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SIMPLIFYING THE JACOBIAN CRITERION FOR PRECLUDING MULTISTATIONARITY IN CHEMICAL REACTION NETWORKS*

BADAL JOSHI† AND ANNE SHIU‡

Abstract. Chemical reaction networks taken with mass-action kinetics are dynamical systems that arise in chemical engineering and systems biology. In general, determining whether a chemical reaction network admits multiple steady states is difficult, as this requires determining existence of multiple positive solutions to a large system of polynomials with unknown coefficients. However, in certain cases, various easy criteria can be applied. One such test is the Jacobian criterion, due to Craciun and Feinberg, which gives sufficient conditions for ruling out the possibility of multiple steady states. A chemical reaction network is said to pass the Jacobian criterion if all terms in the determinant expansion of its parametrized Jacobian matrix have the same sign. In this article, we present a procedure which simplifies the application of the Jacobian criterion, and as a result, we identify a new class of networks for which multiple steady states is precluded: those in which all chemical species have total molecularity of at most two. The total molecularity of a species refers to the sum of all of its stoichiometric coefficients in the network. We illustrate our results by examining enzyme catalysis networks.

Key words. chemical reaction networks, mass-action kinetics, multiple steady states, Jacobian criterion, injectivity, species-reaction graph

AMS subject classifications. 65H04, 37C25, 34A34

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1. Introduction.

This article concerns an important class of dynamical systems arising in chemical engineering and systems biology, namely, chemical reaction networks taken with mass-action kinetics. As bistable chemical systems are thought to be the underpinnings of biochemical switches, a key question is to determine which systems admit multiple steady states. However, the systems of interest are typically high-dimensional, nonlinear, and parametrized by many unknown reaction rate constants. Therefore, determining whether a chemical reaction network admits multiple steady states is difficult: it requires determining existence of multiple positive solutions to a large system of polynomials with unknown coefficients. However, various criteria have been developed that often can answer this question more easily. For example, the deficiency, advanced deficiency, and higher deficiency theories developed by Ellison [12], Feinberg [14], Horn and Jackson [18], and Ji [19] in many cases can affirm that a network admits multiple steady states or can rule out the possibility. These results have been implemented in the CRNT Toolbox, computer software developed by Feinberg and improved by Ellison and Ji, which is freely available [13]. Related software programs include BioNetX [22, 23] and Chemical Reaction Network Software for Mathematica [21]. For systems for which the software approaches are inconclusive, Conradi et al. [4] advocate a two-pronged approach: they first determine whether certain subnetworks admit multiple steady states and, if so, test whether these instances

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†Department of Mathematics, Duke University, Box 90320, Durham, NC 27708-0320 (joshi@math.duke.edu). This author was partially supported by a National Science Foundation grant (EF-1038593).
‡Department of Mathematics, University of Chicago, Chicago, IL 60637 (annejls@math.uchicago.edu). This author was supported by a National Science Foundation postdoctoral fellowship (DMS-1004380).

can be lifted to the original network. Another criterion for lifting multistationarity from subnetworks appears in [20]. For systems whose steady state locus is defined by binomials, a criterion for multistationarity is given by Pérez Millán et al. in [24]. Finally, a degree theory method which can establish multiple steady states can be found in work of Craciun, Helton, and Williams [9].

A criterion for answering the question of multistationarity is the "mass-action injectivity test." An algorithm for deciding whether a network passes this test is given in the recent thesis of Ji and is implemented in the CRNT Toolbox [13, 19]. One case of this test is the Jacobian criterion, which is the focus of this article. The criterion, which is due to Craciun and Feinberg, gives sufficient conditions for precluding multiple steady states [5, 6, 7, 8]. This work has been extended by Banaji and Craciun [1, 2] and by Feliu and Wiuf [15]. A chemical reaction network is said to pass the Jacobian criterion if all terms in the determinant expansion of its parametrized Jacobian matrix have the same sign (see Lemma 3.4). We prove that for a given network the Jacobian criterion can be checked by examining certain subnetworks (Theorem 4.9).

Although software implementations exist for checking the Jacobian criterion, they cannot handle large networks. As a remedy, our main result (Algorithm 6.1) simplifies the application of the criterion in two ways: first, the network is reduced to a simpler network, and then the number of subnetworks that must be examined is decreased. As an important consequence, *we identify a new class of networks for which multiple steady states is precluded: those networks in which each chemical species has total molecularity of less than or equal to two* (Theorem 5.1). The total molecularity of a species refers to how many nonflow reactions it appears in, where reversible reactions are counted only once and each occurrence of the species is counted with its stoichiometric coefficient; see Definition 2.2. More generally, Algorithm 6.1 allows us in certain cases to rule out the possibility of multiple steady states simply by inspecting the network. In addition, the reduction in the number of subnetworks that must be examined lends support to the observation that typically very few terms (if any) in the Jacobian determinant have anomalous sign [5]; this topic is explored by Helton, Klep, and Gomez [17] and Craciun, Helton, and Williams [9]. A proposed application of our work is to the problem of enumerating networks that admit multiple steady states: our algorithm quickly removes from consideration many small networks that are computationally expensive to enumerate (see Remark 6.4). Finally, we provide examples which demonstrate that our approach is complementary to that of the species-reaction graph theorem of Craciun and Feinberg [7, 10].

We note that our results are stated for *continuous-flow stirred-tank reactors (CFSTRs)*, chemical reaction networks in which all chemical species are removed at rates proportional to their concentrations (see Definition 2.2). However, even for more general networks in which not all species take part in the outflows, our results nevertheless often apply in one of the following two ways. First, one can augment a network by any missing outflows so that it becomes a CFSTR, and then apply our results to determine whether it passes the Jacobian criterion. Then, under mild assumptions on the original network (such as weak-reversibility; see Definition 2.2), the work of Craciun and Feinberg allows us to conclude that the original network also precludes multiple steady states [8, Corollary 8.3]. A second approach is due to work of Feliu and Wiuf. Their extended definition of the Jacobian criterion applies to all networks [15], thereby bypassing the need to augment a network by missing outflows. This definition coincides with that of Craciun and Feinberg for "injective" networks [8, Definition 6.1]; as noted above, the CRNT Toolbox can decide whether a network is injective (including outside of the CFSTR setting) [13]. In addition, we emphasize

that the Jacobian criterion applies for more general dynamics than mass-action kinetics [1, 9]; our results also may be used in those settings. Finally, we note that injective networks (and therefore, CFSTRs that pass the Jacobian criterion) not only preclude multistationarity, but also exhibit a certain robustness of the resulting steady-state fluxes under perturbations of rate constants; these results are due to Shinar et al. [26].

This article is organized as follows. Section 2 provides an introduction to chemical reaction systems. Section 3 describes the Jacobian criterion, and section 4 lays the groundwork for simplifying its implementation. The main result of section 5 (Theorem 5.1) precludes multistationarity from any network in which each chemical species has total molecularity at most two. Section 6 presents Algorithm 6.1, our procedure which simplifies the application of the Jacobian criterion. Finally, section 7 contains examples such as enzyme catalysis networks which illustrate our main results.

2. Chemical reaction network theory. In this section we review the standard notation and define "total molecularity." An example of a *chemical reaction* is denoted by the following:

$$2X_1 + X_2 \;\rightarrow\; X_3.$$

The X_i are called chemical *species*, and $2X_1 + X_2$ and X_3 are called chemical *complexes*. Assigning the *reactant* complex $2X_1 + X_2$ to the vector $y = (2, 1, 0)$, and the *product* complex X_3 to the vector $y' = (0, 0, 1)$, we rewrite the reaction as $y \rightarrow y'$. In general we let s denote the total number of species X_i, and we consider a set of m reactions, each denoted by

$$y_k \rightarrow y'_k$$

for $k \in \{1, 2, \ldots, m\}$, and $y_k, y'_k \in \mathbb{Z}^s_{\geq 0}$, with $y_k \neq y'_k$. We write each such vector y_k as $y_k = (y_{k1}, y_{k2}, \ldots, y_{ks}) \in \mathbb{Z}^s_{\geq 0}$, and we call y_{ki} the *stoichiometric coefficient* of species X_i in complex y_k. For ease of notation, when there is no need for enumeration we will drop the subscript k from the notation for the complexes and reactions.

A *self-catalyzing reaction* will refer to a reaction, such as $A + B \rightarrow 2A + C$, in which there is a species (here, A) that is a *self-catalyst*: it appears with a higher stoichiometric coefficient in the product complex than in the reactant complex.

