Analyzing Multistationarity in Chemical Reaction Networks using the Determinant Optimization Method

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Abstract

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 nondegeneracy 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.

Keywords: Mass-action kinetics, chemical reaction networks, multistationarity, determinant optimization method, steady states, degeneracy

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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

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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 parametrized 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.

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 cataloguing the multistationary networks which contain no embedded multistationary networks, because all larger multistationary networks contain at least one embedded multistationary subnetwork from the catalogue.

As a step toward such a catalogue, Joshi and Shiu identified a certain infinite family of chemical reaction networks $\widetilde{K}_{m,n}$ 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 $\widetilde{K}_{m,n}$ is multistationary for all integers $m \geq 2$ and odd integers $n \geq 3$ [4]. Furthermore, they conjectured that these networks can exhibit multiple *nonde-*

generate 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 $\widetilde{K}_{2,n}$ 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, §4]. Also, because trimolecular reactants/products are rather uncommon in chemistry and $\widetilde{K}_{2,n}$ 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 $\widetilde{K}_{m,3}$ 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 $\widetilde{K}_{m,n}$, 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 Sections 4 and 5. Finally, a discussion appears in Section 6.

Notation. We denote the positive real numbers by $\mathbb{R}_+ := \{x \in \mathbb{R} \mid x > 0\}$,

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[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.

and the standard inner product in $\mathbb{R}^n$ by $\langle -, - \rangle$. The ith 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 $\widetilde{K}_{m,n}$.

2.1. Mass-action kinetics systems

Definition 2.1. *A chemical reaction network $G = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ consists of three finite sets:*

1. *a set of* species $\mathcal{S} = \{X_1, X_2, \ldots, X_s\}$,
2. *a set $\mathcal{C}$ of* complexes, *which are non-negative integer linear combinations of the species, and*
3. *a set $\mathcal{R} \subseteq \mathcal{C} \times \mathcal{C}$ of* reactions.

Example 2.2. *The following chemical reaction network:*
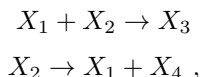
$$X_1 + X_2 \to X_3$$
$$X_2 \to X_1 + X_4 \ ,$$

is entirely defined by:

1. *the set of species $\mathcal{S} = \{X_1, \ X_2, \ X_3, \ X_4\}$,*
2. *the set of complexes $\mathcal{C} = \{X_1 + X_2, \ X_3, \ X_2, \ X_1 + X_4\}$, and*
3. *the set of reactions $\mathcal{R} = \{(X_1 + X_2, X_3), (X_2, X_1 + X_4)\}$.*

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

$$\widetilde{G} := \left\{ \mathcal{S}, \ \mathcal{C} \cup \mathcal{S} \cup \{0\}, \ \mathcal{R} \cup \{X_i \leftrightarrow 0\}_{X_i \in \mathcal{S}} \right\} \ . \tag{1}$$

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

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 describes, for each species, how its concentration changes as a function of time. This ODE system is described by the *stoichiometric matrix* Γ and the *reactant vector* $R(x)$, which is a vector-valued function of the vector of species concentrations $\mathbf{x}$.

Definition 2.3. *Let $G = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a network, and let $\{y_1 \to y_1', y_2 \to y_2', \ldots, y_{|\mathcal{R}|} \to y_{|\mathcal{R}|}'\}$ be an ordering of the reactions.*

1. *The* reaction vector *of the reaction $y_i \to y_i'$ is the vector $y_i' - y_i$, viewed in $\mathbb{R}^{|\mathcal{S}|}$. Note that $y_i \to y_i'$ is a slight abuse of notation, used to denote the reaction $y_i \cdot X \to y_i' \cdot X$ 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 $|\mathcal{S}| \times |\mathcal{R}|$ matrix Γ whose k^{th} column is the reaction vector of $y_k \to y_k'$.*

3. *The* reactant vector *$R(x)$ is the vector of length $|\mathcal{R}|$ whose k^{th} entry is the (monomial) product:*
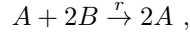
$$r_k x_1^{(y_k)_1} x_2^{(y_k)_2} \cdots x_{|\mathcal{S}|}^{(y_k)_{|\mathcal{S}|}} \ ,$$

where $r_k \in \mathbb{R}_+$ is the reaction rate *of the kth reaction.*

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

$$\frac{d\mathbf{x}}{dt} = \Gamma \cdot R(\mathbf{x}) \ . \tag{2}$$

Example 2.5. *For the following network:*

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

$\Gamma = \begin{bmatrix} 1 \\ -2 \end{bmatrix}$ *and $R(x) = \left(r x_A x_B^2\right)$, so the mass-action kinetics system (2) is:*

$$\begin{bmatrix} \frac{dx_A}{dt} \\ \frac{dx_B}{dt} \end{bmatrix} = \Gamma \cdot R(x) = \begin{bmatrix} r x_A x_B^2 \\ -2 r x_A x_B^2 \end{bmatrix} \ .$$

