_{y \rightarrow y'} \prod_{\mathcal{V} \in \mathcal{S}} c_{\mathcal{V}}^{y_{\mathcal{V}}}. \quad (4)$$

Note that a mass action kinetics for a network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is specified completely by an assignment to each reaction of a positive rate constant, so we can identify a particular mass action kinetics with an element $k \in \mathbb{R}_+^{\mathcal{R}}$. With this in mind, we shall sometimes refer to the mass action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$.

Definition 2.2. For a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ the species formation rate function $r: \overline{\mathbb{R}}_+^{\mathcal{S}} \rightarrow \mathbb{R}^{\mathcal{S}}$ is defined by

$$r(c) = \sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{N}_{y \rightarrow y'}(c) (y' - y). \quad (5)$$

The interpretation of $r(\cdot)$ is as follows: In a mixture of composition c , $r_{\mathcal{J}}(c)$ is the molar production rate per unit volume of species \mathcal{J} due to the occurrence of all chemical reactions. To see this, note that

$$r_{\mathcal{J}}(c) = \sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{N}_{y \rightarrow y'}(c) (y_{\mathcal{J}'} - y_{\mathcal{J}}) \quad (6)$$

and that $y_{\mathcal{J}'} - y_{\mathcal{J}}$ is the net number of molecules of species \mathcal{J} produced with each occurrence of reaction $y \rightarrow y'$. Thus, the right side of (6) is the sum of all the per-unit-volume reaction occurrence rates, each weighted by the net gain in molecules of \mathcal{J} with each occurrence of the corresponding reaction.

Note that, for a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{N}\}$ the species-formation-rate function takes values in the span of the set

$$\{y' - y \in \mathbb{R}^{\mathcal{S}} : y \rightarrow y' \in \mathcal{R}\}. \quad (7)$$

Elements of the set (7) are the reaction vectors of the network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$. The stoichiometric subspace for a reaction network, which we denote by \mathbf{S} , is the span of its reaction vectors:

$$\mathbf{S} := \text{span}\{y' - y \in \mathbb{R}^{\mathcal{S}} : y \rightarrow y' \in \mathcal{R}\}. \quad (8)$$

For a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{N}\}$, in the context of a well-stirred mixture filling a constant-volume cell for which there is no mass transfer to or from the cell, the differential equations governing the species concentrations reduce to

$$\dot{c} = r(c), \quad (9)$$

where $r(\cdot)$ is the species-formation rate function. Note that, in this context, \dot{c} is invariably points along the stoichiometric subspace for the underlying reaction network.

3. The Entrapped Species Model. Now we consider the situation in which enzymes (and enzyme-bound substances such as SE in Section 1) are entrapped within the cell, while all other species (small metabolites) are free to diffuse across the cell boundary. To be somewhat more general, we suppose only that, for the operative reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, the species set \mathcal{S} is partitioned into two subsets, \mathcal{E} and \mathcal{M} , called the entrapped species and the mobile species, respectively. For the application we have in mind, we envision \mathcal{E} to be the enzymatic species and \mathcal{M} to be the small metabolites, but this will not be important to the mathematics. Rather, the species subset \mathcal{E} can be construed simply as the set of all members of \mathcal{S} denied passage through the cell boundary, while \mathcal{M} is the complement of \mathcal{E} in \mathcal{S} . For the simple entrapped enzyme example described in Section 1, we have $\mathcal{E} = \{E, SE\}$ and $\mathcal{M} = \{S, P\}$.

Hereafter, when we speak of an entrapped species model, it will be understood that there is a specified partition of the species set \mathcal{S} into two subsets \mathcal{E} and \mathcal{M} . We denote by $\Gamma_{\mathcal{E}}$ and $\Gamma_{\mathcal{M}}$ the linear subspaces of $\mathbb{R}^{\mathcal{S}}$ consisting of vectors having supports in \mathcal{E} and \mathcal{M} , respectively. That is,

$$\Gamma_{\mathcal{E}} := \{x \in \mathbb{R}^{\mathcal{S}} : i \notin \mathcal{E} \Rightarrow x_i = 0\} \quad \text{and} \quad \Gamma_{\mathcal{M}} := \{x \in \mathbb{R}^{\mathcal{S}} : i \notin \mathcal{M} \Rightarrow x_i = 0\}.$$

Note that $\mathbb{R}^{\mathcal{S}} = \Gamma_{\mathcal{E}} \oplus \Gamma_{\mathcal{M}}$. We denote by $P_{\mathcal{E}} : \mathbb{R}^{\mathcal{S}} \rightarrow \Gamma_{\mathcal{E}}$ and $P_{\mathcal{M}} : \mathbb{R}^{\mathcal{S}} \rightarrow \Gamma_{\mathcal{M}}$ the projections onto $\Gamma_{\mathcal{E}}$ and $\Gamma_{\mathcal{M}}$ respectively.

