Analyzing multistationarity in chemical reaction networks using the determinant optimization method

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A B S T R A C T

Multistationary chemical reaction networks are of interest to scientists and mathematicians alike. While some criteria for multistationarity exist, obtaining explicit reaction rates and steady states that exhibit multistationarity for a given network—in order to check non-degeneracy or determine stability of the steady states, for instance—is nontrivial. Nonetheless, we accomplish this task for a certain family of sequestration networks. Additionally, our results allow us to prove the existence of nondegenerate steady states for some of these sequestration networks, thereby resolving a subcase of a conjecture of Joshi and Shiu. Our work relies on the determinant optimization method, developed by Craciun and Feinberg, for asserting that certain networks are multistationary. More precisely, we implement the construction of reaction rates and multiple steady states which appears in the proofs that underlie their method. Furthermore, we describe in detail the steps of this construction so that other researchers can more easily obtain, as we did, multistationary rates and steady states.

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1. Introduction

Although many dynamical systems arising in applications exhibit bistability, there is no complete characterization of such systems. Even for the subclass of chemical kinetics systems and even under the assumption of mass-action kinetics, which is the focus of this work, the problem is difficult.

Here we consider the simpler, yet still challenging, question: which chemical reaction networks are multistationary, i.e. which have the capacity to exhibit two or more steady-state concentrations with the same reaction rates? Mathematically, this asks: among certain parameterized families of polynomial systems, which admit multiple positive roots? Therefore, this is a real algebraic geometry problem, and we do not expect an easy answer in general.

The first partial answers to this question are due to Feinberg, Horn, and Jackson in the 1970s. Their results in chemical reaction network theory [1,2] (specifically, deficiency theory [3]) can preclude or guarantee multistationarity for certain classes of networks. For a survey of these and other methods, see [4].

Our work pertains to two related results: (1) a method for “lifting” multiple steady states from small networks to larger ones, and (2) the so-called determinant optimization method for certifying that a given network is multistationary.

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The lifting result, stated informally, is as follows: if a chemical reaction network contains an “embedded” network that is multistationary, then the entire reaction network also is multistationary under certain hypotheses [5]. Therefore we are interested in cataloging the multistationary networks which contain no embedded multistationary networks, because all larger multistationary networks contain at least one embedded multistationary subnetwork from the catalog.

As a step toward such a catalogue, Joshi and Shiu identified a certain infinite family of chemical reaction networks $\tilde{K}_{m,n}$ to be of particular interest among all networks that include inflow and outflow reactions [4]. This family is minimal, in that it has no embedded subnetworks (with inflow and outflow reactions) that exhibit multistationarity. To analyze these networks, Joshi and Shiu used the second method for analyzing multistationarity mentioned above.

Developed by Craciun and Feinberg, the determinant optimization method can assert that a network is multistationary [6,7]; as such, it is a partial converse to their results on “injective” reaction networks which guarantee that a network is not multistationary. This topic of injectivity has seen much interest in recent years (see [6,8,9] and the references therein); however, the determinant optimization method has garnered comparatively little attention. The only related results that we are aware of are due to Banaji and Pantea [8], Feliu [10], and Müller et al. [9].

Using the determinant optimization method, Joshi and Shiu proved that $\tilde{K}_{m,n}$ is multistationary for all integers $m \geq 2$ and odd integers $n \geq 3$ [4]. Furthermore, they conjectured that these networks can exhibit multiple nondegenerate steady states. (It is not guaranteed that the determinant optimization method produces nondegenerate steady states; we show this for the first time in Remark 3.11.) The significance of the conjecture is that if it is true, then $\tilde{K}_{2,n}$ would be the first example of an infinite family of chemical reaction networks with inflow and outflow reactions and at-most-bimolecular reactants and products—that is, minimal with respect to the embedding relation among all such networks which have the capacity to exhibit multiple nondegenerate steady states. Nondegeneracy is important because results that “lift” multiple steady states from embedded subnetworks or other typically smaller networks require the steady states to be nondegenerate; a summary of such results appears in [4, Section 4]. Also, because trimolecular reactants/products are rather uncommon in chemistry and $\tilde{K}_{2,n}$ is at most bimolecular, this family of networks is of particular interest in chemical applications.

In the current work, we resolve the conjecture for the case $n = 3$ and all $m \geq 2$; in other words, we prove that $\tilde{K}_{m,3}$ has the capacity to admit multiple nondegenerate steady states for all $m \geq 2$ (Theorem 4.5). To accomplish this, we need information beyond the mere existence of multiple steady states; we also need precise values (or at least estimates) for the rates and steady states. By applying the proofs underlying the determinant optimization method in Craciun and Feinberg’s work to the networks $\tilde{K}_{m,n}$, we obtain (via standard methods for analyzing recurrence relations) explicit closed forms for multistationary rates and steady states. Then we use these closed forms to verify that the steady states are nondegenerate for small values of $m$ and $n$.

Finally, recognizing the usefulness of generating closed forms (or at least estimates$^1$) for rates and steady states for any reaction network that satisfies the hypotheses of the determinant optimization method, in Section 3 we outline the steps of the method with enough generality to be used in other contexts. These steps are present in Craciun and Feinberg’s work but are spread out over several proofs, so our contribution here is to reorganize the method into a concise procedure.

An outline of our work is as follows. Section 2 introduces chemical systems and the main conjecture. Section 3 describes the determinant optimization method in detail. We use this method to resolve some cases of the main conjecture in Section 4 and 5. Finally, a discussion appears in Section 6.

**Notation.** We denote the positive real numbers by $\mathbb{R}_+ := \{x \in \mathbb{R} \mid x > 0\}$, and the standard inner product in $\mathbb{R}^n$ by $(\cdot, \cdot)$.

The $i$th entry of a vector $x$ is denoted $x_i$.

2. **Background**

This section introduces chemical reaction networks, their corresponding mass-action kinetics systems, and the main object of our paper: the sequestration network $\tilde{K}_{m,n}$.

2.1. **Mass-action kinetics systems**

**Definition 2.1.** A chemical reaction network $G = (S, C, R)$ consists of three finite sets:
1. a set of species $S = \{X_1, X_2, \ldots, X_s\}$,
2. a set $C$ of complexes, which are non-negative integer linear combinations of the species, and
3. a set $R \subseteq C \times C$ of reactions.

**Example 2.2.** The following chemical reaction network:

$X_1 + X_2 \rightarrow X_3$

$X_2 \rightarrow X_1 + X_4$,

is entirely defined by:

$^1$ For general networks, the determinant optimization method need not yield closed forms for the rates and steady states, but one can nonetheless obtain estimates.
1. the set of species $S = \{X_1, X_2, X_3, X_4\}$.
2. the set of complexes $C = \{X_1 + X_2, X_3, X_2, X_1 + X_4\}$, and
3. the set of reactions $R = \{(X_1 + X_2, X_3), (X_2, X_1 + X_4)\}$.

Any reaction network $G = (S, C, R)$ is contained in the fully open extension network $\tilde{G}$ obtained by including all inflow and outflow reactions:

$$\tilde{G} := \{S, C \cup S \cup \{0\}, R \cup \{X_i \rightarrow 0\}_{X \in S}\}.$$  \hspace{1cm} (1)

In other words, the fully open extension of any network is obtained by adding the reactions $X \rightarrow 0$ (outflow) and $0 \rightarrow X$ (inflow) for all $X \in S$.