Definition 2.1. *Let $\mathcal{S} = \{X_i\}$, $\mathcal{C} = \{y\}$, and $\mathcal{R} = \{y \rightarrow y'\}$ denote finite sets of species, complexes, and reactions, respectively. The triple $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is called a* chemical reaction network *if it satisfies the following:*

1. *for each complex $y \in \mathcal{C}$, there exists a reaction in $\mathcal{R}$ for which y is the reactant complex or y is the product complex, and*
2. *for each species $X_i \in \mathcal{S}$, there exists a complex $y \in \mathcal{C}$ that contains X_i.*

We say that a species X_i is a *reactant species* (respectively, *product species*) if it appears in a reactant (respectively, product) complex of some reaction. A subset of the reactions $\mathcal{R}' \subset \mathcal{R}$ defines the *subnetwork* $\{\mathcal{S}|_{\mathcal{C}|_{\mathcal{R}'}}, \mathcal{C}|_{\mathcal{R}'}, \mathcal{R}'\}$, where $\mathcal{C}|_{\mathcal{R}'}$ denotes the set of complexes that appear in the reactions $\mathcal{R}'$, and $\mathcal{S}|_{\mathcal{C}_{\mathcal{R}'}}$ denotes the set of species that appear in those complexes. A network *decouples* if there exist nonempty sets of reactions $\mathcal{R}'$ and $\mathcal{R}''$ that contain disjoint sets of species and such that $\mathcal{R} = \mathcal{R}' \dot{\cup} \mathcal{R}''$. Otherwise, we say that the network is *coupled*.

Definition 2.2.

1. *A chemical reaction network is* weakly reversible *if for each reaction $y \rightarrow y' \in \mathcal{R}$ there exists a sequence of reactions in $\mathcal{R}$ from y' to y (that is, reactions $y' \rightarrow y_1 \rightarrow y_2 \rightarrow \cdots \rightarrow y_k \rightarrow y$). A reaction $y \rightarrow y' \in \mathcal{R}$ is* reversible *if its reverse reaction $y' \rightarrow y$ is also a reaction in $\mathcal{R}$.*

2. *A* flow reaction *contains only one molecule; such a reaction is either an* inflow reaction $0 \to X_i$ *or an* outflow reaction $X_i \to 0$. *A* nonflow reaction *is any reaction that is not a flow reaction.*
3. *A chemical reaction network is a* continuous-flow stirred-tank reactor (CFSTR) network *if it contains all outflow reactions* $X_i \to 0$ *(for all* $X_i \in \mathcal{S}$*).*
4. *Consider a chemical reaction network whose set of nonflow reactions is given by*

$$\begin{array}{cccc} y_1 \to y_1' & y_2 \to y_2' & \cdots & y_l \to y_l' \\ y_{l+1} \leftrightarrows y_{l+1}' & y_{l+2} \leftrightarrows y_{l+2}' & \cdots & y_{l+k} \leftrightarrows y_{l+k}', \end{array}$$

where none of the first l *reactions is reversible. The* total molecularity *of a species* X_i *in the network, denoted by* $\mathrm{TM}(X_i)$*, is the following integer:*

$$\mathrm{TM}(X_i) = \sum_{j=1}^{l+k} \left(y_{ji} + y_{ji}'\right),$$

where y_{ji} *is the stoichiometric coefficient of species* X_i *in the complex* y_j*.*

Note that Craciun and Feinberg use the term "feed reactions" for inflow reactions and "true reactions" for nonflow reactions. In chemical engineering, a CFSTR refers to a tank in which reactions occur. An inflow reaction represents the flow of species (at a constant rate) into the tank in which the nonflow reactions take place, and an outflow reaction represents the removal of a species (at a rate proportional to its concentration).

2.1. Dynamics. The concentration vector $x(t) = \left(x_1(t), x_2(t), \ldots, x_{|\mathcal{S}|}(t)\right)$ will track the concentration $x_i(t)$ of the species X_i at time t. For a chemical reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R} = \{y_k \to y_k'\}\}$, *mass-action kinetics* defines the following system of ordinary differential equations:

$$\dot{x}(t) = \sum_{k=1}^{|\mathcal{R}|} \kappa_k x(t)^{y_k} (y_k' - y_k) =: f(x(t)), \tag{2.1}$$

where the last equality is a definition. Here, we make use of multi-index notation: $x(t)^{y_k} := x_1(t)^{y_{k1}} x_2(t)^{y_{k2}} \cdots x_s(t)^{y_{ks}}$. (By convention, $x_i^0 := 1$.) Also, the κ_k's denote unknown (positive) *reaction rate constants*. A concentration vector $\overline{x} \in \mathbb{R}^s_{>0}$ is a (positive) *steady state* of the mass-action system (2.1) if $f(\overline{x}) = 0$.

Remark 2.3. We can scale each concentration x_i so that each outflow reaction (a reaction $y_k \to y_k'$ with $y_k = X_j$ and $y_k' = 0$) has outflow rate equal to one ($\kappa_k = 1$ for such a reaction) [5, Remark 2.9]. We will make this assumption beginning with the next example.

In the following example and all others in this article, we will label species by distinct letters such as $A, B, \ldots$ rather than $X_1, X_2, \ldots$.

Example 2.4. Consider the following CFSTR:

$$0 \underset{k_2=1}{\overset{k_1}{\rightleftarrows}} A, \qquad 0 \underset{k_4=1}{\overset{k_3}{\rightleftarrows}} B, \qquad A + B \overset{k_5}{\to} 2A.$$

The total molecularity of species A and B are three and one, respectively. The mass-

action differential equations (2.1) for this network are the following:

$$\frac{d}{dt}x_A = k_1 - x_A + k_5 x_A x_B \ ,$$
$$\frac{d}{dt}x_B = k_3 - x_B - k_5 x_A x_B \ .$$

DEFINITION 2.5. *A CFSTR is said to admit* multiple steady states *or is* multistationary *if there exist reaction rate constants* $\kappa_i \in \mathbb{R}_{>0}$ *whose resulting system* (2.1) *admits two or more positive steady states.*

2.2. Square networks. Throughout this article, we will be interested in subnetworks in which the number of species and the number of reactions are equal. We will call such subnetworks "square."

DEFINITION 2.6.

1. *A chemical reaction network is said to be* n-square *if it contains exactly* n *species and exactly* n *directed reactions. A network is said to be* square *if it is* n-square *for some* $n \in \mathbb{Z}_{\geq 0}$. *(A* 0*-square network is the empty network.)*
2. *For an* n*-square chemical reaction network* $N = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ *with reaction set* $\mathcal{R} = \{y_i \to y_i' \mid i = 1, 2, \ldots, n\}$, *the* reactant matrix *and* reaction matrix *are the integer* $(n \times n)$*-matrices*

$$M_N := \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix} \quad \textit{and} \quad R_N := \begin{bmatrix} y_1 - y_1' \\ y_2 - y_2' \\ \vdots \\ y_n - y_n' \end{bmatrix}, \quad \textit{respectively.}$$

3. *For a square chemical reaction network* N, *we define its* orientation $\mathrm{Or}(N) \in \{-1, 0, 1\}$ *as follows:*

$$\mathrm{Or}(N) := \mathrm{sign}\left(\det(M_N)\det(R_N)\right).$$

As defined, our reactant and reaction matrices are the transposes of those of Craciun and Feinberg [5]. However, the orientation of a network is unaffected by transposing either matrix. Note that a reaction $y_i \to y_i'$ is encoded by the vector $y_i - y_i'$ in the reaction matrix R_N, which is the negative of its reaction vector $y_i' - y_i$ which appears in the mass-action differential equations (2.1).

3. The Jacobian criterion. The Jacobian criterion concerns the determinant expansion of the Jacobian matrix of the differential equations (2.1) of a chemical reaction network.

DEFINITION 3.1. *Consider a chemical reaction network* $\mathfrak{G}$ *with* s *species. The* Jacobian matrix *associated with* $\mathfrak{G}$ *is the* $s \times s$*-matrix whose* (i, j)*th entry is the partial derivative* $\frac{\partial}{\partial x_j} f_i(x)$, *where* f_i *is the* i*th differential equation in* (2.1). *The* Jacobian determinant *of* $\mathfrak{G}$, *denoted by* $\mathrm{Jac}(\mathfrak{G})$, *refers to the determinant of the negative of the Jacobian matrix, which is a polynomial in the variables* x_i *(for* $i = 1, 2, \ldots, s$*) and* κ_i.