Consider an entrapped species model that derives from a chemistry specified by the kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{N}\}$, with $\mathcal{S} = \mathcal{E} \sqcup \mathcal{M}$. Formulation of the corresponding entrapped species differential equations (analogous to eqs.(3)) requires specification of certain additional parameters apart from the kinetics - in particular, a mass transfer coefficient $\alpha_m > 0$ and an ambient molar concentration $c_m^0 \geq 0$ for each species $m \in \mathcal{M}$. In effect, then, specification of an entrapped species model amounts to specification of a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{N}\}$, a partition of \mathcal{S} into \mathcal{E} and \mathcal{M} , and specification of two elements $\alpha \in \mathbb{R}_+^{\mathcal{M}}$ and $c^0 \in \overline{\mathbb{R}}_+^{\mathcal{M}}$.

Taking account of both chemical reactions and diffusive fluxes across the cell boundary, the entrapped-species-model differential equations governing the concentrations within the cell become

$$\dot{c} = g(c) \quad (10)$$

where $g(\cdot) : \bar{\mathbb{R}}_+^{\mathcal{S}} \rightarrow \mathbb{R}^{\mathcal{S}}$ is defined by

$$\begin{aligned} g(c) &:= r(c) + \sum_{m \in \mathcal{M}} \alpha_m (c_m^0 - c_m) m \\ &= \sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{H}_{y \rightarrow y'}(c) (y' - y) + \sum_{m \in \mathcal{M}} \alpha_m (c_m^0 - c_m) m. \end{aligned} \quad (11)$$

Note that $g(\cdot)$ takes values in the linear subspace

$$\bar{\mathcal{S}} := \text{span} (\{y' - y \in \mathbb{R}^{\mathcal{S}} : y \rightarrow y' \in \mathcal{R}\} \cup \mathcal{M}) = \mathcal{S} + \Gamma_{\mathcal{M}}. \quad (12)$$

For the entrapped species model, then, \dot{c} is no longer constrained to lie in the stoichiometric subspace for the underlying reaction network (as in the closed cell situation). Rather, \dot{c} is constrained to lie in the somewhat larger linear subspace $\bar{\mathcal{S}}$. Still, $\bar{\mathcal{S}}$ will typically remain a proper subspace of $\mathbb{R}^{\mathcal{S}}$, for the reaction vectors will normally reflect certain intracellular conservation conditions among the entrapped (enzymatic) species - conditions that remain operative despite the transport of the mobile species (metabolites) across the cell boundary.

Example. Consider the simple entrapped enzyme example discussed in Section 1. For network (1), the reaction vectors are

$$\{\text{ES} - \text{E} - \text{S}, \text{E} + \text{S} - \text{ES}, \text{P} + \text{E} - \text{ES}\}.$$

Moreover, $\mathcal{E} = \{\text{E}, \text{ES}\}$ and $\mathcal{M} = \{\text{S}, \text{P}\}$. Thus,

$$\bar{\mathcal{S}} := \text{span}\{\text{ES} - \text{E} - \text{S}, \text{E} + \text{S} - \text{ES}, \text{P} + \text{E} - \text{ES}, \text{S}, \text{P}\} \subset \mathbb{R}^{\mathcal{S}}.$$

Although $\dim \mathbb{R}^{\mathcal{S}} = 4$, it is not difficult to see that $\dim \bar{\mathcal{S}} = 3$, so $\bar{\mathcal{S}}$ is a proper linear subspace of $\mathbb{R}^{\mathcal{S}}$. In fact, it is apparent that $\text{E} + \text{ES} \in \mathbb{R}^{\mathcal{S}}$ is or-

thogonal to $\bar{\mathcal{S}}$. This orthogonality, taken together with the fact that \dot{c} takes values in $\bar{\mathcal{S}}$, imply that $\dot{c}_E + \dot{c}_{ES} = 0$, which in turn reflects the constancy of $c_E + c_{ES}$ along solutions of eq.(10).