An important characteristic of mass-action kinetics systems is that they may or may not have the capacity to admit (positive) steady states:
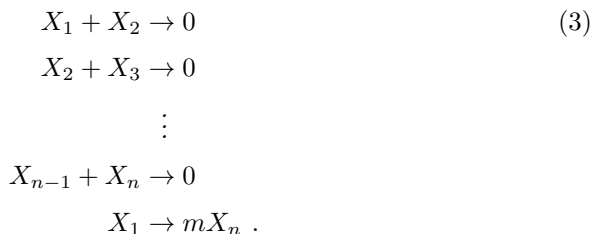
Definition 2.6. *A* positive steady state *is a vector $\mathbf{x}^* \in \mathbb{R}_{>0}^{|\mathcal{S}|}$ such that $\Gamma \cdot R(\mathbf{x}^*) = 0$. A steady state $\mathbf{x}^*$ is* nondegenerate *if $\text{Im}(df(\mathbf{x}^*)|_{\text{Im}(\Gamma)}) = \text{Im}(\Gamma)$, where $df(\mathbf{x}^*)$ denotes the Jacobian matrix of the mass-action kinetics system at $\mathbf{x}^*$.*

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

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:*

$$
\begin{aligned}
X_1 + X_2 &\to 0 \qquad\qquad\qquad\qquad (3) \\
X_2 + X_3 &\to 0 \\
&\vdots \\
X_{n-1} + X_n &\to 0 \\
X_1 &\to mX_n \ .
\end{aligned}
$$

$\widetilde{K}_{m,n}$ *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 $\widetilde{K}_{2,n}$ [11, Table 1], as did Craciun and Feinberg [6, Table 1.1]. Joshi and Shiu introduced the version of the sequestration 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 $\widetilde{K}_{m,n}$ admits multiple positive steady states.*

For $m = 1$ or n even, the network $\widetilde{K}_{m,n}$ is "injective" and therefore not multistationary [4, §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 $\widetilde{K}_{m,n}$ admits multiple* nondegenerate *steady states.*

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

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[2]The focus of this work is on certain networks $\widetilde{K}_{mn,}$ that include all flow reactions. For net-works that do not include all flow reactions, the definition of multistationary must incorporate the conservation relations in the network, if any.

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* $\widetilde{K}_{2,n}$, *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 $\widetilde{K}_{m,n}$. We order the reactions as follows: first, we enumerate the n *internal* (or *true*) reactions listed in (3) (so, the first reaction is $X_1 + X_2 \to 0$, and so on), next are the n outflow reactions (so, the $(n+1)$st reaction is $X_1 \to 0$, and so on), and then we have the n inflow reactions (so, the $(2n+1)$st reaction is $0 \to X_1$, and so on). We will refer to the sets of internal (true), outflow, and inflow reactions as $\mathcal{R}_T$, $\mathcal{R}_O$, and $\mathcal{R}_I$, respectively.

The stoichiometric matrix for $\widetilde{K}_{m,n}$ is:

$$
\Gamma = \left[
\begin{array}{cccccc|c|c}
-1 & 0 & 0 & \ldots & 0 & -1 & & \\
-1 & -1 & 0 & \ldots & 0 & 0 & & \\
0 & -1 & -1 & \ddots & \vdots & \vdots & -I^n & I^n \\
0 & 0 & -1 & \ddots & \vdots & \vdots & & \\
\vdots & \vdots & \ddots & \ddots & -1 & 0 & & \\
0 & 0 & 0 & \ldots & -1 & m & &
\end{array}
\right], \tag{4}
$$

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

$$
R(\mathbf{x}) = \left[
\begin{array}{c}
r_1 x_1 x_2 \\
r_2 x_2 x_3 \\
\vdots \\
r_{n-1} x_{n-1} x_n \\
\hline
r_n x_1 \\
\hline
r_{n+1} x_1 \\
r_{n+2} x_2 \\
\vdots \\
r_{2n} x_n \\
\hline
r_{2n+1} \\
\vdots \\
r_{3n}
\end{array}
\right],
$$

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:

$$\dot{x}_1 = -r_1 x_1 x_2 - r_n x_1 - r_{n+1} x_1 + r_{2n+1}$$
$$\dot{x}_i = -r_{i-1} x_{i-1} x_i - r_i x_i x_{i+1} - r_{n+i} x_i + r_{2n+i} \qquad \text{for } 2 \leq i \leq n-1$$
$$\dot{x}_n = -r_{n-1} x_{n-1} x_n + m r_n x_1 - r_{2n} x_n + r_{3n} .$$

Thus the Jacobian matrix, $df(\mathbf{x})$, is the following $(n \times n)$-matrix

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

$$(5)$$

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 $\widetilde{K}_{m,n}$ (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, §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

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[3]Related techniques for establishing multistationarity appear in work of Banaji and Pantea [8, §4], Feliu [10, §2], and Müller *et al.* [9, §3.2].

for some networks, such as $\widetilde{K}_{m,n}$, the method constructs closed forms for the steady states and rates.