As all the reactions take place, the concentrations of each of the species will change. We make use of mass-action kinetics to define a system of ordinary differential equations that, for each species, how its concentration changes as a function of time. This ODE system is described by the stoichiometric matrix $\Gamma$ and the reactant vector $R(x)$, which is a vector-valued function of the vector of species concentrations $x$.

**Definition 2.3.** Let $G = (S, C, R)$ be a network, and let $\{y_1 \rightarrow y'_1, y_2 \rightarrow y'_2, \ldots, y_{|R|} \rightarrow y'_{|R|}\}$ be an ordering of the reactions.

1. The reaction vector of the reaction $y_i \rightarrow y'_i$ is the vector $y'_i - y_i$, viewed in $\mathbb{R}^{|S|}$. Note that $y_i \rightarrow y'_i$ is a slight abuse of notation, used to denote the reaction $y_i \cdot X \rightarrow y'_i \cdot X$ where $X$ is the vector of all species. Explicitly, the vectors $y_i$ and $y'_i$ only contain species coefficients.
2. The stoichiometric matrix of $G$ is the $|S| \times |R|$ matrix $\Gamma$ whose $k$th column is the reaction vector of $y_k \rightarrow y'_k$.
3. The reactant vector $R(x)$ is the vector of length $|R|$ whose $k$th entry is the (monomial) product:

$$r_k x_1^{(y_1)_k} x_2^{(y_2)_k} \cdots x_{|S|}^{(y_{|S|})_k},$$

where $r_k \in \mathbb{R}^+$ is the reaction rate of the $k$th reaction.

**Definition 2.4.** The mass-action kinetics system of a network $G = (S, C, R)$ and a vector of reaction rates $(r_k) \in \mathbb{R}^{|R|}_+$ is defined by the following system of ordinary differential equations:

$$\frac{dx}{dt} = \Gamma \cdot R(x).$$  \hspace{1cm} (2)

**Example 2.5.** For the following network:

$$A + 2B \xrightarrow{r} 2A,$$

$$\Gamma = \begin{bmatrix} 1 \\ -2 \end{bmatrix}$$

and $R(x) = (rx_A x_B^2)$, so the mass-action kinetics system (2) is:

$$\begin{bmatrix} \frac{dx_1}{dt} \\ \frac{dx_2}{dt} \end{bmatrix} = \Gamma \cdot R(x) = \begin{bmatrix} rx_A x_B^2 \\ -2rx_A x_B^2 \end{bmatrix}.$$

An important characteristic of mass-action kinetics systems is that they may or may not have the capacity to admit (positive) steady states:

**Definition 2.6.** A positive steady state is a vector $x^* \in \mathbb{R}^{|S|}_{>0}$ such that $\Gamma \cdot R(x^*) = 0$. A steady state $x^*$ is nondegenerate if $\text{Im}(df(x^*)|_{\text{Im}(\Gamma)}) = \text{Im}(\Gamma)$. where $df(x^*)$ denotes the Jacobian matrix of the mass-action kinetics system at $x^*$.

**Definition 2.7.** A network that includes all inflow and outflow reactions is multistationary\(^2\) if there exist two distinct concentration vectors $x^*, x^#$ and positive reaction rates such that $\Gamma \cdot R(x^*) = \Gamma \cdot R(x^#) = 0$.

2.2. The sequestration network $K_{m,n}$

The main object of our paper is the fully open extension of the network $K_{m,n}$:

**Definition 2.8.** For positive integers $n \geq 2$ and $m \geq 2$, the sequestration network $K_{m,n}$ is:

$$X_1 + X_2 \rightarrow 0$$

$$X_2 + X_3 \rightarrow 0$$

$$\vdots$$

\(^2\) The focus of this work is on certain networks $\tilde{R}_m$, that include all flow reactions. For networks that do not include all flow reactions, the definition of multistationary must incorporate the conservation relations in the network, if any.
\[ X_{n-1} + X_n \to 0 \]
\[ X_1 \to mX_n. \]

\( \tilde{K}_{m,n} \) is the fully open extension of \( K_{m,n} \), obtained by adjoining all inflow and outflow reactions, as in \( (1) \).

Schlosser and Feinberg analyzed variations of \( \tilde{K}_{2,n} \) [11, Table 1], as did Craciun and Feinberg [6, Table 1.1]. Joshi and Shiu introduced the version of the sequesteration networks in Definition 2.8, and proved that some of them are multistationary:

**Proposition 2.9.** [4, Lemma 6.9] For positive integers \( m \geq 2 \) and \( n \geq 3 \), if \( n \) is odd, then \( \tilde{K}_{m,n} \) admits multiple positive steady states.

For \( m = 1 \) or \( n \) even, the network \( \tilde{K}_{m,n} \) is “injective” and therefore not multistationary [4, Section 6]. Joshi and Shiu conjectured that Proposition 2.9 extends as follows:

**Conjecture 2.10.** For positive integers \( m \geq 2 \) and \( n \geq 3 \), if \( n \) is odd, then \( \tilde{K}_{m,n} \) admits multiple nondegenerate steady states.

To resolve Conjecture 2.10, we must show that \( \text{Im}(df(x^*) ) = \text{Im}(\Gamma^*) \) for two distinct positive steady states \( x^* \). We will see in \( (4) \) below that \( \Gamma^* \) is full rank, so we need only show that \( \det(df(x^*)) \neq 0 \) for two positive steady states \( x^* \).

**Remark 2.11.** As mentioned in the introduction, networks in which all reactants and products are at most bimolecular—that is, each complex has the form \( 0, X, X + Y, \) or \( 2X \)—are the norm in chemistry. This is the case for the networks \( \tilde{K}_{2,n} \), so that the \( n \)th internal reaction is \( X_1 \to 2X_n \).

We end this section by displaying the matrices that define the mass-action kinetics system \( (2) \) defined by \( \tilde{K}_{m,n} \). We order the reactions as follows: first, we enumerate the \( n \) internal (or true) reactions listed in \( (3) \) (so, the first reaction is \( X_1 + X_2 \to 0 \)), next are the \( n \) outflow reactions (so, the \((n+1)\)st reaction is \( X_1 \to 0 \)), and then we have the \( n \) inflow reactions (so, the \((2n+1)\)st reaction is \( 0 \to X_1 \)). We will refer to the sets of internal (true), outflow, and inflow reactions as \( R_T, R_O, \) and \( R_I \), respectively.