Note that Craciun and Feinberg use the notation $\det\left(\frac{\partial p_{\mathfrak{G}}}{\partial x}(x, \kappa)\right)$ for $\mathrm{Jac}(\mathfrak{G})$ [5]; here $p_{\mathfrak{G}}$ denotes the negative of the mass-action differential equations for the network obtained from $\mathfrak{G}$ by removing all inflow reactions.

Example 3.2. We now return to the CFSTR examined in Example 2.4, which we denote by $\mathfrak{G}$. Its Jacobian matrix is

$$\begin{pmatrix} -1 + k_5 x_B & k_5 x_A \\ -k_5 x_B & -1 - k_5 x_A \end{pmatrix}.$$

Therefore, the Jacobian determinant of $\mathfrak{G}$ is the following polynomial:

$$\mathrm{Jac}(\mathfrak{G}) = \det \begin{pmatrix} 1 - k_5 x_B & -k_5 x_A \\ k_5 x_B & 1 + k_5 x_A \end{pmatrix} = k_5 x_A - k_5 x_B + 1. \tag{3.1}$$

The following result, which is due to Craciun and Feinberg [5], states that the determinant expansion of the Jacobian matrix has at most one term in the case of a square chemical reaction network.

LEMMA 3.3 (Theorem 3.2 of [5]). *Let N be a chemical reaction network that contains s species and s directed reactions. Then the Jacobian determinant of N, $\mathrm{Jac}(N)$, is either zero or a monomial (in the variables x_i and κ_i) whose coefficient is given by the following product:*

$$\det(M_N) \cdot \det(R_N),$$

where M_N is the reactant matrix and R_N is the reaction matrix.

The following result is due to Craciun and Feinberg.

LEMMA 3.4 (Theorems 3.1 and 3.3 of [5]). *Let $\mathfrak{G} = \mathfrak{G}^{s,r,r_o,r_i}$ be a chemical reaction network that contains s species, r nonflow reactions, r_o outflow reactions, and r_i inflow reactions. Let $\mathrm{Jac}(\mathfrak{G})$ denote the Jacobian determinant associated with $\mathfrak{G}$, which is a polynomial in variables $x_1, x_2, \ldots, x_s$, $\kappa_1, \kappa_2, \ldots, \kappa_r$. Let $G_1, G_2, \ldots, G_{\binom{r+r_o+r_i}{s}}$ enumerate the subnetworks of $\mathfrak{G}$ that contain exactly s reactions such that the first $\binom{r+r_o}{s}$ do not contain inflow reactions.*

1. *Each term in the expansion of $\mathrm{Jac}(\mathfrak{G})$ is the Jacobian determinant of a unique subnetwork G_i. Conversely, the Jacobian determinant of each subnetwork G_i is either zero or is a monomial in the Jacobian determinant of $\mathfrak{G}$. In other words,*

$$\mathrm{Jac}(\mathfrak{G}) = \sum_{i=1}^{\binom{r+r_o+r_i}{s}} \mathrm{Jac}(G_i) = \sum_{i=1}^{\binom{r+r_o}{s}} \mathrm{Jac}(G_i).$$

2. *Moreover, if $\mathfrak{G}$ is a CFSTR, then the following are equivalent:*
   - *The polynomial $\mathrm{Jac}(\mathfrak{G})$ is positive for all $x \in \mathbb{R}^s_{>0}$ and $\kappa_i > 0$.*
   - *Each term $\mathrm{Jac}(G_i)$ is either zero or a positive monomial.*
   - *Each subnetwork G_i has nonnegative orientation: $\mathrm{Or}(G_i) \geq 0$.*

   If these equivalent conditions hold, then $\mathfrak{G}$ does not admit multiple steady states.

One of the main results in the next section is Theorem 4.9, which will extend the equivalent conditions that appear in part 2 of Lemma 3.4 so that instead of checking whether the subnetworks G_i have nonnegative orientation, we need only check the orientations of certain smaller "embedded" networks (Definition 4.6). If any of these conditions hold for a CFSTR $\mathfrak{G}$, for instance if $\mathrm{Jac}(\mathfrak{G})$ has only positive terms, then we say that $\mathfrak{G}$ *passes the Jacobian criterion* (or $\mathfrak{G}$ is "injective").

Remark 3.5. $\mathrm{Jac}(\mathfrak{G})$ always has at least one positive term when $\mathfrak{G}$ contains all outflow reactions (such as for a CFSTR). This arises because one of the subnetworks G_i consists of the s outflow reactions. Clearly, the reactant and reaction matrices of this subnetwork are both the identity matrix, so its orientation is indeed positive.

The aim of this article is to simplify the computation that must be performed in determining whether a CFSTR passes the Jacobian criterion, that is, whether all other terms in the determinant expansion are positive. In fact, there are typically few negative terms [5]; this topic is explored in [9, 17].

NOTATION 3.6. *Although Feliu and Wiuf have extended the definition of the Jacobian criterion to apply also to non-CFSTR networks* [15]*, the main focus of this article is the case when the chemical reaction network* $\mathfrak{G}$ *is a CFSTR. The subnetwork of* $\mathfrak{G}$ *containing all the nonflow reactions but none of the flow reactions will be called the* nonflow subnetwork of $\mathfrak{G}$ *and denoted by* G*. Conversely, any reaction network* $N = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ *is contained in a CFSTR obtained by including all outflow reactions: we denote this CFSTR by*

$$\tilde{N} := \{\mathcal{S},\ \mathcal{C} \cup \mathcal{S} \cup \{0\},\ \mathcal{R} \cup \{X_i \to 0\}_{X_i \in \mathcal{S}}\}.$$

Example 3.7. We now return to the CFSTR examined in Examples 2.4 and 3.2, which has $s = 2$ species and $r = 1$ nonflow reaction. Its nonflow subnetwork is

$$G := \{A + B \to 2A\}.$$

The $\binom{r+r_o}{s} = \binom{3}{2} = 3$ 2-square subnetworks of $\tilde{G} = \mathfrak{G} = \{A \leftrightarrows 0, B \leftrightarrows 0, A+B \to 2A\}$ that do not contain inflow reactions are the following:

$$\begin{aligned} G_1 &= \{A \to 0,\ B \to 0\}, \\ G_2 &= \{A \to 0,\ A + B \to 2A\}, \quad \text{and} \\ G_3 &= \{B \to 0,\ A + B \to 2A\}. \end{aligned}$$

As noted earlier, the subnetwork comprised of all outflow reactions, G_1, has positive orientation: $\mathrm{Or}(G_1) = 1$. Now we examine the reactant and reaction matrices of the remaining subnetworks G_2 and G_3:

$$\begin{aligned} M_{G_2} &= \begin{pmatrix} 1 & 0 \\ 1 & 1 \end{pmatrix}, & R_{G_2} &= \begin{pmatrix} 1 & 0 \\ -1 & 1 \end{pmatrix}, \\ M_{G_3} &= \begin{pmatrix} 0 & 1 \\ 1 & 1 \end{pmatrix}, & R_{G_3} &= \begin{pmatrix} 0 & 1 \\ -1 & 1 \end{pmatrix}. \end{aligned} \tag{3.2}$$

We see that the orientations of the two networks are $\mathrm{Or}(G_2) = \mathrm{sign}\,(\det(M_{G_2})\det(R_{G_2})) = 1$ and $\mathrm{Or}(G_3) = \mathrm{sign}\,(\det(M_{G_3})\det(R_{G_3})) = -1$. Therefore, Lemma 3.4 implies that the CFSTR $\mathfrak{G} = \tilde{G}$ fails the Jacobian criterion. This is consistent with the computation of the Jacobian determinant (3.1) in Example 3.2, which we saw contains one negative term.

3.1. Reactant and reaction rank. The Jacobian criterion requires that each s-square subnetwork G_i of $\mathfrak{G}$ either have a singular reactant matrix, have a singular reaction matrix, or both matrices have nonzero determinants with the same sign. We now give a name to these cases in the following definitions.