DETERMINANT OPTIMIZATION METHOD (CONSTRUCTIVE VERSION)

Input: Any chemical reaction network G with $n = |\mathcal{S}|$ species that contains all n inflow reactions such that there exist n reactions $y_1 \to y_1'$, $y_2 \to y_2'$, ..., $y_n \to y_n'$ among the internal (true) and outflow reactions $\mathcal{R}_T \cup \mathcal{R}_O$ of G for which

(I) $\det(y_1, y_2, ..., y_n) \cdot \det((y_1 - y_1'), (y_2 - y_2'), ..., (y_n - y_n')) < 0$, and
(II) there exists a vector $\tilde{\eta} \in \mathbb{R}_+^n$ such that $\Sigma_{i=1}^n \tilde{\eta}_i(y_i - y_i') \in \mathbb{R}_+^n = \mathbb{R}_+^{|S|}$.

Output: A certificate of multistationarity of G: (approximations of) a positive reaction rate vector $(r_{y \to y'}) \in \mathbb{R}_+^{|\mathcal{R}|}$ and two positive concentration vectors x^* and $x^{\#}$ which are both steady states of the mass-action system defined by G and $(r_{y \to y'})$.
Steps: Described below.

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

Example 3.1. *For $\widetilde{K}_{m,n}$ (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 $y_i \to y_i'$ 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_η; specifically, for $\eta \in \mathbb{R}^{\mathcal{R}_T \cup \mathcal{R}_O}$, the linear transformation $T_\eta : \mathbb{R}^{|\mathcal{S}|} \to \mathbb{R}^{|\mathcal{S}|}$ is defined by:

$$T_\eta(\delta) \;=\; \sum_{y \to y' \in \mathcal{R}_T \cup \mathcal{R}_O} \eta_{y \to y'}(y \cdot \delta)(y - y') \; . \tag{6}$$

Equivalently, the matrix representation of T_η 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 η (and any choice of inflows: they do not appear in the Jacobian matrix) at the concentration vector $(1, 1, \ldots, 1)$.

Example 3.2. *For $\widetilde{K}_{m,n}$, the matrix representation of T_η is:*

$$\begin{bmatrix} \eta_1 + \eta_n + \eta_{n+1} & \eta_1 & 0 & \cdots & 0 \\ \eta_1 & \eta_1 + \eta_2 + \eta_{n+2} & \eta_2 & \cdots & 0 \\ 0 & \eta_2 & \eta_2 + \eta_3 + \eta_{n+3} & \eta_3 & 0 \\ \vdots & 0 & \ddots & \ddots & \vdots \\ 0 & \vdots & \cdots & \eta_{n-2} + \eta_{n-1} + \eta_{2n-1} & \eta_{n-1} \\ -m\eta_n & 0 & \cdots & \eta_{n-1} & \eta_{n-1} + \eta_{2n} \end{bmatrix} \tag{7}$$

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 $r_i = \eta_i$.

210   **The first step** is to construct a (strictly positive) vector $\eta^- \in \mathbb{R}_+^{|\mathcal{R}_T \cup \mathcal{R}_O|}$, indexed by all internal (true) and outflow reactions, such that

(I) $\det(T_{\eta^-}) < 0$, and
(II) $\sum\limits_{y \to y' \in \mathcal{R}_T \cup \mathcal{R}_O} \eta^-_{y \to y'}(y - y') \in \mathbb{R}_+^{|\mathcal{S}|}$.

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

$$\eta^-_{y \to y'} = \begin{cases} \lambda \tilde{\eta}_{y \to y'} & \text{if } y \to y' \in \{y_i \to y'_i \mid i \in [n]\} \\ \epsilon & \text{else }, \end{cases}$$

where λ is sufficiently large and ϵ is sufficiently small [6, proof of Theorem 4.2].

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

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

215   **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 η^- exists, as an optimization problem.*
220   *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 $\eta^0 \in \mathbb{R}_+^{|\mathcal{R}_T \cup \mathcal{R}_O|}$ for which:

(I′) $\det(T_{\eta^0}) = 0$, and
225   (II′) $\sum\limits_{y \to y' \in \mathcal{R}_T \cup \mathcal{R}_O} \eta^0_{y \to y'}(y - y') \in \mathbb{R}_+^{|\mathcal{S}|}$.

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

$$\eta^+_{y \to y'} = \begin{cases} \lambda^+ & \text{if } y \to y' \in \mathcal{R}_O \\ \epsilon^+ & \text{if } y \to y' \in \mathcal{R}_T , \end{cases}$$

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

Then, by interpolating between this vector η^+ and the vector η^- 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 η^0 with the required
230   properties. Moreover, such a suitable η^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.

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Remark 3.5 (Interpretation of the second step and subsequent steps). *What the second step does is to find reaction rates (given by η_0 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 $(\widetilde{r}_{y \to y'})$ is any positive vector of reaction rates at which some concentration vector $\widetilde{c}$ is a degenerate positive steady state, then the vector $\eta \in \mathbb{R}_+^{|\mathcal{R}_T \cup \mathcal{R}_O|}$ defined coordinate-wise by $\eta_{y \to y'} = \widetilde{r}_{y \to y'}\widetilde{c}^y$ satisfies the second step. So, a reader who has already found a degenerate positive steady state of their system could start the determinant optimization method at the second step. In other words, one could begin applying the method by immediately searching for a suitable η^0 (without first generating η^- and η^+). One strategy for doing this is described in Remark 3.6, which we employ for $\widetilde{K}_{m,n}$ beginning in Example 3.7.

