DISCRETE AND CONTINUOUS
DYNAMICAL SYSTEMS SERIES B

doi:10.3934/dcdsb.2020165

BISTABILITY OF SEQUESTRATION NETWORKS

Xiaoxian Tang

School of Mathematical Sciences, Beihang University
Beijing, China

Jie Wang*

Laboratoire d'Analyse et d'Architecture des Systèmes (LAAS-CNRS)
Toulouse, France

(Communicated by Tomas Gedeon)

Abstract. We solve a conjecture on multiple nondegenerate steady states, and prove bistability for sequestration networks. More specifically, we prove that for any odd number of species, and for any production factor, the fully open extension of a sequestration network admits three nondegenerate positive steady states, two of which are locally asymptotically stable. In addition, we provide a non-empty open set in the parameter space where a sequestration network admits bistability, and we present a procedure for computing a witness for bistability.

1. Introduction.

Bistability is an important problem to determine for given dynamical systems arising under mass-action kinetics from biochemical reaction networks [4, 6, 10]. Biologically, bistability is crucial for understanding basic phenomena such as decision-making process in cellular sigaling [1, 13, 30]. Mathematically, identifying parameter values/regions for which a system exhibits two (or more) stable steady states is a challenging problem in computational real algebraic geometry [20]. A necessary condition for bistability is multistationarity (the system has at least two distinct steady states). In practice, one way to experimentally observe bistability is finding multistationarity. In many lucky cases, a witness for multistationarity gives at least three distinct steady states, two of which are stable (see [4, 21]). Criterions for multistationarity have been widely studied, and many structured networks are well-understood (such as "smallest" networks with a few species or reactions [18], (linearly) binomial networks [9, 23, 24], conservative networks without boundary steady states [3] and MESSI networks [22]). However, given a general network, it is not always true that multistationarity guarantees bistability.

Here we use algebraic methods to study both multistationarity and bistability for a family of important networks arising from biology: the fully open extensions

2010 *Mathematics Subject Classification.* 92C40, 92C45.

Key words and phrases. Multistationarity, bistability, chemical reaction networks, mass-action kinetics, sequestration networks.

XT was partially funded by NSF (DMS-1752672). JW was supported by China Postdoctoral Science Foundation under grants 2018M641055.

* Corresponding author: jwang@laas.fr.

of sequestration networks (see [17], and variations in [5, 25]), i.e., sequestration networks with all inflow and outflow reactions:

$$\begin{aligned} X_1 + X_2 &\xrightarrow{r_1} 0 \\ &\vdots \\ X_{n-1} + X_n &\xrightarrow{r_{n-1}} 0 \\ X_1 &\xrightarrow{r_n} mX_n \end{aligned} \tag{1}$$

$$X_i \xrightarrow{r_{n+i}} 0, \quad 0 \xrightarrow{r_{2n+i}} X_i, \quad i = 1, \ldots, n. \tag{2}$$

We are the first to prove the following results.

(I) For any production factor $m \geq 2$, and for any odd order $n \geq 3$, the fully open extension of a sequestration network admits three nondegenerate steady states (Theorem 4.4).

(II) For any production factor $m \geq 2$, and for any odd order $n \geq 3$, the fully open extension of a sequestration network admits bistability (Theorem 4.5).

(III) For any production factor $m \geq 2$, and for any odd order $n \geq 3$, we provide an open region in the parameter space where the fully open extension of a sequestration network admits bistability (Theorem 4.6).

The fully open extensions of sequestration networks were first introduced in [17], which were motivated by biochemical networks studied in [5, 25]. Our main result (I) solves Conjecture 6.10 proposed in [17] (see [12, Conjecture 2.10]), which generalizes the statement [12, Theorem 4.5] from a fixed order $n = 3$ to any odd order $n \geq 3$. Our method is fundamentally different from the method in the proof of [12, Theorem 4.5], and our proof also applies to the special case for $n = 3$. There are many well-known criteria for multistationarity by applying positive parametrization (e.g., [16, 28]) and examining the sign change of determinant of the Jacobian matrix (e.g., [2, 3, 7, 9, 11, 20, 26, 29]). Under some assumptions, one of these results [3, Theorem 1] (or [9, Theorem 3.12]), proved by the Brouwer degree theory, guarantees an odd number of steady states when a network exhibits multistationarity. But in general there was no proof showing at least three of these steady states are nondegenerate. Here, we use a strong algebraic technique to construct three nondegenerate steady states for sequestration networks $\widetilde{K}_{m,n}$ (see Lemma 5.3, Lemma 5.5, and Theorem 4.4). More specifically, we first select a family of subnetworks, for which we can figure out (by the elimination method) two nondegenerate steady states and one "special" steady state going to infinity. Then we show these three steady states can be lifted to the original sequestration networks.

A standard algebraic tool for studying stability for dynamical systems is the Routh-Hurwitz criterion (see [15]), or alternatively the Liénard-Chipart criterion (see [8]). Using these criteria, one examines the positivity of some gigantic determinants, which is computationally challenging (e.g., [21]). Here, we discover a nice structure of the Jacobian matrices of $\widetilde{K}_{m,n}$ at two of those three nondegenerate steady states we constructed; specifically, they are similar to diagonally dominant matrices. So, we are able to use the Gershgorin circle theorem to conclude stability (see Lemma 5.8, Lemma 5.9, and Theorem 4.5). We remark that the Gershgorin circle theorem can be used to study stability for more general reaction networks (see Theorem 3.6). Also, we derive an open region in the parameter space for bistability, which is described by a set of positive solutions of finitely many polynomial inequalities in terms of rate constants (see Theorem 4.6). We provide a procedure for computing a witness based on these inequalities and the proofs of Theorem 4.5.

Our results on sequestration networks highly depend on the special structures of the mass-action equations and the corresponding Jacobian matrices. However, our work is related to the following widely open questions: If a network admits multiple positive steady states, does this guarantee that the network admits multiple nondegenerate positive steady states? (See Nondegeneracy Conjecture [18, 27].) If a network admits multiple nondegenerate positive steady states, under which condition does the network admit multiple stable positive steady states? It is very difficult to answer these questions for general reaction networks. The two key ideas in our work (i.e., studying the special subnetwork with steady states at infinity and using the Gershgorin circle theorem to conclude stability), which are first used for biochemical reaction networks, can be also applied to more general dynamical systems.

The rest of this paper is organized as follows. In Section 2, we introduce mass-action kinetics systems arising from reaction networks. In Section 3, we introduce an algebraic criterion for stability (Theorem 3.6), which is deduced by the classical Gershgorin circle theorem (Theorem 3.2). In Section 4, we recall a family of sequestration networks defined in [17], and present our main results (I–III) (Theorems 4.4, 4.5 and 4.6). In Section 5, we prove the main results in details. We end with a summary in Section 6.

2. Reaction networks.

In this section, we briefly recall the standard notions and definitions on reaction networks, see [3, 9] for more details. A *reaction network* G (or *network* for short) consists of a set of s species $\{X_1, X_2, \ldots, X_s\}$ and a set of m reactions:

$$\alpha_{1j}X_1 + \alpha_{2j}X_2 + \cdots + \alpha_{sj}X_s \xrightarrow{r_j} \beta_{1j}X_1 + \beta_{2j}X_2 + \cdots + \beta_{sj}X_s, \text{ for } j = 1, 2, \ldots, m,$$

where all α_{ij} and β_{ij} are non-negative integers. We call the $s \times m$ matrix with (i, j)-entry equal to $\beta_{ij} - \alpha_{ij}$ the *stoichiometric matrix* of G, denoted by N. We call the image of N the *stoichiometric subspace*, denoted by S.

We denote by $x_1, x_2, \ldots, x_s$ the concentrations of the species $X_1, X_2, \ldots, X_s$, respectively. Under the assumption of *mass-action kinetics*, we describe how these concentrations change in time by following system of ODEs:

$$\dot{x} \;=\; f(x) \;:=\; N \cdot \begin{pmatrix} r_1\, x_1^{\alpha_{11}} x_2^{\alpha_{21}} \cdots x_s^{\alpha_{s1}} \\ r_2\, x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}} \\ \vdots \\ r_m\, x_1^{\alpha_{1m}} x_2^{\alpha_{2m}} \cdots x_s^{\alpha_{sm}} \end{pmatrix}, \tag{3}$$

where x denotes the vector $(x_1, x_2, \ldots, x_s)$, and each $r_j \in \mathbb{R}_{>0}$ is called a *reaction rate constant*. By considering the rate constants as a vector $r = (r_1, r_2, \ldots, r_m)$, we have polynomials $f_i \in \mathbb{Q}[r, x]$, for $i = 1, 2, \ldots, s$.

