Published in final edited form as:

Math Biosci. 2015 April ; 262: 10–27. doi:10.1016/j.mbs.2015.01.002.

Sharper Graph-Theoretical Conditions for the Stabilization of Complex Reaction Networks

Daniel Knight^{a,1}, Guy Shinar^{b,2}, and Martin Feinberg^{a,c,1,*}

Daniel Knight: knight.326@osu.edu; Guy Shinar: shinarg@gmail.com; Martin Feinberg: feinberg.14@osu.edu

^aThe William G. Lowrie Department of Chemical & Biomolecular Engineering, Koffolt Laboratories, Ohio State University, Columbus, OH, USA 43210

^bJavelin Medical Ltd., 4 Pekeris St., Rehovot 76702, Israel

^cDepartment of Mathematics, Ohio State University, Columbus, OH, USA 43210

Abstract

Across the landscape of all possible chemical reaction networks there is a surprising degree of stable behavior, despite what might be substantial complexity and nonlinearity in the governing differential equations. At the same time there are reaction networks, in particular those that arise in biology, for which richer behavior is exhibited. Thus, it is of interest to understand network-structural features whose presence enforces dull, stable behavior and whose absence permits the dynamical richness that might be necessary for life. We present conditions on a network's *Species-Reaction Graph* that ensure a high degree of stable behavior, so long as the kinetic rate functions satisfy certain weak and natural constraints. These graph-theoretical conditions are considerably more incisive than those reported earlier.

Keywords

reaction network; bistability; multistability; concordant; systems biology; Species-Reaction Graph

1. Introduction

In two recent articles [1, 2] we described a subtle structural attribute, *concordance* (Definition 6.5), that enforces a degree of stable behavior for all chemical reaction networks having that attribute, so long as the kinetic rate functions satisfy certain mild constraints (e.g., weak monotonicity [1]). In some respects, the concordance condition captures completely a network's capacity for particular kinds of behavior.

*Corresponding author. Phone 614-688-4883.

¹MF and DK were supported by NSF grant EF-1038394 and NIH grant 1R01GM086881-01.

²This work was initiated while GS was in the Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

For example, it is *precisely* the concordant reaction networks for which the species-formation-rate function is injective for *all* choices of weakly monotonic kinetics.³ (Among other things, injectivity precludes the possibility of two distinct stoichiometrically compatible positive equilibria.⁴) Moreover, among the fully open reaction networks that have the capacity to admit a positive equilibrium, it is *precisely* the concordant ones for which no differentially monotonic kinetics can give rise to an instability resulting from a positive real eigenvalue. In addition, for every discordant weakly reversible [3] network there invariably exists a differentially monotonic kinetics — in fact a polynomial kinetics — that engenders an unstable positive equilibrium having a positive real eigenvalue. It was in [1] that we discussed the stability-enforcing properties of concordant networks and also the consequences of discordance.

In [2] we connected concordance of a network with properties of the network's *Species-Reaction Graph (SR Graph)*, which resembles the diagram often used for the depiction of biochemical pathways. In particular, we showed that, when a nondegenerate⁵ network's SR Graph satisfies fairly weak conditions, concordance of the network is ensured. Consequently, one can deduce directly from properties of a network's SR Graph the regular, stable behavior that derives from concordance, even in the absence of finely detailed information about the kinetics. Although the concordance of a reaction network can be decided computationally by means of easy-to-use freely available software [4, 5], the SR Graph theorems in [2] have the added virtue of providing insight into the extremely subtle network-structural features that make for concordance or discordance.

The SR Graph theorems in [2] are quite robust in the networks for which they affirm concordance. There are, however, many examples of networks for which computations, via [5], establish concordance but for which the graphical theorems in [2] are silent. (All of the examples in Section 4 are of this kind.) These examples point to the existence of graph-theoretical theorems more incisive than those provided in [2].

It is the purpose of this article to provide SR Graph theorems that subsume the earlier ones and that give concordance information about networks for which the theorems in [2] say nothing. Proofs of the broader theorems presented here turn out to be considerably simpler than the proofs of the narrower ones given in [2].

Readers interested only in the rich dynamical information carried by a network's SR Graph can proceed directly to Theorems 4.1 and 5.1 after reading Section 3 and, to a lesser extent, Section 2. Although network concordance underlies their proofs, those theorem statements make no reference to the concordance idea.

Remark 1.1: See [1] and [2] for a discussion of earlier work [6–12] that connects properties of the Species-Reaction Graph (or the Species-Complex-Linkage Graph) to qualitative

³Appendix A provides a brief review of some vocabulary from [1, 2]. For the purposes of this article, however, the most essential terminology is introduced in the main text.

⁴In fact, in the class of networks with positively dependent reaction vectors, it is precisely the discordant ones for which there exists a weakly monotonic kinetics that admits two distinct stoichiometrically compatible positive equilibria. See Appendix B.

⁵See §2 and Appendix C.

dynamics, in particular to the preclusion of multiple equilibria. Here it is worth pointing out once again that the earlier SR Graph results were confined to mass action kinetics until the surprising papers of Banaji and Craciun [11, 12].

Remark 1.2: In this paper we will impose a fairly inconsequential restriction that was also imposed in [2, 12]: It will be understood that, in connection with the SR Graph theorems, we consider only networks in which no species appears as both a reactant and a product in the same reaction. For example, we preclude from consideration a network containing the reaction $A + B \rightarrow 2A$, but we do not preclude a network containing the reactions $A + B \rightarrow C \rightarrow 2A$.

Remark 1.3: A formal definition of a *weakly monotonic kinetics* [1] for a network is provided in Appendix A. In less formal terms, weak monotonicity reflects a natural restriction on the relationship between mixture composition and the rates of a network's various reactions: For each reaction, an increase in its occurrence rate requires an increase in the concentration of at least one of its *reactant* species. Mass action kinetics provides an example of a weakly monotonic kinetics, but the weakly monotonic class is far wider. For example, the reaction $A + B \rightarrow C$ might be governed by a rate function such as

$$\frac{\alpha c_A c_B}{\beta + \gamma c_A + \delta c_B},$$

where α, β, γ , and δ are positive.

In Section 5, we will also make reference to *two-way weakly monotonic kinetics*, which is defined formally in [1] and which is similar to what Banaji and Craciun [11, 12] call NAC kinetics. The two-way weakly monotonic class of kinetics extends the weakly monotonic class to admit reaction-rate functions consistent with the possibility of product inhibition: For each reaction, an increase in its rate requires an increase in the concentration of at least one of its reactant species or a decrease in the concentration of at least one of its product species. Thus, for example, the reaction $A \rightarrow B$ might be governed by a rate function such as

$$\frac{\alpha c_A}{\beta + \gamma c_B}.$$

2. Prelude: Fully Open and Nondegenerate Networks

A reaction network is *fully open* if, for each species s in the network, there is a reaction of the form $s \rightarrow 0$ (s reacts to zero). Such a reaction is often introduced to model either the degradation of species s to inconsequential products or the physical effusion of s from the reacting mixture. (The network might also contain reactions of the form $0 \rightarrow s$ to model the synthesis or infusion of species s .)

Fully open reaction networks are, in some respects, easier to study than other networks. They have certain features that make for some simplicity in the mathematics; in particular,

constraints imposed by stoichiometry become less consequential. The *fully open extension* of a given reaction network is the network obtained by adding all reactions of the form $s \rightarrow 0$ that are not already present. In some instances, properties of a network's fully open extension are inherited by the network itself.

In fact, apart from certain degenerate networks discussed below (and more fully in Appendix C), a network is concordant if the network's fully open extension is concordant. For this reason, it is of interest to determine whether a network's fully open extension is concordant. This is so not only because fully open networks are easier to study but also because concordance of the network's fully open extension actually gives important dynamical information beyond that given by concordance of the network itself. In particular, when a network's fully open extension is concordant and when the kinetics is differentially monotonic, not only are multiple positive stoichiometrically compatible equilibria impossible for the original network, but also all real eigenvalues at any positive equilibrium are strictly negative [1].

We say that a network is *nondegenerate* if, for the network, there is *even one* choice of a differentially monotonic kinetics such that there exists *some* positive composition (*not necessarily an equilibrium*) at which the derivative of the species-formation rate function is nonsingular [2]. Otherwise, we say that the network is *degenerate*. Note that in this context nondegeneracy (or degeneracy) is a property of a *network*.

Degenerate networks make for poor models of real behavior, for they typically lack robustness. For example, a mass action model derived from a degenerate network might admit multiple stoichiometrically compatible equilibria, but the multiplicity of equilibria can disappear if the model is perturbed just slightly, say by adding the reverse of an existing reaction and assigning to it a *vanishingly small* rate constant.⁶ An example is provided in Appendix C.

The nondegenerate networks are *precisely* the ones for which concordance of the fully open extension ensures concordance of the network itself. Especially among networks that have the capacity to admit a positive equilibrium, degeneracy is rare. In fact, *every reversible network is nondegenerate* (as is every weakly reversible network), but reversibility (or, more generally, weak reversibility) is far from necessary for nondegeneracy.

Because chemists often insist that every naturally occurring network of chemical reactions is reversible, if only to a small extent⁷, they might regard degeneracy of a particular reaction network model to have roots in the improper neglect of reverse reactions that should have, in fact, been taken into account. Indeed, any degenerate reaction network model becomes nondegenerate when it is perturbed by the addition of sufficiently many reverse reactions,

⁶Perverse mathematical phenomena of this kind should not be confused with other model perturbations involving the addition of a reverse reaction but in which changes of behavior require a substantial rate constant for the reaction added. Perturbations of this second kind appear in Section 4.

⁷With mass action kinetics, for example, the reverse rate constant might be extremely small.

usually few in number.⁸ Moreover, *every fully open network is nondegenerate, regardless of what the reactions are* (Remark C.6).

In Appendix C we provide a fuller discussion of network nondegeneracy, including characterizations of non-degeneracy in terms of network structure alone. In [5] we provide a tool to decide a network's nondegeneracy computationally. Because a nondegenerate reaction network inherits concordance from its fully open extension and because fully open networks are, in important respects, easier to study, our development of SR Graph conditions for concordance will, as in [2], focus largely on fully open networks.

In Theorems 4.1 and 5.1 we connect the structure of a network's SR Graph directly to dynamical properties of kinetic systems that derive from that network, without the concordance idea playing an intermediary role. Those theorems are stated for nondegenerate networks, not necessarily fully open ones. Readers who wish to do so can replace *nondegenerate* in the theorem statement with the more tangible *fully open, reversible, or weakly reversible*.

Material from Section 6 onward is devoted entirely to proofs.

3. The Species-Reaction Graph

Here we review the construction of the Species-Reaction Graph for a network. The ideas, which are slightly different from those in [2], are illustrated by means of reaction network (1). The same example will serve to illustrate one difference between results given here and those in [2]. The SR Graph for network (1) is shown in Figure 1.

The Species-Reaction Graph (SR Graph) of a reaction network is constructed from *species vertices*, *reaction vertices*, and *edges* connecting species vertices to reaction vertices: For each species in the network there is precisely one species vertex, labeled by the species's name. Similarly, the reaction vertices are associated with the various reactions of the network, but with the understanding that, for a reversible reaction pair, there is only one reaction vertex, associated with both reactions of the pair. An edge is drawn connecting a species and a reaction vertex if the species participates in the reaction, and the edge is labeled with the name of the complex in which the species appears. (*Complexes* are the objects on either side of the reaction arrow; for example, reaction $A + B \rightarrow C + F$ has two complexes, $A + B$ and $C + F$.⁹) The arrows appearing on some of the edges in Figure 1 will be explained shortly; for the moment we shall consider all edges to be undirected.

⁸It is sufficient for nondegeneracy, but certainly not necessary, that there be r linearly independent reactions that are reversible, where r is the rank of the network (Definition 6.3). See Proposition C.22 in Appendix C.

⁹Because, as in [13] and [2], we consider only networks in which no species appears on both sides of a reaction, there is no ambiguity in the complex in which the species appears.

If two edges adjacent to the same reaction vertex carry identical complex labels, the two edges constitute a *complex-pair*, or *c-pair*. Thus, for example, the two edges labeled $A + B$ in Figure 1 constitute a c-pair. In the figure, there are four c-pairs.¹⁰

Note that Figure 1 contains three cycles — the cycles labeled I and II and also the large outer cycle that traverses species A , B , C , and E . An *odd-cycle* (*even-cycle*) in an SR Graph is a cycle containing an odd (even) number of c-pairs. In Figure 1, cycle I is odd, while cycle II and the large outer cycle are even.

A *fixed-direction edge* is an edge connecting an irreversible reaction at one end and, at the other end, a species that is either (i) a product of the reaction or (ii) the sole reactant. For example, an edge connecting an irreversible reaction $2A \rightarrow B+C$ to species A would be a fixed-direction edge because A is a sole reactant species. The edge connecting that same reaction to species B would also be a fixed direction edge because B is a product of the reaction. On the other hand, an edge connecting an irreversible reaction $D+E \rightarrow F$ to species D would not be a fixed-direction edge because D is not the sole reactant species.

Directionality is given for all fixed-direction edges in the following manner: the edge is directed from species to reaction if the species appears as the (sole) reactant in the given reaction, and the edge is directed from reaction to species when the species appears as a product of said reaction. Note that this prescription is subtly different from the one given in [2] for a fixed-direction edge-pair, a term we do not use here. The SR graph is complete when all edges and vertices are drawn, labeled, and given direction as described. See Figure 1 for the SR graph for network (1).¹¹

Remark 3.1: When a network under study contains “degradation reactions” or “synthesis reactions” of the form $s \rightarrow 0$ or $0 \rightarrow s$ it is understood that the SR Graph is drawn for the network with those reactions removed. In the event that there are reactions of the form $2A \rightarrow 0$, containing a single species on one side of the reaction, such reactions are also removed. A reaction such as $A + B \rightarrow 0$, however, is retained. The SR Graph for network (1) is the same as the SR Graph for the network’s fully open extension.

A cycle in an SR Graph, say $s_1 R_1 s_2 R_2 \dots s_n R_n s_1$, is *orientable* if an assignment of directions, either

$$s_1 \rightarrow R_1 \rightarrow s_2 \rightarrow R_2 \rightarrow \dots \rightarrow s_n \rightarrow R_n \rightarrow s_1$$

or

$$s_1 \leftarrow R_1 \leftarrow s_2 \leftarrow R_2 \leftarrow \dots \leftarrow s_n \leftarrow R_n \leftarrow s_1$$

¹⁰For readers with access to color, c-pair edges in SR graph displays are identically colored.

¹¹An elegant internet-based tool for online-drawing of a variant of the SR Graph is available in [14].

is consistent with the fixed-direction edges in the cycle. If a cycle has no fixed-direction edges, it is orientable in either direction. In Figure 1, cycles I and the large outer cycle are orientable, but only in the clockwise direction. Cycle II is orientable in either direction. Were the reversible reactions $A + B \rightleftharpoons C + F$ replaced by the irreversible reaction $C + F \rightarrow A + B$ then only cycle II would be orientable, in the clockwise direction.