In the next steps, the determinant optimization method constructs a certain vector δ 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 η^0 (without using η^- and η^+). 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) $\displaystyle\sum_{y \to y' \in (\mathcal{R}_T \cup \mathcal{R}_O) \setminus \{y_i \to y_i'\}} \eta_{y \to y'}^0 (y - y') \in \mathbb{R}_+^{|\mathcal{S}|}$, *and*

(b) $y_i - y_i' \in \mathbb{R}_{\geq 0}^{|\mathcal{S}|}$ *(this holds, for instance, if $y_i \to y_i'$ is an outflow reaction).*

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

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

$$
\eta_i^0 = \begin{cases}
\lambda & \text{if } 1 \leq i \leq n-2 \text{ or } i = n \\
(m+1)\lambda & \text{if } i = n-1 \\
\epsilon & \text{if } n+1 \leq i \leq 2n-1 \\
\frac{(m+1)(m\lambda^n + \lambda^2(m+1)\daleth_{n-2})}{(\lambda(m+2)+\epsilon)\daleth_{n-2} - \lambda^2\daleth_{n-3}} - \lambda(m+1) & \text{if } i = 2n \ ,
\end{cases}
\tag{8}
$$

where $\lambda > 0$ and $\epsilon > 0$ are such that η_{2n}^0 is positive[4] (thus, all coordinates of η^0 are

---

[4]We checked that such λ, ϵ exist for $n = 3, 5, 7, 9, 11$ (and $m \geq 2$), and we furthermore conjecture that for larger n, choosing λ sufficiently large and ϵ sufficiently small will suffice.

positive), and $\daleth_i$ is i^{th} principal minor of the matrix representation of T_{η^0} displayed below in (9), i.e. $\daleth_i$ is the determinant of the $i \times i$ (tridiagonal) upper-left submatrix of (9) (also, $\daleth_0 := 1$).

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

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

$$
\begin{bmatrix}
2\lambda + \epsilon & \lambda & 0 & & \cdots & & 0 \\
\lambda & 2\lambda + \epsilon & \lambda & 0 & \cdots & & 0 \\
0 & \lambda & \ddots & \ddots & & & \vdots \\
\vdots & 0 & \ddots & & & & 0 \\
0 & \vdots & & & \lambda + \lambda(m+1) + \epsilon & \lambda(m+1) \\
-m\lambda & 0 & \cdots & 0 & \lambda(m+1) & \lambda(m+1) + \eta^0_{2n}
\end{bmatrix} . \quad (9)
$$

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

$$
\det(T_{\eta^0}) = (-1)^n m(m+1)\lambda^n - \lambda^2(m+1)^2 \daleth_{n-2} + (\lambda(m+1) + \eta^0_{2n})\daleth_{n-1} , \quad (10)
$$

where we recall that $\daleth_i$ is i^{th} principal minor of T_{η^0}.

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

$$
\daleth_{i+2} = (2\lambda + \epsilon)\daleth_{i+1} - \lambda^2 \daleth_i ,
$$

with initial values $\daleth_0 := 1$ and $\daleth_1 = 2\lambda + \epsilon$. Notice that $\daleth_{n-1}$ must be treated separately, because the $(n-1)^{st}$ row contains η^0_{n-1}, which is a function of m. Using standard methods we can get the generating function of the recurrence.

$$
\daleth_i = \frac{1}{2^{i+1} c_1} * \left(-c_2(c_2 - c_1)^i + c_1(c_2 - c_1)^i + c_2(c_1 + c_2)^i + c_1(c_1 + c_2)^i \right) ,
$$

where $c_1 = (\epsilon)^{\frac{1}{2}}(\epsilon + 4\lambda)^{\frac{1}{2}}$ and $c_2 = \epsilon + 2\lambda$. Notice here that $\daleth_i$ is always positive for sufficiently small ϵ. A formula for $\daleth_{n-1}$ is given using the formula for tridiagonal matrices [12]:

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

With this recurrence solved, we set equation (10) to zero and then derive the explicit function for η^0_{2n} from (10) in terms of m, λ, and ϵ; this is the formula in (8).

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

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

$$\delta_k = \begin{cases} \delta_1 & \text{if } k = 1 \\ \frac{-(2\lambda+\epsilon)}{\lambda}\delta_{k-1} - \delta_{k-2} & \text{if } 2 \leq k \leq n-1 \\ \frac{-(\lambda(m+2)+\epsilon)}{\lambda(m+1)}\delta_{n-1} - \frac{1}{m+1}\delta_{n-2} & \text{if } k = n \end{cases} \tag{11}$$

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, δ_n, has a different formula, because the $(n-1)^{st}$ row in (9) used to define δ_n contains terms dependent on m that do not satisfy the recurrence.