A *positive steady state* (or, simply *steady state*)[1] of (3) is a concentration-vector $x^* \in \mathbb{R}^s_{>0}$ at which $f(x)$ on the right-hand side of the ODEs (3) vanishes, i.e., $f(x^*) = 0$. We say a steady state x^* is *nondegenerate* if the image of $\mathrm{Jac}(f)(x^*)|_S$ is equal to the stoichiometric subspace S, where $\mathrm{Jac}(f)(x^*)$ denotes the Jacobian

[1] Usually, a steady state is defined as a non-negative vector $x \in \mathbb{R}^s_{\geq 0}$. In our setting, we do not consider boundary steady states (i.e., steady states with zero coordinates). So all steady states in our context are positive.

matrix of f, with respect to x, at x^*. Notice that when the stoichiometric matrix N is full rank, a steady state x^* is nondegenerate if $\mathrm{Jac}(f)(x^*)$ is full rank. A steady state x^* is said to be *Liapunov stable* if for any $\epsilon > 0$ and for any $t_0 > 0$, there exists $\delta > 0$ such that $\| x(t_0) - x^* \| < \delta$ implies $\| x(t) - x^* \| < \epsilon$ for any $t \geq t_0$. A steady state x^* is said to be *locally asymptotically stable* if it is Liapunov stable, and there exists $\delta > 0$ such that $\| x(t_0) - x^* \| < \delta$ implies $\lim_{t\to\infty} x(t) = x^*$. It is well known that a steady state x^* is locally asymptotically stable if all eigenvalues of $\mathrm{Jac}(f)(x^*)$ have negative real parts [19, Theorem 5.5].

3. **A criterion for stability of matrices.**

Definition 3.1. Let $A = (a_{ij}) \in \mathbb{R}^{n\times n}$ be a matrix. For every $i = 1, \ldots, n$, define the i-th *row Gershgorin disc* of A in the complex plane as the set

$$R_i := \{z \in \mathbb{C} : \ |z - a_{ii}| \leq \sum_{j\neq i} |a_{ij}| \ \}.$$

Similarly, define the i-th *column Gershgorin disc* of A in the complex plane as the set

$$C_i := \{z \in \mathbb{C} : |z - a_{ii}| \leq \sum_{j\neq i} |a_{ji}|\}.$$

Theorem 3.2. [14, Gershgorin circle theorem] *The eigenvalues of a matrix $A = (a_{ij}) \in \mathbb{R}^{n\times n}$ lie in the union of row Gershgorin discs $\cup_i^n R_i$, and also lie in the union of column Gershgorin discs $\cup_i^n C_i$.*

Definition 3.3. Let $A = (a_{ij}) \in \mathbb{R}^{n\times n}$ be a matrix. If for every $i = 1, \ldots, n$, $|a_{ii}| \geq \sum_{j\neq i} |a_{ij}|$ (or, $|a_{ii}| \geq \sum_{j\neq i} |a_{ji}|$), then A is *row diagonally dominant* (or, *column diagonally dominant*).

Definition 3.4. A matrix $A = (a_{ij}) \in \mathbb{R}^{n\times n}$ is said to be *stable* if all of its eigenvalues have negative real parts.

For a diagonally dominant matrix, we have a simple sufficient condition for its stability by virtue of the Gershgorin circle theorem.

Lemma 3.5. *Let $A = (a_{ij}) \in \mathbb{R}^{n\times n}$ be a (row or column) diagonally dominant matrix. If $a_{ii} < 0$ for every $i = 1, \ldots, n$, then every nonzero eigenvalue of A has a negative real part.*

Proof. Let λ be a nonzero eigenvalue of A. Denote respectively the real and imaginary parts of λ by $Re(\lambda)$ and $Im(\lambda)$. Then $Re(\lambda) \neq 0$, or $Im(\lambda) \neq 0$. Without loss of generality, assume A is row diagonally dominant. Note for any i, $a_{ii} < 0$. So if $Re(\lambda) \geq 0$, then for any i, we have

$$|\lambda - a_{ii}| = \sqrt{(Re(\lambda) - a_{ii})^2 + Im(\lambda)^2} > a_{ii} \geq \sum_{j\neq i} |a_{ij}|,$$

which is a contradiction to Theorem 3.2. Hence, we must have $Re(\lambda) < 0$. □

Theorem 3.6. *If a matrix is similar to a (row or column) diagonally dominant matrix with negative diagonal entries, then all the nonzero eigenvalues have negative real parts.*

Proof. The conclusion directly follows from Lemma 3.5 and the fact that similar matrices have the same eigenvalues. □

4. Sequestration networks and main results.

4.1. Preliminary.
In this section, we recall sequestration networks $K_{m,n}$ [17, Definition 6.3] and their fully open extensions $\widetilde{K}_{m,n}$ [17, Definition 2.3].

Definition 4.1. For any integer $m \geq 1$, and for any integer $n \geq 2$, the *sequestration network* $K_{m,n}$ of order n with production factor m is defined to be the network (1). If we add into (1) all inflow reactions and outflow reactions (2), then we obtain the *fully open extension* of $K_{m,n}$, denoted by $\widetilde{K}_{m,n}$.

According to (3), the mass-action ODEs $\dot{x} = f(x)$ of $\widetilde{K}_{m,n}$ are given by:

$$\begin{cases} f_1 &= -r_1x_1x_2 - r_nx_1 - r_{n+1}x_1 + r_{2n+1}, \\ f_i &= -r_{i-1}x_{i-1}x_i - r_ix_ix_{i+1} - r_{n+i}x_i + r_{2n+i}, \quad \text{for } \ 2 \leq i \leq n-1, \\ f_n &= -r_{n-1}x_{n-1}x_n + mr_nx_1 - r_{2n}x_n + r_{3n}. \end{cases} \tag{4}$$

The Jacobian matrix of f with respect to $x_1, \ldots, x_n$ below is simply denoted by J:

$$\begin{bmatrix} -r_1x_2 - r_n - r_{n+1} & -r_1x_1 & \cdots & 0 & 0 \\ -r_1x_2 & -r_1x_1 - r_2x_3 - r_{n+2} & \cdots & \vdots & \vdots \\ 0 & -r_2x_3 & \ddots & 0 & 0 \\ \vdots & 0 & \ddots & -r_{n-2}x_{n-2} & 0 \\ 0 & \vdots & \ddots & -r_{n-2}x_{n-2} - r_{n-1}x_n - r_{2n-1} & -r_{n-1}x_{n-1} \\ mr_n & 0 & \cdots & -r_{n-1}x_n & -r_{n-1}x_{n-1} - r_{2n} \end{bmatrix}. \tag{5}$$

Definition 4.2. The network $\widetilde{K}_{m,n}$ is *multistationary* if, for some choice of positive rate-constant vector $r \in \mathbb{R}^{3n}_{>0}$, there exist two or more positive steady states of (17).

Definition 4.3. The network $\widetilde{K}_{m,n}$ is *bistable* if, for some choice of positive rate-constant vector $r \in \mathbb{R}^{3n}_{>0}$, there exist at least three positive steady states of (17), among which two are locally asymptotically stable and one is unstable.

It is known that for any integers $m \geq 1$, and for any integers $n \geq 2$, $\widetilde{K}_{m,n}$ is multistationary if and only if $m > 1$ and n is odd [17, Theorem 6.4]. Below, we recall a conjecture proposed in [17] on the number of nondegenerate steady states.

Conjecture. [17, Conjecture 6.10] For any integers $m \geq 2$, and for odd integers $n \geq 3$, $\widetilde{K}_{m,n}$ admits multiple nondegenerate steady states.

Notice that the stoichiometric matrix N of $\widetilde{K}_{m,n}$ is full rank (e.g., see [12, Formula (4)]), so the conjecture says for some rate-constant vector $r^* \in \mathbb{R}^{3n}_{>0}$, there exist at least two positive steady states $x^{(1)}$ and $x^{(2)}$ such that $\det J|_{r=r^*, x=x^{(i)}} \neq 0, i = 1, 2$. For any integers $m \geq 2$, and for $n = 3$, the conjecture was resolved in [12, Theorem 4.5]. For $m = 2, 3, 4, 5$, and for $n = 5, 7, 9, 11$, the conjecture was proved in [12, Theorem 5.1].

4.2. Main results.
Our main results are a proof of [17, Conjecture 6.10] (see Theorem 4.4) and a bistability result for sequestration networks (see Theorem 4.5). Also, we provide an open region in the parameter space where $\widetilde{K}_{m,n}$ admits bistability (see Theorem 4.6). The proofs of these results are given later in Section 5. A procedure for computing a witness for bistability is presented (see **Procedure Witness**). A concrete example of $\widetilde{K}_{m,n}$ with two locally asymptotically stable

steady states is given (see Example 1), which is not covered by [12, Theorem 4.5] or [12, Theorem 5.1]

Theorem 4.4. *For any integer $m \geq 2$, if for any odd integer $n > 3$, the rate-constant vector $(r_1, \ldots, r_n, r_{n+2}) \in \mathbb{R}^{n+1}_{>0}$ belongs to the open set determined by the following polynomial inequalities (6)–(11)*

$$\begin{aligned}
(r_1 + r_n)r_{n+2} &\neq (m-1)r_1 r_n, && (6)\\
r_{n-1} &> mr_n, && (7)\\
(m-1)r_1 &> r_{n+2}, && (8)\\
(m-1)r_1(r_{i-1} + (-1)^i m r_n) &> (-1)^i m(r_1 + r_n)r_{n+2}, \quad i = 3, \ldots, n, && (9)\\
r_1 + r_{n+2} &> r_{n-2}, && (10)\\
r_i &> r_{n-2}, \qquad\qquad i = 3, 5, \ldots, n-4, && (11)
\end{aligned}$$

or, if for $n = 3$, the rate-constant vector $(r_1, r_2, r_3, r_5) \in \mathbb{R}^4_{>0}$ belongs to the open set determined by the polynomial inequalities (6)–(9), then there exist rate constants $r_{n+1}, r_{n+3}, \ldots, r_{3n} > 0$ such that $\widetilde{K}_{m,n}$ has three nondegenerate steady states. Moreover, the above open set in $\mathbb{R}^{n+1}_{>0}$ is non-empty.