DEFINITION 3.8. *A square chemical reaction network* N *is said to have* full reactant-rank *if* $\det(M_N) \neq 0$*, and is said to have* full reaction-rank *if* $\det(R_N) \neq 0$*.*

If a square network has both full reactant-rank and full reaction-rank, then it clearly has *nonzero orientation.*

DEFINITION 3.9. *In a square network* N*, we say that species* X_i *is* changed *in reaction* j *if* $(R_N)_{ji} \neq 0$*.*

In other words, species X_i is changed in reaction j if the concentration of species X_i is changed when reaction j takes place. For example, species A is changed in the reaction $A \to B + C$ and in the reaction $A + B \to 2A + B$. On the other hand, species B and C are changed only in the first reaction.

LEMMA 3.10. *If* N *is a square network with full reactant-rank, then the following hold:*

1. *Each species of N is a reactant species.*
2. *No reaction of N is an inflow reaction or a generalized inflow reaction of the form $0 \to \sum_i a_i X_i$ (where $a_i \geq 0$).*
3. *No two reactions of N share the same reactant complex.*

If N is an n-square network with full reaction-rank, then we have the following:

a. *Each species of N is changed in some reaction.*
b. *N does not contain both a reaction and its reverse.*

Proof. This result follows because nonsingular square matrices cannot have a zero column, a zero row, two identical rows, or a row and its negative. ∎

3.2. Known tests for passing the Jacobian criterion. Known sufficient conditions on the reaction network for passing the Jacobian criterion arise from the following results: the SCL graph theorem of Schlosser and Feinberg [25], the species-reaction graph theorem of Craciun and Feinberg [7, 8] and the related work of Banaji and Craciun [1, 2], and results of Helton, Klep, and Gomez [17]. In this article, we present a complementary test (Algorithm 6.1).

4. Analysis of subnetworks. Theorems 4.9 and 4.11 in this subsection form the basis of the procedure (Algorithm 6.1) that we will introduce to simplify the implementation of the Jacobian criterion. The following example motivates our results.

Example 4.1. We return to the network G in Example 3.7. Note that in both the reactant and product matrices (3.2) of the subnetwork G_2, the first row is the vector $(1, 0)$. So, the orientation of G_2 depends only on the entries in the lower-right of the two matrices. In other words, by letting N_2 denote the 1-square network obtained from G_2 by "removing" the outflow reaction $A \to 0$ and removing the species A from the nonflow reaction $A + B \to 2A$,

$$N_2 = \{B \to 0\},$$

it follows that $\mathrm{Or}(G_2) = \mathrm{Or}(N_2)$. Similarly, for the network obtained from G_3 by removing $B \to 0$ and the species B from the nonflow reaction,

$$N_3 = \{A \to 2A\},$$

we have $\mathrm{Or}(G_3) = \mathrm{Or}(N_3)$.

In this section, we make the simplifications performed in Example 4.1 formal: the first rows in the matrices M_{G_2} and R_{G_2} (3.2) will be called "pivotal rows" (Definition 4.2), Theorem 4.9 will permit us to restrict our attention to the square "embedded" networks N_i rather than the larger s-square subnetworks G_i, and Theorem 4.11 will allow us to remove the species B from the network. In general the networks N_i will be smaller and fewer in number, thereby simplifying the Jacobian criterion.

4.1. Analysis of row and column pivots.

Definition 4.2.

1. *For any matrix A, if there exists a row i with one positive entry, $A_{ij} > 0$ (respectively, < 0) for some j, and all other entries zero, $A_{ik} = 0$ for $k \neq j$, we say that A_{ij} is a positive (respectively, negative)* row pivot *and the ith row is a* pivotal row.
2. *For any matrix A, if $A_{ij} > 0$ (respectively, < 0) for some i and $A_{kj} = 0$ for all $k \neq i$, we say that A_{ij} is a positive (respectively, negative)* column pivot *and the jth column is a* pivotal column.

NOTATION 4.3. *For an $s \times s$-matrix A and indices $1 \leq i, j \leq s$, we define the associated matrix $A^{\widehat{ij}}$ which is obtained from A by removing the ith row and the jth column.*

In the following theorem, we translate pivot conditions for reactant and reaction matrices into the language of reaction networks. Note that reactant matrices M are nonnegative matrices, so their pivots are necessarily positive pivots.

PROPOSITION 4.4. *Let N be a square chemical reaction network with reactant matrix M and reaction matrix R. Then the following hold:*

1. *M_{ij} is a row pivot if and only if the ith reactant contains only species j (in other words, the ith reaction is $kX_j \to \cdot$).*
2. *M_{ij} is a column pivot if and only if species j appears in the reactant complex of the ith reaction and in no other reactant complex.*
3. *R_{ij} is a row pivot if and only if only species j is changed in reaction i.*
4. *R_{ij} is a column pivot if and only if species j is changed only in reaction i.*
5. *Both M_{ij} and R_{ij} are row pivots if and only if the ith reaction is of the form*
$$a_j X_j \to b_j X_j, \qquad \text{where } 0 \neq a_j \neq b_j.$$
Moreover, R_{ij} is a positive pivot if $a_j > b_j$ and a negative pivot if $a_j < b_j$. If M_{ij} and R_{ij} are positive row pivots, then we will say that i is a pivotal reaction.
6. *Both M_{ij} and R_{ij} are column pivots if and only if species X_i appears only in reaction j. Moreover, R_{ij} is a negative column pivot if and only if X_i is a self-catalyst in reaction j.*
7. *Suppose that for some i, j, both M_{ij} and R_{ij} are positive pivots. Then*
$$\mathrm{Or}(N) = \mathrm{sign}(\det(M)\det(R)) = \mathrm{sign}(\det(M^{\widehat{ij}})\det(R^{\widehat{ij}})).$$
If M_{ij} is a (positive) pivot and R_{ij} is a negative pivot, then
$$\mathrm{Or}(N) = \mathrm{sign}(\det(M)\det(R)) = -\,\mathrm{sign}(\det(M^{\widehat{ij}})\det(R^{\widehat{ij}})).$$

Proof. This proposition follows easily from definitions and elementary linear algebra. ∎

4.2. Analysis of embedded networks. Theorem 4.9 in this section will tell us that instead of analyzing all s-square subnetworks G_i of a network $\mathfrak{G}$, we can remove the outflow reactions from G_i and then remove the outflow species from the remaining reactions, and analyze those simpler embedded networks. First we must define what is meant by removing a reaction or a species.

DEFINITION 4.5. *Let $\mathfrak{G} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a chemical reaction network.*

1. *Consider a subset of the species $S \subset \mathcal{S}$, a subset of the complexes $C \subset \mathcal{C}$, and a subset of the reactions $R \subset \mathcal{R}$.*
   - *The* restriction of R to S, *denoted by $R|_S$, is the multiset of reactions obtained by taking the reactions in R and removing all species not in S.*
   - *The* restriction of C to R, *denoted by $C|_R$, is the set of (reactant and product) complexes of the reactions in R.*
   - *The* restriction of S to C, *denoted by $S|_C$, is the set of species that are in the complexes in C.*
2. *The network obtained from $\mathfrak{G}$ by* removing a subset of species $\{X_i\} \subset \mathcal{S}$ *is the network*
$$\left\{\mathcal{S} \setminus \{X_i\},\ \mathcal{C}|_{\mathcal{R}|_{\mathcal{S}\setminus\{X_i\}}},\ \mathcal{R}|_{\mathcal{S}\setminus\{X_i\}}\right\}.$$

3. *The network obtained from* $\mathfrak{G}$ *by* removing a set of reactions $\{y \to y'\} \subset \mathcal{R}$ *is the subnetwork*

$$\left\{\mathcal{S}|_{\mathcal{C}|_{\mathcal{R}\setminus\{y\to y'\}}},\ \mathcal{C}|_{\mathcal{R}\setminus\{y\to y'\}},\ \mathcal{R}\setminus\{y\to y'\}\right\}.$$

Note that $R|_S$ is viewed as a multiset (a generalized set in which an element may appear more than once), so that in the following definition, embedded networks are indeed square.

DEFINITION 4.6. *Let* $\mathfrak{G} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ *be a chemical reaction network with* s *species, and let* G *be its nonflow subnetwork. For* $k = 0, 1, \ldots, s$, *a* k-square embedded network *of* $\mathfrak{G}$ *(or of* G*), which is defined by two sets, a* k*-subset of the species,* $S = \{X_{i_1}, X_{i_2}, \ldots, X_{i_k}\} \subset \mathcal{S}$, *and a* k*-subset of the nonflow reactions,* $R = \{R_{j_1}, R_{j_2}, \ldots, R_{j_k}\} \subset \mathcal{R}$, *that involve all species of* S, *is the* k*-square network* $(S, \mathcal{C}|_{R|_S}, R|_S)$ *consisting of the* k *reactions* $R|_S$.