To see that δ is a nonzero vector in the nullspace of T_{η^0}, notice that the conditions on δ that state that its inner product with each of the the first $n-1$ rows of T_{η^0} coincide precisely with the $n-1$ recurrences in the definition of δ (11). We claim that the last row of T_η is linearly dependent on the other rows, and from this we will conclude that the last row of T_{η_0} automatically has zero inner product with δ. To see this, we recall that $\det T_{\eta^0} = 0$ by construction, so we need only show that the first $n-1$ rows of T_{η^0} are linearly independent. Assume, to the contrary, that there is a non-trivial linear combination of the first $n-1^{st}$ rows of T_{η^0} that adds to 0. Note that in the first $n-1$ rows only the last one contains an entry in the last column, namely η_{n-1}^0. Since $\eta_{n-1}^0 \neq 0$, 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, δ is in the nullspace of T_{η^0}.

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

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

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

The final step is to use the vectors η^0 and δ (or, as we will see, a sufficiently scaled version of δ) 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:

$$r_{y \to y'} = \frac{\langle y, \delta \rangle}{e^{\langle y, \delta \rangle} - 1} \eta_{y \to y'}^0 \qquad \text{for all } \ y \to y' \in \mathcal{R}_T \cup \mathcal{R}_O \,,$$

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

$$(r_{0 \to X_i}) \ = \ \sum_{y \to y'} \eta_{y \to y'}^0 (y - y') \ \in \ \mathbb{R}_+^{|\mathcal{S}|} \,, \tag{12}$$

and the two steady states are:

$$\mathbf{x}^* = (1, 1, ..., 1) \qquad \text{and} \qquad \mathbf{x}^\# = (e^{\delta_1}, e^{\delta_2}, ..., e^{\delta_{|\mathcal{S}|}}) \,.$$

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

Example 3.9. *For $\widetilde{K}_{m,n}$, 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 305 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 η^0 be as is (8)[5]. Then, for the following internal (true) and outflow reaction rates:*

$$r_i = \frac{\langle y_i, \delta \rangle}{e^{\langle y_i, \delta \rangle} - 1} \eta_i^0 \qquad \text{for all} \ \ i \in \{1, 2, ..., 2n\} \ ,$$

and the following inflow reaction rates:

$$r_{2n+1} = r_1 + r_n + r_{n+1}$$
$$r_{2n+i} = r_{i-1} + r_i + r_{n+i} \qquad \text{for all} \ \ 2 \leq i \leq n - 1$$
$$r_{3n} = r_{n-1} + r_{2n} - mr_n \ ,$$

the concentrations:

$$\mathbf{x}^* \ = \ (1, 1, ..., 1) \qquad \text{and} \qquad \mathbf{x}^{\#} = (e^{\delta_1}, e^{\delta_2}, ..., e^{\delta_n}) \tag{13}$$

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

Remark 3.11. *One may wonder whether or not the determinant optimization method* 310 *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 $\widetilde{K}_{2,3}$ as in Theorem 3.10. By letting ϵ be a free variable we compute the parametrized determinants $\det(df(\mathbf{x}^*))$ and $\det(df(\mathbf{x}^{\#}))$ as 315 *functions of ϵ. Both functions are easily checked to be continuous for positive values of ϵ, and from the graph (Figure 1), we can see easily that there exist choices of ϵ 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 $\epsilon \in (1.159, 1.160)$.*

---

[5]In fact, this theorem will hold for any larger n for which the last coordinate of η^0 as defined in (8) can be made to be positive.

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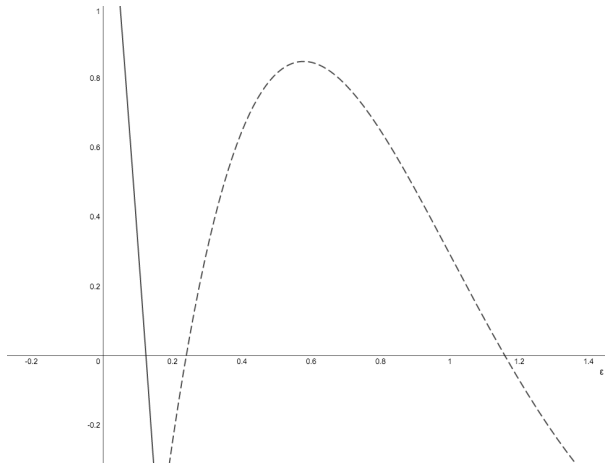Figure 1: Graphs of $\det(df(\mathbf{x}^*))$ *solid* and $\det(df(\mathbf{x}^\#))$ *dashed* as functions of ϵ

.