Remark 1. As mentioned before, for $n = 3$, the original conjecture ([17, Conjecture 6.10]) was already proved in [12, Theorem 4.5]. However, we still provide a self-contained proof in Section 5 because we need the construction of three nondegenerate steady states shown in our proof to demonstrate the bistability result (see Theorem 4.5).

Remark 2. If we replace the condition (6) listed in Theorem 4.4 with the inequality (12) below, then we can conclude that there are two stable steady states among the three nondegenerate steady states stated in Theorem 4.4, where one of the two stable steady states is $(1, 1, \ldots, 1)$ (see Theorem 4.5).

Theorem 4.5 (Bistability)**.** *For any integer $m \geq 2$, if for any odd integer $n > 3$, the rate-constant vector $(r_1, \ldots, r_n, r_{n+2}) \in \mathbb{R}^{n+1}_{>0}$ belongs to the open set determined by the polynomial inequalities (7)–(11) and the following inequality*

$$(r_1 + r_n)r_{n+2} > (m-1)r_1 r_n, \tag{12}$$

or, if for $n = 3$, the rate-constant vector $(r_1, r_2, r_3, r_5) \in \mathbb{R}^4_{>0}$ belongs to the open set determined by the polynomial inequalities (7)–(9) and (12), then there exist rate constants $r_{n+1}, r_{n+3}, \ldots, r_{3n} > 0$ such that $\widetilde{K}_{m,n}$ has two locally asymptotically stable steady states, and one of these two steady states is $(1, 1, \ldots, 1)$. Moreover, the above open set in $\mathbb{R}^{n+1}_{>0}$ is non-empty.

In Theorem 4.5, it is obvious that the set of positive solutions of the inequalities (7)–(12) for $n > 3$ (or, the inequalities (7)–(9) and (12) for $n = 3$) is an open set in $\mathbb{R}^{n+1}_{>0}$. In order to make it more obvious to see that the open set is non-empty, we provide Theorem 4.6, which explicitly describe the positive solutions of the inequalities stated in Theorem 4.5.

Theorem 4.6. *For any integer $m \geq 2$, and for $n = 3$, the open set in $\mathbb{R}^{n+1}_{>0}$ determined by the inequalities by (7)–(9) and (12) in Theorem 4.5 is equivalent to the following set:*

$$(r_1, r_2, r_3, r_5) \in \mathbb{R}^4_{>0} : \begin{cases} r_5 < (m-1)r_1, \\ r_2 > mr_3. \end{cases} \tag{13}$$

For any integer $m \geq 2$, and for any odd integer $n > 3$, the open set in $\mathbb{R}^{n+1}_{>0}$ determined by the inequalities (7)–(12) in Theorem 4.5 is equivalent to the following set:

$$(r_1, \ldots, r_n, r_{n+1}) \in \mathbb{R}^{n+1}_{>0} : \begin{cases} r_{n+2} \; < \; (m-1)r_1, \\ r_n \; < \; \frac{r_1 r_{n+2}}{(m-1)r_1 - r_{n+2}}, \\ r_{n-1} \; > \; m r_n, \\ \frac{m((r_1+r_n)r_{n+2}-(m-1)r_1 r_n)}{(m-1)r_1} \; < \; r_{n-2} < r_1 + r_{n+2}, \\ r_i \; > \; r_{n-2}, \qquad \textit{for } i = 3, 5, \ldots, n-4. \end{cases} \tag{14}$$

Remark 3. By the inequalities in (13) and (14), one can easily choose a rate-constant vector such that the conditions of Theorem 4.5 are satisfied. In fact, for any integer $m \geq 2$, if $n = 3$, then for any fixed $r_1, r_3 > 0$, there alway exist $r_5, r_2 > 0$ such that the two inequalities in (13) are satisfied. If $n > 3$, notice that the inequalities in (14) have a "triangular" shape. More specifically, first, for any fixed $r_1 > 0$, one can always choose $r_{n+2} > 0$ such that the first inequality is satisfied. Second, for the chosen $r_1, r_{n+2} > 0$ in the first step, one can find $r_n > 0$ such that the second inequality is satisfied. Third, for the chosen $r_n > 0$ in the second step, one can find $r_{n-1} > 0$ such that the third inequality is satisfied. Similarly, we can find r_{n-2} and r_i for $i = 3, 5, \ldots, n-4$ by the last two inequalities (notice that in the fourth inequality, there exists $r_{n-2} > 0$ between the two numbers $\frac{m((r_1+r_n)r_{n+2}-(m-1)r_1 r_n)}{(m-1)r_1}$ and $r_1 + r_{n+2}$. In fact, after multiplying by the denominator $(m-1)r_1 > 0$, the inequality $\frac{m((r_1+r_n)r_{n+2}-(m-1)r_1 r_n)}{(m-1)r_1} < r_1 + r_{n+2}$ is equivalent to $r_{n+2}(r_1 + m r_n) < (m-1)r_1(r_1 + m r_n)$, which is equivalent to the first inequality $r_{n+2} < (m-1)r_1$ in (14) under our setting $r_1 + m r_n > 0$). Notice that $r_2, r_4, \ldots, r_{n-3}$ do not appear in the inequalities (14). We can choose any values for them. For instance, we give the following choices.

For $n = 3$, we can choose $r_1 = 2, r_2 = m+1, r_3 = 1$, and $r_5 = m-1$ such that the inequalities (7)–(9) and (12) in Theorem 4.5 are satisfied.

For any odd integer $n > 3$, we can choose $r_1 = 2, r_2 = r_4 = \cdots = r_{n-3} = 1, r_3 = r_5 = \cdots = r_{n-4} = m+1, r_{n-2} = m, r_{n-1} = m+1, r_n = 1$, and $r_{n+2} = m-1$ such that the inequalities (7)–(12) in Theorem 4.5 are satisfied.

Based on Theorem 4.6 and the proofs of Theorems 4.4 and 4.5 (in Section 5), we provide a procedure (**Procedure Witness**) for computing a witness for bistability. Notice that Step 1 in the procedure below can be carried out according to Remark 3. We give a more concrete example later for $m = 6$ and $n = 5$; see Example 1.

Procedure Witness. Input. $m \geq 2$, and odd $n \geq 3$; Output. $r_1, \ldots, r_{3n} > 0$ such that $\widetilde{K}_{m,n}$ is bistable.

Step 1. For $n = 3$, find values for $r_1, r_2, r_3, r_5 > 0$ by (13) such that the inequalities (7)–(9) and (12) are satisfied. For $n > 3$, find values for $r_1, \ldots, r_n, r_{n+2} > 0$ by (14) such that the inequalities (7)–(12) are satisfied.

Step 2. Let $r_{n+1} = r_{n+3} = \ldots = r_{2n} = \epsilon > 0$.

Step 3. Compute values for $r_{2n+1}, \ldots, r_{3n}$ by the equalities:

$$\begin{cases} r_{2n+1} &= r_1 + r_n + r_{n+1}, \\ r_{2n+i} &= r_{i-1} + r_i + r_{n+i}, \quad \text{for} \quad 2 \le i \le n-1, \\ r_{3n} &= r_{n-1} - m r_n + r_{2n}. \end{cases} \tag{15}$$

Step 4. Compute steady states of $\widetilde{K}_{m,n}$ and check their stability (for instance, by `Mathematica`). If $\widetilde{K}_{m,n}$ is bistable, then output $r_1, \ldots, r_{3n}$. Otherwise, go back to Step 2, make ϵ smaller and repeat Steps 2–4 until $\widetilde{K}_{m,n}$ is bistable.