The stoichiometric matrix for \( \tilde{K}_{m,n} \) is:

\[
\Gamma = \begin{bmatrix}
-1 & 0 & 0 & \ldots & 0 & -1 \\
-1 & -1 & 0 & \ldots & 0 & 0 \\
0 & -1 & -1 & \ddots & \vdots & \vdots \\
0 & 0 & -1 & \ddots & \vdots & \vdots \\
\vdots & \vdots & \ddots & \ddots & -1 & 0 \\
0 & 0 & 0 & \ldots & -1 & m \\
\end{bmatrix}.
\]

where \( I^n \) is the \( n \times n \) identity matrix. The reactant vector is:

\[
R(x) = \begin{bmatrix}
r_1 x_1 x_2 \\
r_2 x_2 x_3 \\
\vdots \\
r_{n-1} x_{n-1} x_n \\
r_n x_1 \\
r_{n+1} x_1 \\
r_{n+2} x_2 \\
\vdots \\
r_{2n+1} x_n \\
\vdots \\
r_{3n} \\
\end{bmatrix},
\]

where the \( r_i \in \mathbb{R}_+ \) are the reaction rates and each \( x_i \in \mathbb{R}_+ \) is the concentration of each species \( X_i \). The mass-action ODEs \( (2.4) \) are:

\[
\begin{align*}
\dot{x}_1 &= -r_1 x_1 x_2 - r_n x_1 - r_{n+1} x_1 + r_{2n+1} \\
\dot{x}_i &= -r_{i-1} x_{i-1} x_i - r_i x_i x_{i+1} - r_{n+1} x_i + r_{2n+1} & \text{for } 2 \leq i \leq n-1 \\
\dot{x}_n &= -r_{n-1} x_{n-1} x_n + m r_n x_1 - r_{2n} x_n + r_{3n} 
\end{align*}
\]
Thus the Jacobian matrix, \( df(x) \), is the following \((n \times n)\)-matrix

\[
\begin{bmatrix}
-r_1 x_2 - r_n - r_{n+1} & -r_1 x_1 & 0 & \ldots & 0 & 0 \\
-r_1 x_2 & -r_1 x_1 - r_2 x_3 - r_{n+2} & -r_2 x_2 & \ldots & \vdots & \vdots \\
0 & -r_2 x_3 & -r_2 x_2 - r_3 x_4 - r_{n+3} & \ddots & 0 & 0 \\
\vdots & \vdots & \vdots & \ddots & -r_{n-2} x_{n-2} & 0 \\
0 & \vdots & \vdots & \cdots & -r_{n-2} x_{n-2} - r_{n-1} x_n - r_{2n-1} & -r_{n-1} x_{n-1} \\
m r_n & 0 & 0 & \cdots & -r_{n-1} x_n & -r_{n-1} x_{n-1} - r_{2n-1}
\end{bmatrix}
\]

(5)

3. Constructing multiple steady states via the determinant optimization method

The determinant optimization method\(^3\) was developed by Craciun and Feinberg to show that certain chemical reaction networks are multistationary [6]. More precisely, the method guarantees that some networks (such as those that satisfy the ‘Input’ conditions below) are necessarily multistationary. For instance, Joshi and Shiu showed that the networks \( \bar{K}_{m,n} \) (for \( m \geq 2 \) and odd \( n \geq 3 \)) satisfy the ‘Input’ conditions, and thus concluded these networks are multistationary (Proposition 2.9).

In fact, the determinant optimization method also applies to some networks that do not satisfy the ‘Input’ conditions. To determine if this is the case for a given network, one must check whether a certain optimization problem has a solution (see Remark 3.4). If so, then the method guarantees that the network is multistationary.

However, in many applications, it is useful not only to know that a network is multistationary but also to have explicit steady-state concentrations and reaction rates that are witnesses to multistationarity. For instance, here we would like to determine whether the steady states are degenerate, whereas in other settings one might like to perform stability analysis.

Fortunately, the proofs in [6, Section 4] that underlie the determinant optimization method are constructive, up to one use of the Intermediate Value Theorem, so one can generate or at least approximate steady states and rates. This section describes the step-by-step procedure to do this; following our steps is easier than (although equivalent to) “backtracking” through the proofs. That is, our contribution here is to re-package the determinant optimization method into a constructive algorithm. We will see that for some networks, such as \( \bar{K}_{m,n} \), the method constructs closed forms for the steady states and rates.

**Determinant optimization method (constructive version)**

**Input:** Any chemical reaction network \( G \) with \( n = |S| \) species that contains all \( n \) inflow reactions such that there exist \( n \) reactions \( y_1 \rightarrow y'_1, y_2 \rightarrow y'_2, \ldots, y_n \rightarrow y'_n \) among the internal (true) and outflow reactions \( R_I \cup R_O \) of \( G \) for which

(I) \( \text{det}(y_1, y_2, \ldots, y_n) \cdot \text{det}(y_1 - y'_1, y_2 - y'_2, \ldots, y_n - y'_n) < 0 \), and

(II) there exists a vector \( \tilde{\eta} \in \mathbb{R}^n_+ \) such that \( \sum_{i=1}^{n} \tilde{\eta}(y_i - y'_i) \in \mathbb{R}_+^S \).

**Output:** A certificate of multistationarity of \( G \): (approximations of) a positive reaction rate vector \( (r_{y \rightarrow y'}) \in \mathbb{R}_+^{|R_I|} \) and two positive concentration vectors \( x^\circ \) and \( x^\circ \) which are both steady states of the mass–action system defined by \( G \) and \( (r_{y \rightarrow y'}) \).

**Steps:** Described below.

With an eye toward resolving Conjecture 2.10, \( \bar{K}_{m,n} \) will be our ongoing example.

**Example 3.1.** For \( \bar{K}_{m,n} \) (with \( m \geq 2 \) and \( n \geq 3 \) odd), hypothesis (II) is satisfied by the vector \( \tilde{\eta} = (1, 1, \ldots, 1, m + 1, 1) \) [4, Lemma 6.9]. Hypothesis (I) was proven in [4, Lemma 6.7], where the \( n \) reactions are precisely the \( n \) internal (true) reactions (3). Conveniently, these are the reactions labeled \( y_i \rightarrow y'_i \) of the sequestration network, for \( 1 \leq i \leq n \), so our notation for the first \( n \) reactions—as well as the use of \( n \) for the number of species—matches that of the determinant optimization method.

The steps below involve a certain linear transformation \( T_{\tilde{\eta}} \); specifically, for \( \eta \in \mathbb{R}^{R_I \cup R_O} \), the linear transformation \( T_{\tilde{\eta}} : \mathbb{R}^{[S]} \rightarrow \mathbb{R}^{[S]} \) is defined by:

\[
T_{\tilde{\eta}}(\delta) = \sum_{y \rightarrow y' \in R_I \cup R_O} \tilde{\eta}_{y \rightarrow y'}(y \cdot \delta)(y - y').
\]

(6)

Equivalently, the matrix representation of \( T_{\tilde{\eta}} \) is \( d(-f)(1, 1, \ldots, 1) \) where the rates are given by \( r_i = \eta_i \). In other words, this matrix is the Jacobian matrix of the mass-action system (2.4) defined by the internal and outflow reaction rates \( \eta \) (and any choice of inflows: they do not appear in the Jacobian matrix) at the concentration vector \((1, 1, \ldots, 1)\).

---

\(^3\) Related techniques for establishing multistationarity appear in work of Banaji and Pantea [8, Section 4], Félix [10, Section 2], and Müller et al. [9, Section 3.2].
Example 3.2. For $\tilde{K}_{n,n}$, the matrix representation of $T_y$ is:

$$
\begin{bmatrix}
\eta_1 + \eta_n + \eta_{n+1} & \eta_1 & 0 & \cdots & 0 \\
\eta_1 & \eta_1 + \eta_2 + \eta_{n+2} & \eta_2 & \cdots & 0 \\
0 & \eta_2 & \eta_2 + \eta_3 + \eta_{n+3} & \eta_3 & 0 \\
\vdots & \vdots & \ddots & \ddots & \vdots \\
0 & \vdots & \cdots & \eta_{n-2} + \eta_{n-1} + \eta_{2n-1} & \eta_{n-1} \\
-m\eta_n & 0 & \cdots & \eta_{n-1} & \eta_{n-1} + \eta_{2n-1}
\end{bmatrix}
$$

(7)

From the Jacobian matrix (5), it is clear that this matrix (7) equals $d(-f)(1, 1, \ldots, 1)$, where the reaction rates are given by $r_i = \eta_i$.