4. Resolving Conjecture 2.10 for the $n = 3$ case

Recall that Conjecture 2.10 asserts that $\widetilde{K}_{m,n}$ 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 $\widetilde{K}_{m,n}$ 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 $\widetilde{K}_{m,3}$ 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_{η^0}:

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

Thus, Theorem 3.10 specializes to:

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Proposition 4.1. *Consider any integer $m \geq 2$, and the following internal, outflow, and internal reaction rates:*

$$r_1 = \frac{-1.1}{e^{-1.1}-1} \approx 1.65 \qquad r_2 = \frac{1.31}{e^{\frac{1.31}{m+1}}-1} \qquad r_3 = \frac{1}{e-1} \approx .58$$

$$r_4 = \frac{.1}{e-1} \approx .06 \qquad r_5 = \frac{-.21}{e^{-2.1}-1} \approx .24 \qquad r_6 = \frac{m-1.31}{e^{\frac{2.1m+3.41}{m+1}}-1} \qquad (14)$$

$$r_7 = r_1 + r_3 + r_4 \approx 2.29 \qquad r_8 = r_1 + r_2 + r_5 \qquad r_9 = r_2 + r_6 - mr_3 .$$

Then for the mass-action kinetics system defined by the fully open sequestration network $\widetilde{K}_{m,3}$ and the above rate constants r_i, both $\mathbf{x}^* = (1,1,1)$ and $\mathbf{x}^{\#} = \left(e, e^{-2.1}, e^{\frac{2.1m+3.41}{m+1}}\right)$ are positive steady states.

Note that in Proposition 4.1, only $\mathbf{x}_3^{\#}$, 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:*

$$r_9 \;=\; r_2 + r_6 - mr_3 \;>\; r_2 + 0 - m\left(\frac{1}{e-1}\right) \;\geq\; m - m\left(\frac{1}{e-1}\right) \;>\; 0 \,,$$

where the second-to-last inequality follows from Lemma 4.3 below.

4.2. Bounding rates and steady states of $\widetilde{K}_{m,3}$

Here we give upper and lower bounds which we will use to prove that $\mathbf{x}^*$ and $\mathbf{x}^{\#}$ are nondegenerate. The following bounds are on the third coordinate of $\mathbf{x}^{\#}$:

$$e^{\frac{2.1y+3.41}{y+1}} \;\geq\; \mathbf{x}_3^{\#} = e^{\frac{2.1m+3.41}{m+1}} \;>\; e^{2.1} \qquad \text{for all } m \;\geq\; y \;\geq 0 \,. \qquad (15)$$

The first inequality in (15) follows from the easy fact that $e^{\frac{2.1m+3.41}{m+1}}$ 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:*

$$0.14m \;>\; r_6 \;>\; 0.13m - 0.5 \,,$$

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 $\widetilde{K}_{m,3}$

345   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 $\widetilde{K}_{m,3}$ has the capacity to admit multiple nondegenerate positive steady states.*

We will prove Theorem 4.5 by showing that $\mathbf{x}^*$ and $\mathbf{x}^{\#}$ in Proposition 4.1 are nondegenerate, i.e. we must prove that the image of the 3×3 Jacobian matrix $df(\mathbf{x})$
350   at each of the steady states is equal to the image of the 3×9 matrix Γ. As stated earlier (after Conjecture 2.10), since Γ is full rank, our problem reduces to showing that $\det(df(\mathbf{x})) \neq 0$ for both steady states and for all integers $m \geq 2$.

We begin by displaying the Jacobian matrix (5) of $\widetilde{K}_{m,3}$:

$$df(\mathbf{x}) = \begin{bmatrix} -r_1 x_2 - r_3 - r_4 & -r_1 x_1 & 0 \\ -r_1 x_2 & -r_1 x_1 - r_2 x_3 - r_5 & -r_2 x_2 \\ m r_3 & -r_2 x_3 & -r_2 x_2 - r_6 \end{bmatrix} .$$

Thus, our goal is to show that the following determinants (obtained by strategically cancelling and rearranging terms) are nonzero for all integers $m \geq 2$:

$$
\begin{aligned}
D_1 &:= \det(df(\mathbf{x}^*)) = r_2 r_1 r_3 m - (r_2 + r_6)(r_1 r_3 + r_1 r_4 + r_1 r_5 + r_3 r_5 + r_4 r_5) \\
&\qquad - r_2 r_6 (r_1 + r_3 + r_4) \qquad\qquad\qquad\qquad\qquad\qquad\qquad (16) \\
D_2 &:= \det(df(\mathbf{x}^{\#})) = r_2 x_2^{\#}((r_1 x_2^{\#} + r_3 + r_4)(r_2 x_3^{\#}) + r_1 x_1^{\#} m r_3) \\
&\qquad - (r_2 x_2^{\#} + r_6)(r_1 x_2^{\#} + r_3 + r_4)(r_1 x_1^{\#} + r_2 x_3^{\#} + r_5) \\
&\qquad + (r_2 x_2^{\#} + r_6)(r_1 x_1^{\#} r_1 x_2^{\#}) \qquad\qquad\qquad\qquad\qquad (17)
\end{aligned}
$$

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
355   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 evaluating D_1 and D_2 at the remaining
360   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:

$$
\begin{aligned}
D_1 &> m^2 r_1 r_3 - ((m+1) + 0.14 m)(r_1 r_3 + r_1 r_4 + r_1 r_5 + r_3 r_5 + r_4 r_5) \\
&\quad - (m+1)(0.14 m)(r_1 + r_3 + r_4) .
\end{aligned}
$$

---

[6]Analogous graphs for larger n appear in Appendix B.