Example 1. We give a concrete example of $\widetilde{K}_{6,5}$ with two locally asymptotically stable steady states. Let $r_1 = 2$, $r_2 = r_5 = 1$, $r_3 = 6$, $r_4 = 7$, $r_7 = 5$, $r_6 = r_8 = r_9 = r_{10} = 0.006$, $r_{11} = 3.006$, $r_{12} = 8$, $r_{13} = 7.006$, $r_{14} = 13.006$, and $r_{15} = 1.006$. Here, the values of rate constants $r_1, \ldots, r_5$ and r_7 are chosen by the method described in Remark 3, which satisfy the inequalities (7)–(12). By the proof of Theorem 4.5 (see Section 5.2), the values for r_6, r_8, r_9 and r_{10} are chosen to be the same small number 0.006. After we choose these values for $r_1, \ldots, r_{10}$, the values of $r_{11}, \ldots, r_{15}$ are computed by the equalities (15). The specialized system f in (4) is given by

$$\begin{cases} f_1 &= -2x_1x_2 - 1.006x_1 + 3.006, \\ f_2 &= -2x_1x_2 - x_2x_3 - 5x_2 + 8, \\ f_3 &= -x_2x_3 - 6x_3x_4 - 0.006x_3 + 7.006, \\ f_4 &= -6x_3x_4 - 7x_4x_5 - 0.006x_4 + 13.006, \\ f_5 &= -7x_4x_5 + 6x_1 - 0.006x_n + 1.006. \end{cases}$$

It can be verified by `Mathematica` that the above system $f = 0$ has three positive solutions:

$$\hat{x}^{(1)} = (1, 1, 1, 1, 1), \quad \hat{x}^{(2)} \approx (1.69795, 0.382186, 12.5363, 0.028445, 54.5727)$$

and $\hat{x}^{(3)} \approx (1.92826, 0.276459, 20.0808, 0.0110718, 150.601)$, where $\hat{x}^{(1)}$ and $\hat{x}^{(3)}$ are locally asymptotically stable. Indeed, the Jacobian matrix at $\hat{x}^{(1)}$ has five negative eigenvalues, which are approximately

$$-19.7034, \ -9.28405, \ -6.17915, \ -2.78462, \ -0.07275,$$

and the Jacobian matrix at $\hat{x}^{(3)}$ has five negative eigenvalues, which are approximately

$$-1174.78, \ -29.2068, \ -1.49192, \ -0.151575, \ -0.00198971.$$

Remark 4. In Theorem 4.5, if we replace the inequality (12) with its opposite

$$(r_1 + r_n) r_{n+2} \ < \ (m-1) r_1 r_n, \tag{16}$$

one can still prove (in a similar way with the proof of Theorem 4.5) that $\widetilde{K}_{m,n}$ admits two locally asymptotically stable steady states, and one of the two stable steady states is close to $(\delta_1, \ldots, \delta_n)$ given in (19) (Section 5). For instance, for any integer $m \ge 2$, when $n = 3$, we can choose $r_1 = 3, r_2 = 3m, r_3 = 2, r_5 = m-1$ such that the inequalities (7)–(9) and (16) are satisfied, and when $n > 3$ is odd, we can choose $r_1 = 3, r_2 = r_4 = \cdots = r_{n-3} = m, r_3 = r_5 = \cdots = r_{n-4} = m+1, r_{n-2} = m, r_{n-1} = 3m, r_n = 2, r_{n+2} = m-1$ such that the inequalities (7)–(11) and (16) are satisfied. We give another example to illustrate this case; see Example 2.

Example 2. Again, we consider the network $\widetilde{K}_{6,5}$. Let $r_1 = 3$, $r_2 = r_3 = 6$, $r_4 = 18$, $r_5 = 2$, $r_7 = 5$, $r_6 = r_8 = r_9 = r_{10} = 0.06$, $r_{11} = 5.06$, $r_{12} = 14$, $r_{13} = 12.06$, $r_{14} = 24.06$, and $r_{15} = 6.06$. This time, these rate constants satisfy the inequalities (7)–(11) and (16).It can be verified by **Mathematica** that there are three positive steady states:

$$\hat{x}^{(1)} = (1, 1, 1, 1, 1), \quad \hat{x}^{(2)} \approx (0.932124, 1.12282, 0.778704, 1.44839, 0.659961)$$

and $\hat{x}^{(3)} \approx (1.68739, 0.312906, 5.77995, 0.0248477, 51.8643)$, where $\hat{x}^{(2)}$ and $\hat{x}^{(3)}$ are locally asymptotically stable. Indeed, the Jacobian matrix at $\hat{x}^{(2)}$ has five negative eigenvalues, which are approximately

$$-40.2232, \ -20.8642, \ -7.79735, \ -7.20777, \ -0.0343658,$$

and the Jacobian matrix at $\hat{x}^{(3)}$ has five negative eigenvalues, which are approximately

$$-968.734, \ -46.3232, \ -2.9517, \ -0.5785, \ -0.0443525.$$

Remark 5. Numerical experiments show that if the conditions of Theorem 4.4 are not satisfied, then it is possible for $\widetilde{K}_{m,n}$ to admit either one or three nondegenerate steady states. We have never seen more than three nondegenerate steady states. We always observe bistability whenever three nondegenerate steady states are found. So, we propose Conjecture 1 below[2].

Conjecture 1. *For any integer $m \geq 2$, and for any odd integer $n \geq 3$, the maximum number of nondegerate steady states of $\widetilde{K}_{m,n}$ is three, and the network $\widetilde{K}_{m,n}$ is multistationary if and only if it is bistable.*

5. **Proofs of main results.** The goal of this section is to prove Theorem 4.4, Theorem 4.5 and Theorem 4.6. Our first step is to apply the specializations of parameters (15)[3] to the network. Substituting (15) into the system f (4), the system can be rewritten as

$$\begin{cases} f_1 = -r_1x_1x_2 - r_nx_1 - r_{n+1}x_1 + r_1 + r_n + r_{n+1}, \\ f_i \ = -r_{i-1}x_{i-1}x_i - r_ix_ix_{i+1} - r_{n+i}x_i + r_{i-1} + r_i + r_{n+i}, \ \text{for } 2 \leq i \leq n-1, \\ f_n = -r_{n-1}x_{n-1}x_n + mr_nx_1 - r_{2n}x_n + r_{n-1} - mr_n + r_{2n}. \end{cases} \tag{17}$$

Note that by the equalities (15), $x^{(1)} := (1, \ldots, 1)$ is always a positive solution to the system (17). Note also that this substitution does not change the Jacobian matrix of f with respect to x since $r_{2n+1}, \ldots, r_{3n}$ are constant terms in (4).

Under the equalities (15), we only need to find rate constants $r_1, \ldots, r_{2n} > 0$ such that the system $f = 0$ in (17) has three distinct simple positive solutions. Then by (15), we can find positive values for rate constants $r_{2n+1}, \ldots, r_{3n}$. Remark that in order to ensure $r_{3n} > 0$, we need to require $r_{n-1} + r_{2n} - mr_n > 0$. Here, we require a stronger condition

$$r_{n-1} > mr_n \qquad \text{(i.e., the inequality (7) in Theorem 4.4).}$$

In fact, if we have $r_{n-1} > mr_n$, then for any $r_{2n} > 0$, we can make sure $r_{3n} > 0$. We make this stronger requirement on r_{n-1} and r_n because we need more flexibility on r_{2n} later when we prove Theorem 4.4.

[2]The **Mathematica** code for the numerical experiments can be found on the website: https://wangjie212.github.io/jiewang/code.html.

[3]These specializations are inspired by the proof of [12, Theorem 4.5].

5.1. **Nondegenerate multistationarity.** In this subsection, we prove Theorem 4.4. We give an outline of the proof below.

First, we consider a simpler network. For $i \neq 2$, we remove the inflow reactions $X_i \to 0$ from $\widetilde{K}_{m,n}$ and obtain a subnetwork. Notice that for the subnetwork, we have $r_{n+1} = r_{n+3} = \cdots = r_{2n} = 0$ in f (17) on the right-hand side of mass-action ODEs. We show in Lemma 5.3 that for this special choice of rate constants, the system $f = 0$ has two nondegenerate positive solutions under conditions (6) and (8)–(9). In order to prove Lemma 5.3, we need two results from linear algebra; see Lemmas 5.1 and 5.2.

Second, for $r_{n+1} = r_{n+3} = \cdots = r_{2n} = 0$, besides two solutions $x^{(1)}$ and $x^{(2)}$ shown in Lemma 5.3, the system $f = 0$ (17) has a "special" solution $x^{(3)}$ with its last coordinate $x_n = +\infty$. We make this third solution "visible" by applying a variable substitution to the system f (see (21)–(22)). Equivalently, we show the resulting system g in (22) has a nondegenerate positive solution (under the condition (8) for $n = 3$, or the conditions (9)–(11) for $n > 3$), which gives the third solution $x^{(3)}$ to $f = 0$; see Lemma 5.4 for $n = 3$ and Lemma 5.5 for $n > 3$.

Finally, we set $r_{n+1} = r_{n+3} = \cdots = r_{2n} = \epsilon$. By the previous steps and the implicit function theorem, we show that the original system $f = 0$ has three nondegenerate positive solutions if ϵ is a sufficiently small positive number; see Lemma 5.6 and the proof of Theorem 4.4.

Remark 6. Our proof shows that for a specific choice of reaction constants (i.e., $r_{n+1}, r_{n+3}, \cdots, r_{2n}$ are sufficiently small), the network $\widetilde{K}_{m,n}$ has at least three nondegerate steady states. However, this special choice of parameters might be unrealistic for the real life applications. Also, it is still unknown if it is possible to get more positive steady states for some other parameter values. The maximum number of possible steady states is another open problem in the area of chemical reaction networks.

Lemma 5.1. *For any $n \geq 3$, the determinant of the tridiagonal matrix*

$$\begin{bmatrix} a_1 + b_1 & a_2 & & & \\ b_1 & a_2 + b_2 & a_3 & & \\ & \ddots & \ddots & \ddots & \\ & & b_{n-2} & a_{n-1} + b_{n-1} & a_n \\ & & & b_{n-1} & a_n \end{bmatrix}$$

is equal to $a_1 a_2 \ldots a_n$.