The first step is to construct a (strictly positive) vector $\eta^- \in \mathbb{R}_+^{[R_T \cup R_O]}$, indexed by all internal (true) and outflow reactions, such that

(I) $\det(T_{\eta^-}) < 0$, and

(II) $\sum_{y \mapsto y' \in R_T \cup R_O} \eta^-_{y \mapsto y'} (y - y') \in \mathbb{R}_+^{|S|}$.

Craciun and Feinberg proved that these conditions (I) and (II) are satisfied by a vector $\eta^-$ of the following form:

$$
\eta^-_{y \mapsto y'} = \begin{cases} 
\lambda \eta^-_{y \mapsto y'} / \epsilon & \text{if } y \mapsto y' \in \{y_i \mapsto y_i' \mid i \in \eta \} \\
\epsilon & \text{else},
\end{cases}
$$

where $\lambda$ is sufficiently large and $\epsilon$ is sufficiently small [6, proof of Theorem 4.2].

Example 3.3. For $\tilde{K}_{m,n}$ (with $m \geq 2$ and $n \geq 3$ odd), we define $\eta^-$ as follows:

$$
\eta^-_i = \begin{cases} 
\lambda & \text{if } 1 \leq i \leq n - 2 \text{ or } i = n \\
(m + 1)\lambda & \text{if } i = n - 1 \\
\epsilon & \text{if } n + 1 \leq i \leq 2n.
\end{cases}
$$

Remark 3.4 (Stronger versions of the determinant optimization method). This section describes the simplest version of the determinant optimization method. In fact, even if a network does not satisfy the hypotheses in the input given above, the method can still apply: [6, Remark 4.1] describes, in this setting, how to implement the above first step, i.e. how to test whether a suitable $\eta^-$ exists, as an optimization problem. Specifically, this is a polynomial optimization problem with linear constraints over a compact set. Therefore, one can use any applicable optimization method. Additionally, see Remark 3.5 for how one can begin the algorithm at the second step.

The second step is to construct a (strictly positive) vector $\eta^0 \in \mathbb{R}_+^{[R_T \cup R_O]}$ for which:

(I) $\det(T_{\eta^0}) > 0$, and

(II) $\sum_{y \mapsto y' \in R_T \cup R_O} \eta^0_{y \mapsto y'} (y - y') \in \mathbb{R}_+^{|S|}$.

Craciun and Feinberg proved that this can be accomplished as follows [6, proof of Theorem 4.1]. First, construct an $\eta^+ \in \mathbb{R}_+^{[R_T \cup R_O]}$ such that $\det(T_{\eta^+}) > 0$; do this by assigning a large value to outflow reactions and a small value to internal reactions:

$$
\eta^+_{y \mapsto y'} = \begin{cases} 
\lambda^+ & \text{if } y \mapsto y' \in R_O \\
\epsilon^+ & \text{if } y \mapsto y' \in R_T,
\end{cases}
$$

where $\lambda^+ > 0$ is large and $\epsilon^+ > 0$ is small.

Then, by interpolating between this vector $\eta^+$ and the vector $\eta^-$ from the previous step, the Intermediate Value Theorem (plus the fact that the set of vectors satisfying condition (II) is convex) guarantees the existence of an $\eta^0$ with the required properties. Moreover, such a suitable $\eta^0$ can be numerically approximated, and, with careful tracking of error, one can use this approximation to generate steady states and concentrations in the following steps.

Remark 3.5 (Interpretation of the second step and subsequent steps). What the second step does is to find reaction rates (given by $\eta_i$ for the internal and outflow rates, and the vector in (II′) for the inflow rates) at which the concentration vector $(1, 1, \ldots, 1)$ is a degenerate steady state: degeneracy is by (I′), and being a steady state comes from (II′).

Equivalently, if $\tilde{\eta}_{y \mapsto y'}$ is any positive vector of reaction rates at which some concentration vector $\tilde{c}$ is a degenerate positive steady state, then the vector $\eta \in \mathbb{R}_+^{[R_T \cup R_O]}$ defined coordinate-wise by $\eta_{y \mapsto y'} = \tilde{\eta}_{y \mapsto y'} \tilde{c}$ satisfies the second step. So,
a reader who has already found a degenerate positive steady state of their system could start the deterministic optimization method at the second step. In other words, one could begin applying the method by immediately searching for a suitable \( \eta^0 \) (without first generating \( \eta^- \) and \( \eta^+ \)). One strategy for doing this is described in Remark 3.6, which we employ for \( \bar{K}_{m,n} \) beginning in Example 3.7.

In the next steps, the deterministic optimization method constructs a certain vector \( \delta \) so that \( |\delta| \) is a suitable bifurcation parameter: for \( |\delta| \) small but positive, the degenerate steady state breaks into two nondegenerate steady states.

**Remark 3.6.** Here is one strategy for constructing a suitable \( \eta^0 \) (without using \( \eta^- \) and \( \eta^+ \)). First, identify (if possible) an \( \eta \in \mathbb{R}^{|\mathcal{S}|} \) and a reaction \( y_i \to y'_i \) among the internal (true) and outflow reactions such that:

(a) \( \sum_{y 
rightarrow y'} \eta_{y 
rightarrow y'} (y - y') \in \mathbb{R}_{y_i} \), and

(b) \( y_i - y'_i \in \mathbb{R}_{y_i} \) (this holds, for instance, if \( y_i \to y'_i \) is an outflow reaction).

One could see whether \( \eta^+ \) or \( \eta^- \) might work (we use \( \eta^- \) in Example 3.7 below). Then, define \( \eta \) as follows: let the entry \( \eta_j \) (corresponding to the same \( i \)th reaction) be free, and fix \( \eta_j = \eta_j^0 \) for \( j \neq i \). Then, solve the (univariate polynomial) equation \( \det(T_{\eta^0}) = 0 \). If there is a positive solution for \( \eta_j^0 \), then the resulting vector \( \eta^0 \) is positive, and \( \ell' \) holds by construction. Furthermore, \((\ell')\) holds because the sum in \((\ell')\) is precisely the sum of a positive vector (namely, the sum in (a)) and a non-negative vector (namely, \( \eta^0_i(y_i - y_i') \)). However, \( \eta^0 \) is not guaranteed to be positive, so this strategy may fail.

**Example 3.7.** For \( \bar{K}_{m,n} \) (with \( m \geq 2 \) and \( n = 3, 5, 7, 9, 11 \)), the following choice of \( \eta^0 \) satisfies the requirements of the second step:

\[
\eta_j = \begin{cases} 
\lambda & \text{if } 1 \leq i \leq n - 2 \text{ or } i = n \\
(m+1)\lambda & \text{if } i = n - 1 \\
\epsilon & \text{if } n + 1 \leq i \leq 2n - 1 \\
\frac{(m+1)(m+2)(2m+1)\gamma_{m-1} - \lambda(m+1)}{(m+1)(m+2)(m+1)\gamma_{m-1}} - \lambda(m+1) & \text{if } i = 2n,
\end{cases}
\]

where \( \lambda > 0 \) and \( \epsilon > 0 \) are such that \( \eta^0_2n \) is positive \(^4\) (thus, all coordinates of \( \eta^0 \) are positive), and \( \gamma_i \) is \( i \)th principal minor of the matrix representation of \( T_{\eta^0} \) displayed below in (9), i.e., \( \gamma_i \) is the determinant of the \( i \times i \) (tridiagonal) upper-left submatrix of (9) (also, \( \gamma_0 := 1 \)).