Because \dot{c} invariably points along $\bar{\mathcal{S}}$, it is not difficult to see that a composition c' can evolve from a composition c only if $c' - c$ is contained in $\bar{\mathcal{S}}$. From (12) it follows that this condition is satisfied precisely when $P_{\mathcal{E}}(c' - c) \in P_{\mathcal{E}}(S)$, the latter being the projection of the stoichiometric subspace into $\Gamma_{\mathcal{E}}$. This is to say that the composition change reflected in $c' - c$ must, for the entrapped species, derive only from the occurrence of chemical reactions. These considerations motivate the following definition.

Definition 3.1. Consider an entrapped species model in which the underlying reaction network is $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, with $\mathcal{S} = \mathcal{E} \sqcup \mathcal{M}$, and let $\bar{\mathcal{S}}$ be as in eq.(12). Two compositions c and c' in $\bar{\mathbb{R}}_+^{\mathcal{S}}$ are entrapped-species-compatible (denoted $c \approx c'$) if $c' - c$ lies in $\bar{\mathcal{S}}$. The equivalence relation \approx serves to partition $\bar{\mathbb{R}}_+^{\mathcal{S}}$ into entrapped-species-compatibility classes. (These are parallels of $\bar{\mathcal{S}}$, restricted to $\bar{\mathbb{R}}_+^{\mathcal{S}}$.)

Compositions along a solution of eq. (10) that begins within a particular entrapped-species-compatibility class lie entirely within the same compatibility class. Indeed, each such compatibility class is the union of composition trajectories, and one can associate a flow, deriving from eq. (10), with each class. Each of the various compatibility classes will usually have one or more equilibria of its own. Thus, questions about the existence of multiple equilibria should properly be construed as questions about the existence of more than one equilibrium within a compatibility class.

An equilibrium of a vector field is sometimes said to be degenerate if the derivative of the vector field at that equilibrium is singular. Without some qualification, every positive equilibrium of eq. (10) would typically be degenerate for the following reason: An equilibrium composition, say c^* ,

within a particular compatibility class will typically lie on a manifold of equilibria, each nearby point of which corresponds to an equilibrium within a different (nearby) compatibility class. Thus, $dg(c^*)$, the derivative of g at c^* , will be singular, for it will have in its kernel a vector tangent to the equilibrium manifold at c^* . For the situation at hand, then, a more appropriate notion of degeneracy would require that the singularity correspond to directions along the compatibility class containing c^* , not transverse to it. With this in mind we posit the following definition:

Definition 3.2. For an entrapped species model, an equilibrium $c^* \in \mathbb{R}_+^{\mathcal{S}}$ of eq. (10) is nondegenerate if $(\ker dg(c^*)) \cap \bar{\mathcal{S}} = \{0\}$; otherwise, c^* is degenerate. We say that the model admits multiple nondegenerate equilibria if there are at least two distinct nondegenerate equilibria, say c^* and $c^{**} \in \mathbb{R}_+^{\mathcal{S}}$, such that $c^{**} - c^* \in \bar{\mathcal{S}}$. (The last requirement ensures that c^* and c^{**} are entrapped-species-compatible.)

4. The Fully Diffusive Model. Here we consider a chemistry described by the kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{N}\}$, but now we imagine that all species are permitted passage through the cell boundary. For an ambient-medium composition $\bar{c}^0 \in \bar{\mathbb{R}}_+^{\mathcal{S}}$ and a specification of mass transfer coefficients $\bar{\alpha} \in \mathbb{R}_+^{\mathcal{S}}$, the fully diffusive model differential equations, analogous to eqns. (2), take the form

$$\dot{c} = h(c), \tag{13}$$

where

$$h(c) := \sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{N}_{y \rightarrow y'}(c) (y' - y) + \sum_{\mathcal{S} \in \mathcal{S}} \bar{\alpha}_{\mathcal{S}} (\bar{c}_{\mathcal{S}}^0 - c_{\mathcal{S}}). \tag{14}$$

In contrast to the situation for the entrapped species model, the range of $h(\cdot)$ will not usually be contained in a proper linear subspace of $\mathbb{R}^{\mathcal{S}}$. In this case, there will often be just one equilibrium in all of $\mathbb{R}_+^{\mathcal{S}}$, and, when there are more than one positive equilibrium, these will typically be few in number. This is different from the entrapped species model, for which manifolds of equilibria typically pass through the different entrapped-species-compatibility classes transversely.