Proof. We transform the matrix into an upper triangular matrix by applying the Gaussian elimination starting from the last row to the first row:

$$\begin{bmatrix} a_1 + b_1 & a_2 & & & \\ b_1 & a_2 + b_2 & a_3 & & \\ & \ddots & \ddots & \ddots & \\ & & b_{n-2} & a_{n-1} + b_{n-1} & a_n \\ & & & b_{n-1} & a_n \end{bmatrix}$$

$$\longrightarrow \begin{bmatrix} a_1+b_1 & a_2 & & & \\ b_1 & a_2+b_2 & a_3 & & \\ & \ddots & \ddots & \ddots & \\ & & b_{n-2} & a_{n-1} & 0 \\ & & & b_{n-1} & a_n \end{bmatrix}$$

$$\longrightarrow \cdots \longrightarrow \begin{bmatrix} a_1 & 0 & & & \\ b_1 & a_2 & 0 & & \\ & \ddots & \ddots & \ddots & \\ & & b_{n-2} & a_{n-1} & 0 \\ & & & b_{n-1} & a_n \end{bmatrix}.$$

Thus, the determinant is $a_1 a_2 \ldots a_n$. $\square$

Lemma 5.2. *For any integer $m \geq 2$, and for any odd integer $n \geq 3$, if*

$$r_{n+1} = 0 \text{ and } r_{n+i} = 0, \text{ for } 3 \leq i \leq n, \tag{18}$$

then the determinant of J in (5) is

$$r_2 \cdots r_{n-1} x_2 \cdots x_{n-1}((m-1)r_1 r_n x_1 - (r_n + r_1 x_2) r_{n+2}).$$

Proof. We expand $\det J|_{r_{n+1}=0 \text{ and } r_{n+i}=0,\, 3\leq i\leq n}$ with respect to the first row and obtain $\det J = -(r_1x_2 + r_n)\det J_1 + r_1 x_1 \det J_2$, where

$$J_1 = \begin{bmatrix} -r_1x_1 - r_2x_3 - r_{n+2} & \cdots & 0 & 0 \\ -r_2x_3 & \ddots & \vdots & \vdots \\ 0 & \ddots & -r_{n-2}x_{n-2} & 0 \\ \vdots & \ddots & -r_{n-2}x_{n-2} - r_{n-1}x_n & -r_{n-1}x_{n-1} \\ 0 & \cdots & -r_{n-1}x_n & -r_{n-1}x_{n-1} \end{bmatrix}$$

and

$$J_2 = \begin{bmatrix} -r_1x_2 & -r_2x_2 & \cdots & 0 \\ 0 & -r_2x_2 - r_3x_4 & \ddots & \vdots \\ \vdots & -r_3x_4 & \ddots & 0 \\ 0 & \ddots & -r_{n-2}x_{n-2} - r_{n-1}x_n & -r_{n-1}x_{n-1} \\ mr_n & \cdots & -r_{n-1}x_n & -r_{n-1}x_{n-1} \end{bmatrix}.$$

By Lemma 5.1, $\det J_1 = r_2x_2 \cdots r_{n-1}x_{n-1}(r_1x_1 + r_{n+2})$. Again, we expand $\det J_2$ with respect to the first column: $\det J_2 = -r_1x_2 \det J_3 - mr_n \det J_4$, where

$$J_3 = \begin{bmatrix} -r_2x_2 - r_3x_4 & -r_3x_3 & \cdots & 0 \\ -r_3x_4 & -r_3x_3 - r_4x_5 & \ddots & \vdots \\ 0 & \ddots & \ddots & 0 \\ \vdots & \ddots & -r_{n-2}x_{n-2} - r_{n-1}x_n & -r_{n-1}x_{n-1} \\ 0 & \cdots & -r_{n-1}x_n & -r_{n-1}x_{n-1} \end{bmatrix}$$

and

$$J_4 = \begin{bmatrix} -r_2x_2 & 0 & \cdots & 0 \\ -r_2x_2 - r_3x_4 & -r_3x_3 & \ddots & \vdots \\ -r_3x_4 & \ddots & 0 & 0 \\ \vdots & \ddots & -r_{n-2}x_{n-2} & 0 \\ 0 & \cdots & -r_{n-2}x_{n-2} - r_{n-1}x_n & -r_{n-1}x_{n-1} \end{bmatrix}.$$

By Lemma 5.1, $\det J_3 = -r_2x_2 \cdots r_{n-1}x_{n-1}$. Clearly, $\det J_4 = -r_2x_2 \cdots r_{n-1}x_{n-1}$. Thus

$$\begin{aligned} \det J &= -(r_1x_2 + r_n)r_2x_2 \ldots r_{n-1}x_{n-1}(r_1x_1 + r_{n+2}) \\ &\quad + r_1x_1(r_1x_2 + mr_n)r_2x_2 \ldots r_{n-1}x_{n-1} \\ &= r_2 \cdots r_{n-1}x_2 \cdots x_{n-1}((m-1)r_1r_nx_1 - (r_n + r_1x_2)r_{n+2}). \end{aligned}$$

□

Lemma 5.3. *For any integer $m \geq 2$, and for any odd integer $n \geq 3$, if the rate constants $r_{n+1}, r_{n+3}, \ldots, r_{2n}$ satisfy the condition (18), and if the positive rate constants $r_1, \ldots, r_n, r_{n+2}$ satisfy the inequalities (6) and (8)–(9), then the system f in (17) has two distinct positive solutions*

$$x^{(1)} = (1, 1, \ldots, 1) \quad \textit{and} \quad x^{(2)} = (\delta_1, \delta_2, \ldots, \delta_n), \tag{19}$$

where

$$\delta_1 \;:=\; \frac{(r_1 + r_n)r_{n+2}}{(m-1)r_1r_n}, \quad \delta_2 \;:=\; \frac{((m-1)r_1 - r_{n+2})r_n}{r_1r_{n+2}},$$

and

$$\delta_i \;:=\; \frac{(m-1)r_1(r_{i-1} + (-1)^i mr_n) + (-1)^{i-1}m(r_1 + r_n)r_{n+2}}{(m-1)r_1r_{i-1}\delta_{i-1}}, \quad i = 3, \ldots, n,$$

and the Jacobian matrix J in (5) has full rank at both solutions.

Proof. First, it is straightforward to check that if the rate constants satisfy condition (15), then $x^{(1)} = (1, 1, \ldots, 1)$ is a positive solution to $f(x) = 0$ for f in (17).

Below, we show how to obtain the other solution $x^{(2)}$ to $f(x) = 0$. Note that under the condition (18), we have

$$\sum_{j=1}^{n} (-1)^{j-1} f_j = (m-1)r_nx_1 + r_{n+2}x_2 - (m-1)r_n - r_{n+2}.$$

We solve for x_2 from $\sum_{j=1}^{n} (-1)^{j-1} f_j = 0$, substitute the expression into $f_1 = 0$, and obtain a quadratic equation in terms of only x_1:

$$(m-1)r_1r_nx_1^2 - ((m-1)r_1r_n + (r_1 + r_n)r_{n+2})x_1 + (r_1 + r_n)r_{n+2} = 0,$$

which indeed has two solutions: $x_1^{(1)} = 1$ and $x_1^{(2)} = \delta_1$. We substitute $x_1^{(2)} = \delta_1$ into $f_1 = 0$ and solve that $x_2^{(2)} = \delta_2$. Note that for $3 \leq i \leq n$, under the condition (18), we have

$$\sum_{j=1}^{i-1} (-1)^{j-1} f_j = (-1)^{i-1} r_{i-1}x_{i-1}x_i + (-1)^i r_{i-1} - r_n(x_1 - 1) + r_{n+2}(x_2 - 1).$$

So, we can substitute $x_1^{(2)} = \delta_1$ and $x_2^{(2)} = \delta_2$ into $\sum_{j=1}^{i-1} (-1)^{j-1} f_j = 0$ and solve $x_i^{(2)} = \delta_i$ for $i = 3, \ldots, n$. Hence $x^{(2)} = (\delta_1, \delta_2, \ldots, \delta_n)$ is also a solution to $f(x) = 0$

under the condition (18). If the rate constants satisfy conditions (8)–(9), then $x^{(2)}$ is clearly positive. Obviously, under the condition (6), we have $1 \neq \delta_1$, and hence $x^{(1)} \neq x^{(2)}$.

Below, we show that if the inequality (6) is satisfied, then at both solutions $x^{(1)}$ and $x^{(2)}$, the Jacobian matrix J has nonzero determinants. In fact, by Lemma 5.2,

$$\det J \;=\; r_2 \cdots r_{n-1} x_2 \cdots x_{n-1}((m-1)r_1 r_n x_1 - (r_n + r_1 x_2) r_{n+2}).$$

Therefore,

$$\det J|_{x=x^{(1)}} = r_2 \cdots r_{n-1}((m-1)r_1 r_n - (r_1 + r_n) r_{n+2})$$

and

$$\det J|_{x=x^{(2)}} = r_2 \cdots r_{n-1} \delta_2 \cdots \delta_{n-1}((r_1 + r_n) r_{n+2} - (m-1) r_1 r_n),$$

which are nonzero if (6) holds. □

As mentioned before, for the special choice of rate-constant values in the condition (18), besides two solutions $x^{(1)}$ and $x^{(2)}$ shown in Lemma 5.3, the polynomial system $f = 0$ in (17) has a "special" solution with its last coordinate $x_n = +\infty$. In order to make this third solution "visible", we need to apply a variable substitution to the system f.