To show that this choice of \( \eta^0 \) satisfies the two conditions of the second step, we first note that \((\ell')\) is straightforward to verify.

Satisfying \((\ell')\) only requires \( \eta^0 \) to satisfy one (determinantal) equation, so allowing one free variable is sufficient. We choose \( \eta^0_{2n} \) as this free variable, and we will recover the formula in (8). Namely, we let all other coordinates \( \eta_j^0 \) have the form given above (for \( 1 \leq i \leq 2n - 1 \)), and then, recalling (7), the matrix representation of \( T_{\eta^0} \) is:

\[
\begin{bmatrix}
2\lambda + \epsilon & \lambda & 0 & \cdots & 0 \\
\lambda & 2\lambda + \epsilon & \lambda & 0 & \cdots & 0 \\
0 & \lambda & \ddots & \ddots & \ddots & \ddots \\
\vdots & 0 & \ddots & \ddots & \ddots & \ddots \\
-\lambda m & \cdots & 0 & \lambda (m + 1) + \epsilon & \lambda (m + 1) \\
-\lambda m & \cdots & 0 & \lambda (m + 1) + \epsilon & \lambda (m + 1) + \eta^0_{2n}
\end{bmatrix}
\]

Expanding (9) along the bottom row, we obtain the determinant of \( T_{\eta^0} \):

\[
\det(T_{\eta^0}) = (-1)^n m(m + 1) \lambda^n - \lambda^2 (m + 1)^2 \gamma_{n-2} + (\lambda (m + 1) + \eta^0_{2n}) \gamma_{n-1},
\]

where we recall that \( \gamma_i \) is \( i \)th principal minor of \( T_{\eta^0} \).

The determinant of tridiagonal matrices can be solved recursively \([12]\). For our matrix, it is easy to verify the following recursion for \( i \leq n - 2 \), which is independent of \( m \):

\[
\gamma_{i+2} = (2\lambda + \epsilon) \gamma_{i+1} - \lambda^2 \gamma_i,
\]

with initial values \( \gamma_0 := 1 \) and \( \gamma_1 = 2\lambda + \epsilon \). Notice that \( \gamma_{n-1} \) must be treated separately, because the \((n - 1)^{\text{st}}\) row contains \( \eta^0_{n-1} \), which is a function of \( m \). Using standard methods we can get the generating function of the recurrence.

\[
\gamma_i = \frac{1}{2^{i+1} C_i} \ast (-c_2(c_2 - c_1)^i + c_1(c_2 - c_1)^i + c_2(c_1 + c_2)^i + c_1(c_1 + c_2)^i).
\]

\(^4\) We checked that such \( \lambda, \epsilon \) exist for \( n = 3, 5, 7, 9, 11 \) (and \( m \geq 2 \)), and we furthermore conjecture that for larger \( n \), choosing \( \lambda \) sufficiently large and \( \epsilon \) sufficiently small will suffice.
where $c_1 = (\epsilon)^{\frac{1}{2}} (\epsilon + 4\lambda)^{\frac{1}{2}}$ and $c_2 = \epsilon + 2\lambda$. Notice here that $\gamma_0$ is always positive for sufficiently small $\epsilon$. A formula for $\gamma_{n-1}$ is given using the formula for tridiagonal matrices [12]:

$$\gamma_{n-1} = \gamma_{n-2}(\lambda(m+2) + \epsilon) - \lambda^2 \gamma_{n-3}.$$ 

With this recurrence solved, we set Eq. (10) to zero and then derive the explicit function for $\eta_0^2$ from (10) in terms of $m, \lambda$, and $\epsilon$; this is the formula in (8).

The third step is to construct a nonzero vector $\delta \in \mathbb{R}^{[S]}$ in the nullspace of $T_{\varphi}$, i.e. such that $T_{\varphi} \cdot \delta = 0$. (Such a vector $\delta$ exists because det$(T_{\varphi}) = 0$.)

**Example 3.8.** For our example $\tilde{K}_{m,n}$ (with $m \geq 2$ and $n \geq 3$ odd), we claim that the vector $\delta$ whose coordinates are defined as follows is in the nullspace of (9):

$$\delta_k = \begin{cases} 
\frac{1}{\lambda} \delta_{k-1} - \delta_{k-2} & \text{if } k = 1 \\
\frac{-(\lambda(m+2)+\epsilon)}{\lambda(m+1)} \delta_{n-1} - \frac{1}{m+1} \delta_{n-2} & \text{if } k = n, \\
-(\lambda m+\epsilon) \delta_{k-1} - \delta_{k-2} & \text{if } 2 \leq k \leq n-1 
\end{cases}$$

(11)

where we introduce $\delta_0 = 0$ for convenience in solving the recurrence relation, and $\delta_1 \neq 0$ is our free variable. Note that the last coordinate, $\delta_n$, has a different formula, because the $(n-1)$st row in (9) used to define $\delta_n$ contains terms dependent on $m$ that do not satisfy the recurrence.

To see that $\delta$ is a nonzero vector in the nullspace of $T_{\varphi}$, notice that the conditions on $\delta$ that state that its inner product with each of the first $n-1$ rows of $T_{\varphi}$ coincide precisely with the $n-1$ recurrences in the definition of $\delta$ (11). We claim that the last row of $T_{\varphi}$ is linearly dependent on the other rows, and from this we will conclude that the last row of $T_{\varphi}$ automatically has zero inner product with $\delta$. To see this, we recall that det$(T_{\varphi}) = 0$ by construction, so we need only show that the first $n-1$ rows of $T_{\varphi}$ are linearly independent. Assume, to the contrary, that there is a non-trivial linear combination of the first $(n-1)$st rows of $T_{\varphi}$ that adds to 0. Note that in the first $n-1$ rows only the last one contains an entry in the last column, namely $\eta^0_{n-1}$. Since $\eta^0_{n-1} \neq 0$, the corresponding scalar of the $(n-1)$st row should be 0, thus annihilating the entire row. In the same manner, the corresponding scalar for the $(n-2)$nd row would be equal to 0. Repeating the process shows that the only linear combination that adds to 0 is the trivial one, thus, arriving at a contradiction. So, $\delta$ is in the nullspace of $T_{\varphi}$.

Again, by using standard techniques for analyzing recurrences, we find the generating function for each of the first $n-1$ entries of $\delta$:

$$\delta_k = \delta_1 \lambda \cdot \frac{(\sqrt{4\lambda \epsilon + \epsilon^2} - (2\lambda + \epsilon))^k - (-\sqrt{4\lambda \epsilon + \epsilon^2} - (2\lambda + \epsilon))^k}{2^{k+1} \sqrt{4\lambda \epsilon + \epsilon^2}},$$

for $1 \leq k \leq n-1$.

The final step is to use the vectors $\eta^0$ and $\delta$ (or, as we will see, a sufficiently scaled version of $\delta$) from the previous two steps to construct a certificate of multistationarity [6, proof of Lemma 4.1]; namely, the internal (true) and outflow reaction rates are:

$$r_y = \frac{\langle y, \delta \rangle}{e^{\langle y, \delta \rangle} - 1} \eta^0_y \quad \text{for all } y \to y' \in R_T \cup R_O.$$ 

The inflow reaction rates are the coordinates of the following vector:

$$\left( r_0 \to x \right) = \sum_{y \to y'} \eta^0_{y \to y'} (y - y') \in \mathbb{R}^{[S]}_+,$$

(12)

and the two steady states are:

$$x^* = (1, 1, \ldots, 1) \quad \text{and} \quad x^\# = (e^{\delta_1}, e^{\delta_2}, \ldots, e^{\delta_n}).$$

Craciun and Feinberg showed that for sufficiently small scaling of $\delta$, all inflow rates (12) are positive [6].