The intersection of two cycles¹² might contain a nonempty set of edges. If both cycles can be assigned an orientation such that the shared edges are traversed in the same direction, then the cycles have a *consistent orientation*. Each pair of cycles in Figure 1 admits a consistent orientation.

With every edge in an SR Graph we can associate its *stoichiometric coefficient*, which, for the edge connecting species s and reaction R , is the stoichiometric coefficient of species s in reaction R . For example, an edge connecting reaction $A + B \rightarrow 2C$ to species C has a stoichiometric coefficient of two; the edge connecting the same reaction to species A has a stoichiometric coefficient of one.

Consider an oriented cycle, with each species-to-reaction edge ($s \rightarrow R$) and each reaction-to-species edge ($R \rightarrow s$) having associated stoichiometric coefficients $e_{s \rightarrow R}$ and $f_{R \rightarrow s}$, respectively. A cycle is *stoichiometrically expansive* relative to a given orientation $R_1 \rightarrow s_1 \rightarrow R_2 \rightarrow s_2 \rightarrow \dots s_n \rightarrow R_1$ if

$$\frac{f_{R_1 \rightarrow s_1} f_{R_2 \rightarrow s_2} \cdots f_{R_n \rightarrow s_n}}{e_{s_1 \rightarrow R_2} e_{s_2 \rightarrow R_3} \cdots e_{s_n \rightarrow R_1}} > 1. \quad (2)$$

Note that every edge in Figure 1 has a stoichiometric coefficient of one. This will often be the case in SR Graphs for naturally occurring chemical reaction networks. As a result, cycles in the SR Graph that are stoichiometrically expansive relative to a given orientation are not so common; it will most often be the case that the ratio in inequality (2) is equal to 1.

4. A Species-Reaction Graph theorem: Dynamical consequences when the kinetics is weakly monotonic

Here we state a principal theorem of this paper, one that connects properties of a nondegenerate network's SR Graph to dynamical properties of kinetic systems that derive from the network. The theorem asserts that, when fairly mild graphical conditions are satisfied, network structure enforces a certain dullness of dynamical behavior (e.g., the impossibility of bistable switching between two stoichiometrically compatible positive equilibria), notwithstanding what might be considerable complexity in the network or substantial nonlinearity in the kinetics, so long as the kinetics satisfies very weak and natural constraints.

¹²By the intersection of two cycles, we mean the subgraph consisting of edges and vertices common to both.

When we refer to the differential equations for a network endowed with a kinetics, we mean the differential equations formulated in the usual way [3, 15]; terminology in the theorem statement is the same as in [1].

Theorem 4.1—Consider a nondegenerate reaction network for which the Species-Reaction Graph has the following properties:

- i. No even cycle admits a stoichiometrically expansive orientation.
- ii. *No two consistently oriented even cycles have as their intersection a **single** directed path **originating at a species vertex and terminating at a reaction vertex**.*

Then, for any choice of weakly monotonic kinetics, the resulting differential equations cannot admit two distinct stoichiometrically-compatible equilibria, at least one of which is positive. If the kinetics is differentiably monotonic, then every real eigenvalue¹³ associated with a positive equilibrium is strictly negative.

If, in addition, the network is weakly reversible then the following also hold true: For each choice of kinetics (not necessarily weakly monotonic¹⁴) no nontrivial stoichiometric compatibility class has an equilibrium on its boundary. If the network is also conservative then, for any choice of a continuous weakly monotonic kinetics, there is precisely one equilibrium in each nontrivial stoichiometric compatibility class, and it is positive.

Theorem 4.1 and Remark 4.3 follow directly, on one hand, from consequences of network concordance established in [1] and, on the other hand, from Proposition 4.2 below, proof of which is a principal objective of this article.

Proposition 4.2—A nondegenerate reaction network is concordant if its Species-Reaction Graph satisfies conditions (i) and (ii) of Theorem 4.1.

Remark 4.3: (*The all or nothing property.*) Whether or not a network is nondegenerate, we shall see in Section 6 that conditions (i) and (ii) serve to ensure that the network's fully open extension is concordant. Nondegeneracy then ensures that the network itself is concordant. Thus, when the SR Graph for a nondegenerate network satisfies conditions (i) and (ii), one has not only concordance of the network but also concordance of the network's fully open extension. As a result, one can say considerably more than is actually said in Theorem 4.1.

For a concordant network with a concordant fully open extension, one can make statements about compositions that aren't necessarily equilibria [1]: For *every* differentiably monotonic kinetics, at *every* positive composition, *not necessarily an equilibrium*, every real eigenvalue of the derivative of the species formation function is negative. In particular, at *every* positive composition the derivative is nonsingular. (The last assertion derives from concordance of the network itself, whether or not the fully open extension is concordant.)

¹³We are referring here to eigenvalues associated with eigenvectors in the network's stoichiometric subspace.

¹⁴We assume here only that the kinetics satisfies the very weak conditions of Definition A.1 in Appendix A.

Thus, for any network with a concordant fully open extension (in particular for any network whose SR Graph satisfies conditions (i) and (ii)), one has the following “all or nothing” [2] situation. Either (a) the network is non-degenerate, in which case the network itself is concordant, whereupon for *every* differentially monotonic kinetics the derivative of the species-formation rate function is nonsingular at *every* positive composition or (b) the network is degenerate, in which case for *no* choice of differentially monotonic kinetics is the derivative of the species formation rate function nonsingular at *any* positive composition. In the second case, the network is discordant [2].

See also Appendix C for more on the “all or nothing” property of networks with a concordant fully open extension.

Remark 4.4: The “all or nothing” property has some striking consequences. Consider a reaction network having a concordant fully open extension, perhaps because it has an SR Graph that satisfies conditions (i) and (ii). Suppose that, for the reaction network, there is *some* set of positive rate constants such that the resulting mass action species formation rate function has a nonsingular derivative at *some* positive equilibrium. Then the derivative must be nonsingular at *all* positive equilibria, in particular those residing in other stoichiometric compatibility classes. This same situation will obtain for *every other assignment of rate constants* and, indeed, *for every other assignment of a differentially monotonic kinetics, not necessarily mass action, and at every positive composition*. Moreover, the network inherits all of the dynamical consequences of concordance, in particular those described in Theorem 4.1.

Contrast this with the behavior of network (3). No matter how rate constants are assigned to the two reactions, the derivative of the mass action species

formation rate function will be singular at one positive equilibrium and nonsingular at others. (A phase portrait is shown in [3].) Network (3) has a discordant full open extension.

The principal sharpening that Proposition 4.2 and Theorem 4.1 afford relative to the analogous assertions in [2] lies in the nature of condition (ii). There are two ways in which the sharpening in (ii) is exerted:

First, the assertions in [2] give no information when two even cycles have an intersection consisting of *multiple* disjoint paths as its connected components, each with a species at one end and a reaction at the other. In Theorem 4.1 condition (ii) is indifferent to the presence of *multiple* such paths; it is violated only if there is *precisely one*.

Second, the assertions in [2] stand silent when two even cycles have *any* “species-to-reaction intersection,” *regardless of its direction*. (In [2] the term “species-to-reaction intersection” does not connote a direction.) In condition (ii) of Theorem 4.1 it is only a (single) path *directed from a species to a reaction* that results in a violation; a path directed from a reaction to a species does not.¹⁵

Some examples will illustrate the sharpening afforded by Theorem 4.1.

Example 4.5: (*Two even cycles intersecting in multiple directed species-to-reaction paths.*)

Consider network (4), which for the purposes of this discussion we shall imagine to model the true chemical reactions operative in a classical fully open continuous-flow stirred-tank reactor (CF-STR). In addition to their presence in the effluent stream, we shall suppose that all species are supplied at a constant rate in the feed stream. As indicated earlier, the full reaction network of interest is network (4) taken together with a “degradation” reaction of the form $s \rightarrow 0$ and a “synthesis” reaction of the form $0 \rightarrow s$ for each of the nine species. Because the augmented network is fully open, it is non-degenerate. (Network (4) is, by itself, also nondegenerate, as can be ascertained by means described in Appendix C and implemented in [5].)

The SR Graph for the augmented CFSTR network (which is identical to the SR Graph for the smaller network (4) of “true” chemical reactions) is shown in Figure 2. Note that the large outer cycle, passing through species G , A , C , and D contains no c-pairs, so it is even. The innermost cycle, labeled II, has two c-pairs, so it too is even. The two cycles, which can only be oriented clockwise, have as their intersection *two* directed species-to-reaction paths, each consisting of just one edge, originating at A and D respectively. Such an intersection would cause the analogous theorem in [2] to be silent, *but here condition (ii) of Theorem 4.1 is not violated.*

In fact, the SR Graph shown in Figure 2 satisfies both conditions (i) and (ii) of Theorem 4.1, in which case the theorem’s dynamical consequences obtain. In particular, no matter what weakly monotonic kinetics is presumed for the various reactions, the quite complicated system of differential equations for the fully open CFSTR cannot admit more than one equilibrium.

The same is *not* true when reactions $A \rightarrow B + D$ and $D \rightarrow F + G$ are made reversible. Indeed, when the kinetics is mass action (and therefore weakly monotonic) computations via [5] indicate parameter values such that the resulting CFSTR differential equations admit multiple positive equilibria. In this case, condition (ii) is no longer satisfied: The two even cycles II and III admit a consistent orientation, with cycle II counterclockwise and cycle III clockwise. Their intersection is a single path directed from species D to reaction(s) $A \rightleftharpoons B + C$.

Example 4.6: (*Two even cycles intersecting in a directed reaction-to-species path.*) Here we return to network (1). As in Example 4.5, we will imagine that network (1) amounts to a

¹⁵Directionality was, however, invoked in the Remark in Section 6 of [9], an article restricted to mass action kinetics. In that Remark, though, there was concern with directions along what might be *multiple* paths comprising the intersection of two even cycles.

display of the true chemical reactions operative in a classical fully open continuous-flow stirred tank reactor, with all species in both the effluent and feed streams. The full network of interest, then, is (1) taken together with a degradation ($s \rightarrow 0$) and a synthesis ($0 \rightarrow s$) reaction for every species. Because the augmented network is fully open, it is nondegenerate. (The original network (1) is, by itself, also nondegenerate [5].)

The SR Graph for the augmented network, which is identical to the SR Graph for network (1), was displayed in Figure 1. In the figure there are just two even cycles, cycle II and the large outer cycle. They admit only one consistent orientation, with both cycles oriented clockwise. The intersection of the two cycles consists of a single path with the reaction(s) $A + B \rightleftharpoons C + F$ at one end and species C at the other, an intersection that would cause results in [2] to remain silent. Note, however, that with respect to the clockwise orientation, *the single path intersection is directed from the reaction end to the species end*, so condition (ii) of Theorem 4.1 is not violated.

Indeed, both conditions (i) and (ii) of Theorem 4.1 are satisfied, in which case, with very little said about the details of the kinetics, the theorem ensures that the resulting complex CFSTR differential equations, however nonlinear, can admit behavior only of a very proscribed and largely mundane kind. This would *not* be true were the reaction $C \rightarrow E$ reversible: There would be no directions to any of the edges in cycle I of the SR Graph. The large outer cycle and cycle II could then be given counterclockwise orientations. Those two even cycles would be consistently oriented, with an intersection consisting of the directed (single) path originating at species C , passing through species B , and terminating at reaction(s) $A + B \rightleftharpoons C + F$. Thus, condition (ii) is violated.

In fact, when $C \rightarrow E$ is reversible and when the kinetics is mass action, there are parameter values such that the resulting CFSTR equations admit multiple positive equilibria [5].

Example 4.7: *A two-enzyme network.* There is another way in which Proposition 4.2 and Theorem 4.1 sharpen results in [2], this time through the compulsory orientation in the SR Graph of *product* c-pairs adjacent to irreversible reactions. (That orientation was not prescribed in [2].) The sharpening can be illustrated by means of network (5).

In network (5) $E1$ is an enzyme that serves to convert a substrate S to a product P . A second enzyme $E2$ serves to cleave P into two molecules of Q . In turn, Q binds reversibly to $E1$ and thereby inhibits its action on S . As in Examples 1 and 2, we imagine network (5) to model the chemical reactions operative in a classical continuous-flow stirred tank reactor, this time with S , $E1$, and $E2$ in the feed stream. Alternatively, we can imagine that all species are degrading to inconsequential products via first order reactions while S , $E1$, and $E2$ are synthesized at constant rates. In any case, the reaction network of real interest is network (5) taken together with degradation reactions of the form $s \rightarrow 0$ for all of the various species and

also synthesis reactions $0 \rightarrow S$, $0 \rightarrow E1$, and $0 \rightarrow E2$. Because the augmented network is fully open it is nondegenerate. (The original network (5) is also nondegenerate.)

The SR Graph for the augmented network, which is identical to the SR Graph for network (5), is shown in Figure 3. Note that there are several cycles, but not all of them are orientable. The cycles labeled I, II, and III are even, as is the cycle consisting of the outer perimeter of the union of II and III. All other cycles are odd. Of the even cycles, only I and III are orientable, and they have no intersection at all. Thus, condition (ii) of Theorem 4.1 and Proposition 4.2 is satisfied. In this example, unlike the others, there is a stoichiometric coefficient that is not 1. This raises the possibility that condition (i) might be violated. However, the sole edge having stoichiometric co-efficient 2 (and labeled as such) appears in no orientable even cycle, so condition (i) is satisfied.

Thus, with very little said about details of the kinetics, we can be sure that the resulting CFSTR differential equations can admit only the dull behavior described in Theorem 4.1. The same would *not* be true if the reaction $E1S \rightarrow E1 + P$ were reversible. In that case, the formerly compulsory arrows leading to and from that reaction would not be present in the SR Graph. Cycle II would then be orientable and stoichiometrically expansive relative to its only orientation; condition (i) would fail. Condition (ii) would fail as well: the two even cycles I and II admit a consistent orientation (with cycle I oriented counterclockwise and cycle II oriented clockwise) such that their intersection is the directed edge from species $E1$ to the (now reversible) reaction(s) $E1S \rightleftharpoons E1 + P$. The failure of either condition causes Theorem 4.1 to remain silent.

In fact, when the kinetics is mass action there is a choice of parameter values such that the resulting CFSTR equations admit multiple positive equilibria [5].

5. Another Species-Reaction Graph theorem: Dynamical consequences when the kinetics admits product inhibition

In this section we state a theorem that extends Theorem 4.1 to give dynamical information when the kinetics is two-way monotonic — that is, when one or more reaction rate functions might reflect product inhibition. (Recall Remark 1.3.) Because the two-way monotonic class is broader than the monotonic class, we should expect that conclusions similar to those in Theorem 4.1 will obtain for a narrower class of networks, in particular for networks whose SR Graph satisfies more stringent conditions.