First, define a map $\varphi : \mathbb{R}^n \to \mathbb{R}^n$ as follows:

$$\varphi(y_1, \ldots, y_n) \;=\; \left(y_1, \ldots, y_{n-2}, \frac{r_{2n} y_{n-1}}{y_n}, \frac{y_n}{r_{2n}}\right). \tag{20}$$

We substitute $x = \varphi(y)$ into f in (17) and view $y_1, \ldots, y_n$ as new variables. We define the resulting rational functions as

$$p(y_1, \ldots, y_n; r_1, \ldots, r_{2n}) := f|_{x=\varphi(y)} \in \mathbb{Q}(r_1, \ldots, r_{2n}, y_1, \ldots, y_n). \tag{21}$$

Then, substitute (18) into p, and define the resulting polynomials as

$$g(y_1, \ldots, y_n; r_1, \ldots, r_n, r_{n+2}) := p|_{r_{n+1}=0,\; r_{n+i}=0\; (3 \leq i \leq n)}. \tag{22}$$

Denote by J_p and J_g respectively the Jacobian matrix of p and g with respect to variables $y_1, \ldots, y_n$.

When $n = 3$, the system g in (22) is given by the polynomials:

$$\begin{cases} g_1 &= r_1 + r_3 - r_3 y_1, \\ g_2 &= r_1 + r_2 + r_5 - r_2 y_2, \\ g_3 &= r_2 - m r_3 - r_2 y_2 + m r_3 y_1 - y_3. \end{cases} \tag{23}$$

Lemma 5.4. *For any integer $m \geq 2$, if the positive rate constants r_1 and r_5 satisfy,*

$$(m-1) r_1 - r_5 \;>\; 0, \tag{24}$$

then for any positive rate constant r_2 and r_3, the system $g = 0$ in (23) has a positive solution $\xi = (\xi_1, \xi_2, \xi_3)$ such that $\det J_g|_{y=\xi} \neq 0$.

Proof. Solve the system $g = 0$ from (23) for the variables y_1, y_2, y_3 over $\mathbb{Q}(r)$, and obtain a solution in terms of r:

$$\xi_1 \;:=\; \frac{r_1 + r_3}{r_3}, \;\; \xi_2 \;:=\; \frac{r_1 + r_2 + r_5}{r_2}, \;\; \xi_3 \;:=\; (m-1) r_1 - r_5.$$

Clearly, if $(m-1) r_1 - r_5 > 0$, then for any positive r_2 and r_3, the above solution is positive. It is straightforward to compute that $\det J_g|_{y=\xi} = -r_2 r_3 \neq 0$. □

Remark 7. The inequality (24) is a specific case of the inequality (8) for $n = 3$.

Now, we focus on the case when $n \geq 5$. Explicitly, the form of p in (21) for $n \geq 5$ is given below:

$$\begin{cases} p_1 &= -r_1y_1y_2 - r_ny_1 - r_{n+1}y_1 + r_1 + r_n, \\ p_2 &= -r_1y_1y_2 - r_2y_2y_3 - r_{n+2}y_2 + r_1 + r_2 + r_{n+2}, \\ p_i &= -r_{i-1}y_{i-1}y_i - r_iy_iy_{i+1} - r_{n+i}y_i + r_{i-1} + r_i + r_{n+i}, \qquad \text{for } 2 \leq i \leq n-3, \\ p_{n-2} &= -r_{n-3}y_{n-3}y_{n-2} - r_{n-2}y_{n-2}\frac{r_{2n}y_{n-1}}{y_n} - r_{2n-2}y_{n-2} + r_{n-3} + r_{n-2} + r_{2n-2}, \\ p_{n-1} &= -r_{n-2}y_{n-2}\frac{r_{2n}y_{n-1}}{y_n} - r_{n-1}y_{n-1} - r_{2n-1}y_{n-1} + r_{n-2} + r_{n-1} + r_{2n-1}, \\ p_n &= -r_{n-1}y_{n-1} + mr_ny_1 - y_n + r_{n-1} + r_{2n} - mr_n. \end{cases}$$

Explicitly, the form of g in (22) for $n \geq 5$ is given below:

$$\begin{cases} g_1 &= r_1 + r_n - r_ny_1 - r_1y_1y_2, \\ g_2 &= r_1 + r_2 + r_{n+2} - r_1y_1y_2 - r_2y_2y_3 - r_{n+2}y_2, \\ g_i &= r_{i-1} + r_i - r_{i-1}y_{i-1}y_i - r_iy_iy_{i+1}, \qquad \text{for } 3 \leq i \leq n-3, \\ g_{n-2} &= r_{n-3} + r_{n-2} - r_{n-3}y_{n-3}y_{n-2}, \\ g_{n-1} &= r_{n-2} + r_{n-1} - r_{n-1}y_{n-1}, \\ g_n &= r_{n-1} - mr_n + mr_ny_1 - r_{n-1}y_{n-1} - y_n. \end{cases} \tag{25}$$

Lemma 5.5. *For any integer $m \geq 2$, and for any odd integer $n > 3$, if the positive rate constants $r_1, \ldots, r_n, r_{n+2}$ satisfy the inequalities (10)–(11) and*

$$(m-1)r_1r_{n-2} + m(m-1)r_1r_n \;>\; m(r_1 + r_n)r_{n+2}, \tag{26}$$

then the system $g = 0$ in (25) has a positive solution $\xi = (\xi_1, \ldots, \xi_n)$ such that $\det J_g|_{y=\xi} \neq 0$.

Proof. The goal is to find a positive solution $\xi = (\xi_1, \ldots, \xi_n)$ to the equations

$$g_1(y; r_1, \ldots, r_n, r_{n+2}) = \cdots = g_n(y; r_1, \ldots, r_n, r_{n+2}) = 0$$

for positive parameter values $r_1, \ldots, r_n, r_{n+2}$. First, we solve for y_{n-1} from $g_{n-1} = 0$ over $\mathbb{Q}(r)$, and we get

$$y_{n-1} \;=\; \frac{r_{n-1} + r_{n-2}}{r_{n-1}}. \tag{27}$$

Second, we substitute (27) into g_n, and then we solve for y_n from $g_n = 0$ over $\mathbb{Q}(r, y_1)$:

$$y_n \;=\; mr_n(y_1 - 1) - r_{n-2}. \tag{28}$$

Now, we show how to solve for $y_2, \ldots, y_{n-2}$ from (25) over $\mathbb{Q}(r, y_1)$. For this purpose, for every $i = 2, \ldots, n-2$, let $h_i \;=\; \Sigma_{k=i}^{n-2}(-1)^k g_k$. Notice that n is odd. So, explicitly, we obtain

$$\begin{aligned} h_2 &= r_1 + r_{n+2} - r_1y_1y_2 - r_{n+2}y_2 - r_{n-2}, \qquad \text{and} \\ h_i &= (-1)^i\left(r_{i-1} - r_{i-1}y_{i-1}y_i\right) - r_{n-2} \qquad \text{for } i = 3, \ldots, n-2. \end{aligned}$$

We solve for y_i from $h_i = 0$, and we have

$$\begin{aligned} y_2 &= \frac{r_1 - r_{n-2} + r_{n+2}}{r_1y_1 + r_{n+2}}, \qquad \text{and} \\ y_i &= \frac{r_{i-1} - (-1)^i r_{n-2}}{r_{i-1}y_{i-1}} \qquad \text{for } i = 3, \ldots, n-2. \end{aligned} \tag{29}$$

We substitute (29) into g_1 and remove the denominator. Then we obtain a quadratic polynomial in y_1:

$$h_1 \;:=\; r_1r_ny_1^2 + (r_1r_{n+2} + r_nr_{n+2} - r_1r_{n-2} - r_1r_n)y_1 - (r_1 + r_n)r_{n+2}.$$

It is straightforward to check by the discriminant and Vieta's formulas that for any positive parameters $r_1, r_{n-2}, r_n, r_{n+2}$, the quadratic equation $h_1(y_1) = 0$ has two real roots, and only one of these two roots is positive. Let ξ_1 be this positive root. Substituting ξ_1 back to (27), (28), and (29), we obtain a solution $\xi = (\xi_1, \ldots, \xi_{n-1}, \xi_n)$ of $g = 0$ in (25), where

$$\xi_2 = \frac{r_1 + r_{n+2} - r_{n-2}}{r_1\xi_1 + r_{n+2}}, \quad \xi_i = \frac{r_{i-1} - (-1)^i r_{n-2}}{r_{i-1}\xi_{i-1}}, i = 3, \ldots, n-2$$

and

$$\xi_{n-1} = \frac{r_{n-2} + r_{n-1}}{r_{n-1}}, \quad \xi_n = mr_n(\xi_1 - 1) - r_{n-2}.$$

We show the positivity of this solution. Clearly, if (10) holds, then $\xi_2 > 0$. Also, if (11) holds, then for every $i = 3, \ldots, n-2$, $\xi_i > 0$ holds. Note that $\xi_n > 0$ if $\xi_1 > \frac{r_{n-2}}{mr_n} + 1$. Note also ξ_1 is the only positive root of $h_1(y_1) = 0$. So, if $h_1(\frac{r_{n-2}}{mr_n} + 1) < 0$, then $\xi_1 > \frac{r_{n-2}}{mr_n} + 1$. Note

$$h_1(\frac{r_{n-2}}{mr_n} + 1) = -\frac{r_{n-2}}{m^2 r_n}\left((m-1)r_1 r_{n-2} + m\left((m-1)r_1 r_n - (r_1 + r_n)r_{n+2}\right)\right).$$

So for positive m, r_{n-2} and r_n, $h_1(\frac{r_{n-2}}{mr_n} + 1) < 0$ is equivalent to (26). Thus, if (10)–(11) and (26) are satified, then ξ is positive.