The 1-square embedded networks with negative orientation are precisely reactions of the form $aX_j \to bX_j$ with $1 \le a < b$. Thus, a network contains a negatively oriented 1-square embedded reaction if and only if it contains a self-catalyzing reaction.

The following proposition will be used in the proofs of Theorems 4.9 and 4.11.

PROPOSITION 4.7. *Let* N *be a square network that contains one of the following:*

1. *an outflow reaction* $X_i \to 0$ *or a generalized outflow reaction* $aX_i \to bX_i$ *(where* $0 \le b < a$*), or*
2. *a species that appears in exactly one reaction, and the reaction is not self-catalyzing.*

Then $\mathrm{Or}(N) = 0$, *or the network* N^{red} *obtained from* N *by removing the aforementioned reaction and species is a square network and* $\mathrm{Or}(N) = \mathrm{Or}(N^{\mathrm{red}})$.

Proof. Part 1 follows easily from Proposition 4.4, as such a reaction is a pivotal reaction. Now, part 2 is the case when some species X_j appears in exactly one reaction $y \to y'$ in N. If X_j is in the product complex y', then $\mathrm{Or}(N) = 0$ by Lemma 3.10. In the remaining case (X_j is in the reactant complex y), then $y \to y'$ is a pivotal reaction, so $\mathrm{Or}(N) = \mathrm{Or}(N^{\mathrm{red}})$. ∎

The following lemma generalizes what we saw in Example 4.1 in the CFSTR setting: each s-square subnetwork G_i of $\mathfrak{G}$ defines an embedded network N_i of G, and they have the same orientation.

LEMMA 4.8. *Let* $\mathfrak{G}$ *be a chemical reaction network with* s *species, and let* G *denote the subnetwork of nonflow reactions. Consider the map from* s*-square subnetworks* G_i *of* $\mathfrak{G}$ *that do not contain inflow reactions to square embedded networks of* G, *which maps* G_i *to the network* N_i *obtained from* G_i *by first removing all outflow reactions* $X_{i_l} \to 0$ *(for* $l = 1, 2, \ldots, s-k$*) and then removing those outflow species* X_{i_l} *from the remaining nonflow reactions of* G_i. *Then this map is surjective, and*

$$\mathrm{Or}(G_i) = \mathrm{Or}(N_i). \tag{4.1}$$

(If N_i *is empty, its orientation is defined to be* $+1$*.)*

Proof. The surjection is clear: both objects are defined by a subset of $\mathcal{S}$ and a subset of $\mathcal{R}_G$ whose cardinalities sum to s. Equation (4.1) follows from noting that the outflow reactions are pivotal reactions, and then applying Proposition 4.7. ∎

From Lemma 4.8, we obtain the following theorem, which will be used in Step III in Algorithm 6.1.

THEOREM 4.9. *For a CFSTR* $\mathfrak{G}$, *with* G *its subnetwork of nonflow reactions, the following are equivalent:*

1. $\mathfrak{G}$ *passes the Jacobian criterion.*
2. *Each square embedded network of* G *has nonnegative orientation.*
3. G *has no self-catalyzing reaction, and each square embedded network* N *of* G *which satisfies the following properties has nonnegative orientation:*
   - N *has no outflows, inflows, generalized inflow reactions* $0 \to \sum_i a_i X_i$, *or generalized outflows* $aX_i \to bX_i$ *(where* $0 \leq b \leq a$*).*
   - *Each species appears in at least two reactions (where a pair of reversible reactions is counted only once).*

Proof. Parts 1 and 2 are equivalent by Lemmas 3.4 and 4.8. Clearly, part 2 implies part 3, so it remains only to show the converse. It suffices to show that if N is a square embedded network which violates one of the conditions of part 3, then either it has zero orientation or we can find a "smaller" embedded network which satisfies part 3 and has the same orientation as N. In the case of inflows and generalized inflows, the orientation of N is zero. All remaining cases are handled by Proposition 4.7. ∎

The following corollary follows easily from Theorem 4.9.

COROLLARY 4.10. *If a CFSTR contains a self-catalyzing reaction, then it fails the Jacobian criterion. Conversely, if a CFSTR does not contain a self-catalyzing reaction, then the only square embedded networks in Theorem* 4.9 *that must be checked are those of size at least two.*

Corollary 4.10 allows us to see quickly by inspection that the network introduced in Example 2.4 (and analyzed in Examples 3.2, 3.7, and 4.1) fails the Jacobian criterion.

4.3. A network reduction theorem. This subsection concerns those reactions or species that can be removed from a CFSTR without affecting whether the network passes the Jacobian criterion. The following theorem will be the foundation of Step II of Algorithm 6.1.

THEOREM 4.11. *Let* $\mathfrak{G}$ *be a CFSTR, and let* $\mathfrak{G}^{\text{red}}$ *be a network obtained from* $\mathfrak{G}$ *by performing any of the following operations:*

1. *Remove any inflow or outflow reactions.*
2. *Remove any generalized inflow reactions* $0 \to \sum_i a_i X_i$.
3. *Remove reactions containing only one species* $aX_i \to bX_i$ *for which* $0 \leq b < a$.
4. *Remove any species that is not a reactant species.*
5. *Remove any species that appears in exactly one reaction (or a pair of reversible reactions) unless the species is a self-catalyst of the reaction.*

Then $\mathfrak{G}$ *passes the Jacobian criterion if and only if all square embedded networks of* $\mathfrak{G}^{\text{red}}$ *have nonnegative orientation.*

Proof. Let G (respectively, G^{red}) denote the subnetwork of nonflow reactions of $\mathfrak{G}$ (respectively, $\mathfrak{G}^{\text{red}}$). By Theorem 4.9, we need only show that all square embedded networks of $\mathfrak{G}$ have nonnegative orientation if and only if all square embedded networks of G^{red} do. First, we consider the operations 1 through 3, in which $\mathfrak{G}^{\text{red}}$ is obtained from $\mathfrak{G}$ by removing a reaction $y \to y'$. In this case,

$$\{\text{SENs of } G\} = \{\text{ SENs of } G^{\text{red}}\} \cup \{\text{ SENs of } G \text{ that involve } y \to y'\}\ , \tag{4.2}$$

where "SEN" is shorthand for "square embedded network." So, we need only show that if N_i is a square embedded network of G, then either N_i has zero orientation or there exists a square embedded network N^i_{red} of G^{red} which has the same orientation as N_i. In the case that the reaction $y \to y'$ is an inflow or a generalized inflow, $\text{Or}(N_i) = 0$. So, the remaining cases are when $y \to y'$ is an outflow reaction $X_j \to 0$ or is of the form $aX_j \to bX_j$ (where $1 \leq b < a$); this is handled by Proposition 4.7.

Now, only parts 4 and 5 remain to be proven. Here, $\mathfrak{G}^{\text{red}}$ is obtained from $\mathfrak{G}$ by removing a species X_j. The analogue to (4.2) now is that the set of square embedded networks of G^{red} are precisely the square embedded networks of G that do not involve species X_j. Again, we must show that either a square embedded network N_i of G has zero orientation or there exists a square embedded network N^i_{red} of G^{red} with the same orientation as N_i. If X_j is not a reactant species of G (in the case of part 4), then N_i has zero orientation by Lemma 3.10. Finally, if X_j satisfies the condition in part 5, then X_j appears in exactly one reaction $y \to y'$ in N_i. This case is completed by applying Proposition 4.7. This completes the proof. ∎

5. Networks with total molecularity at most two do not admit multiple steady states. In this section, we prove the following theorem, which concerns networks in which the maximum total molecularity (Definition 2.2) is at most two.

THEOREM 5.1. *If all species of a CFSTR have total molecularity less than or equal to two, then it passes the Jacobian criterion and therefore does not admit multiple steady states.*

Theorem 5.1 has the following corollary.

COROLLARY 5.2. *For any weakly reversible chemical reaction network (not necessarily a CFSTR) in which all species have total molecularity less than or equal to two, the network does not admit multiple steady states.*

Remark 5.3. Theorem 5.1 cannot be improved to higher total molecularity. For example, a CFSTR that contains the reaction $A \to 2A$ fails the Jacobian criterion (by Corollary 4.10).