5. A Relationship between the Entrapped Species Model and the Fully Diffusive Model. Our aim in this section is to prove a theorem that will extend results in [1] and [2] to entrapped species models.

Theorem 5.1. Suppose that, for a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, there are no values of $\bar{c}^0 \in \bar{\mathbb{R}}_+^{\mathcal{S}}$ and $\bar{\alpha} \in \mathbb{R}_+^{\mathcal{S}}$ such that the fully diffusive differential equations (13)-(14) admit multiple positive equilibria. Then, for a specified partition $\mathcal{S} = \mathcal{E} \sqcup \mathcal{M}$, there are no values $\alpha \in \mathbb{R}_+^{\mathcal{M}}$ and $c^0 \in \bar{\mathbb{R}}_+^{\mathcal{M}}$ such that the entrapped-species differential equations (10)-(11) admit multiple nondegenerate (positive) equilibria in the sense of Definition 3.2.

Proof. Suppose on the contrary that for $\alpha \in \mathbb{R}_+^{\mathcal{M}}$ and $c^0 \in \bar{\mathbb{R}}_+^{\mathcal{M}}$ equations (10)-(11) admit multiple nondegenerate equilibria. That is, suppose that there are $c^*, c^{**} \in \mathbb{R}_+^{\mathcal{S}}$ such that

$$c^{**} - c^* \in \bar{\mathcal{S}}, \quad (15)$$

$$g(c^*) = 0, \quad g(c^{**}) = 0, \quad (16)$$

$$(\ker dg(c^*)) \cap \bar{\mathcal{S}} = \{0\}, \text{ and } (\ker dg(c^{**})) \cap \bar{\mathcal{S}} = \{0\}. \quad (17)$$

Let $c^*_{\mathcal{E}}$ be the projection of c^* into $\Gamma_{\mathcal{E}}$. Because $\text{supp}(c^* - c^*_{\mathcal{E}})$ is contained in \mathcal{M} and because $\Gamma_{\mathcal{M}}$ is contained in $\bar{\mathcal{S}}$, it follows that $c^* - c^*_{\mathcal{E}}$ is contained in $\bar{\mathcal{S}}$. Now we define $\tilde{h}(\cdot, \cdot): \mathbb{R}_+^{\mathcal{S}} \times \mathbb{R} \rightarrow \mathbb{R}^{\mathcal{S}}$ by

$$\begin{aligned} \tilde{h}(c, \theta) &:= g(c) + \theta \sum_{e \in \mathcal{E}} (c_e^* - c_e) e \\ &= \sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{K}_{y \rightarrow y'}(c) (y' - y) + \sum_{m \in \mathcal{M}} \alpha_m (c_m^0 - c_m) m + \theta \sum_{e \in \mathcal{E}} (c_e^* - c_e) e. \end{aligned}$$

Our aim will be to show that there is a $\theta^\dagger > 0$ and distinct \tilde{c}^* and \tilde{c}^{**} such that $\tilde{h}(\tilde{c}^*, \theta^\dagger) = 0$ and $\tilde{h}(\tilde{c}^{**}, \theta^\dagger) = 0$. This will contradict the hypothesis that the fully diffusive model does not have the capacity for multiple equilibria. (In particular the distinct positive equilibria \tilde{c}^* and \tilde{c}^{**} will correspond to the fully-diffusive-model parameter values $\bar{\alpha}_m = \alpha_m$, $\bar{c}_m^0 = c_m^0$ for

all $m \in \mathcal{M}$ and $\bar{c}_e^0 = c_e^*$, $\bar{a}_e = \theta^\dagger$, for all $e \in \mathcal{E}$.