Finally, we show $\det J_g|_{y=\xi} \neq 0$. In fact, the Jacobian matrix of $g_1, \ldots, g_n$ with respect to $y_1, \ldots, y_n$ is

$$J_g = \begin{bmatrix} -r_1 y_2 - r_n & -r_1 y_1 & \cdots & 0 & 0 & 0 \\ -r_1 y_2 & -r_1 y_1 - r_2 y_3 - r_{n+2} & \ddots & \vdots & \vdots & \vdots \\ 0 & -r_2 y_3 & \ddots & -r_{n-3} y_{n-3} & 0 & 0 \\ \vdots & 0 & \ddots & -r_{n-3} y_{n-3} & 0 & 0 \\ 0 & \vdots & \ddots & 0 & -r_{n-1} & 0 \\ mr_n & 0 & \cdots & 0 & -r_{n-1} & -1 \end{bmatrix}.$$

Expanding $\det J_g$ with respect to the first row and taking advantage of Lemma 5.1, we have

$$\det J_g = -r_2 \cdots r_{n-3} r_{n-1} y_2 \cdots y_{n-3}(r_n r_{n+2} + r_1 r_n y_1 + r_1 r_{n+2} y_2)$$

which is obviously nonzero at any positive solution $y = \xi$. □

Remark 8. The inequality (26) is a specific case of the inequality (9) for $i = n-1$.

Lemma 5.6. *If for a choice of the rate constants $r_1, \ldots, r_{2n}$, the system $p = 0$ has a solution $\xi = (\xi_1, \ldots, \xi_n)$ such that $\xi_n \neq 0$ and $\det J_p|_{y=\xi} \neq 0$, and if the rate constant $r_{2n} \neq 0$, then for the same choice of rate constants, $\hat{x} := (\xi_1, \ldots, \xi_{n-2}, \frac{r_{2n}\xi_{n-1}}{\xi_n}, \frac{\xi_n}{r_{2n}})$ is a solution to f such that $\det J|_{x=\hat{x}} \neq 0$.*

Proof. By the definition of the map φ (20), and by the definition of the system p (21), $\hat{x}$ is a solution to f. Note also, the Jacobian matrix of φ with respect to $y_1, \ldots, y_n$ is

$$J_\varphi := \begin{bmatrix} \mathrm{I}_{n-2} & & \\ & \frac{y_n}{r_{2n}} & \frac{r_{2n} y_{n-1}}{y_n} \\ & & r_{2n} \end{bmatrix},$$

where I_{n-2} denotes the identity matrix of size $(n-2)\times(n-2)$. So, if $\xi_n \neq 0$ and $r_{2n} \neq 0$, $J_{\varphi}|_{y=\xi}$ is invertible. By (21), we have $J_p = J \cdot J_{\varphi}$. So we conclude that $\det J|_{x=\hat{x}} \neq 0$. □

Proof of Theorem 4.4. Let $r_{n+1} = \epsilon$, and for $i = 3, \ldots n$, let $r_{n+i} = \epsilon$. For $n = 3$, choose values for the rate constants r_1, r_2, r_3, r_5 such that the conditions (6)–(9) are satisfied. For instance, we can choose

$$r_1 = 2, \quad r_2 = m+1, \quad r_3 = 1, \quad \text{and} \quad r_5 = m-1. \tag{30}$$

For any odd integer $n > 3$, choose values for the rate constants $r_1, \ldots, r_n, r_{n+2}$ such that the conditions (6)–(11) are satisfied. For instance, we can choose

$$\begin{aligned} &r_1 = 2, \quad r_2 = r_4 = \cdots = r_{n-3} = r_n = 1, \\ &r_3 = r_5 = \cdots = r_{n-4} = r_{n-1} = m+1, \quad r_{n-2} = m, \quad r_{n+2} = m-1. \end{aligned} \tag{31}$$

Here, by the values in (30) (or, the values in (31)), we see the open set determined by the inequalities (6)–(9) for $n = 3$ (or, the inequalities (6)–(11) for $n > 3$) is non-empty. For $i = 2n+1, \ldots, 3n$, set r_i as (15).

By Lemma 5.3, if $\epsilon = 0$, then $f = 0$ in (17) has two distinct positive solutions $x^{(1)} = (1, 1, \ldots, 1)$ and $x^{(2)}$ in (19). So, by the implicit function theorem, if ϵ is a sufficiently small positive number, then $f = 0$ has two distinct positive solutions $\hat{x}^{(1)}$ and $\hat{x}^{(2)}$ with $\det J|_{x=\hat{x}^{(i)}} \neq 0, (i = 1, 2)$, where $\hat{x}^{(1)} = x^{(1)}$ (since $x^{(1)}$ is always a solution to $f = 0$ in (17)), and $\hat{x}^{(2)}$ is sufficiently close to $x^{(2)}$. That means $\widetilde{K}_{m,n}$ has at least two distinct nondegenerate steady states.

By Lemmas 5.4–5.5, for the rate constants (30) when $n = 3$, or respectively for the rate constants (31) when $n > 3$, the system $g = 0$ in (22) has a positive solution $\xi = (\xi_1, \ldots, \xi_n)$ such that $\det J_g|_{y=\xi} \neq 0$. By the definition of g in (22), when $\epsilon = 0$, ξ is also a positive solution of the system $p = 0$ in (21) such that $\det J_p|_{y=\xi} \neq 0$ for the same choice of $r_1, \ldots, r_n, r_{n+2}$. Therefore, by the implicit function theorem, if ϵ is a sufficiently small positive number, then $p = 0$ has a positive solution, say $\hat{\xi} = (\hat{\xi}_1, \ldots, \hat{\xi}_n)$, which is close to ξ, such that $\det J_p|_{y=\hat{\xi}} \neq 0$. Let $\hat{x}^{(3)} = (\hat{\xi}_1, \ldots, \hat{\xi}_{n-2}, \frac{\epsilon\hat{\xi}_{n-1}}{\hat{\xi}_n}, \frac{\hat{\xi}_n}{\epsilon})$. By Lemma 5.6, $\hat{x}^{(3)}$ is a positive solution to the system $f = 0$ such that $\det J|_{x=\hat{x}^{(3)}} \neq 0$. So $\hat{x}^{(3)}$ is the third nondegenerate steady state of $\widetilde{K}_{m,n}$.

5.2. **Bistability.** Here, we prove that two of those three steady states stated in Theorem 4.4 are stable if we replace the condition (6) in Theorem 4.4 with the condition (12) (see Theorem 4.5). The main idea is to show the Jacobian matrices at two steady states are similar to column diagonally dominant matrices (see Lemmas 5.7-5.9). Then, we can conclude bistability by Theorem 3.6.

Lemma 5.7. *For any integer $m \geq 2$, and for any odd integer $n \geq 3$, if the rate constants $r_{n+1}, r_{n+3}, \ldots, r_{2n}$ satisfy the condition (18), and if the rate constants r_1, r_n, r_{n+2} satisfy the inequality (12), then for $x^{(1)} = (1, 1, \ldots, 1)$, the matrix $J|_{x=x^{(1)}}$ is similar to a column diagonally dominant matrix.*

Proof. Since the condition (18) holds, for any $r_1, \ldots, r_n, r_{n+2}$, the Jacobian matrix J is as follows:

$$J \;=\; \begin{bmatrix} -r_1x_2 - r_n & -r_1x_1 & \cdots & 0 & 0 \\ -r_1x_2 & -r_1x_1 - r_2x_3 - r_{n+2} & \cdots & \vdots & \vdots \\ 0 & -r_2x_3 & \ddots & 0 & 0 \\ \vdots & 0 & \ddots & -r_{n-2}x_{n-2} & 0 \\ 0 & \vdots & \ddots & -r_{n-2}x_{n-2} - r_{n-1}x_n & -r_{n-1}x_{n-1} \\ mr_n & 0 & \cdots & -r_{n-1}x_n & -r_{n-1}x_{n-1} \end{bmatrix}.$$