Remark 5.4. Theorem 5.1 can be proven by applying the species-reaction graph theorem of Craciun and Feinberg [7, Theorem 1.1]. Rather than introducing the terminology and results of species-reaction graphs, we give a direct proof here.

Our proof of Theorem 5.1 will proceed by showing that for a chemical reaction network that has total molecularity at most two, if a square embedded network has nonzero orientation, then it in fact has positive orientation. The proof requires the following lemmata, which will be used to reduce to studying square embedded networks and then to elucidate what values can be taken by the determinants of the reactant and reaction matrices of such networks.

LEMMA 5.5. *Let $\mathfrak{G}$ be a chemical reaction network whose species all have total molecularity less than or equal to some $m \in \mathbb{Z}_{>0}$. Then the same holds for any embedded network of $\mathfrak{G}$.*

The proof of Lemma 5.5 follows immediately from the definitions of total molecularity and embedded networks.

LEMMA 5.6. *Let N be a coupled s-square network with at least two reactions (none of which are inflows or outflows) such that all species have total molecularity at most two. Assume that N has nonzero orientation. Then each reaction of N has the form of $A + B \to 0$ or $A \to B$.*

Proof. We first claim that if N is a coupled, s-square network in which all species have total molecularity two; then each reaction of N must contain exactly two molecules, because no reaction can have only one molecule (because inflows and outflows are not allowed). So, each of the s reactions must have exactly two molecules, and it follows that the total molecularity of each species must be two. Now, a reaction of the form $0 \to A + B$ would yield zero orientation for the network (because the corresponding row of the reactant matrix would be zero), and a reaction $2A \to 0$ is not allowed, because then the network would decouple into this reaction and the remaining subnetwork that does not contain A. So, only two types of reactions are

possible: $A + B \to 0$ and $A \to B$. ∎

LEMMA 5.7. *Consider an $n \times n$-matrix $Q \in \{-1, 0, 1\}^{n\times n}$ such that*

1. *all diagonal entries are $+1$,*
2. *the entries above the diagonal (mod n), $Q_{j,j+1(mod\ n)}$, are 0 or ± 1, and*
3. *all other entries are 0.*

Then $\det(Q) \geq 0$. *(In fact, the determinant is 0, 1, or 2.)*

Proof. The determinant is $\det(Q) = 1 \pm \prod_{i=1}^{n}(Q_{i,i+1(mod\ n)}) \in \{0, 1, 2\}$. ∎

We now can prove Theorem 5.1 and Corollary 5.2.

Proof of Theorem 5.1. By Theorem 4.9 and Lemmas 4.8 and 5.5, we need only show that if N is a square network with nonzero orientation and with maximum total molecularity at most two, then $\det(M_N)\det(R_N) \geq 0$. Let s denote the number of species of N. Now, if N decouples, then we could reduce to analyzing the two subnetworks separately, both of which have fewer than s species; the network N passes the Jacobian criterion if and only if the two subnetworks do. The $s = 1$ case is trivial (only $2A \to 0$ is allowed, and in this case both reaction and reactant matrices are the same, so their determinants are the same), so we may proceed by induction on s in the case of a coupled network N.

Now Lemma 5.6 applies, so we know that the reactions of N are of the form $A + B \to 0$ and $A \to B$. By relabelling the species, we can ensure that the first reaction contains species X_1 and X_2, the second reaction contains X_2 and X_3, and so on. Therefore, both the reactant matrix and the reaction matrix R_N now have the form of the matrix in Lemma 5.7. By the lemma, both determinants are nonnegative, so the orientation of the network N is either zero or positive.

Finally, Lemma 3.4 implies that a CFSTR network that passes the Jacobian criterion does not admit multiple steady states. ∎

Proof of Corollary 5.2. For a weakly reversible network G that has total molecularity at most two, we consider the CFSTR $\tilde{G}$ obtained by augmenting the network G by all outflows. Theorem 5.1 implies that $\tilde{G}$ passes the Jacobian criterion. Finally, results due to Craciun and Feinberg allow us to conclude that the such a network precludes multiple steady states [8, Theorem 8.2 and Corollary 8.3]. ∎

6. Procedure for simplifying the Jacobian criterion. Algorithm 6.1 is the main result of this article. It combines the results in previous sections into a procedure that simplifies the implementation of the Jacobian criterion. After preprocessing in Step I, the algorithm reduces a chemical reaction network to a simpler network in Step II, and then decreases the number of embedded networks of the reduced network that must be examined in Step III.

ALGORITHM 6.1 (simplifying the Jacobian criterion).

Step I (preprocessing step).

Input: a CFSTR $\mathfrak{G}$.

Output: either whether $\mathfrak{G}$ passes the Jacobian criterion, or $\mathfrak{G}$ (to be passed to Step II).

1. If $\mathfrak{G}$ has a self-catalyzing reaction (an embedded reaction $aX_j \to bX_j$ with $1 \leq a < b$), then $\mathfrak{G}$ automatically fails the Jacobian criterion.
2. If each species of $\mathfrak{G}$ has total molecularity at most two, then $\mathfrak{G}$ automatically passes the Jacobian criterion.

If neither applies, then pass $\mathfrak{G}$ to Step II.

Step II (reduction step).

Input: a CFSTR $\mathfrak{G}$ that passes Step I.

Output: a nonflow chemical reaction network G_{red} such that the CFSTR $\tilde{G}_{\text{red}}$ passes

the Jacobian criterion if and only if $\mathfrak{G}$ does.
Perform the following operations on the network $\mathfrak{G}$, in any order, as long as any one can be completed:

1. Remove any inflow or outflow reaction.
2. Remove any generalized inflow reaction $0 \to \sum_i a_i X_i$.
3. Remove any reaction that contains only one species.
4. Remove any species that is not a reactant species.
5. Remove any species that appears in only one reaction (or in a pair of reversible reactions and in no other reactions). In particular, remove any species whose total molecularity is one.
6. Remove one copy of any repeated reactions.

If the reduced network is the empty network or all species in the reduced network have total molecularity at most two, then $\mathfrak{G}$ passes the Jacobian criterion. Otherwise, pass the reduced network G^{red} to Step III.

Step III (analysis of embedded networks step).
Input: a chemical reaction network G (such as the output of Step II) which passes Step I.
Output: whether $\tilde{G}$ passes the Jacobian criterion (if G is the output of Step II, then this step will determine whether the input $\mathfrak{G}$ of Step II passes the Jacobian criterion).
Consider all coupled square embedded networks N of G with the following properties:

1. N contains none of the following reactions:
   - inflow or outflow reactions,
   - generalized inflow reactions $0 \to \sum_i a_i X_i$,
   - reactions containing only one species,
   - both a reaction and its reverse reaction,
   - two reactions that share the same reactant complex.
2. N has at least two species.
3. Each species of N is a reactant species.
4. Each species appears in at least two reactions (where pairs of reversible reactions are counted only once).
5. At least one species has total molecularity three or more.
6. N has nonzero orientation.

Output: The network $\tilde{G}$ passes the Jacobian criterion if and only if all such square embedded networks N have nonnegative orientation.

Proof of correctness of Algorithm 6.1. Part 1 of Step I follows from Corollary 4.10, and part 2 follows from Theorem 5.1. Step II is valid by Theorem 4.11. Parts 1 through 4 of Step III follow from Lemma 3.10, Corollary 4.10, and Theorem 4.9. Part 5 of Step III follows from Theorem 5.1, and part 6 simply avoids embedded networks with zero orientation. ∎

Remark 6.2. Instead of applying Step III to a reduced network that is the output of Step II, one can apply other tests to check the Jacobian criterion. For instance, one can apply the species-reaction graph criterion [7] to the reduced network or directly compute the expansion of the Jacobian determinant using available software [13, 21, 22]. The reduced network typically contains fewer species and fewer reactions than the original network, so computer computation will be more tractable.