**Example 3.9.** For $\tilde{K}_{m,n}$, with $m \geq 2$ and $n = 3, 5, 7, 9, 11$, we checked that $\delta_1 = 1$ suffices. Details for the $n = 3$ case are provided in Remark 4.2.

Summarizing what we accomplished above, we have closed-form expressions for reaction rate constants and steady states that show that the sequestration network is multistationary:

**Theorem 3.10.** Consider positive integers $m \geq 2$ and $n \in \{3, 5, 9, 11\}$. Let $\delta \in \mathbb{R}^n$ be as in (11) with $\delta_1 = 1$. Also, let $\eta^0$ be as is (8). Then, for the following internal (true) and outflow reaction rates:

$$r_i = \frac{\langle y_i, \delta \rangle}{e^{\langle y_i, \delta \rangle} - 1} \eta^0_i \quad \text{for all } i \in \{1, 2, \ldots, 2n\},$$

In fact, this theorem will hold for any larger $n$ for which the last coordinate of $\eta^0$ as defined in (8) can be made to be positive.
and the following inflow reaction rates:

\[ r_{2n+1} = r_1 + r_n + r_{n+1} \]
\[ r_{2n+i} = r_{i-1} + r_1 + r_{n+i} \quad \text{for all} \quad 2 \leq i \leq n-1 \]
\[ r_{3n} = r_{n-1} + r_{2n} - m r_n. \]

the concentrations:

\[ \mathbf{x}^* = (1, 1, \ldots, 1) \quad \text{and} \quad \mathbf{x}^\# = (e^{\delta_1}, e^{\delta_2}, \ldots, e^{\delta_n}) \]

both are positive steady states of the mass-action kinetics system defined by \( \tilde{K}_{m,n} \) and the reaction rates \( r_i \) above.

**Remark 3.11.** One may wonder whether or not the determinant optimization method has the potential to create degenerate steady states. Indeed, if we could prove that this method always constructs nondegenerate steady states, then this would resolve **Conjecture 2.10.** However, this is not the case.

We determined this by analyzing \( \tilde{K}_{2,3} \) as in **Theorem 3.10.** By letting \( \epsilon \) be a free variable we compute the parameterized determinants \( \det(df(\mathbf{x}^*)) \) and \( \det(df(\mathbf{x}^\#)) \) as functions of \( \epsilon \). Both functions are easily checked to be continuous for positive values of \( \epsilon \), and from the graph (Fig. 1), we can see easily that there exist choices of \( \epsilon \) for which one of the two steady states is degenerate. More precisely, \( \det(df(\mathbf{x}^*)) = 0 \) for some \( \epsilon \in (0.12, 0.125) \) and \( \det(df(\mathbf{x}^\#)) = 0 \) for some \( \epsilon \in (0.240, 0.241) \) and some \( \epsilon \in (1.159, 1.160) \).

4. Resolving **Conjecture 2.10** for the \( n = 3 \) case

Recall that **Conjecture 2.10** asserts that \( \tilde{K}_{m,n} \) admits multiple nondegenerate positive steady states, for integers \( m \geq 2 \) and \( n \geq 3 \) with \( n \) odd. The main result of this section (**Theorem 4.5**) resolves the conjecture when \( n = 3 \). To accomplish this, we first write down rate constants for this \( n = 3 \) case for which there are two steady states \( \mathbf{x}^* \) and \( \mathbf{x}^\# \); these values were obtained by the determinant optimization method in the previous section for the general \( \tilde{K}_{m,n} \) case (**Proposition 4.1**). We then resolve the conjecture for \( n = 3 \) by proving that \( \mathbf{x}^* \) and \( \mathbf{x}^\# \) are nondegenerate.

4.1. Reaction rate constants for which \( \tilde{K}_{m,3} \) is multistationary

**Proposition 4.1** below specializes **Theorem 3.10** to the \( n = 3 \) case. Following the description in **Section 3**, \( \lambda = 1 \) and \( \epsilon = 0.1 \) will suffice, and then we obtain

\[ \eta_0 = \left( \lambda, \lambda (m + 1), \lambda, \epsilon, \epsilon, \frac{m^2 - 0.31m - 1.31}{2.1m + 3.41} \right)^T \]

from the second step of the determinant optimization method. Next, in the third step, we find that the following vector spans the nullspace of \( T_{\mathbf{\eta}^0} \):

\[ \delta = \left( 1, -2.1, \frac{2.1m + 3.41}{m + 1} \right)^T. \]

Thus, **Theorem 3.10** specializes to:

![Fig. 1. Graphs of \( \det(df(\mathbf{x}^*)) \) solid and \( \det(df(\mathbf{x}^\#)) \) dashed as functions of \( \epsilon \).](image_url)
Proposition 4.1. Consider any integer \( m \geq 2 \), and the following internal, outflow, and internal reaction rates:
\[
\begin{align*}
    r_1 &= \frac{1}{e - 1} \approx 1.65 &
    r_2 &= \frac{\sqrt[3]{m+4}}{e - 1} \approx 1.31 &
    r_3 &= \frac{1}{e - 1} \approx 0.58 \\
    r_4 &= \frac{1}{e - 1} \approx 0.06 &
    r_5 &= \frac{\sqrt[3]{m+4}}{e - 1} \approx 0.24 &
    r_6 &= \frac{m-1.31}{e - 1} \\
    r_7 &= r_1 + r_3 + r_4 \approx 2.29 &
    r_8 &= r_1 + r_2 + r_5 &
    r_9 &= r_2 + r_6 - m r_3.
\end{align*}
\]

Then for the mass-action kinetics system defined by the fully open sequestration network \( \tilde{K}_{m,3} \) and the above rate constants \( r_i \), both \( x^* = (1, 1, 1) \) and \( x^\# = (e^{-2.1}, e^{-1.341}, e^{-1.341}) \) are positive steady states.

Note that in Proposition 4.1, only \( x^*_1, r_2, r_6, r_8, \) and \( r_9 \) depend on \( m \).

Remark 4.2. The only reaction rate in (14) that is not obviously positive is the inflow rate \( r_9 \), so we verify it here:
\[
    r_9 = r_2 + r_6 - m r_3 > r_2 + 0 - m \left( \frac{1}{e - 1} \right) \geq m - m \left( \frac{1}{e - 1} \right) > 0,
\]
where the second-to-last inequality follows from Lemma 4.3 below.

4.2.Bounding rates and steady states of \( \tilde{K}_{m,3} \)

Here we give upper and lower bounds which we will use to prove that \( x^* \) and \( x^\# \) are nondegenerate. The following bounds are on the third coordinate of \( x^\# \):
\[
e^{\sqrt{\frac{3n+4}{m+1}}} \geq x^\#_3 = e^{\sqrt{\frac{3n+4}{m+1}}} > e^{2.1} \quad \text{for all} \quad m \geq y \geq 0.
\]

The first inequality in (15) follows from the easy fact that \( e^{\sqrt{\frac{3n+4}{m+1}}} \) is a decreasing function when \( m > 0 \), and the second inequality is straightforward.