In preparation for Theorem 5.1 we note that a cycle in the SR Graph will be stoichiometrically non-expansive relative to *both* clockwise and counterclockwise orientations only if, relative to one such orientation, the ratio in inequality (2) is 1, whereupon it will be 1 relative to the other orientation as well. In the SR Graph literature such cycles are called *s*-cycles: an *s*-cycle in an SR Graph is a cycle such that with respect to some orientation $R_1 \rightarrow s_1 \rightarrow R_2 \rightarrow s_2 \rightarrow \dots s_n \rightarrow R_1$, either clockwise or counterclockwise,

$$\frac{f_{R_1 \rightarrow s_1} f_{R_2 \rightarrow s_2} \cdots f_{R_n \rightarrow s_n}}{e_{s_1 \rightarrow R_2} e_{s_2 \rightarrow R_3} \cdots e_{s_n \rightarrow R_1}} = 1. \quad (6)$$

In Theorem 5.1 conditions (i) and (ii) essentially amount to orientation-free versions of their counterparts in Theorem 4.1. In contrast to Theorem 4.1, conditions imposed on even cycles in Theorem 5.1 refer to all even cycles, whether or not they are orientable.

Theorem 5.1—Consider a nondegenerate network for which the Species-Reaction Graph has the following properties:

- i. Every even cycle is an s-cycle.
- ii. No two even cycles have as their intersection a single path with a species vertex at one end and a reaction vertex at the other.

For such a network the conclusions of Theorem 4.1 obtain with “weakly monotonic” replaced by “two-way weakly monotonic” and “differentiably monotonic” replaced by “differentiably two-way monotonic [1].”

A reaction network is *strongly concordant* if it is concordant and has certain additional properties defined in §6.11. Theorem 5.1 derives, on one hand, from consequences of strong concordance established in [1], and, on the other hand, from Proposition 5.2 below, proof of which is given in §6.11:

Proposition 5.2—A nondegenerate network is strongly concordant if its Species-Reaction Graph satisfies conditions (i) and (ii) of Theorem 5.1.

The following example¹⁶ demonstrates how condition (ii) of Theorem 5.1 sharpens the corresponding condition in earlier SR Graph theorems appearing elsewhere [2, 11, 12, 16].

Example 5.3: Here we consider network (7). Because the network is reversible it is nondegenerate. The SR Graph for network (7) is shown in Figure 4.

(7)

There are several cycles in the figure, all of them even. Because every stoichiometric coefficient is 1, condition (i) of Theorem 5.1 is satisfied. Although various pairs of even cycles intersect, the intersections in several cases consist of single paths having species at both ends or reactions at both ends; these do not constitute violations of condition (ii). The intersection of the central circular cycle with the large outer cycle is comprised of *two*

¹⁶The example was also used in [9] to make a different point.

components, each of which is a single-edge path having a species vertex at one end and a reaction vertex at the other end. Because there are *two* such paths, there is no contradiction of conditions (ii) in Theorem 5.1. *That same intersection does, however, violate the corresponding condition of theorems in [2, 11, 12, 16]. In those theorems a forbidden “S-to-R intersection” of two even cycles can be comprised of multiple connected components, each of which is a path having a species and a reaction at its ends.* In such cases, the previous theorems give no information.

Because conditions (i) and (ii) of Theorem 5.1 are satisfied, the dynamical consequences of the theorem obtain for the differential equations resulting from network (7), this time for two-way monotonic kinetics. In fact, the same dynamical consequences obtain for the differential equations that describe a continuous flow stirred tank reactor in which network (7) is the operative chemistry: The SR Graph for the (nondegenerate) fully open CFSTR network is precisely the one shown in Figure 4.

6. Proofs

The remainder of this article is devoted to proofs of Propositions 4.2 and 5.2. Those propositions, taken with consequences of concordance given in [1, 2], lead directly to Theorems 4.1 and 5.1. In fact, Propositions 4.2 and 5.2 amount to corollaries of Theorem 6.10 below. Theorem 6.10 ensures concordance of a fully open network whose SR Graph satisfies conditions that are substantially weaker but more complicated than those given in Theorem 4.1 (and Proposition 4.2), in which case all of the dynamical consequences of concordance given in Theorem 4.1 accrue to the network.

Because parts of the proof of Theorem 6.10, especially in its beginning, are identical to arguments given in [2], we have merely summarized briefly that common material here, emphasizing instead aspects of the proof that differ substantially from [2].

6.1. Some definitions

We begin with some definitions taken from [1, 2, 15], where more discussion and motivation can be found; the notation is the same as in those papers. In particular, when I is a finite set (for example, a set of species), we denote the vector space of real-valued functions with domain I by $\mathbb{R}^I$. If x is a member of $\mathbb{R}^I$, we denote by x_i the value that x takes on element $i \in I$; the number x_i will sometimes be called the i^{th} component of x . The standard basis for $\mathbb{R}^I$ is denoted $\{\omega_i\}_{i \in I}$; that is, ω_i is the vector of $\mathbb{R}^I$ that has 1 for its i^{th} component and 0 for its other components. Thus, every $x \in \mathbb{R}^I$ has the representation

$$x = \sum_{i \in I} x_i \omega_i.$$

If u and v are members of $\mathbb{R}^I$ we denote by uv the member of $\mathbb{R}^I$ such that $(uv)_i = u_i v_i, \forall i \in I$.

When I is the set $\mathcal{S}$ of species in a reaction network, we shall (especially in Appendix C) choose to replace symbols for the standard basis vectors $\{\omega_s\}_{s \in \mathcal{S}}$ for $\mathbb{R}^{\mathcal{S}}$ by symbols for the species themselves, $\{s\}_{s \in \mathcal{S}}$. In this way, every vector $x \in \mathbb{R}^{\mathcal{S}}$ has a representation

$$x = \sum_{s \in \mathcal{S}} x_s s,$$

and $\mathbb{R}^{\mathcal{S}}$ can be identified with the vector space of formal linear combinations of the species. In this case, when A and B are species, $A + B$ can be regarded as a vector in $\mathbb{R}^{\mathcal{S}}$, as can $2B - A$.

The subset of $\mathbb{R}^I$ consisting of vectors having only positive (nonnegative) components is denoted $\mathbb{R}_+^I$ ($\overline{\mathbb{R}}_+^I$). By the *support* of $x \in \mathbb{R}^I$, denoted $\text{supp } x$, we mean the set of indices $i \in I$ for which x_i is different from zero. When ξ is a real number, the symbol $\text{sgn } (\xi)$ denotes the sign of ξ .

Definition 6.1—A *chemical reaction network* consists of three finite sets:

- i. a set $\mathcal{S}$ of distinct *species* of the network;
- ii. a set $\mathcal{C} \subset \overline{\mathbb{R}}_+^{\mathcal{S}}$ of distinct *complexes* of the network;
- iii. a set $\mathcal{R} \subset \mathcal{C} \times \mathcal{C}$ of distinct *reactions*, with the following properties:
 - a. $(y, y) \notin \mathcal{R}$ for any $y \in \mathcal{C}$;
 - b. for each $y \in \mathcal{C}$ there exists $y' \in \mathcal{C}$ such that $(y, y') \in \mathcal{R}$ or such that $(y', y) \in \mathcal{R}$.

If (y, y') is a member of the reaction set $\mathcal{R}$, we say that y *reacts to* y' , and we write $y \rightarrow y'$ to indicate the reaction whereby complex y reacts to complex y' . The complex situated at the tail of a reaction arrow is the *reactant complex* of the corresponding reaction, and the complex situated at the head is the reaction's *product complex*.

Definition 6.2—A reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is *fully open* if $\mathcal{C}$ contains the zero complex (i.e., the zero vector of $\mathbb{R}^{\mathcal{S}}$) and if, for each $s \in \mathcal{S}$, $\mathcal{R}$ contains the reaction $s \rightarrow 0$. (Reactions of the form $s \rightarrow 0$, $s \in \mathcal{S}$, are the network's *degradation reactions*.)

Definition 6.3—The *reaction vectors* for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ are the members of the set

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

The *rank* of a reaction network is the rank of its set of reaction vectors.

Definition 6.4—The *stoichiometric subspace* S of a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is the linear subspace of $\mathbb{R}^{\mathcal{S}}$ defined by

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

Note that the dimension of the stoichiometric subspace is identical to the rank of the network. If the network is fully open, then both are equal to the number of species, and $S = \mathbb{R}^{\mathcal{S}}$.

In preparation for the definition of reaction network concordance [1], we consider a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ with stoichiometric subspace $S \subset \mathbb{R}^{\mathcal{S}}$, and we let $L : \mathbb{R}^{\mathcal{R}} \rightarrow S$ be the linear map defined by

$$L\alpha = \sum_{y \rightarrow y' \in \mathcal{R}} \alpha_{y \rightarrow y'} (y' - y). \quad (9)$$

Definition 6.5—The reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is *discordant* if there exist an $\alpha \in \ker L$ and a nonzero $\sigma \in S$ having the following properties:

- i. For each $y \rightarrow y'$ such that $\alpha_{y \rightarrow y'} \neq 0$, $\text{supp } y$ contains a species s for which $\text{sgn } \sigma_s = \text{sgn } \alpha_{y \rightarrow y'}$.
- ii. For each $y \rightarrow y'$ such that $\alpha_{y \rightarrow y'} = 0$, $\sigma_s = 0$ for all $s \in \text{supp } y$ or else $\text{supp } y$ contains species s and s' for which $\text{sgn } \sigma_s = -\text{sgn } \sigma_{s'}$, both not zero.

A network is *concordant* if it is not discordant.

For the purposes of this article we shall find it convenient to introduce the following definition:

Definition 6.6—A *discordance* for a network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is a pair $\{\alpha, \sigma\}$, with $\alpha \in \ker L$ and σ a nonzero member of the stoichiometric subspace, that satisfies conditions (i) and (ii) in Definition 6.5.

Clearly, discordances exist only for discordant reaction networks.

Remark 6.7: For a user-specified reaction network *The Chemical Reaction Network Toolbox* [5] will test for concordance. When the network is not concordant, it will provide an example of a discordance.

6.2. The Simple Core Theorem, from which Propositions 4.2 and 5.2 follow

In this section we state Theorem 6.10, a theorem that will give rise to Propositions 4.2 and 5.2 as corollaries. Subsequent sections are then devoted to proof of Theorem 6.10. Its statement and proof invoke fairly standard graph-theoretical language (e.g., strongly connected, non-separable, ear, block), such as that used in [17]. Much of the required terminology is reviewed informally in [2].

In what follows, we shall often suppose that a subgraph of the SR Graph has been oriented, which is to say that each edge in the subgraph has been assigned a direction, even if it is not a fixed-direction edge. *When this is the case, it will be understood that the directions assigned are consistent with the directions imposed on the fixed-direction edges.*

Definition 6.8—An *even cycle cluster* is a nontrivial subgraph of a Species-Reaction Graph, taken with an orientation of the subgraph's edges such that the resulting directed subgraph is strongly connected and all of its directed cycles are even. An even cycle cluster is *complete* if it is not a subgraph of a larger even cycle cluster having the same vertices.

Definition 6.9—An oriented subgraph, G , of the SR Graph has a *simple core* if G contains a subgraph, G^* , consisting perhaps of G itself, for which the following are true:

- i. G^* is strongly connected;
- ii. every reaction vertex in G^* is, within G^* , adjacent to precisely two species;
- iii. G contains no edge external to G^* that terminates in a species vertex of G^* .

These ideas are illustrated in Figure 5, drawn in connection with the simple network (10). In that network the substrate S is converted to product P after binding with an enzyme E . The enzyme E has an inactive variant E^* , and there is a spontaneous reversible conversion of one enzyme-form to another. The conversion of E to E^* also proceeds via another path, in which the substrate S acts as a catalyst.

In Figure 5(a) we show the network's SR Graph. In Figures 5(b) and 5(c) we show two even cycle clusters for the SR Graph. Note that Figure 5(b) is a subgraph of Figure 5(c) and has the same vertices. Thus, Figure 5(b) is not a *complete* even cycle cluster. On the other hand, Figure 5(c) is complete. Figure 5(d) is a simple core for the complete even cycle cluster in Figure 5(c): viewed as a subgraph of Figure 5(c), it is strongly connected, each reaction vertex is adjacent to precisely two species in the subgraph, and no species vertex in that subgraph has, in Figure 5(c), an incoming edge that is not in the subgraph.

We are now in a position to state Theorem 6.10.

Theorem 6.10—(The Simple Core Theorem). A fully open network is concordant if its SR graph satisfies the following conditions:

- i. No even cycle admits a stoichiometrically expansive orientation.
- ii. Every complete even cycle cluster has a simple core.

Remark 6.11: Recall that an even cycle cluster is, among other things, a subgraph of the SR Graph taken with an orientation of the edges (consistent with the fixed edge directions). Thus, condition (ii) should be understood in the following sense: if a subgraph of the SR

Graph can be oriented so that the requirements of a complete even cycle cluster are met, then the resulting oriented subgraph should have a simple core.

Remark 6.12: Note that we do not require that an even cycle cluster be non-separable [17]. That is, it might be the union of blocks joined at separating vertices. It can be shown that an even cycle cluster has a simple core if each of its blocks has a simple core. Thus, condition (ii) will be satisfied if and only if every *nonseparable* complete even cycle cluster has a simple core.

Corollary 6.13—A nondegenerate network is concordant if its SR Graph satisfies conditions (i) and (ii) of Theorem 6.10.

Proof: Consider a (not necessarily fully open) network whose SR Graph satisfies conditions (i) and (ii) of Theorem 6.10. Because the network's SR Graph is the same as the SR Graph for the network's fully open extension, Theorem 6.10 asserts that the fully open extension is concordant. Because the original network is nondegenerate, it too is concordant by virtue of Theorem C.4 in Appendix C. (See also [1, 2].)

The following corollary merely draws on some of the consequences of concordance given in [1].

Corollary 6.14—For a nondegenerate network whose SR Graph satisfies conditions (i) and (ii) of Theorem 6.10 all of the conclusions of Theorem 4.1 obtain.

Example 6.15: (*A network for which Theorem 6.10 gives information but for which Theorem 4.1 and Proposition 4.2 are silent.*) Consider a continuous flow stirred tank reactor in which the chemistry in network (10) is operative; we suppose that S and E are supplied at fixed rate. The SR Graph for the corresponding fully open network (which is identical to the SR graph for network (10)) is shown in Figure 5(a). Note that the SR Graph does not satisfy condition (ii) of Theorem 4.1 (or of Proposition 4.2). In particular, the small even cycle labeled I and the large outer cycle, also even, admit a consistent orientation, with both oriented clockwise, such that the cycles have an intersection consisting of a single directed path, beginning at a species and terminating at a reaction.

We turn instead to the broader Theorem 6.10. Because every stoichiometric coefficient is 1, it is evident that condition (i) of Theorem 6.10 is satisfied. Condition (ii) is satisfied as well: Recall that Figure 5(c) is an example of a complete even cycle cluster of the SR Graph and that it has a simple core, shown in 5(d). There are other complete even cycle clusters of the SR Graph — for example the single directed cycle labeled I in 5(c), and it too has a simple core, the cycle itself. In fact, every complete even cycle cluster of the SR Graph has a simple core. (Note that the even cycle cluster shown in 5(b) does not have a simple core. There is, however, no violation of condition (ii) because that even cycle cluster is not complete.)