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

$$
\begin{aligned}
D_1 \ &> \ m^2(0.95) - ((m+1) + 0.14m)1.61 - (m+1)(0.14m)2.29 \\
&= \ 0.6294m^2 - 2.156m - 1.61 \ .
\end{aligned}
$$

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.

m			2	3	4	5	6	7	8	9	10
D_1	$=$	$\det(df(\mathbf{x}^*))$	0.336	2.784	6.525						
D_2	$=$	$\det(df(\mathbf{x}^\#))$	-1.063	-3.811	-7.85	-13.19	-19.8	-27.71	-36.89	-47.36	-59.11
m			11	12	13	14	15	16	17	18	19
D_2	$=$	$\det(df(\mathbf{x}^\#))$	-72.14	-86.4	-102	-118.9	-137.1	-156.5	-177.2	-199.2	-222.5

Table 1: Determinants of the Jacobian matrices (16–17) at the two steady states $\mathbf{x}^*$ and $\mathbf{x}^\#$ for the values of $m \geq 2$ before the proven bounds are valid. All of these determinants are nonzero, so the corresponding steady states are nondegenerate.

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:

$$
\begin{aligned}
D_2 \ &< \ (m+1)x_2^\#((r_1 x_2^\# + r_3 + r_4)((m+1)x_3^\#) + r_1 x_1^\# m r_3) \\
&\quad - \ (mx_2 + (.13m - .5))(r_1 x_2^\# + r_3 + r_4)(r_1 x_1^\# + m x_3^\# + r_5) \\
&\quad + \ ((m+1)x_2^\# + .14m)(r_1 x_1^\# r_1 x_2^\#) \ .
\end{aligned}
$$

Note that we used the lower bound on r_6, 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:

$$
\begin{aligned}
D_2 \ &< \ (m+1).13((.85)((m+1)8.7) + 2.61m) - (.25m - .5)(.84)(4.72 + 8.16m) \\
&\quad + \ (.13(m+1) + .14m)(.91) \\
&= -0.41295m^2 + 4.9437m + 3.06205 \ .
\end{aligned}
$$

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:

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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 $\widetilde{K}_{m,n}$ 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 $\delta_1 = 1$, $\lambda = 1$, and $\epsilon = 0.001$ satisfy all necessary hypotheses. Thus, we obtain two steady states, $x^* = (1, 1, \ldots, 1)$ and $x^\#$ defined in (13). Then, as in the proof of Theorem 3.10, we verify that the determinants $\det(df(x^*))$ and $\det(df(x^\#))$ 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 n, namely $n = 5, 7, 9, 11$, i.e. for $m > 5$ as well.) □

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 $\widetilde{K}_{m,n}$ by using the determininant 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

$$\log\left(\left(1 + \frac{1.31}{m+1}\right)^{m+1}\right) \; < \; \log(e^{1.31}) \; = \; 1.31 \qquad \text{for all} \;\; m \geq 0 \; ,$$

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

$$
\begin{aligned}
1.31 \; > \;\; & \log\left(\left(1 + \frac{1.31}{m+1}\right)^{m+1}\right) \\
> \;\; & \log\left(\left(\frac{m+2.31}{m+1}\right)^{m+1}\right) \; = \; (m+1)\log\left(\frac{m+2.31}{m+1}\right) \; ,
\end{aligned}
$$

which implies that

$$
\begin{aligned}
\frac{1.31}{m+1} \; &> \; \log\left(\frac{m+2.31}{m+1}\right) \\
\implies e^{\frac{1.31}{m+1}} \; &> \; \frac{m+2.31}{m+1} \\
\implies e^{\frac{1.31}{m+1}}(m+1) - (m+1) \; &> \; 1.31 \; .
\end{aligned}
$$

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:

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

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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:

$$\log(1 + az) \;\geq\; \frac{a}{z^{-1} + 1} \;=\; \frac{az}{1 + z} \qquad \text{for all } z \in (0, 1/2) \ .$$

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:

$$
\begin{aligned}
\log(1 + az) - \frac{az}{1+z} \;&=\; \int_0^{az} \left(\frac{1}{1+t} - \frac{1}{1+z} \right) dt \\
&=\; \int_0^{bz} \frac{z - t}{(1+z)(1+t)} dt + \int_{bz}^{z} \left(\frac{1}{1+t} - \frac{1}{1+z} \right) + \int_z^{az} \frac{z - t}{(1+z)(1+t)} dt \ . \quad (19)
\end{aligned}
$$

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:

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

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

Lemma 6.2 (Lemma 4.4). *The function* $r_6(m) = \frac{m - 1.31}{e^{\frac{2.1m + 3.41}{m+1}} - 1}$ *satisfies:*

$$0.14m \;>\; r_6(m) \;>\; 0.13m - 0.5 \ , \qquad (20)$$

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), $(e^{\frac{2.1m + 3.41}{m+1}} - 1) > 0$ for $m \geq 2$. Thus our desired upper bound in (20) is equivalent to the following:

$$m(0.14 e^{\frac{2.1m + 3.41}{m+1}} - 1.14) \;>\; -1.31 \ ,$$

which holds (for positive m) whenever $(0.14 e^{\frac{2.1m + 3.41}{m+1}} - 1.14) > 0$. 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:

$$1.13m - 1.81 \;\geq\; e^{\frac{2.1m + 3.41}{m+1}} (0.13m - 0.5) \ .$$

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

$$
\begin{aligned}
1.13m - 1.81 \;&\geq\; 8.692(0.13m - 0.5) \\
&>\; e^{\frac{2.1(20) + 3.41}{(20)+1}} (0.13m - 0.5) \;\geq\; e^{\frac{2.1m + 3.41}{m+1}} (0.13m - 0.5) \ ,
\end{aligned}
$$

and the final inequality holds for $m \geq 20$ because $e^{\frac{2.1m + 3.41}{m+1}}$ is a decreasing function for positive values of m. $\qquad \square$

Appendix B: graphs for the proof of Thoeorem 5.1

Figures 2a–3a 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 $\widetilde{K}_{m,n}$ 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].

(a) $\det(df(\mathbf{x}^*))$
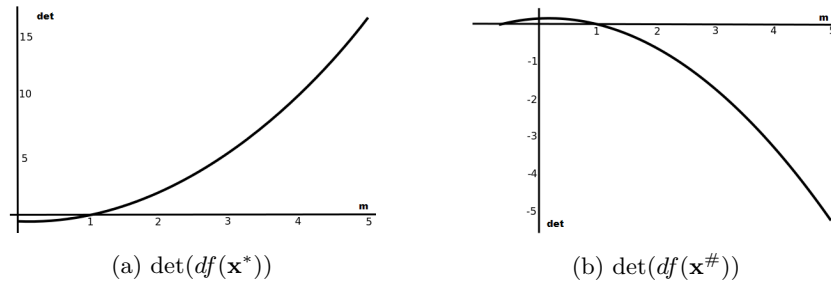

(b) $\det(df(\mathbf{x}^{\#}))$
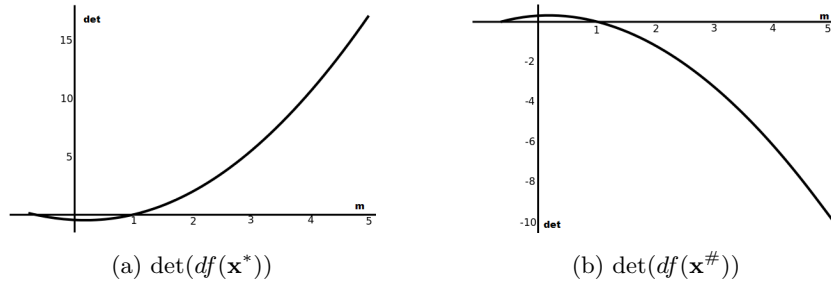
Figure 2: $\widetilde{K}_{m,5}$

(a) $\det(df(\mathbf{x}^*))$

(b) $\det(df(\mathbf{x}^{\#}))$

Figure 3: $\widetilde{K}_{m,7}$

(a) $\det(df(\mathbf{x}^*))$

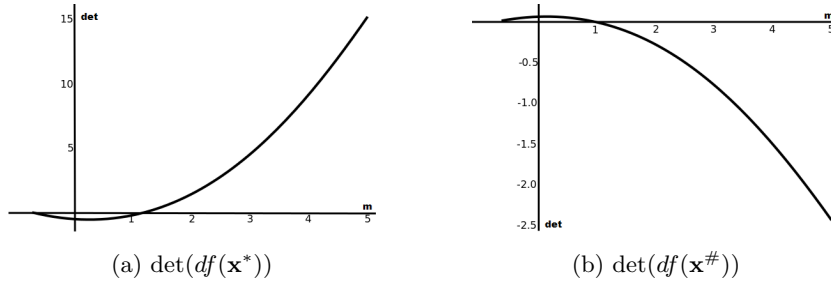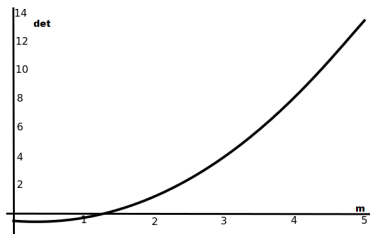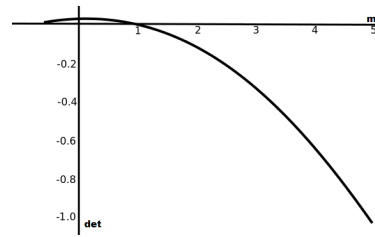(b) $\det(df(\mathbf{x}^{\#}))$

Figure 4: $\widetilde{K}_{m,9}$

(a) $\det(df(\mathbf{x}^*))$

(b) $\det(df(\mathbf{x}^{\#}))$

Figure 5: $\widetilde{K}_{m,11}$