Remark 6.3. In practice, the number of square embedded networks that must be examined in Step III of Algorithm 6.1 is relatively small compared to the number of s-square subnetworks G_i in Lemma 3.4. Comparisons of the number of square subnetworks G_i versus the number of square embedded networks to be examined appear in the last two columns of Table 6.1 and in the comments of Table 6.2. In

TABLE 6.1

Networks examined in Table 1 of Schlosser and Feinberg [25]. Listed are the nonflow networks G, whether the CFSTR network $\tilde{G}$ passes the Jacobian criterion (JC), whether $\tilde{G}$ precludes multiple steady states (MSS), additional comments, the number of s-square networks G_i of $\tilde{G}$ that do not contain inflow reactions (here, $r_o = s$; see Lemma 3.4), and the number of square embedded networks (SENs) which must be considered in Algorithm 6.1. The entry in the last column is left blank (−) for networks that can be determined to pass or to fail the Jacobian criterion due to a self-catalyzing reaction or low total molecularity. Note that the number of such SENs is much lower than the number of subnetworks G_i, resulting in a substantial reduction in the number and size of the orientations that must be computed. Many of these networks were also analyzed by Craciun and Feinberg [5, Table 1.1], Banaji, Donnell, and Baigent [3], and Craciun, Helton, and Williams [9].

Network	(G)	$\tilde{G}$ passes JC	$\tilde{G}$ does not admit MSS	Remarks	Number of G_i, $\binom{r+r_o}{s}$	Number of SENs to examine
(i)	$A+B \leftrightarrows P$ $B+C \leftrightarrows Q$ $C \leftrightarrows 2A$	No	No	See Ex. 7.4	$\binom{6+5}{5} = 462$	2
(ii)	$A+B \leftrightarrows P$ $B+C \leftrightarrows Q$ $C+D \leftrightarrows R$ $D \leftrightarrows 2A$	Yes	Yes	See Ex. 7.4	$\binom{8+7}{7} = 6435$	2
(iii)	$A+B \leftrightarrows P$ $B+C \leftrightarrows Q$ $C+D \leftrightarrows R$ $D+E \leftrightarrows S$ $E \leftrightarrows 2A$	No	No	See Ex. 7.4	$\binom{10+9}{9} = 92378$	2
(iv)	$A+B \leftrightarrows P$ $B+C \leftrightarrows Q$ $C \leftrightarrows A$	Yes	Yes	TM ≤ 2; see Ex. 7.2	$\binom{6+5}{5} = 462$	–
(v)	$A+B \leftrightarrows F$ $A+C \leftrightarrows G$ $C+D \leftrightarrows B$ $C+E \leftrightarrows D$	No	No	See Ex. 7.5	$\binom{8+7}{7} = 6435$	3
(vi)	$A+B \leftrightarrows 2A$	No	Yes	Self-catalyzing; see Ex. 7.1	$\binom{2+2}{2} = 6$	–
(vii)	$2A+B \leftrightarrows 3A$	No	No	Self-catalyzing; see Ex. 7.1	$\binom{2+2}{2} = 6$	–
(viii)	$A+2B \leftrightarrows 3A$	No	Yes	Self-catalyzing; see Ex. 7.1	$\binom{2+2}{2} = 6$	–

addition, the determinants of such square embedded networks (as given in Lemma 3.3) that must be computed are of smaller matrices than those of G_i.

Remark 6.4. We propose that Algorithm 6.1 could aid in enumerating chemical reaction networks that admit multiple steady states. Recall that failing the Jacobian criterion is a necessary condition for admitting multiple steady states (Lemma 3.4), so let us consider the problem of identifying the set of networks that fail the Jacobian criterion. More specifically, we now define a *bimolecular* network to be a network in which all complexes contain at most two molecules; we then pose the following question: which bimolecular CFSTRs fail the Jacobian criterion?

Accordingly, we enumerated all bimolecular CFSTRs that contain two pairs of reversible nonflow reactions, and we found that there are 386 such networks. Of these 386 networks, 142 have a maximum total molecularity of two or fewer and thus immediately pass the Jacobian criterion. It is interesting to note that for these 142 networks, several distinct species typically have the same total molecularity. Networks

TABLE 6.2

The enzyme catalysis networks examined in Table 1 of Craciun, Tang, and Feinberg [10]. Listed are the underlying networks G, whether the CFSTR network $\tilde{G}$ passes the Jacobian criterion (JC), whether $\tilde{G}$ precludes multiple steady states (MSS), and additional comments, including the number of square embedded networks (SENs) that need to be examined for those networks for which total molecularity (TM) is not sufficient for verifying the Jacobian criterion.

Network	(G)	$\tilde{G}$ passes JC	$\tilde{G}$ does not admit MSS	Remarks
(i)	$E+S \leftrightarrows ES \to E+P$	Yes	Yes	Total molecularity ≤ 2; see Ex. 7.2
(ii)	$E+S \leftrightarrows ES \to E+P$ $E+I \leftrightarrows EI$	Yes	Yes	Reduced network has TM ≤ 2; see Ex. 7.3
(iii)	$E+S \leftrightarrows ES \to E+P$ $ES+I \leftrightarrows ESI$	Yes	Yes	Reduced network has TM ≤ 2; see Ex. 7.3
(iv)	$E+S \leftrightarrows ES \to E+P$ $E+I \leftrightarrows EI$ $ES+I \leftrightarrows ESI \leftrightarrows EI+S$	No	No	Only 9 SENs to examine; see Example 7.5
(v)	$E+S_1 \leftrightarrows ES_1$ $S_2+ES_1 \leftrightarrows ES_1S_2 \to E+P$	Yes	Yes	Total molecularity ≤ 2; see Ex. 7.2
(vi)	$E+S_1 \leftrightarrows ES_1 \quad E+S_2 \leftrightarrows ES_2$ $S_2+ES_1 \leftrightarrows ES_1S_2 \leftrightarrows S_1+ES_2$ $ES_1S_2 \to E+P$	No	No	Only 14 SENs to examine; see Example 7.5
(vii)	$S_1+E_1 \leftrightarrows E_1S_1$ $S_2+E_1S_1 \leftrightarrows E_1S_1S_2 \to P_1+E_1$ $E_2+S_2 \leftrightarrows E_2S_2 \to 2S_1+E_2$	Yes	Yes	Only 1 SEN to examine; see Example 7.5
(viii)	$S_1+E_1 \leftrightarrows E_1S_1$ $S_2+E_1S_1 \leftrightarrows E_1S_1S_2 \to P_1+E_1$ $S_2+E_2 \leftrightarrows E_2S_2$ $S_3+E_2S_2 \leftrightarrows E_2S_2S_3 \to P_2+E_2$ $S_3+E_3 \leftrightarrows E_3S_3 \to 2S_1+E_3$	No	No	Only 1 SEN to examine; see Example 7.5
(ix)	$S_1+E_1 \leftrightarrows E_1S_1$ $S_2+E_1S_1 \leftrightarrows E_1S_1S_2 \to P_1+E_1$ $S_2+E_2 \leftrightarrows E_2S_2$ $S_3+E_2S_2 \leftrightarrows E_2S_2S_3 \to P_2+E_2$ $S_3+E_3 \leftrightarrows E_3S_3$ $S_4+E_3S_3 \leftrightarrows E_3S_3S_4 \to P_3+E_3$ $S_4+E_4 \leftrightarrows E_4S_4 \to 2S_1+E_4$	Yes	Yes	Only 1 SEN to examine; see Example 7.5

that have such symmetry are computationally the most expensive to enumerate [11]. As we are interested in listing only those networks that fail the Jacobian criterion, we can avoid these computationally expensive networks.

Moreover, of the remaining 244 networks, applying Step II of Algorithm 6.1 immediately reduces the number of networks that need to be examined. In other words, many networks are related in whether or not they pass the Jacobian criterion, and thus studying all 244 networks is redundant. When we discard networks in which some species has total molecularity equal to one, we are left with only 55 networks whose multistationarity needs to be investigated. Details of our enumeration of bimolecular CFSTRs appear in [20]. Note that a similar enumeration of small bimolecular reaction networks was undertaken by Deckard, Bergmann, and Sauro [11], from which Pantea and Craciun sampled networks to compute the fraction of such networks that pass the Jacobian criterion [23, Figure 1].

7. Examples. The examples in this section illustrate that Algorithm 6.1 in some cases can determine whether a network passes the Jacobian criterion more quickly than

applying the species-reaction graph criterion of Craciun and Feinberg [7], deficiency theory [14], or the results of Banaji and Craciun [2]. Moreover, we are able to analyze examples in [5, 7, 10, 25], which appear in Tables 6.1 and 6.2. In addition, we demonstrate that instead of checking the orientations of all the s-square networks G_i from Lemma 3.4 (which entails computing determinants of many $s \times s$-matrices), we need only compute the orientations of certain square embedded networks, which are both smaller and fewer in number. The final columns in both Tables 6.1 and 6.2 illustrate the great reduction in the number of subnetworks that must be examined. We begin with examples in which no orientations must be computed, as checking the Jacobian criterion sometimes can be performed by inspection.