Thus, we have concordance of the fully open network appropriate to the continuous flow stirred-tank reactor under consideration. In turn, we can be sure that the dynamical consequences of concordance, such as those stated in Theorem 4.1, are inherited by the

CFSTR differential equations, so long as the kinetics conforms to natural and quite mild constraints.

6.3. Beginning the proof of Theorem 6.10

To prove Theorem 6.10 we will suppose that a given fully open network is discordant and then show that its SR Graph cannot satisfy both conditions (i) and (ii) of the theorem statement. For the given network one or more non-degradation reactions might be reversible. It is considerably easier to work with a network in which every non-degradation reaction is irreversible. The following lemma, taken from [2], permits us to do that:

Lemma 6.16—If a fully open network is discordant, it is possible to choose from each reversible pair of non-degradation reactions at least one (and sometimes both) of the reactions for removal such that the resulting fully open subnetwork is again discordant.

Given the original discordant fully open network, we will work with the discordant network whose existence is guaranteed by Lemma 6.16 and show that its SR Graph could not satisfy conditions (i) and (ii) of Theorem 6.10. Violation of either of these conditions amounts to the existence of certain disagreeable objects in the SR Graph for the network containing only irreversible non-degradation reactions. From there it is not difficult to argue that the same disagreeable objects are present in the SR Graph for the original network. In that case the SR Graph for the original network, with reversible non-degradation reactions, would also violate condition (i) or (ii).

Hereafter, then, we suppose that $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is a discordant fully open network in which each non-degradation reaction is irreversible. Furthermore, we suppose that $\{a, \sigma\}$ is a particular discordance for the network.

6.4. The sign-causality graph corresponding to the discordance $\{a, \sigma\}$

For the putative discordance $\{a, \sigma\}$, Definition 6.5 requires that σ be nonzero so that, for one or more species $s \in \mathcal{S}$, we must have $\sigma_s \neq 0$. We say that such species are *signed* relative to the discordance¹⁷. A signed species $s \in \mathcal{S}$ is *positive* or *negative* according to whether σ_s is positive or negative. Similarly, for the fully open network under consideration, it is a consequence of Definition 6.5 that a must also be nonzero. A reaction $y \rightarrow y'$ is *signed* if $a_{y \rightarrow y'}$ is not zero, and we say that $y \rightarrow y'$ is *positive* or *negative* according to whether $a_{y \rightarrow y'}$ is positive or negative.

The *sign-causality graph* [2] induced by the discordance $\{a, \sigma\}$ is a directed graph constructed in the following way: The vertices are the signed species and signed (non-degradation) reactions. An edge $\leadsto$ is drawn from a signed species s to a signed reaction $y \rightarrow y'$ whenever s is contained in $\text{supp } y$ and the two signs agree; the edge is then labeled with the complex y . An edge $\leadsto$ is drawn from a signed reaction $y \rightarrow y'$ to a signed species s in either of the following situations: (i) s is contained in $\text{supp } y'$ and the sign of s agrees with the sign of the reaction; in this case the edge carries the label y' or (ii) s is contained in $\text{supp } y$

¹⁷Hereafter, the qualifier “relative to the discordance” will be taken as understood.

y and the sign of s disagrees with the sign of the reaction; in this case the edge carries the label y . It is understood that the signed species and the signed reactions are labeled by their corresponding signs. A *c-pair* in the sign-causality graph is a pair of edges adjacent to the same reaction node that carries the same complex label.

Stoichiometric coefficients associated with edges in the sign-causality graph are designated much as they were in the Species-Reaction Graph: For a species-to-reaction edge $s \rightsquigarrow R$ of the sign-causality graph we denote by $e_{s \rightsquigarrow R}$ the (positive) stoichiometric coefficient of species s in the corresponding edge-labeling complex. For a reaction-to-species edge $R \rightsquigarrow s$ we denote by $f_{R \rightsquigarrow s}$ the (positive) stoichiometric coefficient of species s in its edge-labeling complex.

6.5. Sources in the sign-causality graph corresponding to the discordance $\{\alpha, \sigma\}$

In the sign-causality graph associated with the putative discordance $\{\alpha, \sigma\}$, a *source* [2] is a strongly connected component of the sign-causality graph whose vertices have no incoming edges originating at vertices outside that strong component. Because the sign-causality graph has a finite number of vertices, it is clear that every component of the sign-causality graph has at least one source. In particular, the sign-causality graph corresponding to the putative discordance $\{\alpha, \sigma\}$ must itself have at least one source.

Hereafter we focus on one such source—We denote by $\mathcal{S}_0$ its species vertices and by $\mathcal{R}_0$ its reaction vertices. Moreover, for each species $s \in \mathcal{S}_0$ we denote by $\mathcal{R}_0 \rightsquigarrow s$ the set of all edges of the source that are incoming to s and by $s \rightsquigarrow \mathcal{R}_0$ the set of all edges of the source that are outgoing from s . From arguments in [2] it follows that the stoichiometric coefficients and the $\alpha_{y \rightarrow y'}$ corresponding to $y \rightarrow y' \in \mathcal{R}_0$ must satisfy the inequality system (11).

$$\sum_{\mathcal{R}_0 \rightsquigarrow s} f_{R \rightsquigarrow s} |\alpha_R| - \sum_{s \rightsquigarrow \mathcal{R}_0} e_{s \rightsquigarrow R} |\alpha_R| > 0, \quad \forall s \in \mathcal{S}_0.$$

(11)

6.6. The counterpart in the SR Graph of a source in the sign-causality graph

A source in the sign-causality graph (corresponding to the putative discordance $\{\alpha, \sigma\}$ for the network under consideration) can, to some extent, be identified with a subgraph G of the network's SR Graph having the same vertices and edges. It should be kept in mind, however, that the sign-causality graph is directed, while in the SR Graph there is a direction thus far imparted only to its fixed-direction edges. In fact, we can make the identification complete if we give each edge of G a direction (denoted $\rightarrow$) identical to its $\rightsquigarrow$ -direction in the sign-causality graph.

Recall that a source, viewed as a subgraph of the sign-causality graph, is strongly connected, so that the source, viewed as a directed subgraph of the SR Graph, is also strongly connected.

In the following proposition we summarize some of what we have already said, but we also say considerably more. Much of the supporting argument, but not all of it, appeared in [2].

Proposition 6.17—Given a discordance for a reaction network, consider a source in the corresponding sign-causality graph. Let G be the subgraph of the network's SR Graph having the same vertices and edges as the source, with each edge in G given a direction “ $\rightarrow$ ” identical to its $\rightsquigarrow$ -direction in the source. Then the resulting directed subgraph of the SR Graph has edge directions consistent with the fixed direction edges of the SR Graph, and it is a complete even cycle cluster.

Proof: If one of the edges of G has an SR Graph fixed-direction, the coincidence of this direction with the $\rightsquigarrow$ -direction in the sign-causality graph follows from analysis of the rules for assigning fixed-directions in the SR Graph and the rules for assigning $\rightsquigarrow$ -directions to edges in the sign-causality graph. That G has the properties of an even cycle cluster follows from arguments already given in [2]. What was not argued there, however, is that the even cycle cluster must be complete. We establish the necessity of completeness in Appendix D.

With the identification given by Proposition 6.17 we shall henceforth regard the putative source under consideration to reside in the SR Graph. It will be understood that the edges of the source carry directions inherited from the sign-causality graph induced by the presumed discordance. The inequality system (11) can then be viewed as rooted in the SR Graph, and it can be rewritten as (12), in which “ $\rightsquigarrow$ ” in (11) has been replaced by “ $\rightarrow$.”

$$\sum_{\mathcal{R}_0 \rightarrow s} f_{R \rightarrow s} |\alpha_R| - \sum_{s \rightarrow \mathcal{R}_0} e_{s \rightarrow R} |\alpha_R| > 0, \quad \forall s \in \mathcal{S}_0.$$

(12)

The proof of Theorem 6.10 will amount to showing that, when the conditions of the theorem are satisfied, the system (12) cannot admit a solution. That is, we will show that the putative discordance giving rise to the sign-causality graph source under consideration cannot exist.

6.7. Proof strategy—Suppose that M is a non-zero member of $\overline{\mathbb{R}}_+^{\mathcal{S}}$ with support in $\mathcal{S}_0$, to be chosen later. If (12) holds then so must the single inequality (13).

$$\sum_{s \in \mathcal{S}_0} M_s \left(\sum_{\mathcal{R}_0 \rightarrow s} f_{R \rightarrow s} |\alpha_R| - \sum_{s \rightarrow \mathcal{R}_0} e_{s \rightarrow R} |\alpha_R| \right) > 0. \quad (13)$$

If, for each reaction $R \in \mathcal{R}_0$, we denote by $R \rightarrow \mathcal{S}_0$ the set of all edges of the source that are outgoing from R and by $\mathcal{S}_0 \rightarrow R$ the set of all edges of the source that are incoming to R , then (13) can be rewritten as (14).

$$\sum_{R \in \mathcal{R}_0} \left(\sum_{R \rightarrow \mathcal{S}_0} M_s f_{R \rightarrow s} - \sum_{\mathcal{S}_0 \rightarrow R} M_s e_{s \rightarrow R} \right) |\alpha_R| > 0. \quad (14)$$

Clearly if, for the putative source under consideration, we can choose M to satisfy (15) then we will have a contradiction. Proof that such an M exists will be our goal.

$$\sum_{R \rightarrow \mathcal{S}_0} M_s f_{R \rightarrow s} - \sum_{\mathcal{S}_0 \rightarrow R} M_s e_{s \rightarrow R} \leq 0, \quad \forall R \in \mathcal{R}_0 \quad (15)$$

6.8. Key propositions in the proof of Theorem 6.10

The following proposition will be central to the proof of Theorem 6.10.

Proposition 6.18—Consider a directed strongly connected subgraph G_* of the SR Graph having species set $\mathcal{S}_*$ and reaction set $\mathcal{R}_*$. Suppose that no directed cycle in G_* is stoichiometrically expansive and that, within G_* , every reaction vertex is adjacent to precisely two species vertices. Then there is a set of positive numbers $\{M_s\}_{s \in \mathcal{S}_*}$ that satisfies the following system of inequalities:

$$M_{s'} f_{R \rightarrow s'} - M_s e_{s \rightarrow R} \leq 0, \quad \forall R \in \mathcal{R}_*. \quad (16)$$

Remark 6.19: By virtue of the hypothesis of Proposition 6.18, each reaction vertex in G_* has, within G_* , precisely one incoming edge and one outgoing edge. In (16) $f_{R \rightarrow s'}$ and $e_{s \rightarrow R}$ are the stoichiometric coefficients associated with the pair of edges, respectively, outgoing from and incoming to reaction vertex R .

Proof: The proof of Proposition 6.18 is, in essence, identical to the proof of Proposition 5.12 in [2]. Although Proposition 5.12 was stated for a block (i.e., a *nonseparable* strongly connected subgraph), the interest in blocks was driven by context in [2]. Nonseparability played no role in the proof.

Proposition 6.20—Suppose that a directed subgraph G_0 of the SR Graph, with species set $\mathcal{S}_0$ and reaction set $\mathcal{R}_0$, has a simple core. Suppose also that no directed cycle within the simple core is stoichiometrically expansive. Then there is a set of non-negative numbers $\{M_s\}_{s \in \mathcal{S}_0}$, not all zero, such that

$$\sum_{R \rightarrow \mathcal{S}_0} M_s f_{R \rightarrow s} - \sum_{\mathcal{S}_0 \rightarrow R} M_s e_{s \rightarrow R} \leq 0, \quad \forall R \in \mathcal{R}_0. \quad (17)$$

In fact, if $\mathcal{S}_*$ is the species set of the simple core, one can choose M_s to be positive for all $s \in \mathcal{S}_*$ and zero for all $s \notin \mathcal{S}_*$.

In the proposition statement $R \rightarrow \mathcal{S}_0$ denotes the set of all edges of G_0 that originate at reaction R and terminate at a species of $\mathcal{S}_0$. Similarly, $\mathcal{S}_0 \rightarrow R$ denotes the set of all edges of G_0 that originate at a species of $\mathcal{S}_0$ and terminate at reaction R .

Proof: We denote by G_* the simple core and by $\mathcal{S}_*$ and $\mathcal{R}_*$ its species and reaction sets. From the definition of a simple core, G_* is strongly connected and each reaction vertex of G_* is, within G_* , adjacent to precisely two species vertices of G_* . Moreover, the hypothesis of the proposition requires that no directed cycle in G_* is stoichiometrically expansive.

Proposition 6.18 then ensures the existence of positive numbers $\{M_s^*\}_{s \in \mathcal{S}_*}$ that satisfy (18).

$$M_{s'}^* f_{R \rightarrow s'} - M_s^* e_{s \rightarrow R} \leq 0, \quad \forall R \in \mathcal{R}_*, \quad (18)$$

where, for each $R \in \mathcal{R}_*$, s' and s are the two species of $\mathcal{S}_*$ adjacent to R in G_* .

Now we take the set of non-negative numbers $\{M_s\}_{s \in \mathcal{S}_0}$ as follows: $M_s = M_s^*$ for all $s \in \mathcal{S}_*$ and $M_s = 0$ for all $s \notin \mathcal{S}_*$. It remains to be shown that this choice satisfies (17). Note that, in view of the choice, (17) reduces to (19), where it is understood that a sum over the empty set is zero.

$$\sum_{R \rightarrow \mathcal{S}_*} M_s^* f_{R \rightarrow s} - \sum_{\mathcal{S}_* \rightarrow R} M_s^* e_{s \rightarrow R} \leq 0, \quad \forall R \in \mathcal{R}_0. \quad (19)$$

First suppose that R is a reaction of the simple core G_* . In this case there are precisely two edges within G_* adjacent to R , one outgoing ($R \rightarrow s'$) and one incoming ($s \rightarrow R$). Moreover, by properties of a simple core there can be no edge in G_0 external to G_* that terminates in a member of $\mathcal{S}_*$. Therefore, $R \rightarrow s'$ is the only member of $R \rightarrow \mathcal{S}_*$. Thus, for the particular $R \in \mathcal{R}_*$ under study the left side of (19) takes the form (20):

$$M_{s'}^* f_{R \rightarrow s'} - M_s^* e_{s \rightarrow R} - \sum_{\{\mathcal{S}_* \setminus \{s\}\} \rightarrow R} M_s^* e_{s \rightarrow R} \quad (20)$$

From (18) this is clearly non-positive.

Next suppose that R is not a reaction of the simple core. In this case, properties of a simple core ensure that $R \rightarrow \mathcal{S}_*$ is empty, whereupon the first sum in (19) is zero, in which case the inequality in (19) corresponding to the particular R under study is clearly satisfied.