To show the contradiction we first let Ω be a (relatively) open neighborhood of 0 in $\bar{\mathcal{S}}$ such that $c^* + \gamma$ and $c^{**} + \gamma$ lie in $\mathbb{R}_+^{\mathcal{J}}$ for all $\gamma \in \Omega$. Then we let $\tilde{h}^*: \Omega \times \mathbb{R} \rightarrow \bar{\mathcal{S}}$ be defined as follows: For all $\gamma \in \Omega$ and all $\theta \in \mathbb{R}$

$$\tilde{h}^*(\gamma, \theta) = g(c^* + \gamma) + \theta \sum_{e \in \mathcal{E}} (c_e^* - (c_e^* + \gamma_e)) e$$

That $\tilde{h}(\cdot, \cdot)$ does indeed take values in $\bar{\mathcal{S}}$ can be seen in the following way: Note that the sum following θ is the same as $P_{\mathcal{M}}(\gamma) - \gamma$. Recall that $P_{\mathcal{M}}(\cdot)$ takes values in $\Gamma_{\mathcal{M}}$, which in turn is contained in $\bar{\mathcal{S}}$. Since each γ takes values in $\bar{\mathcal{S}}$, so then does $P_{\mathcal{M}}(\gamma) - \gamma$. Finally, recall that $g(\cdot)$ takes values in $\bar{\mathcal{S}}$.

By supposition

$$\tilde{h}^*(0, 0) = g(c^*) = 0.$$

Moreover, $d_\gamma \tilde{h}^*(0, 0) \sigma = dg(c^*) \sigma$, $\forall \sigma \in \bar{\mathcal{S}}$. From this and eq. (17) it follows that $d_\gamma \tilde{h}^*(0, 0)$ is nonsingular. From the Implicit Function Theorem, then, there is a $\theta^* > 0$ such that, for all θ in the interval $(-\theta^*, \theta^*)$, there exists a $\gamma^*(\theta)$ satisfying (18), where $\tilde{c}^*(\theta) = c^* + \gamma^*(\theta)$.

$$0 = \tilde{h}^*(\gamma^*(\theta), \theta) = g(c^* + \gamma^*(\theta)) + \theta \sum_{e \in \mathcal{E}} (c_e^* - (c_e^* + \gamma_e^*(\theta))) e = \tilde{h}(\tilde{c}^*(\theta), \theta) \quad (18)$$

Now let $\tilde{h}^{**}: \Omega \times \mathbb{R} \rightarrow \bar{\mathcal{S}}$ be defined as follows: For all $\gamma \in \Omega$ and all $\theta \in \mathbb{R}$

$$\tilde{h}^{**}(\gamma, \theta) = g(c^{**} + \gamma) + \theta \sum_{e \in \mathcal{E}} (c_e^* - (c_e^{**} + \gamma_e)) e.$$

In this case, to see that $\tilde{h}^{**}(\cdot, \cdot)$ does indeed take values in $\bar{\mathcal{S}}$ is slightly more complicated. Note that

$$\sum_{e \in \mathcal{E}} (c_e^* - (c_e^{**} + \gamma_e)) e = \sum_{e \in \mathcal{E}} (c_e^* - c_e^{**}) e - \sum_{e \in \mathcal{E}} \gamma_e e \quad (19)$$

As before, the second sum on the right of (19) is a member of $\bar{\mathcal{S}}$. On the other hand, the first term on the right is identical to $(c^* - c^{**}) - P_{\mathcal{M}}(c^* - c^{**})$. By supposition $c^* - c^{**}$ is a member of $\bar{\mathcal{S}}$. Moreover, $P_{\mathcal{M}}(\cdot)$ take values in $\Gamma_{\mathcal{M}}$, which is contained in $\bar{\mathcal{S}}$. All of this, taken with the fact that $g(\cdot)$ takes values in $\bar{\mathcal{S}}$, implies that $\tilde{h}^{**}(\cdot, \cdot)$ takes values in $\bar{\mathcal{S}}$.

Then we can argue, just as we argued for $\tilde{h}^*(\cdot, \cdot)$, that there is a $\theta^{**} > 0$ such that, for all θ in the interval $(-\theta^{**}, \theta^{**})$, there exists a $\gamma^{**}(\theta)$ satisfying

$$\begin{aligned} 0 &= \tilde{h}^{**}(\gamma^{**}(\theta), \theta) \\ &= g(c^{**} + \gamma^{**}(\theta)) + \theta \sum_{e \in \mathcal{E}} (c_e^* - (c_e^{**} + \gamma_e^{**}(\theta))) e = \tilde{h}(\tilde{c}^{**}(\theta), \theta), \end{aligned} \quad (20)$$

where $\tilde{c}^{**}(\theta) = c^{**} + \gamma^{**}(\theta)$.