The proofs of the following two upper/lower bounds are in Appendix A:

Lemma 4.3. (Bounds on \( r_2 \)). When \( \lambda = 1 \) and \( \epsilon = 0.1 \), the rate constant \( r_2 \) defined in (14) satisfies the following inequalities for all \( m \geq 2 \):
\[
m + 1 > r_2 \geq m.
\]

Lemma 4.4. (Bounds on \( r_6 \)). When \( \lambda = 1 \) and \( \epsilon = 0.1 \), the rate constant \( r_6 \) defined in (14) satisfies the following inequalities:
\[
0.14 m > r_6 > 0.13 m - 0.5,
\]
where the upper bound holds for \( m \geq 2 \), and the lower bound holds for \( m \geq 20 \).

4.3. Proving nondegeneracy of steady states for the network \( \tilde{K}_{m,3} \)

The main result of this section is:

Theorem 4.5. (Resolution of Conjecture 2.10 when \( n = 3 \)). For integers \( m \geq 2 \), the network \( \tilde{K}_{m,3} \) has the capacity to admit multiple nondegenerate positive steady states.

We will prove Theorem 4.5 by showing that \( x^* \) and \( x^\# \) in Proposition 4.1 are nondegenerate, i.e. we must prove that the image of the \( 3 \times 3 \) Jacobian matrix \( df(x) \) at each of the steady states is equal to the image of the \( 3 \times 3 \) matrix \( \Gamma \).

As stated earlier (after Conjecture 2.10), since \( \Gamma \) is full rank, our problem reduces to showing that \( det(df(x)) \neq 0 \) for both steady states and for all integers \( m \geq 2 \).

We begin by displaying the Jacobian matrix (5) of \( \tilde{K}_{m,3} \):
\[
df(x) = \begin{bmatrix}
    -r_1 x_2 - r_3 - r_4 & -r_1 x_1 & 0 \\
    -r_1 x_2 & -r_1 x_1 - r_2 x_3 - r_5 & -r_2 x_2 \\
    m r_3 & -r_2 x_2 & -r_2 x_2 - r_6
\end{bmatrix}.
\]

Thus, our goal is to show that the following determinants (obtained by strategically cancelling and rearranging terms) are nonzero for all integers \( m \geq 2 \):
\[
D_1 := det(df(x^*)) = r_2 r_1 r_3 m - (r_2 + r_6)(r_1 r_3 + r_1 r_4 + r_1 r_5 + r_3 r_5 + r_4 r_5) \\
- r_2 r_6 (r_1 + r_3 + r_4)
\]
\[
D_2 := det(df(x^\#)) = r_2 x^\#_2 ((r_1 x^\#_2 + r_3 + r_4)(r_2 x^\#_3) + r_4 x^\#_4 m r_3) \\
- (r_2 x^\#_2 + r_6)(r_1 x^\#_2 + r_3 + r_4)(r_1 x^\#_3 + r_2 x^\#_3 + r_5) + (r_2 x^\#_2 + r_6)(r_1 x^\#_3 r_1 x^\#_2)
\]
From its graph,\(^6\) \(D_1\) appears to be increasing quadratically as a function of \(m\). So, to prove that \(D_1\) is nonzero for integer values of \(m \geq 2\), we will bound it from below by a quadratic function. Similarly, \(D_2\) appears to be decreasing quadratically, so we will bound it from above by another quadratic function. Using these bounds, we will then conclude that \(D_1\) and \(D_2\) are strictly positive and negative (respectively) after certain cutoff points of \(m\), effectively showing nondegeneracy of both steady states beyond the cutoffs. Finally, we will complete the proof by estimating \(D_1\) and \(D_2\) at the remaining integers \(m\) between the 2 and the cutoff points to show that these values are nonzero.

**Proof of Theorem 4.5.** We generate our rates and concentrations as in Proposition 4.1.
Following the description immediately after Theorem 4.5, we need only show that \(D_1 \neq 0\) and \(D_2 \neq 0\) for all integers \(m \geq 2\). First, we bound \(D_1\) by using its formula (16) together with the bounds in Lemmas 4.3 and 4.4:

\[
D_1 > m^2 r_1 r_3 - ((m + 1) + 0.14m)(r_1 r_3 + r_1 r_4 + r_1 r_5 + r_2 r_3 + r_4 r_5) - (m + 1)(0.14m)(r_1 + r_3 + r_4).
\]

Next, estimating the remaining rates \(r_i\), which are constants (recall Eqs. (14)), by appropriate upper or lower bounds, we obtain:

\[
D_1 > m^2 (0.95) - ((m + 1) + 0.14m)1.61 - (m + 1)(0.14m)2.29
\]

\[
= 0.6294m^2 - 2.156m - 1.61.
\]

It is easy to show that the quadratic function which bounds \(D_1\) above is always positive for integers \(m > 4\). So, \(D_1 > 0\) for \(m > 4\). Thus, it remains only to show that \(D_1 \neq 0\) at \(m = 2, 3, 4\); indeed, those values are nonzero and are listed in Table 1.

Now we proceed to bound \(D_2\). Again, we use its formula (17) together with the bounds in (15) and Lemmas 4.3 and 4.4:

\[
D_2 < (m + 1)x_2^w ((r_1 x_2^w + r_3 + r_4)((m + 1)x_2^w)+ r_1 x_2^w m r_3)
- (m x_2 + (0.13m - .5))(r_1 x_2^w + r_3 + r_4)(r_1 x_2^w + m x_2 + r_5)
+ ((m + 1)x_2^w + 0.14m)(r_1 x_2^w r_1 x_2^w).
\]

Note that we used the lower bound on \(r_5\), so the above inequality holds for \(m \geq 20\) (and thus we will need to check the values of \(m\) between 2 and 19 separately). In the same manner as before, we approximate all of the constants appropriately for \(m \geq 20\), and then simplify:

\[
D_2 < (m + 1)0.13((m + 1)8.7) + 2.61m - (0.25m - 0.5)(0.84)(4.72 + 8.16m)
+ (0.13(m + 1) + 0.14m)(0.91)
\]

\[
= -0.41295m^2 + 4.9437m + 3.06205.
\]

Therefore, it is easy to see that \(D_2\) is nonzero for \(m \geq 20\). For \(2 \leq m \leq 19\) we again refer to Table 1, which completes our proof. \(\square\)

**5. Resolving Conjecture 2.10 for small values of \(m\) and \(n\)**

The main result of this section extends Theorem 4.5 to \(n \leq 11\), for small \(m\):

**Theorem 5.1.** (Resolution of Conjecture 2.10 for small \(m\) and \(n\)). For \(m = 2, 3, 4, 5\) and \(n = 5, 7, 9, 11\), the network \(\tilde{K}_{m,n}\) has the capacity to admit multiple nondegenerate positive steady states.

**Proof.** We generate our rates and concentrations as in Theorem 3.10: it is straightforward to check that \(\delta = 1\), \(\lambda = 1\), and \(\epsilon = 0.001\) satisfy all necessary hypotheses. Thus, we obtain two steady states, \(x^* = (1, 1, \ldots, 1)\) and \(x^0\) defined in (13). Then, as in the proof of Theorem 3.10, we verify that the determinants \(\det(df(x^*))\) and \(\det(df(x^0))\) are nonzero for \(m = 2, 3, 4, 5\), which is readily seen from their graphs, which appear in Appendix B. (In fact, the graphs strongly suggest that the conjecture holds completely for each of these values of \(m\), namely \(n = 5, 7, 9, 11\), i.e. for \(m > 5\) as well.) \(\square\)

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\(^6\) Analogous graphs for larger \(n\) appear in Appendix B.
6. Discussion

As stated in the Introduction, deciding whether a chemical reaction network is multistationary is not easy in the general case. And even when we can confirm that a network is multistationary, there is no general technique to show that it will admit multiple nondegenerate steady states. Nonetheless, in this paper we succeeded in this task for certain sequestration networks $K_{m,n}$ by using the determinant optimization method to obtain closed forms for reaction rates and steady states.