6.9. Completing the proof of Theorem 6.10

Recall from §6.3 that, for the (presumed discordant) fully open reaction network under consideration, the SR Graph satisfies conditions (i) and (ii) of Theorem 6.10. From Proposition 6.17 the putative source (with species set designated $\mathcal{S}_0$ and reaction set designated $\mathcal{R}_0$) is a complete even cycle cluster, whereupon all of its directed cycles are

even. From condition (i), then, no directed cycle in the source is stoichiometrically expansive. From condition (ii) the source has a simple core. Because no directed cycle in the simple core has a stoichiometrically expansive orientation, it follows from Proposition 6.20 that there is a set of non-negative numbers $\{M_s\}_{s \in S_0}$, not all zero, that satisfies the inequality system (15). Thus, the discordance-denying goal set in §6.7 has been achieved.

6.10. Proposition 4.2 and Theorem 4.1 as a corollaries of Theorem 6.10

Here we show that Proposition 4.2 and Theorem 4.1 emerge as a corollaries of Theorem 6.10. We begin with a proposition, for which we need some vocabulary.

If G is a directed subgraph of the SR Graph, we say that $G_* \subset G$ is an *R-subgraph* of G if it satisfies the first two requirements of a simple core but not necessarily the third; that is, G_* is strongly connected and, within G_* , every reaction vertex is adjacent to precisely two species vertices. Furthermore, we say that G_* has an *R-to-S ear* in G if there exists in G a directed path that begins with a reaction vertex of G_* , ends with a species vertex of G_* , but contains no edge of G_* .

Proposition 6.21—If G is a strongly connected directed subgraph of the SR Graph that has no simple core, then there exists in G an R-subgraph having an R-to-S ear in G .

Proof: We can show the existence of the required R-subgraph by means of an iterative construction. Because G is strongly connected we know that it contains at least one R-subgraph G_0 , which can, for example, be taken to be any directed cycle in G .

Suppose, then, that G_i is an R-subgraph of G , where i is a non-negative integer index label. Because G has no simple core, there must be an edge of G that is not in G_i and that terminates at a species vertex of G_i . Let $R \rightarrow s$ be such an edge. Because G is strongly connected, there must be a directed path P that originates at a vertex of G_i , is edge-disjoint from G_i , and terminates in R . Let E denote the union of P with the edge $R \rightarrow s$. Note that E originates at a vertex of G_i , terminates at the species vertex s , and has no edge in common with G_i . If the originating vertex of E is a reaction vertex of G_i , then G_i can be taken to be the required R-subgraph, with E its R-to-S ear.

Suppose, on the other hand, that the originating vertex of E is a species vertex of G_i , say $\hat{s}$ (which might be s). In this case E is a path (when $\hat{s} \neq s$) or a cycle (when $\hat{s} = s$) having no edge in common with G_i and whose only vertices in common with G_i are species vertices corresponding to members of $\{s, \hat{s}\}$.

Now let $G_{i+1} = G_i \cup E$. Note that G_{i+1} is another, larger R-subgraph of G . The argument applied above to G_i can be applied in the same way to G_{i+1} and, in fact, iteratively. Because G has no simple core the process must end in an R-subgraph that has an R-to-S ear.

The preceding proposition gives rise to the next one, which will bring us just short of the proof of Theorem 4.1.

Proposition 6.22—If G is a strongly connected directed subgraph of the SR Graph that has no simple core, then there exists in G a pair of consistently oriented cycles having as their intersection a single directed path originating at a species vertex and terminating at a reaction vertex.

Proof: From Proposition 6.21 it follows that there exists in G an R-subgraph having an R-to-S ear. We denote that R-subgraph by G^0 , and we denote by R^0 and S^0 the reaction and species vertices at the ends of that ear, denoted E . Because G^0 is strongly connected, there are within G^0 a directed path P originating at R^0 and terminating at S^0 and also a directed path Q originating at S^0 and terminating at R^0 . We show schematically some possibilities in Figure 6.

Because G^0 is an R-subgraph of G , the first vertex along the path P that is also a vertex of Q must be a species vertex. We denote that vertex by S^* (which might be S^0), and we denote by R^0PS^* the segment of P beginning at R^0 and terminating at S^* .

Note that, by virtue of the definition of S^* , there can be no species vertex of R^0PS^* that is also a vertex of Q (apart from S^*). Thus, there can be no internal common vertices of the path R^0PS^* and the directed segment of Q (denoted S^*QR^0) that begins at S^* and terminates at R^0 . Therefore, the union of the two paths, $R^0PS^*QR^0$, is a directed cycle (which we call $C1$).

Note also that the union of the ear E and the path Q is a directed cycle, which can be regarded as the union of E with the two complementary Q -segments S^0QS^* and S^*QR^0 . This second cycle, $C2 := ES^0QS^*QR^0$ and the cycle $C1$ have as their intersection the single path S^*QR^0 , which begins at the species vertex S^* and terminates at the reaction vertex R^0 .

We are now in a position to see that Proposition 4.2 and Theorem 4.1 are consequences of Theorem 6.10. Suppose that the two conditions in the hypothesis of Theorem 4.1 are satisfied. (These are identical to the two conditions in the hypothesis of Proposition 4.2.) Condition (i) of Theorem 4.1 is identical to condition (i) of Theorem 6.10. Now if condition (ii) of Theorem 4.1 is satisfied, condition (ii) of Theorem 6.10 is also satisfied: When condition (ii) of Theorem 4.1 is satisfied, Proposition 6.22 ensures that every strongly connected directed subgraph of the SR Graph has a simple core. In particular, every even cycle cluster has a simple core. Thus, when the hypothesis of Theorem 4.1 is satisfied, so is the hypothesis of Theorem 6.10.

6.11. Proof of Proposition 5.2

Our aim in this section is to provide arguments supporting Proposition 5.2. Theorem 5.1 then follows from Proposition 5.2 by arguments in [1]. For the record, we first provide the definition of strong concordance [1]. In the definition, we consider a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ with stoichiometric subspace S . The linear map $L : S \rightarrow S$ is the same as in (9).

Definition 6.23—A reaction network is *strongly concordant* if there do not exist $a \in \ker L$ and a nonzero $\sigma \in S$ satisfying the following conditions:

- i. For each $y \rightarrow y'$ such that $\alpha_{y \rightarrow y'} > 0$, there exists a species s for which $\text{sgn} \sigma_s = \text{sgn}(y - y')_s = 0$.
- ii. For each $y \rightarrow y'$ such that $\alpha_{y \rightarrow y'} < 0$, there exists a species s for which $\text{sgn} \sigma_s = -\text{sgn}(y - y')_s = 0$.
- iii. For each $y \rightarrow y'$ such that $\alpha_{y \rightarrow y'} = 0$, either (a) $\sigma_s = 0$ for all $s \in \text{supp } y$, or (b) there exist species s, s' for which $\text{sgn} \sigma_s = \text{sgn}(y - y')_s = 0$ and $\text{sgn} \sigma_{s'} = -\text{sgn}(y - y')_{s'} = 0$.

We repeat Proposition 5.2 below:

Proposition 5.2—A nondegenerate network is strongly concordant if its Species-Reaction Graph has the following properties:

- i. Every even cycle is an s-cycle.
- ii. No two even cycles have as their intersection a single path with a species vertex at one end and reaction vertex at the other.

The proof will require the following lemma, which was proved in [2].

Lemma 6.24—Suppose that the fully open extension of reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is not strongly concordant. Then there is another reaction network $\{\mathcal{S}, \bar{\mathcal{C}}, \mathcal{R}\}$ whose fully open extension is discordant and whose SR Graph is identical to a subgraph of the SR Graph for $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, apart perhaps from changes in certain arrow directions within the reaction vertices.

Proof of Proposition 5.2: Suppose that the SR Graph for a nondegenerate reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ satisfies both conditions (i) and (ii) of Proposition 5.2. In this case it follows from Proposition 4.2 that the network and its fully open extension are concordant. (The fully open extension is also nondegenerate, as are all fully open networks (Remark C.6).)

We need to show that network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is strongly concordant. Suppose not. Then, because the network is nondegenerate, its fully open extension is not strongly concordant [1, 2]¹⁸. From Lemma 6.24 there is a reaction network $\{\mathcal{S}, \bar{\mathcal{C}}, \mathcal{R}\}$ whose fully open extension is discordant and whose SR Graph is identical to a subgraph of the SR Graph for $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, apart perhaps from changes in certain arrow directions within the reaction vertices. That subgraph will also satisfy conditions (i) and (ii) of Proposition 5.2, which are independent of reaction arrow directions. When those conditions are satisfied, so too must conditions (i) and (ii) of Theorem 4.1, in which case Proposition 4.2 ensures that the fully open extension of $\{\mathcal{S}, \bar{\mathcal{C}}, \mathcal{R}\}$ is concordant.¹⁹ Thus, we have a contradiction.

References

1. Shinar G, Feinberg M. Concordant chemical reaction networks. *Mathematical Biosciences*. 2012; 240:92–113. [PubMed: 22659063]

¹⁸In particular, it is shown in [1] that a normal network with a strongly concordant fully open extension is itself strongly concordant. In Appendix C we argue that for a network with a concordant fully open extension, which is the case here, there is no distinction between nondegenerate, weakly normal, and normal (Remark C.20).

¹⁹Note that the fully open extension of $\{\mathcal{S}, \bar{\mathcal{C}}, \mathcal{R}\}$ is nondegenerate (Remark C.6) and has the same SR Graph as the network itself.

2. Shinar G, Feinberg M. Concordant chemical reaction networks and the species-reaction graph. *Mathematical Biosciences*. 2013; 241:1–23. [PubMed: 22940368]
3. Feinberg M. Chemical reaction network structure and the stability of complex isothermal reactors I. The deficiency zero and deficiency one theorems. *Chemical Engineering Science*. 1987; 42:2229–2268.
4. Ji, H. PhD thesis. Department of Mathematics, The Ohio State University; 2011. Uniqueness of Equilibria for Complex Chemical Reaction Networks.
5. Ji, H.; Ellison, P.; Knight, D.; Feinberg, M. The chemical reaction network toolbox, version 2.3. 2014. Available at <http://www.crnt.osu.edu/CRNTWin>
6. Schlosser, PM. PhD thesis. University of Rochester; 1988. A Graphical Determination of the Possibility of Multiple Steady States in Complex Isothermal CFSTRs.
7. Schlosser PM, Feinberg M. A theory of multiple steady states in isothermal homogeneous CFSTRs with many reactions. *Chemical Engineering Science*. 1994; 49:1749–1767.
8. Craciun, G. PhD thesis. The Ohio State University; 2002. Systems of Nonlinear Differential Equations Deriving from Complex Chemical Reaction Networks.
9. Craciun G, Feinberg M. Multiple equilibria in complex chemical reaction networks. II. The species-reaction graph. *SIAM Journal on Applied Mathematics*. 2006; 66:1321–1338.
10. Craciun G, Tang Y, Feinberg M. Understanding bistability in complex enzyme-driven reaction networks. *Proceedings of the National Academy of Sciences*. 2006; 103:8697–8702.
11. Banaji M, Craciun G. Graph-theoretic approaches to injectivity and multiple equilibria in systems of interacting elements. *Communications in Mathematical Sciences*. 2009; 7:867–900.
12. Banaji M, Craciun G. Graph-theoretic criteria for injectivity and unique equilibria in general chemical reaction systems. *Advances in Applied Mathematics*. 2010; 44:168–184. [PubMed: 20161590]
13. Craciun G, Feinberg M. Multiple equilibria in complex chemical reaction networks: Semiopen mass action systems. *SIAM Journal on Applied Mathematics*. 2010; 70:1859–1877.
14. Donnell, P.; Banaji, M.; Marginean, A.; Pantea, C. Control: a tool for the analysis of chemical reaction networks. 2014. Available at <http://reaction-networks.net/control/>
15. Feinberg, M. Written version of lectures given at the Mathematical Research Center. University of Wisconsin; Madison, WI: 1979. Lectures on chemical reaction networks. Available at <http://www.crnt.osu.edu/LecturesOnReactionNetworks>
16. Shinar G, Knight D, Ji H, Feinberg M. Stability and instability in isothermal CFSTRs with complex chemistry: Some recent results. *AIChE Journal*. 2013; 59:3403–3411.
17. Bondy, A.; Murty, U. *Graph Theory*. Springer; 2010.
18. Horn F, Jackson R. General mass action kinetics. *Archive for Rational Mechanics and Analysis*. 1972; 47:81–116.
19. Horn F. Necessary and sufficient conditions for complex balancing in chemical kinetics. *Archive for Rational Mechanics and Analysis*. 1972; 49:172–186.
20. Feinberg M. The existence and uniqueness of steady states for a class of chemical reaction networks. *Archive for Rational Mechanics and Analysis*. 1995; 132:311–370.
21. Greub, WH. *Linear Algebra*. 4. Springer; 1981.
22. Craciun G, Feinberg M. Multiple equilibria in complex chemical reaction networks. I. The injectivity property. *SIAM Journal on Applied Mathematics*. 2005; 65:1526–1546.

Appendices

A. Supplementary definitions

In this appendix we repeat a few definitions from [1]. Although the definitions provided here are not essential for the proofs of the results in the main body of this article, they will be especially helpful in the appendices to follow. For a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ a mixture state is generally represented by a *composition vector* $c \in \overline{\mathbb{R}}_+^{\mathcal{S}}$, where, for each $s \in \mathcal{S}$, we

understand c_s to be the molar concentration of s . By a *positive composition* we mean a strictly positive composition — that is, a composition in $\mathbb{R}_+^{\mathcal{S}}$.

Definition A.1

A *kinetics* $\mathcal{K}$ for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is an assignment to each reaction $y \rightarrow y' \in \mathcal{R}$ of a *rate function* $\mathcal{K}_{y \rightarrow y'}: \mathbb{R}_+^{\mathcal{S}} \rightarrow \mathbb{R}_+$ such that

$$\mathcal{K}_{y \rightarrow y'}(c) > 0 \text{ if and only if } \text{supp } y \subset \text{supp } c. \quad (\text{A.1})$$

A *kinetic system* $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ is a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ taken with a kinetics $\mathcal{K}$ for the network.

Definition A.2

A kinetics $\mathcal{K}$ for reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is *weakly monotonic* if, for each pair of compositions c^* and c^{**} , the following implications hold for each reaction $y \rightarrow y' \in \mathcal{R}$ such that $\text{supp } y \subset \text{supp } c^*$ and $\text{supp } y \subset \text{supp } c^{**}$:

- i. $\mathcal{K}_{y \rightarrow y'}(c^{**}) > \mathcal{K}_{y \rightarrow y'}(c^*) \Rightarrow$ there is a species $s \in \text{supp } y$ with $c_s^{**} > c_s^*$.
- ii. $\mathcal{K}_{y \rightarrow y'}(c^{**}) = \mathcal{K}_{y \rightarrow y'}(c^*) \Rightarrow c_s^{**} = c_s^*$ for all $s \in \text{supp } y$ or else there are species $s, s' \in \text{supp } y$ with $c_s^{**} > c_s^*$ and $c_{s'}^{**} < c_{s'}^*$.