By choosing $\theta^\dagger \in (0, \theta^*) \cap (0, \theta^{**})$ we get the desired result. \square

6. A Pathological Example. Note that, when its hypothesis is satisfied, Theorem 5.1 does not entirely preclude multiple positive equilibria for the entrapped-species differential equations; rather, it denies the possibility of two distinct nondegenerate positive equilibria. Our purpose here is to show that an entrapped species model (in fact, a mass action model) can admit multiple degenerate positive equilibria even when the corresponding fully diffusive model cannot admit multiple positive equilibria of any kind. On the other hand, the example is hardly robust. Extremely slight perturbations of the example cause pathologies exhibited by it to vanish.

Consider the reaction network shown in eqns. (21). We take the kinetics to be mass action with every rate constant set to 1. With some effort it can



be shown that, for this kinetic system, the fully diffusive model gives rise to precisely one positive equilibrium for all positive choices of $\bar{c}_A^0, \dots, \bar{c}_E^0$ and $\bar{\alpha}_A, \dots, \bar{\alpha}_E$.

Now, for the same kinetic system, consider an entrapped species model with $\mathcal{E} = \{A, B, C\}$ and $\mathcal{M} = \{D, E\}$. The corresponding entrapped-species-model differential equations become those shown in eqns. (22). Moreover, it

$$\begin{aligned}
\dot{c}_A &= -2c_A + 2c_Bc_C \\
\dot{c}_B &= c_A - c_Bc_C \\
\dot{c}_C &= c_A - c_Bc_C \\
\dot{c}_D &= c_E - c_D + \alpha_D(c_D^0 - c_D) \\
\dot{c}_E &= c_D - c_E + \alpha_E(c_E^0 - c_E)
\end{aligned} \tag{22}$$

is not hard to see that the equilibria of eqns. (22) consist of all compositions satisfying eqns. (23)-(25). Our interest is in deciding whether there

$$c_A = c_Bc_C \tag{23}$$

$$c_D = c_D^{\text{eq}} := \frac{\alpha_E c_E^0 + \alpha_D c_D^0 + \alpha_E \alpha_D c_D^0}{\alpha_E + \alpha_D + \alpha_D \alpha_E} \tag{24}$$

$$c_E = c_E^{\text{eq}} := \frac{\alpha_E c_E^0 + \alpha_D c_D^0 + \alpha_E \alpha_D c_E^0}{\alpha_E + \alpha_D + \alpha_D \alpha_E} \tag{25}$$

can be more than one positive equilibrium in the same entrapped-species-compatibility class. In this case, it is not hard to see that

$$\bar{\mathbf{s}} = \text{span}\{B - A, C - A, D, E\}$$

and that two compositions c and c' are entrapped-species-compatible if and only if $c_A + c_B + c_C = c'_A + c'_B + c'_C$.

In particular, we can study the equilibria residing in the entrapped-species-compatibility class of compositions characterized by the condition

$$c_A + c_B + c_C = 1. \tag{26}$$

From eqns. (23)-(26) it follows that there are an infinite number of equilibrium compositions within this compatibility class: These trace out a curve, parameterized by $c_B \in [0, 1]$, and given by

$$c_A = \frac{c_B(1 - c_B)}{1 + c_B}, \quad c_C = \frac{1 - c_B}{1 + c_B}, \quad c_D = c_D^{\text{eq}}, \quad c_E = c_E^{\text{eq}}. \quad (27)$$

It can be confirmed, however, that all such equilibria are degenerate. In fact, if c^* is a point along the curve given by eqn. (27) then any choice of a (nonzero) vector tangent to the curve at c^* is a member of $\ker(\text{dg}(c^*)) \cap \bar{S}$.

The example itself is highly degenerate, for its capacity for multiple positive equilibria disappears completely when the reactions $A \rightarrow B$ and $A \rightarrow C$ are made very slightly reversible. In particular, consider network (28) and suppose again that the kinetics is mass action with rate constants for reactions in the original network (21) set to 1 and with rate constants for the added reactions $B \rightarrow A$ and $C \rightarrow A$ both set to a very small number ε .