Our work resolved the $n = 3$ case of Conjecture 2.10, and we believe that our results form an important step toward resolving the full conjecture. Specifically, one could use the formulas for rates and steady states given in Theorem 3.10 to analyze the general case, or, perhaps easier, the case of some fixed $m$ and general $n$. Two other possible approaches are to (1) find an alternate method to obtain closed forms for the steady states of a chemical reaction network, or (2) identify criteria that can guarantee that steady states are nondegenerate.

Expanding on the last idea, our ultimate goal is to develop general techniques to assert that steady states of a chemical reaction network are nondegenerate. For instance, our analysis of the Jacobian determinants in this work suggest that even if the determinant optimization method yields a degenerate steady state, then the rate constants can be perturbed slightly so that the degenerate steady state becomes nondegenerate (and the other steady state also remains nondegenerate). Is this true for any network for which the determinant optimization method applies? If so, then this would completely resolve Conjecture 2.10, and, more generally, this would enable us to more readily “lift” multistationarity and thereby enlarge our catalogue of known multistationary networks.

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Appendix A. Proofs of Lemmas 4.3 and 4.4.

Lemma 6.1 (Lemma 4.3). The function $r_2(m) = \frac{1.31}{e^{\frac{1.31}{m+1}} - 1}$ satisfies the following inequalities for all $m \geq 2$:

$$m + 1 > r_2(m) \geq m.$$

Proof. For the upper bound, first observe that

$$\log \left( \left( 1 + \frac{1.31}{m+1} \right)^{m+1} \right) < \log (e^{1.31}) = 1.31$$

for all $m \geq 0$.

since $\lim_{x \to \infty} (1 + \frac{y}{x})^x$ converges to $e^y$ from below for positive values of $y$. Thus:

$$1.31 > \log \left( \left( 1 + \frac{1.31}{m+1} \right)^{m+1} \right)$$

$$> \log \left( \frac{m + 2.31}{m+1} \right)^{m+1} = (m+1) \log \left( \frac{m + 2.31}{m+1} \right),$$

which implies that

$$\frac{1.31}{m+1} > \log \left( \frac{m + 2.31}{m+1} \right)$$

$$\Rightarrow e^{\frac{1.31}{m+1}} > \frac{m + 2.31}{m+1}$$

$$\Rightarrow e^{\frac{1.31}{m+1}} (m+1) - (m+1) > 1.31.$$

and this final inequality implies our desired upper bound: $m + 1 > \frac{1.31}{e^{\frac{1.31}{m+1}} - 1} = r_2(m)$.

Notice that our lower bound is equivalent to the following inequality:

$$\frac{m + 1.31}{m} \geq e^{\frac{1.31}{m+1}} \text{ for all } m \geq 2. \tag{18}$$

We set $a = 1.31$, make use of the change of variables $z = \frac{1}{m}$, and then apply $\log$ to see that our desired inequality (18) is equivalent to:

$$\log(1 + az) \geq \frac{a}{z^{-1} + 1} = \frac{az}{1 + z} \text{ for all } z \in (0, 1/2).$$
We will show that \( \log(1 + az) - \frac{az}{1 + z} \geq 0 \). To this end, define \( b \) by \( 1 - b = a - 1 \), and notice that \( 1 > b > \frac{1}{2} > (a - 1) \). Next, note that we have the following equalities:

\[
\log(1 + az) - \frac{az}{1 + z} = \int_0^z \left( \frac{1}{1 + t} - \frac{1}{1 + z} \right) dt \\
= \int_0^b \frac{z - t}{(1 + z)(1 + t)} dt + \int_{b}^z \left( \frac{1}{1 + t} - \frac{1}{1 + z} \right) dt + \int_z^{az} \frac{z - t}{(1 + z)(1 + t)} dt.
\]

The second integral in (19) is nonnegative (because its integrand is nonnegative), so we complete the proof now by showing that the sum of the first and third integrals in (19) is nonnegative:

\[
\int_0^b \frac{z - t}{(1 + z)(1 + t)} dt + \int_z^{az} \frac{z - t}{(1 + z)(1 + t)} dt \geq \frac{(1 - b)z^2b}{(z + 1)^2} + \frac{-(a - 1)^2z^2}{(z + 1)^2} \geq 0,
\]

where the two inequalities come from recalling that \( b < 1 \), and, respectively, \( 1 - b = (a - 1) \) and \( b \geq (a - 1) \). □

**Lemma 6.2** (Lemma 4.4). The function \( r_6(m) = \frac{m - 1.31}{e^{\frac{2m}{m+1}} - 1} \) satisfies:

\[
0.14m > r_6(m) > 0.13m - 0.5,
\]

where the upper bound holds for \( m \geq 2 \) and the lower bound holds for \( m \geq 20 \).

**Proof.** We first prove the upper bound. By the second inequality in (15), \( (e^{\frac{2m}{m+1}} - 1) > 0 \) for \( m \geq 2 \). Thus our desired upper bound in (20) is equivalent to the following:

\[
m(0.14e^{\frac{2m}{m+1}} - 1.14) > -1.31,
\]

which holds (for positive \( m \)) whenever \( (0.14e^{\frac{2m}{m+1}} - 1.14) > 0 \). This inequality in turn is equivalent to the following (since \( \log \) is an increasing function):

\[
2.1m + 3.41 > (m + 1)\log\left(\frac{1.14}{0.14}\right) \approx (m + 1)(2.10),
\]

which is true for positive \( m \), so the proof of the upper bound is complete.

For the lower bound, by clearing the denominator and gathering exponential terms on the right-hand side, we see that the desired inequality is equivalent to the following:

\[
1.13m - 1.81 \geq e^{\frac{2m}{m+1}} (0.13m - 0.5).
\]

We prove this now. The first inequality below is equivalent to the inequality .00004m \( \geq -2.536 \), which is true for positive \( m \):

\[
1.13m - 1.81 \geq 8.692(0.13m - 0.5)
\]

\[
> e^{\frac{2m}{m+1}} (0.13m - 0.5) \geq e^{\frac{2m}{m+1}} (0.13m - 0.5),
\]

and the final inequality holds for \( m \geq 20 \) because \( e^{\frac{2m}{m+1}} \) is a decreasing function for positive values of \( m \). □

**Appendix B. Graphs for the proof of Theorem 5.1**

Figs. 2 a and 3 a below present the graphs of the determinant of the Jacobian matrix evaluated at the steady states \( x^* \) and \( x^\# \) (as described in the proof of Theorem 5.1) for the network \( K_{m,n} \) for odd \( 5 \leq n \leq 11 \) as functions of \( m \). Note that the graphs are nonzero for \( 2 \leq m \leq 5 \), confirming Conjecture 2.10 for odd \( 5 \leq n \leq 11 \) and those values of \( m \). Also, the graphs strongly suggest that the conjecture holds for larger \( m \) as well. All graphs were made using Desmos Graphing Calculator [13] (Figs. 1–5).
References