We say that the kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ is weakly monotonic when its kinetics $\mathcal{K}$ is weakly monotonic.

Definition A.3

A kinetics $\mathcal{K}$ for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is *differentiably monotonic* at $c^* \in \mathbb{R}_+^{\mathcal{S}}$ if, for every reaction $y \rightarrow y' \in \mathcal{R}$, $\mathcal{K}_{y \rightarrow y'}(\cdot)$ is differentiable at c^* and, moreover, for each species $s \in \mathcal{S}$,

$$\frac{\partial \mathcal{K}_{y \rightarrow y'}}{\partial c_s}(c^*) \geq 0, \quad (\text{A.2})$$

with inequality holding if and only if $s \in \text{supp } y$. A *differentiably monotonic kinetics* is one that is differentiably monotonic at every positive composition.

Definition A.4

The *species formation rate function* for a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ with stoichiometric subspace S is the map $f: \mathbb{R}_+^{\mathcal{S}} \rightarrow S$ defined by

$$f(c) = \sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{K}_{y \rightarrow y'}(c)(y' - y). \quad (\text{A.3})$$

An *equilibrium* of the kinetic system is a composition $c^* \in \bar{\mathbb{R}}_+^{\mathcal{S}}$ such that $f(c^*) = 0$.

Remark A.5

From (A.1) and (A.3) it follows that a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ can admit a positive equilibrium — that is, an equilibrium in $\bar{\mathbb{R}}_+^{\mathcal{S}}$ — only if its reaction vectors are *positively dependent*, which is to say that there are positive numbers $\{\alpha_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}}$ such that

$$\sum_{y \rightarrow y' \in \mathcal{R}} \alpha_{y \rightarrow y'}(y' - y) = 0. \quad (\text{A.4})$$

Definition A.6

Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a reaction network with stoichiometric subspace S . (Recall §6.1.) Two compositions c and c' in $\bar{\mathbb{R}}_+^{\mathcal{S}}$ are *stoichiometrically compatible* if $c' - c$ lies in S .

Definition A.7

A kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ is *injective* if, for each pair of distinct stoichiometrically compatible compositions $c^* \in \bar{\mathbb{R}}_+^{\mathcal{S}}$ and $c^{**} \in \bar{\mathbb{R}}_+^{\mathcal{S}}$, at least one of which is positive, $f(c^*) \neq f(c^{**})$; that is,

$$\sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{K}_{y \rightarrow y'}(c^{**})(y' - y) \neq \sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{K}_{y \rightarrow y'}(c^*)(y' - y). \quad (\text{A.5})$$

B. Discordance and the existence of multiple stoichiometrically compatible equilibria

In Theorem 4.11 of [1] we established that *a reaction network has injectivity in all weakly monotonic kinetic systems derived from it if and only if the network is concordant*. This is to say that the class of concordant networks *coincides* with the class of networks that are injective against every choice of weakly monotonic kinetics. A consequence of this is that, for a concordant network, no choice of weakly monotonic kinetics can result in two distinct stoichiometrically compatible equilibria, at least one of which is positive.

On the other hand, we did not assert that for every *discordant* network there is a weakly monotonic kinetics that results in two distinct stoichiometrically compatible equilibria, at least one of which is positive. In fact, such an assertion would be false, for not every discordant network has the capacity to admit a positive equilibrium. In particular, there are

discordant networks for which the reaction vectors are not positively dependent (see Remark A.5), and for those networks no choice of a kinetics, weakly monotonic or not, can result in even one positive equilibrium.

In this appendix we show that if attention is restricted to networks that do have the capacity to admit a positive equilibrium (i.e., to networks that have positively dependent reaction vectors) then discordance of the network is equivalent to the existence of a weakly monotonic kinetics that results in a pair of distinct stoichiometrically compatible positive equilibria. This is the substance of the Proposition B.1 below, which essentially amounts to an easy corollary of Theorem 4.11 of [1].

Proposition B.1

For a reaction network having positively dependent reaction vectors, the following are equivalent:

- i. The network is discordant.
- ii. There exists for the network a weakly monotonic kinetics for which the species-formation-rate function admits two distinct stoichiometrically compatible positive equilibria.

Proof

That (ii) implies (i) is a straightforward, for when (ii) is satisfied there exists for the network a weakly monotonic kinetics for which the resulting kinetic systems is not injective. From Theorem 4.11 in [1] it follows that the network is discordant.

It remains to be shown that (i) implies (ii). Suppose that network $\{ \mathcal{S}, \mathcal{C}, \mathcal{R} \}$ is discordant. From the proof of Theorem 4.11 in [1]²⁰ there is a weakly monotonic kinetics $\{ \mathcal{K}_{y \rightarrow y'} \}_{y \rightarrow y' \in \mathcal{R}}$ (in fact a power law kinetics) and two distinct stoichiometrically compatible positive compositions c^* and c^{**} at which the species formation rate function takes the same value. That is, for some ξ in the stoichiometric subspace we have

$$\sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{K}_{y \rightarrow y'}(c^{**})(y' - y) = \xi \quad (\text{B.1})$$

and

$$\sum_{y \rightarrow y' \in \mathcal{R}} \mathcal{K}_{y \rightarrow y'}(c^*)(y' - y) = \xi. \quad (\text{B.2})$$

Because the reaction vectors are positively dependent, $-\xi$ has a representation of the form (B.3), in which the numbers $\{\gamma_{y \rightarrow y' \in \mathcal{R}}\}_{y \rightarrow y' \in \mathcal{R}}$ are all positive.

²⁰See in particular the proof of Proposition 4.9 in [1].

$$\sum_{y \rightarrow y' \in \mathcal{R}} \gamma_{y \rightarrow y'}(y' - y) = -\xi. \quad (\text{B.3})$$

Now let $\mathcal{K}$ be a kinetics defined in the following way: For each $y \rightarrow y' \in \mathcal{R}$

$$\begin{aligned} \overline{\mathcal{K}}_{y \rightarrow y'}(c) &= \mathcal{K}_{y \rightarrow y'}(c) + \gamma_{y \rightarrow y'} & \text{if } \text{supp } y \subset \text{supp } c, \\ \overline{\mathcal{K}}_{y \rightarrow y'}(c) &= 0 & \text{if } \text{supp } y \not\subset \text{supp } c. \end{aligned}$$

This kinetics is weakly monotonic, and, moreover, c^* and c^{**} are stoichiometrically compatible positive equilibria for the kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$.

Remark B.2

In Definition A.1 (and similarly in [1]) we did not insist that the reaction rate functions be continuous, for many of the results in [1] do not require it. Indeed, the kinetics $\mathcal{K}$ constructed above will have rate functions that fail to be continuous at places on the boundary of $\overline{\mathbb{R}}_+^{\mathcal{S}}$. Slightly more complicated constructions, serving the same purpose, can be made to result in continuous rate functions. For one such example, we begin by letting $a_s = \min\{c_s^*, c_s^{**}\}$, $\forall s \in \mathcal{S}$, and, for each $y \rightarrow y' \in \mathcal{R}$, we take $\theta_{y \rightarrow y'}: \overline{\mathbb{R}}_+^{\mathcal{S}} \rightarrow [0, 1]$ to be defined by

$$\begin{aligned} \theta_{y \rightarrow y'}(c) &= \prod_{s \in \text{supp } y} \left(\frac{c_s}{a_s}\right) & \text{if } \prod_{s \in \text{supp } y} \left(\frac{c_s}{a_s}\right) \leq 1, \\ \theta_{y \rightarrow y'}(c) &= 1 & \text{if } \prod_{s \in \text{supp } y} \left(\frac{c_s}{a_s}\right) > 1. \end{aligned}$$

Finally, we can replace $\mathcal{K}$ in the proof above by the following continuous kinetics: For each $y \rightarrow y' \in \mathcal{R}$

$$\overline{\mathcal{K}}_{y \rightarrow y'}(c) = \mathcal{K}_{y \rightarrow y'}(c) + \theta_{y \rightarrow y'}(c) \gamma_{y \rightarrow y'}.$$

The kinetics is weakly monotonic and, for the resulting kinetic system, c^* and c^{**} are again stoichiometrically compatible positive equilibria. In a similar way, one can construct differentially monotonic kinetics serving the same purpose.

C. On nondegenerate networks

This appendix is intended as a supplement to the main article in which we elaborate on properties of nondegenerate reaction networks. In particular, we provide a formal statement of what it means for a network to be non-degenerate and also some alternative characterizations of nondegeneracy [2]. Unlike the primary definition, these alternative characterizations make no mention of kinetics but, rather, are intrinsic to the network itself. Finally, for a network that satisfies the nondegeneracy condition, we explore the relationship

between concordance of the network and concordance of the network's fully open extension. It should be kept in mind that in [5] we provide a freely available and easy-to-use computational tool to test for network nondegeneracy and for concordance.

Definition C.1

A reaction network is *nondegenerate* if there exists for it a differentiable monotonic kinetics such that at some positive composition c^* (not necessarily an equilibrium) the derivative of the species-formation-rate function $df(c^*): S \rightarrow S$ is nonsingular. Otherwise, the network is *degenerate*.

Remark C.2

Every weakly reversible network can be assigned a complex balanced mass action kinetics [15, 18, 19]. For such a kinetics there is precisely one positive equilibrium in each nontrivial stoichiometric compatibility class, and, moreover, the derivative of the species formation rate function is nonsingular at every positive equilibrium [20]. Thus *every weakly reversible network (and, therefore, every reversible network) is nondegenerate*.²¹

Example C.3

The network displayed as (C.1) is degenerate. On the other hand, if $B \rightarrow A + C$ is replaced by its reversible version, $B \rightleftharpoons A + C$, the network becomes nondegenerate.

For reasons already given in this article and, more so, in [1, 2], the following theorem has considerable importance.

Theorem C.4

A nondegenerate network is concordant if its fully open extension is concordant. In particular, a weakly reversible network is concordant if its fully open extension is concordant.

The proof of Theorem C.4 was essentially given in [1, 2].²² Here we will outline a very different proof, based on ideas in [13]. This second proof has the virtue of simultaneously pointing to several different equivalent characterizations of the nondegeneracy condition, some of which have attractive computational features (Remark C.19) that are exploited in [5]. These alternative characterizations are described in the following proposition, proved below, in which it is understood that $\mathbb{R}^{\mathcal{S}}$ has the natural scalar product

²¹This was the idea that motivated the proof in [13] that every weakly reversible network (and, therefore, every reversible network) is *normal*. Normality implies nondegeneracy. See Remark C.9.

²²In [1] we showed that a *normal* network (Remark C.9) is concordant if its fully open extension is concordant. The proof for *weakly normal* networks (Remark C.9) or, equivalently, for nondegenerate networks is virtually identical. In fact, as we shall see in Remark C.20 there is no difference between normality and weak normality of a network when the network's fully open extension is concordant.

$$v \cdot w := \sum_{s \in \mathcal{S}} v_s w_s.$$

Proposition C.5

Consider a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ of rank r having stoichiometric subspace S . The following are equivalent:

- i. The network is nondegenerate.
- ii. For each reaction $y \rightarrow y'$, there is a vector $p_{y \rightarrow y'} \in \bar{\mathbb{R}}_+^{\mathcal{S}}$ with $\text{supp } p_{y \rightarrow y'} = \text{supp } y$ such that the linear transformation $T: S \rightarrow S$ defined by

$$T\sigma := \sum_{y \rightarrow y' \in \mathcal{R}} p_{y \rightarrow y'} \cdot \sigma(y' - y) \quad (\text{C.2})$$

is nonsingular (or, equivalently, $\det T \neq 0$).

- iii. There is a choice of distinct species $\{s_i\}_{i=1 \dots r}$ and r reactions $\{y_i \rightarrow y'_i\}_{i=1 \dots r}$ with $s_i \in \text{supp } y_i$, $i = 1 \dots r$, such that the matrix

$$\left[s_i \cdot (y'_j - y_j) \right]_{i,j=1 \dots r} \quad (\text{C.3})$$

has nonzero determinant.

- iv. There is a choice of distinct species $\mathcal{S}^* = \{s_i\}_{i=1 \dots r}$ and r reactions $\{y_i \rightarrow y'_i\}_{i=1 \dots r}$ with $s_i \in \text{supp } y_i$, $i = 1 \dots r$, such that the $\mathcal{S}^*$ -projected reaction vectors $\{\bar{y}'_i - \bar{y}_i\}_{i=1 \dots r}$ are linearly independent.

In item (iv) when we refer to the $\mathcal{S}^*$ -projection of a vector $x \in \mathbb{R}^{\mathcal{S}}$ we mean the vector $\bar{x} \in \mathbb{R}^{\mathcal{S}^*}$ such that $x_s^- = x_s$ for all $s \in \mathcal{S}^*$ and $x_s^- = 0$ for all $s \notin \mathcal{S}^*$.

Remark C.6

Every fully open reaction network is non-degenerate. To see this, suppose that $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is fully open, in which case its rank is the number of species. Then, in (iii) or (iv) we can choose the reaction set to be $\{s \rightarrow 0\}_{s \in \mathcal{S}}$ and $\mathcal{S}^* = \mathcal{S}$.

Example C.7

For the rank 4 network (C.4) we can, in item (iv),

choose $\mathcal{S}^* = \{A, C, D, F\}$, with the corresponding reaction set taken to be $\{A \rightarrow B, A + C \rightarrow B, D \rightarrow E, C + F \rightarrow 0\}$. The projected reaction vectors

$$\{-A, -(A+C), -D, -(C+F)\}$$

are linearly independent. Thus, (C.4) is nondegenerate. No such choice, satisfying the requirements of (iv), can be made for the degenerate network (C.1).

Remark C.8

Taken with mass action kinetics and with a certain assignment of rate constants, the degenerate network (C.1) admits multiple stoichiometrically compatible positive equilibria, as determined by [5]. If those same rate constants are assigned to the corresponding reactions in the nondegenerate network (C.4) the capacity for multiple stoichiometrically compatible positive equilibria disappears, *no matter how small might be the rate constant assigned to the added reaction $A+C \rightarrow B$* . Degenerate networks are poor models for the description of real systems, for phenomena these networks admit can vanish in the presence of arbitrarily small perturbations of the model.

Remark C.9

In [2] a network satisfying condition (ii) was said to be *weakly normal*; it was *normal* if, for some fixed $a \in \mathbb{R}_+^{\mathcal{S}}$ and some set of positive numbers $\{\eta_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}}$, the requirements of condition (ii) are satisfied with the special choice $p_{y \rightarrow y'} = \eta_{y \rightarrow y'} a_y$, $\forall y \rightarrow y' \in \mathcal{R}$. In [2] it was demonstrated that weak normality is equivalent to nondegeneracy; that is, we have the equivalence (i) $\Leftrightarrow$ (ii). In [13] it was shown that every weakly reversible network is normal, from which it follows that every weakly reversible network is nondegenerate. The equivalent conditions (iii) and (iv) also appear in [2] as means to test for nondegeneracy.