In this case, the entrapped-species-model differential equations (corresponding to $\mathcal{E} = \{A, B, C\}$ and $\mathcal{M} = \{D, E\}$) become those shown in (29). It is not difficult to verify that, in contrast to the situation for $\varepsilon = 0$, the

$$\begin{aligned} \dot{c}_A &= -2c_A + 2c_Bc_C + \varepsilon(c_B + c_C) \\ \dot{c}_B &= c_A - c_Bc_C - \varepsilon c_B \\ \dot{c}_C &= c_A - c_Bc_C - \varepsilon c_C \\ \dot{c}_D &= c_E - c_D + \alpha_D(c_D^0 - c_D) \\ \dot{c}_E &= c_D - c_E + \alpha_E(c_E^0 - c_E) \end{aligned} \quad (29)$$

entrapped-species-compatibility class corresponding to $c_A + c_B + c_C = 1$ now contains precisely one positive equilibrium c^* , no matter how small the rate constant $\varepsilon > 0$ might be. In fact, the equilibrium composition c^* is given by

$$c_A^* = (c_B^*)^2 + \varepsilon c_B^*, \quad c_B^* = \frac{-(2 + \varepsilon) + \sqrt{(2 + \varepsilon)^2 + 4}}{2}, \quad c_C^* = c_B^*, \quad c_D^* = c_D^{\text{eq}}, \quad c_E^* = c_E^{\text{eq}},$$

where c_D^{eq} and c_E^{eq} are as in eqns.(24) and (25). We took the rate constants for the added reactions $B \rightarrow A$ and $C \rightarrow A$ to be equal only to indicate the pathology of the original example in a simple way. In fact, uniqueness of positive

equilibria (in the sense of Section 3) for the entrapped-species model obtains no matter what rate constants are assigned to the various reactions in network (28).

It is perhaps useful to summarize just how the example behaves: For network (21), taken with all mass action rate constants set to 1, the fully diffusive model admits precisely one positive equilibrium for all values of the mass transfer coefficients and the ambient species concentrations. On the other hand, an entrapped-species model (with $\mathcal{E} = \{A, B, C\}$ and $\mathcal{M} = \{D, E\}$) for the same kinetic system admits multiple positive equilibria (in fact, an infinite number) within the same entrapped-species-compatibility class. Nevertheless, these are all degenerate, and the behavior is not robust: The capacity for multiple positive equilibria is destroyed by tiny perturbations of the underlying kinetic system.

Remark 6.1. That the elements of \mathcal{E} and \mathcal{M} appear in separate reactions is not consequential to the example, for other similar examples can be constructed in which elements of the two sets interact. The example used here was chosen for its simplicity, in particular so that its equilibria could be calculated easily. The structural origin of the pathology inherent in the example is discussed in Appendix IV of Reference [6]. (For mass action kinetics, such pathologies do not arise when, for example, all reactions are reversible, however small some of the reverse rate constants might be.)

7. Concluding Remarks. As we indicated in the Introduction, our interest is in extending results for fully diffusive models ([1],[2]) to entrapped-species models. Theorem 5.1 does this to the extent that, when the fully diffusive model for a given kinetic system does not have the capacity for multiple positive equilibria, then neither does any entrapped-species model derived from the same kinetic system, except perhaps for degenerate multiple positive equilibria. (That is, if there are multiple positive equilibria, all but at most one are degenerate.)

It should be noted that Theorem 5.1 is written for arbitrary kinetic systems, not necessarily mass action systems. The theorem as it stands is, for the most part, adequate for our purposes. We wish to point out, however, that for mass action systems Theorem 5.1 lends itself to sharpening. In particular, it can be shown without much difficulty that for a reaction network that is injective (in the sense of [1]) the only possible way that multiple posi-

tive steady states might be exhibited is if all are degenerate. (That is, in contrast to the slightly weaker conclusion given by Theorem 5.1, not even one can be nondegenerate.) It should be kept in mind that Section 6 provides an example of a (structurally unstable) mass action system for which an entrapped-species model admits multiple (degenerate) steady states even when the fully diffusive model admits only a unique steady state. Nevertheless, it is possible to prove statements, restricted to certain broad classes of mass action systems, that are sharper than Theorem 5.1 to the extent that the denial of multiple positive equilibria in the entrapped-species model is total, unqualified by issues of degeneracy. We expect this to be the subject of a separate paper.

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