Example 7.1 (networks containing a self-catalyzing reaction). Recall that Corollary 4.10 states that if a chemical reaction network contains a self-catalyzing reaction, then it fails the Jacobian criterion. One such network is the one examined in Examples 3.7 and 4.1. Other networks that are resolved easily by Step I of Algorithm 6.1 include networks (vi), (vii), and (viii) in Table 6.1.

In addition to the self-catalyzing networks, the other networks that are resolved in Step I of Algorithm 6.1 are those with total molecularity at most two.

Example 7.2 (networks with total molecularity at most two). Consider the following network examined by Gnacadja et al. [16]:

$$\begin{aligned} L + R \leftrightarrow LR, \quad A + R \leftrightarrow AR, \\ L + T \leftrightarrow LT, \quad A + T \leftrightarrow AT. \end{aligned} \tag{7.1}$$

This ligand-receptor-antagonist-trap network summarizes the four pairs of binding and dissociating reactions that occur in the presence of two drugs, a "receptor antagonist" and a "trap," which are used to treat patients with rheumatoid arthritis. In their work, Gnacadja et al. apply deficiency theory to determine the existence and uniqueness of a steady state [16, Proposition 2]. If one is interested only in precluding multiple steady states, then the approach advocated here can be performed more quickly. It is easily seen that the total molecularity of each of the eight species, L, R, A, T, LR, AR, LT, and AT, is no more than two. Therefore, Theorem 5.1 implies that a CFSTR version of this network passes the Jacobian criterion and does not admit multiple steady states. Moreover, as the underlying network (7.1) is weakly reversible, we can conclude that multiple steady states are also ruled out in the non-CFSTR setting [8].

Other networks with total molecularity at most two include the network (iv) in Table 6.1 and the enzyme catalysis networks (i) and (iv) in Table 6.2. All of these networks can be seen by inspection to pass the Jacobian criterion without appealing to the species-reaction graph criterion.

The networks in Example 7.2 had total molecularity at most two and so could be seen immediately to pass the Jacobian criterion. The networks in the next example do not have this property until after Step II of Algorithm 6.1 is applied.

Example 7.3 (networks with total molecularity at most two after reduction). Consider the CFSTR introduced by Banaji and Craciun for which the nonflow subnetwork is

$$D \leftrightarrows A + B + C, \qquad E \leftrightarrows A + B + C, \qquad F \leftrightarrows A + B. \tag{7.2}$$

Banaji and Craciun showed that the CFSTR fails the species-reaction graph criterion but nevertheless passes the Jacobian criterion, because the stoichiometric matrix is

"strongly sign determined" [2, section 6.2]. We now show that Step II of Algorithm 6.1 can easily be performed on this network, to conclude that it passes the Jacobian criterion.

In Step I of Algorithm 6.1, we note that the total molecularities of species A and B in network (7.2) are both three, and no self-catalyzing reactions exist. So, we begin Step II by removing species D, E, and F, each of which has total molecularity equal to one. The resulting network is

$$0 \leftrightarrows A + B + C, \qquad 0 \leftrightarrows A + B + C, \qquad 0 \leftrightarrows A + B.$$

Now the first two pairs of reversible reactions are the same, so we remove one copy. Additionally, we remove the remaining two generalized inflow reactions, to obtain

$$0 \leftarrow A + B + C, \qquad 0 \leftarrow A + B.$$

Clearly, each species in this reduced network has total molecularity no more than two, so we conclude that the original CFSTR passes the Jacobian criterion. Two other networks whose reduced networks can be obtained easily and which have total molecularity at most two are the enzyme catalysis networks with inhibition, (ii) and (iii) in Table 6.2.

While the above examples did not need to proceed beyond Step II of Algorithm 6.1, the networks in the following example do require this step. We will see that the algorithm greatly reduces the number of subnetworks that must be checked.

Example 7.4 (networks requiring the full algorithm). Consider the reversible network (i) in Table 6.1; its nonflow subnetwork is

$$A + B \leftrightarrows P, \qquad B + C \leftrightarrows Q, \qquad C \leftrightarrows 2A. \tag{7.3}$$

The output of Step II of Algorithm 6.1 is the following network:

$$A + B \rightarrow 0, \qquad B + C \rightarrow 0, \qquad C \leftrightarrows 2A.$$

As only species A has total molecularity greater than two, each square embedded network we consider must contain both $A + B \rightarrow 0$ and one of the reversible reactions $C \leftrightarrows 2A$ (because we need not consider networks with both directions of a reversible reaction). However, such a 2-square embedded network would have a species (B or C) that appears in only one complex. Therefore, we need consider only the two 3-square embedded networks, which are the following:

$$\{A + B \rightarrow 0 \qquad B + C \rightarrow 0 \qquad C \rightarrow 2A\} \quad \text{and} \tag{7.4}$$

$$\{A + B \rightarrow 0 \qquad B + C \rightarrow 0 \qquad C \leftarrow 2A\}. \tag{7.5}$$

The first embedded network (7.4) has negative orientation, and the second network (7.5) has positive orientation, so a CFSTR version of the original network (7.3) fails the Jacobian criterion. Note that our algorithm reduced the Jacobian criterion from examining the $\binom{6+5}{5} = 462$ s-square subnetworks (the G_i in Lemma 3.4) to two embedded networks. Networks (ii), (iii), and (v) in Table 6.1 can be analyzed similarly: they require examining only two, two, and three square embedded networks, respectively.

Our final example network requires more complicated analysis, but the number of square embedded networks that must be checked is nevertheless small.

Example 7.5. Consider network (iv) in Table 6.2. After removing species P in Step II and then relabeling the six remaining species, the subnetwork of nonflow reactions is

$$A + B \leftrightarrows C, \qquad C \to A, \qquad A + E \leftrightarrows F, \qquad C + E \leftrightarrows G, \qquad G \leftrightarrows F + B.$$

Only one reaction, $C \to A$, is not reversible; we label the remaining four reversible reactions as R_1 through R_4. We now show that instead of checking that the $\binom{16}{7} = 11440$ 7-square subnetworks of the original CFSTR (with seven species and nine nonflow reactions) have nonnegative orientation, Algorithm 6.1 allows us to examine only nine square embedded networks.

Let N denote a square embedded network that must be considered according to Step III of Algorithm 6.1. The total molecularities of the species (A, B, C, E, F, G) are respectively $(3, 2, 3, 2, 2, 2)$. So species A together with all its reactions (R_1, R_2, R_3) or species C together with all its reactions (R_1, R_2, R_4) must be in N. In particular, both reactions R_1 and R_2 must appear in N, and both A and C must take part so that R_2 is not a flow reaction. Next, in order for species C not to appear twice as a reactant, R_1 must appear in N in the forward direction $A + B \to C$.

Now, the rows corresponding to reactions R_1 and R_2 in the reaction matrix of N must be linearly independent, so the species B must be in N. Thus, we also must include reaction R_5 so that B has total molecularity greater than 1. Finally, so that R_5 is not a flow reaction, we must include species F or G.

Therefore, N must be a 4-square or a 5-square embedded network, and the only possible 4-square networks are the following:

$$\begin{aligned} &\{A + B \to C \qquad C \to A \qquad B \to G \qquad G \to C\} \quad \text{and} \\ &\{A + B \to C \qquad C \to A \qquad F + B \to 0 \qquad A \leftrightarrows F\}, \end{aligned}$$

where the second network represents two square embedded networks. Additionally, the only possible 5-square networks are

$$\begin{aligned} &\{A + B \to C \qquad C \to A \qquad A \to F \qquad G \to C \qquad F + B \to G\}, \\ &\{A + B \to C \qquad C \to A \qquad A + E \to 0 \qquad C + E \leftrightarrows G \qquad G \leftrightarrows B\}, \quad \text{and} \\ &\{A + B \to C \qquad C \to A \qquad A + E \leftrightarrows F \qquad C + E \to 0 \qquad F + B \to 0\}. \end{aligned}$$

Here, the second network represents three networks (the network in which G appears twice as a reactant is not permitted), and the third network represents two networks. Therefore, there are nine possible square embedded networks N. Using similar analysis, network (v) of Table 6.1 and networks (vi), (vii), (viii), and (ix) of Table 6.2 can be shown to require checking only 3, 14, 1, 1, and 1 embedded networks, respectively.

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