For arguments underlying Theorem C.4 and Proposition C.5 it will help to have available Proposition C.10 below. In that proposition, we suppose that V is a finite-dimensional real vector space of dimension p with scalar product “ $\cdot$ ”. Moreover, we suppose that $\det[\cdot, \dots, \cdot]$ is a nontrivial determinant function on V . That is, $\det$ is a skew-symmetric p -linear real-valued function on $V \times V \cdots \times V$ (p times) that is not identically zero. We presume further that $\det$ is normalized such that for some orthonormal basis for V , say $\{b_1, b_2, \dots, b_p\}$, $\det[b_1, b_2, \dots, b_p] = 1$. If $T: V \rightarrow V$ is a linear transformation, then by $\det T$ we mean the number $\det[Tb_1, Tb_2, \dots, Tb_p]$.

Finally, let J denote some index set, having at least p elements. When we write $C(J, p)$, we mean the set of all combinations of (distinct) elements of J taken p at a time. If χ is a member of $C(J, p)$, we indicate the p members of χ by symbols $\{\chi(1), \chi(2), \dots, \chi(p)\}$. The numbering imparts an artificial order to members of χ , but that order will have no significance in anything that follows.

Proposition C.10

Let $\{v_j\}_{j \in J}$ and $\{u_j\}_{j \in J}$ be members of V , let $\{\alpha_j\}_{j \in J}$ be real numbers, and let $T: V \rightarrow V$ be defined by

$$Tx := \sum_{j \in J} \alpha_j (v_j \cdot x) u_j. \quad (\text{C.5})$$

Then

$$\det T = \sum_{\chi \in C(J,p)} \left(\prod_{\theta=1}^p \alpha_{\chi(\theta)} \right) \det [v_{\chi(1)}, \dots, v_{\chi(p)}] \det [u_{\chi(1)}, \dots, u_{\chi(p)}]. \quad (\text{C.6})$$

Equivalently,

$$\det T = \sum_{\chi \in C(J,p)} \left(\prod_{\theta=1}^p \alpha_{\chi(\theta)} \right) \text{Det} [v_{\chi(i)} \cdot u_{\chi(j)}]_{i,j=1,2,\dots,p}. \quad (\text{C.7})$$

Remark C.11

In (C.7) $\text{Det} [\cdot]$ denotes the determinant of the indicated $p \times p$ matrix. The equivalence of (C.6) and (C.7) is a result of the identity (C.8). (That identity is proved in [21].)

$$\det [x_1, x_2, \dots, x_p] \det [w_1, w_2, \dots, w_p] = \text{Det} [x_i \cdot w_j]_{i,j=1,2,\dots,p} \quad (\text{C.8})$$

The proof of (C.6) is essentially the same as that given for a similar formula in [22]. In our use of (C.6) below, the vector space V will be identified not with $\mathbb{R}^{\mathcal{S}}$ for a particular network under study, but, instead, with the network's stoichiometric subspace. In [13] the formula (C.6), also adapted to the stoichiometric subspace setting, was used in much the same way that it will be employed here.

In preparation for the remainder of this appendix we will want to recast linear transformations $T: S \rightarrow S$ of the general form (C.2). Here, as before, S is the stoichiometric subspace for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$. We let $\pi: \mathbb{R}^{\mathcal{S}} \rightarrow S$ denote the projection onto S along $S^\perp$. Then (C.2) can be rewritten as in (C.9), where $p_{y \rightarrow y'}^s$ denotes the component of $p_{y \rightarrow y'}$ corresponding to species s .

$$\begin{aligned} T\sigma &:= \sum_{y \rightarrow y' \in \mathcal{R}} \pi p_{y \rightarrow y'} \cdot \sigma(y' - y) \\ &= \sum_{y \rightarrow y' \in \mathcal{R}} \pi \left(\sum_{s \in \text{supp } y} p_{y \rightarrow y'}^s \cdot s \right) \cdot \sigma(y' - y) \quad (\text{C.9}) \\ &= \sum_{y \rightarrow y' \in \mathcal{R}} \left(\sum_{s \in \text{supp } y} p_{y \rightarrow y'}^s \pi s \right) \cdot \sigma(y' - y) \end{aligned}$$

Now for the network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ let J denote the set of all distinct pairs $[s, y \rightarrow y']$, where $y \rightarrow y'$ is a reaction and s is, for that reaction, a *reactant* species. That is,

$$J := \{[s, y \rightarrow y'] : y \rightarrow y' \in \mathcal{R} \text{ and } s \in \text{supp } y\} \quad (\text{C.10})$$

In effect, J is the set of all distinct “roles” played by the network’s species as *reactants*. For each $j \in J$ we denote by s_j the species of the pair and by $(y \rightarrow y')_j$ or $y(j) \rightarrow y'(j)$ the pair’s reaction. Moreover, for each $j \in J$, we write p_j in place of the number $p_{y(j) \rightarrow y'(j)}^{s(j)}$ in (C.9). Then (C.9) can be rewritten as in (C.11).

$$T\sigma = \sum_{j \in J} p_j (\pi s_j) \cdot \sigma(y' - y)_j \quad (\text{C.11})$$

From this and Proposition C.10 it follows that, for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ of rank r with stoichiometric subspace S , the determinant of any linear map $T: S \rightarrow S$ of the form (C.2) can be written as (C.12). Here J is as indicated above, and $\det_S$ is any normed determinant function on S .

$$\det T = \sum_{\chi \in C(J, r)} \left(\prod_{\theta=1}^r p_{\chi(\theta)} \right) (\det_S [\pi s_{\chi(1)}, \dots, \pi s_{\chi(r)}]) \times (\det_S [(y' - y)_{\chi(1)}, \dots, (y' - y)_{\chi(r)}]) \quad (\text{C.12})$$

Note that, by virtue of the identity (C.8), (C.12) can also be written in the form (C.13). In writing (C.13) we have used the fact that, for any $x \in \mathbb{R}^{\mathcal{S}}$ and any $\xi \in S$, $(\pi x) \cdot \xi = x \cdot \xi$.

$$\det T = \sum_{\chi \in C(J, r)} \left(\prod_{\theta=1}^r p_{\chi(\theta)} \right) \text{Det} [s_{\chi(i)} \cdot (y' - y)_{\chi(j)}]_{i,j=1,2,\dots,r} \quad (\text{C.13})$$

Finally, if, for each $\chi \in C(J, r)$, we let

$$\begin{aligned} D_\chi &:= \det_S [\pi s_{\chi(1)}, \dots, \pi s_{\chi(r)}] \det_S [(y' - y)_{\chi(1)}, \dots, (y' - y)_{\chi(r)}] \\ &= \text{Det} [s_{\chi(i)} \cdot (y' - y)_{\chi(j)}]_{i,j=1,2,\dots,r}, \end{aligned} \quad (\text{C.14})$$

then (C.12) and (C.13) can be written in the succinct form (C.15).

$$\det T = \sum_{\chi \in C(J, r)} \left(\prod_{\theta=1}^r p_{\chi(\theta)} \right) D_\chi \quad (\text{C.15})$$

In light of (C.15) we can view $\det T$ as a homogeneous polynomial of degree r in the variables $\{p_j\}_{j \in J}$ with the D_χ as coefficients. The polynomial becomes nontrivial (that is, not identically zero) if and only if at least one of the D_χ is nonzero. This gives the equivalence of (ii) and (iii) in Proposition C.5. The equivalence of (iii) and (iv) is an easy exercise.

The discussion so far yields the following lemma, which we shall use soon.

Lemma C.12

For a network to be nondegenerate it is necessary and sufficient that $D_\chi = 0$ for at least one $\chi \in C(J, r)$.

We turn now to a proof of Theorem C.4. A simple idea used often in [1, 2] is expressed in the following easy lemma:

Lemma C.13

Suppose that $\{\alpha^*, \sigma^*\}$ is a discordance for reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$. Then, for each reaction $y \rightarrow y' \in \mathcal{R}$, there is a vector $p_{y \rightarrow y'} \in \bar{\mathbb{R}}_+^{\mathcal{S}}$ with $\text{supp } p_{y \rightarrow y'} = \text{supp } y$ such that $\alpha^* = p_{y \rightarrow y'} \cdot \sigma^*$.

This leads immediately to the following lemma, which ties discordance to degeneracy.

Lemma C.14

For a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ with stoichiometric subspace S the following are equivalent:

- i. The network is discordant.
- ii. For each reaction $y \rightarrow y'$, there is a vector $p_{y \rightarrow y'} \in \bar{\mathbb{R}}_+^{\mathcal{S}}$ with $\text{supp } p_{y \rightarrow y'} = \text{supp } y$ such that the linear transformation $T: S \rightarrow S$ defined by

$$T\sigma := \sum_{y \rightarrow y' \in \mathcal{R}} p_{y \rightarrow y'} \cdot \sigma(y' - y) \quad (\text{C.16})$$

is singular.

Remark C.15

For a network to be nondegenerate Proposition C.5 requires that, for *some* choice of $\{p_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}}$, with $\text{supp } p_{y \rightarrow y'} = \text{supp } y$, $\forall y \rightarrow y' \in \mathcal{R}$, the map T be non-singular. Lemma C.14 tells us that, for the network to be concordant, T must be nonsingular for *all* such choices. Thus, nondegeneracy is a necessary condition for concordance. This is the content of Proposition 7.9 in [2].

Lemma C.16

For a network to be discordant it is sufficient that there be members, χ^+ and χ^- , of $C(J, r)$ such that D_{χ^+} is positive and D_{χ^-} is negative. If the network is nondegenerate, this same condition is necessary for discordance.

Proof

Discordance is tantamount to requiring that condition (ii) of Lemma C.14 be satisfied. This amounts to the requirement that, for some choice of positive $\{p_j\}_{j \in J}$, the polynomial on the right side of (C.15) take the value zero.

Suppose that we have the existence of χ^+ and χ^- with D_{χ^+} positive and D_{χ^-} negative. If, in the set $\{p_j\}_{j \in J}$, we choose $p_{\chi^+(\theta)} = P > 0$, $\theta = 1, \dots, r$, and all other p_j equal to 1, we can, by taking P to be sufficiently large, force the polynomial on the right side of (C.15) to take a positive value. Exploiting the existence of χ^- we can, in a similar way, choose $\{p_j\}_{j \in J}$ such that the polynomial takes a negative value. Thus, there is some choice of $\{p_j\}_{j \in J}$ for which $\det T = 0$, whereupon the network is discordant.

Now suppose that the network is nondegenerate and discordant. By virtue of nondegeneracy and Lemma C.12, not all the D_{χ} in the polynomial on the right side of (C.15) are zero. For that polynomial to take the value zero for some choice of positive $\{p_j\}_{j \in J}$, it is therefore necessary that there be χ^+ and χ^- with D_{χ^+} positive and D_{χ^-} negative.

To prove Theorem C.4 we want to show that every nondegenerate discordant reaction network has a discordant fully open extension. With $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ denoting the original network, we let $\{\bar{\mathcal{S}}, \bar{\mathcal{C}}, \bar{\mathcal{R}}\}$ be that network's fully open extension. That is,²³

$$\bar{\mathcal{C}} = \mathcal{C} \cup \mathcal{S} \cup \{0\} \quad \text{and} \quad \bar{\mathcal{R}} = \mathcal{R} \cup \{s \rightarrow 0\}_{s \in \mathcal{S}}. \quad (\text{C.17})$$

The stoichiometric subspace for the fully open extension is readily seen to be $\mathbb{R}^{\bar{\mathcal{S}}}$. We denote by S the stoichiometric subspace for the original network, which will typically be smaller than $\mathbb{R}^{\bar{\mathcal{S}}}$. In the following proposition we show that, in examining discordance of the fully open network by means of Lemma C.14, it is enough to regard T there as a map not on $\mathbb{R}^{\bar{\mathcal{S}}}$ (the stoichiometric subspace for the fully open extension) but, rather, on S (the stoichiometric subspace for the original network).

Before proceeding there is a special situation that must be considered: it might happen that, for one or more species $\hat{s}$, the original network contains the reaction $\hat{s} \rightarrow 0$. In this case, that reaction would not be among the ones added to obtain the network's fully open extension. With this in mind, for the original network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, we denote by $\mathcal{E}$ the set of species $s \rightarrow \mathcal{S}$ such that $s \rightarrow 0$ is *not* a member of $\mathcal{R}$. Similarly, we denote by $\mathcal{E} \rightarrow 0$ the set of reactions in the fully open extension that are not members of $\mathcal{R}$. In most applications we will have $\mathcal{E} = \mathcal{S}$.

Proposition C.17

Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a reaction network with stoichiometric subspace S , and let $\{\bar{\mathcal{S}}, \bar{\mathcal{C}}, \bar{\mathcal{R}}\}$ be the network's fully open extension. The following are equivalent:

- i. $\{\mathcal{S}, \bar{\mathcal{C}}, \bar{\mathcal{R}}\}$ is discordant.

²³Here $s \in \mathcal{S}$ is regarded as a member $\mathbb{R}^{\bar{\mathcal{S}}}$. See §6.1.

- ii. There are $\{\bar{p}_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}} \subset \bar{\mathbb{R}}_+^{\mathcal{S}}$, with $\text{supp } \bar{p}_{y \rightarrow y'} = \text{supp } y$, $\forall y \rightarrow y' \in \mathcal{R}$, such that the map $T: \mathbb{R}^{\mathcal{S}} \rightarrow \mathbb{R}^{\mathcal{S}}$ given by (C.18) is singular.

$$\bar{T}\sigma = \sum_{y \rightarrow y' \in \mathcal{R}} \bar{p}_{y \rightarrow y'} \cdot \sigma(y' - y) + \sum_{s \rightarrow 0 \in \mathcal{E} \rightarrow 0} \bar{p}_{s \rightarrow 0} \cdot \sigma(-s) \quad (\text{C.18})$$

- iii. There are $\{p_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}} \subset \bar{\mathbb{R}}_+^{\mathcal{S}}$, with $\text{supp } p_{y \rightarrow y'} = \text{supp } y$, $\forall y \rightarrow y' \in \mathcal{R}$, such that the map $T_S: S \rightarrow S$ given by (C.19) is singular.

$$\bar{T}_S \sigma = \sum_{y \rightarrow y' \in \mathcal{R}} p_{y \rightarrow y'} \cdot \sigma(y' - y) + \sum_{s \rightarrow 0 \in \mathcal{E} \rightarrow 0} s \cdot \sigma(-s) \quad (\text{C.19})$$

Proof

The equivalence of (i) and (ii) is a consequence of Lemma C.14. That (iii) implies (ii) is trivial. To see that (ii) implies (iii), we suppose that (ii) is true with $\bar{\sigma}$ a nonzero vector in the kernel of T (but not necessarily a member of S). That is,

$$\sum_{y \rightarrow y' \in \mathcal{R}} \bar{p}_{y \rightarrow y'} \cdot \bar{\sigma}(y' - y) + \sum_{s \rightarrow 0 \in \mathcal{E} \rightarrow 0} \bar{p}_{s \rightarrow 0} \cdot \bar{\sigma}(-s) = 0. \quad (\text{C.20})$$

From (C.20) it follows that the second sum on the left lies in the span of the reaction vectors for the original network, which is to say that the second sum on the left is equal to a member of S , which we call $-\sigma_{\mathcal{E}}^*$; in fact,

$$(\sigma_{\mathcal{E}}^*)_s = \bar{p}_{s \rightarrow 0}^s \bar{\sigma}_s, \quad \forall s \in \mathcal{E}. \quad (\text{C.21})$$

Moreover, let σ^* be defined by

$$\sigma^* := \sigma_{\mathcal{E}}^* + \sum_{s \in \mathcal{S} \setminus \mathcal{E}} \bar{\sigma}_s s. \quad (\text{C.22})$$

Because, for each $s \in \mathcal{S} \setminus \mathcal{E}$, $s \rightarrow 0$ is a reaction of the original network, it follows that the rightmost sum in (C.22) is a member of S . Thus, σ^* is a nonzero member of S .

Now let $\{p_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}} \subset \bar{\mathbb{R}}_+^{\mathcal{S}}$ be defined as follows: For each $y \rightarrow y' \in \mathcal{R}$

$$p_{y \rightarrow y'}^s := \frac{\bar{p}_{y \rightarrow y'}^s}{\bar{p}_{s \rightarrow 0}^s}, \quad \forall s \in \mathcal{E} \quad (\text{C.23})$$

and

$$p_{y \rightarrow y'}^s := \bar{p}_{y \rightarrow y'}^s \quad \forall s \in \mathcal{S} \setminus \mathcal{E}. \quad (\text{C.24})$$

With this choice, and with T_S^- then taken as in (C.19), it is not difficult to see from (C.20) that $T_S \sigma^* = 0$, whereupon T_S is singular.

Proof of Theorem C.4

Suppose that the network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is a nondegenerate discordant network. Our aim is to show that the network's fully open extension $\{\mathcal{S}, \bar{\mathcal{C}}, \bar{\mathcal{R}}\}$ is also discordant. In particular, we want to show that, for some choice of $\{p_{y \rightarrow y'}\}_{y \rightarrow y' \in \bar{\mathcal{R}}}$, the map T_S in Proposition C.17 has a determinant of zero.

As before, we regard $\bar{\mathcal{R}}$ to be the disjoint union of $\mathcal{R}$ and $\mathcal{E} \rightarrow 0$. Moreover, for the original network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ we let J be as in (C.10). From Lemma C.16 there must be χ^+ and χ^- in $C(J, r)$ such that D_{χ^+} is positive and D_{χ^-} is negative.

Now let $\bar{J}$ be the set of all distinct “roles” played by the species as reactants in the fully open extension. That is,

$$\begin{aligned} \bar{J} := & \{[s, y \rightarrow y'] : y \rightarrow y' \in \mathcal{R} \text{ and } s \in \text{supp } y\} \\ & \cup \{[s, s \rightarrow 0] : s \rightarrow 0 \in \mathcal{E} \rightarrow 0\}. \end{aligned} \quad (\text{C.25})$$

From the discussion preceding Lemma C.12 it follows that we can express the determinant of T_S in the form

$$\det \bar{T}_S = \sum_{\chi \in C(\bar{J}, r)} \left(\prod_{\theta=1}^r p_{\chi(\theta)} \right) D_{\chi}, \quad (\text{C.26})$$

where it is understood that $p_j = 1$ whenever the reaction $(y \rightarrow y')_j$ is a member of $\mathcal{E} \rightarrow 0$.

Because J is a subset of $\bar{J}$ we clearly have $C(J, r) \subset C(\bar{J}, r)$, whereupon χ^+ and χ^- are members of $C(\bar{J}, r)$. Note that no reaction associated with χ^+ or χ^- is a member of $\mathcal{E} \rightarrow 0$. Exploiting the positivity of D_{χ^+} and the negativity of D_{χ^-} , we can now argue as in the proof of Lemma C.14 that $T_S = 0$ for some choice of $\{p_j\}_{j \in \bar{J}}$ — in particular, for a choice consistent with the requirement that $p_j = 1$ whenever the reaction $(y \rightarrow y')_j$ is a member of $\mathcal{E} \rightarrow 0$. Thus, the fully open extension is discordant.

We conclude this appendix with two useful propositions that follow easily from the preceding material.

Proposition C.18 (An “all-or-nothing” proposition)

Consider a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ with stoichiometric subspace S . If the network has a concordant fully open extension — in particular, if its Species-Reaction Graph satisfies the conditions of Theorem 4.1 — the following are equivalent:

- i. There is **some** choice of $\{p_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}} \subset \bar{\mathbb{R}}_+^{\mathcal{S}}$, with $\text{supp } p_{y \rightarrow y'} = \text{supp } y, \forall y \rightarrow y' \in \mathcal{R}$, such that the map $T: S \rightarrow S$ given by (C.27) is nonsingular.

$$T\sigma = \sum_{y \rightarrow y' \in \mathcal{R}} p_{y \rightarrow y'} \cdot \sigma(y' - y) \quad (\text{C.27})$$

- ii. For **every** choice of $\{p_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}} \subset \bar{\mathbb{R}}_+^{\mathcal{S}}$, with $\text{supp } p_{y \rightarrow y'} = \text{supp } y, \forall y \rightarrow y' \in \mathcal{R}$, the map $T: S \rightarrow S$ given by (C.27) is nonsingular.

Proof

When (i) holds, the network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is nondegenerate. From Theorem C.4 that same network is concordant, which is equivalent to (ii). That (ii) implies (i) is trivial.

Remark C.19

Proposition C.18 has important computational uses, as exploited in [5]. If it is known that a network has a concordant fully open extension, the nondegeneracy (and therefore the concordance) of the network itself can be determined definitively by a simple procedure: Choose *any* set of $\{p_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}}$ consistent with (i), and determine the nonsingularity of the resulting T , for example by ascertaining that its determinant is not zero. One such choice is $p_{y \rightarrow y'} = y, \forall y \rightarrow y' \in \mathcal{R}$. The choice will not matter, for every choice must give the same result. If T is nonsingular, the network is both nondegenerate and concordant; if T is singular, the network is degenerate and discordant.

Remark C.20

When a network's fully open extension is concordant, Proposition C.18 tells us that, for the original network, *there is no distinction between weak normality and normality (Remark C.9) or, equivalently, between nondegeneracy and normality*. In particular, if the network is weakly normal, it is also normal. Network (C.28) is weakly normal but not normal [2]. Its fully open extension, however, is discordant.

Remark C.21

In light of network (C.28), readers familiar with some standard ideas and language of chemical reaction network theory [3, 15] might suspect that degeneracy and discordance are related to an excess of terminal strong linkage classes. This is true, but the relationship is subtle. Let δ , ℓ , and t denote, respectively, a network's deficiency, the number of its linkage classes, and the number of its terminal strong linkage classes. Then:

- i. A network for which $t - \ell - \delta > 0$ is discordant.
- ii. Among networks that have positively dependent reaction vectors, those that satisfy $t - \ell > 0$ and $t - \ell - \delta = 0$ are discordant.

- iii. A network for which $t - \ell - \delta > 0$ can nevertheless be nondegenerate, but only if its fully open extension is discordant. (Network (C.28) is an example.)
- iv. A network for which $t - \ell - \delta = 0$ can be degenerate. (Network (C.1) is an example.)

The following proposition asserts that a network can be degenerate only if, in formulation of the network model, certain reactions have been deemed irreversible. Recall that chemists often insist that all reactions should be deemed reversible, albeit with some reverse reactions occurring at very small rates.

Proposition C.22

Suppose that a network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ of rank r is degenerate. Then there is nondegenerate network $\{\mathcal{S}, \mathcal{C}, \overline{\mathcal{R}}\}$, with $\mathcal{R} \subset \overline{\mathcal{R}}$, having no more than r additional reactions, each being the reverse of a reaction in the original network.

Example C.23

Contrast the degenerate network (C.1) with the nondegenerate network (C.4), in which just one reaction of (C.1) has been made reversible. The rank of both networks is four.

Proof

Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}^*\}$ be the network obtained by making every reaction in the network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ reversible. By virtue of Proposition 7.2 in [13] every weakly reversible and, therefore, every reversible network is nondegenerate. With

$$J^* := \{[s, y \rightarrow y'] : y \rightarrow y' \in \mathcal{R}^* \text{ and } s \in \text{supp } y\},$$

it follows from Lemma C.12 that there exists $\chi^* \in C(J^*, r)$ such that $D_{\chi^*} = 0$. Now let

$$\overline{\mathcal{R}} := \mathcal{R} \cup \{(y \rightarrow y')_{\chi^*(\theta)}\}_{\theta=1,2,\dots,r}$$

and

$$\overline{J} := \{[s, y \rightarrow y'] : y \rightarrow y' \in \overline{\mathcal{R}} \text{ and } s \in \text{supp } y\}.$$

Clearly, χ^* is a member of $C(\overline{J}, r)$ with $D_{\chi^*} = 0$. From Lemma C.12 the network $\{\mathcal{S}, \mathcal{C}, \overline{\mathcal{R}}\}$ is nondegenerate.

D. Completion of the Proof of Proposition 6.17

In this appendix we complete the proof of Proposition 6.17, which is repeated here:

Proposition D.1

Given a discordance for a reaction network, consider a source in the corresponding sign-causality graph. Let G be the subgraph of the network's SR Graph having the same vertices and edges as the source, with each edge in G given a direction " $\rightarrow$ " identical to its $\rightsquigarrow$ -direction in the source. Then the resulting directed subgraph of the SR Graph has edge directions consistent with the fixed direction edges of the SR Graph, and it is a complete even cycle cluster.

Arguments in [2] ensure that the graph G described in the proposition statement must be an even cycle cluster. It remains to be shown that G is *complete* in the sense of Definition 6.8.

Suppose, on the contrary, that G is not a complete even cycle cluster. This requires that G be a subgraph of a larger even cycle cluster G^* in the SR Graph having the same vertices as G but also one or more additional edges. Hereafter we denote by S^*R^* one such edge, having S^* and R^* as its species and reaction end vertices. Note that S^*R^* must fail to correspond to an edge in the putative source, for otherwise it would be an edge of G .

Our aim is to show that S^*R^* must in fact correspond to an edge of the source. There are four different cases we will want to consider, two when the edge S^*R^* is, in G^* , directed from S^* to R^* , and two when it is directed from R^* to S^* . These are depicted schematically in Figure D.1.

Figure D.1.

Four cases. The markings “odd” and “even” refer to the number of c-pairs on the path connecting $S^\dagger$ and S^* .

First we will suppose that the direction is from S^* to R^* . Because G is strongly connected, there is a directed path in G from R^* to S^* . We denote by $S^\dagger$ species adjacent to R^* along that path. Because G^* is an even cycle cluster, the directed cycle formed by the directed edge S^*R^* and the path from R^* to S^* is even. Because in G^* the direction of the edge S^*R^* is from S^* to R^* , it must be the case that S^* is a *reactant* species of the reaction R^* . On the other hand, $S^\dagger$ might be a reactant species or a product species of R^* .

If $S^\dagger$ is a reactant species of R^* , the edges S^*R^* and $R^*S^\dagger$ constitute a c-pair, in which case the number of c-pairs along the path connecting $S^\dagger$ to S^* must be odd. (See Figure D.1A.) Arguments in [2] then indicate that, in the source, the signs of S^* and $S^\dagger$ are different. Moreover, in the source an edge can be directed from a reaction (R^*) to one of its reactant species ($S^\dagger$) only if their signs differ. Thus, the sign of S^* must be identical to the sign of R^* . In this case, $S^* \rightsquigarrow R^*$ must be an edge of the source, whereupon S^*R^* must be an edge of G .

If $S^\dagger$ is a product species of R^* , the edges S^*R^* and $R^*S^\dagger$ do not constitute a c-pair, in which case the number of c-pairs along the path connecting $S^\dagger$ to S^* must be even. (See Figure D.

1B.) Arguments in [2] then indicate that, in the source, the signs of S^* and $S^\dagger$ are the same. Moreover, in the source an edge can be directed from a reaction (R^*) to one of its product species ($S^\dagger$) only if their signs are the same. Thus, the sign of S^* must be identical to the sign of R^* . In this case, $S^* \leadsto R^*$ must again be an edge of the source, and S^*R^* must be an edge of G .

Next we suppose that the direction of edge S^*R^* in G^* is from R^* to S^* . Because G is strongly connected, there is a directed path in G from S^* to R^* , and we again denote by $S^\dagger$ species adjacent to R^* along that path. Because G^* is an even cycle cluster, the directed cycle formed by the directed edge S^*R^* and the path from S^* to R^* is even. Because in G the direction of the edge $R^*S^\dagger$ is from $S^\dagger$ to R^* , it must be the case that $S^\dagger$ is a *reactant* species of the reaction R^* . On the other hand, S^* might be a reactant species or a product species of R^* .

Consider first the case in which S^* is a reactant species of R^* . Then the edges S^*R^* and $R^*S^\dagger$ constitute a c-pair, in which case the number of c-pairs in the path connecting S^* to $S^\dagger$ is odd. (See Figure D.1C.) From arguments in [2] it follows that, in the source, the signs of S^* and $S^\dagger$ are different, while the signs of $S^\dagger$ and R^* are the same. Thus, the signs of R^* and S^* are different. This requires that in the source there be an edge $R^* \leadsto S^*$, whereupon S^*R^* is an edge of G .

Finally, suppose that S^* is a product species of R^* . In this case the edges S^*R^* and $R^*S^\dagger$ do not constitute a c-pair, so the number of c-pairs in the path connecting S^* to $S^\dagger$ is even. (See Figure D.1D.) From arguments in [2] it follows that, in the source, the signs of S^* and $S^\dagger$ are identical, while the signs of $S^\dagger$ and R^* are also identical. Thus, the signs of R^* and S^* are the same. This requires that in the source there be an edge $R^* \leadsto S^*$, whereupon S^*R^* is an edge of G .

Highlights

- A new theorem determines stability characteristics of reaction networks.
- Use of the theorem requires only inspection of a networks Species-Reaction Graph.
- Use of the theorem requires only weak assumptions about reaction kinetics.

Figure 1.
An instructive example

Figure 2.
Two consistently oriented even cycles intersecting in two directed S-to-R paths

Math Biosci. Author manuscript; available in PMC 2016 April 01.

Figure 4.
SR Graph for network (7)

Figure 5. Some graphs associated with network (10): (a) The Species-Reaction Graph. (b) An incomplete even cycle cluster. (c) A complete even cycle cluster. (d) A simple core residing in (c).

Figure 6.
Schematic depiction of some possibilities in connection with Proposition 6.22