Let $\alpha = 1 + \frac{r_{n+2}}{r_1x_1}$, and let D be the diagonal matrix $\mathrm{diag}(\alpha, 1, \ldots, 1)$. Note that the matrix $\tilde{J} := DJD^{-1}$ is equal to

$$\tilde{J} \;=\; \begin{bmatrix} -r_1x_2 - r_n & -r_1x_1\alpha & \cdots & 0 & 0 \\ -\frac{r_1x_2}{\alpha} & -r_1x_1 - r_2x_3 - r_{n+2} & \cdots & \vdots & \vdots \\ 0 & -r_2x_3 & \ddots & 0 & 0 \\ \vdots & 0 & \ddots & -r_{n-2}x_{n-2} & 0 \\ 0 & \vdots & \ddots & -r_{n-2}x_{n-2} - r_{n-1}x_n & -r_{n-1}x_{n-1} \\ \frac{mr_n}{\alpha} & 0 & \cdots & -r_{n-1}x_n & -r_{n-1}x_{n-1} \end{bmatrix}.$$

We denote by a_{ij} the (i, j)-entry in $\tilde{J}$. Clearly, for $i > 2$, we have $|a_{ii}| = \sum_{j \neq i} |a_{ij}|$. For $i = 2$, by $\alpha = 1 + \frac{r_{n+2}}{r_1x_1}$, we have

$$|a_{22}| \;=\; r_1x_1 + r_2x_3 + r_{n+2} \;=\; \alpha r_1x_1 + r_2x_3 \;=\; \sum_{j \neq 2} |a_{2j}|.$$

Note that the inequality $|a_{11}| > \sum_{j \neq 1} |a_{1j}|$ is equivalent to

$$\frac{(r_1x_2 + r_n)r_{n+2}}{x_1} \;>\; (m-1)r_1r_n. \tag{32}$$

For $x = x^{(1)} = (1, 1, \ldots, 1)$, the inequality (32) is exactly the inequality (12). □

Remark 9. In analogy with Lemma 5.7, one can prove if the rate constants satisfy the inequality (16), then for $x^{(2)} = (\delta_1, \delta_2, \ldots, \delta_n)$ (19) stated in Lemma 5.3, $J|_{x=x^{(2)}}$ is similar to a column diagonally dominant matrix.

Lemma 5.8. *For any integer $m \geq 2$, and for $n = 3$, if $r_4 = r_6 = \epsilon$, and if $\epsilon > 0$ is sufficiently small, then for any positive rate constants r_1, r_2, r_3, r_5, and for any positive numbers $\hat{\xi}_1, \hat{\xi}_2, \hat{\xi}_3$, the matrix $J|_{x=(\hat{\xi}_1, \frac{\epsilon\hat{\xi}_2}{\hat{\xi}_3}, \frac{\hat{\xi}_3}{\epsilon})}$ is similar to a column diagonally dominant matrix.*

Proof. Let $D = \mathrm{diag}(d_1, 1, d_3)$, where d_1 and d_3 satisfy the equalities:

$$\begin{cases} d_1r_1x_1 + d_3r_2x_3 \;=\; r_1x_1 + r_2x_3 + r_5 \\ \frac{1}{d_3}r_2x_2 \;=\; r_2x_2 + r_6 \end{cases}. \tag{33}$$

We solve for d_1 and d_3 from (33) over $\mathbb{Q}(r, x)$:

$$\begin{cases} d_1 \;=\; \frac{r_1r_2x_1x_2 + r_1r_6x_1 + r_2r_5x_2 + r_2r_6x_3 + r_5r_6}{r_1x_1(r_2x_2 + r_6)}, \\ d_3 \;=\; \frac{r_2x_2}{r_2x_2 + r_6}. \end{cases} \tag{34}$$

Notice that the matrix $\tilde{J} := DJD^{-1}$ is equal to

$$\begin{bmatrix} -r_1x_2 - r_3 - r_4 & -d_1r_1x_1 & 0 \\ -\frac{1}{d_1}r_1x_2 & -r_1x_1 - r_2x_3 - r_5 & -\frac{1}{d_3}r_2x_2 \\ \frac{d_3}{d_1}mr_3 & -d_3r_2x_3 & -r_2x_2 - r_6 \end{bmatrix}.$$

We denote by a_{ij} the (i,j)-entry in $\tilde{J}$. By (33), for $i = 2, 3$, we have $|a_{ii}|= \sum_{j\neq i}|a_{ij}|$. By Definition 3.3, in order to make $\tilde{J}$ to be column diagonally dominant, we only need to ensure $|a_{11}|\leq \sum_{j\neq 1}|a_{1j}|$. That means, it is sufficient to show that for $x = (\hat{\xi}_1, \frac{\epsilon\hat{\xi}_2}{\hat{\xi}_3}, \frac{\hat{\xi}_3}{\epsilon})$, and for $r_4 = r_6 = \epsilon$, the inequality below is true if $\epsilon > 0$ is sufficiently small:

$$\frac{d_3}{d_1}mr_3 + \frac{1}{d_1}r_1x_2 \;\leq\; r_1x_2 + r_3 + r_4. \tag{35}$$

In fact, we substitute (34) into the inequality (35) and obtain

$$r_1x_1x_2(mr_2r_3+r_1(r_2x_2+r_6)) \leq (r_2r_6x_3+(r_1x_1+r_5)(r_2x_2+r_6))(r_1x_2+r_3+r_4). \tag{36}$$

When $x = (\hat{\xi}_1, \frac{\epsilon\hat{\xi}_2}{\hat{\xi}_3}, \frac{\hat{\xi}_3}{\epsilon})$ and $r_4 = r_6 = \epsilon$, the inequality (36) is

$$r_1\hat{\xi}_1\frac{\hat{\xi}_2\epsilon}{\hat{\xi}_3}(mr_2r_3+r_1r_2\frac{\hat{\xi}_2\epsilon}{\hat{\xi}_3}+r_1\epsilon) \leq (r_2\hat{\xi}_3+(r_1\hat{\xi}_1+r_5)(r_2\frac{\hat{\xi}_2\epsilon}{\hat{\xi}_3}+\epsilon))(r_3+r_1\frac{\hat{\xi}_2\epsilon}{\hat{\xi}_3}+\epsilon). \tag{37}$$

Note that both sides of (37) are quadratic functions in ϵ. Note also, at $\epsilon = 0$, the function on the left-hand side evaluates to 0, while the one on the right-hand side is positive. So for sufficiently small $\epsilon > 0$, the inequality (37) holds. □

Lemma 5.9. *For any integer $m \geq 2$, and for any odd integer $n > 3$, if $r_{n+1} = r_{n+3} = \ldots = r_{2n} = \epsilon$, and if $\epsilon > 0$ is sufficiently small, then for any positive rate constants $r_1, \ldots, r_n, r_{n+2}$, and for any positive numbers $\hat{\xi}_1, \ldots, \hat{\xi}_n$, the matrix*

$$J|_{x=(\hat{\xi}_1,\ldots,\hat{\xi}_{n-2},\frac{\epsilon\hat{\xi}_{n-1}}{\hat{\xi}_n},\frac{\hat{\xi}_n}{\epsilon})}$$

is similar to a column diagonally dominant matrix.

Proof. Let $D = \mathrm{diag}(d_1, 1, \ldots, 1, d_{n-1}, d_n)$, where d_1, d_{n-1}, and d_n satisfy the equalities:

$$\begin{cases} d_1r_1x_1 = r_1x_1 + r_{n+2} \\ \frac{1}{d_{n-1}}r_{n-2}x_{n-2} + \frac{d_n}{d_{n-1}}r_{n-1}x_n = r_{n-2}x_{n-2} + r_{n-1}x_n + r_{2n-1} \\ \frac{d_{n-1}}{d_n}r_{n-1}x_{n-1} = r_{n-1}x_{n-1} + r_{2n} \end{cases}. \tag{38}$$

We solve for d_1, d_{n-1}, and d_n from (38) over $\mathbb{Q}(r, x)$:

$$\begin{cases} d_1 &= \frac{r_1x_1+r_{n+1}}{r_1x_1}, \\ d_{n-1} &= \frac{r_{n-1}r_{n-2}x_{n-1}x_{n-2}+r_{2n}r_{n-2}x_{n-2}}{r_{n-1}r_{n-2}x_{n-1}x_{n-2}+r_{2n}r_{n-2}x_{n-2}+r_{2n}r_{n-1}x_n+r_{2n-1}r_{n-1}x_{n-1}+r_{2n}r_{2n-1}}, \\ d_n &= \frac{r_{n-1}r_{n-2}x_{n-1}x_{n-2}}{r_{n-1}r_{n-2}x_{n-1}x_{n-2}+r_{2n}r_{n-1}x_n+r_{2n}r_{n-2}x_{n-2}+r_{2n-1}r_{n-1}x_{n-1}+r_{2n}r_{2n-1}}. \end{cases} \tag{39}$$

Notice that $\tilde{J} := DJD^{-1}$ is equal to

$$\begin{bmatrix} -r_1x_2 - r_n - r_{n+1} & -d_1r_1x_1 & \cdots & 0 & 0 \\ -\frac{1}{d_1}r_1x_2 & -r_1x_1 - r_2x_3 - r_{n+2} & \cdots & \vdots & \vdots \\ 0 & -r_2x_3 & \ddots & 0 & 0 \\ \vdots & 0 & \ddots & -\frac{1}{d_{n-1}}r_{n-2}x_{n-2} & 0 \\ 0 & \vdots & \ddots & -r_{n-2}x_{n-2} - r_{n-1}x_n - r_{2n-1} & -\frac{d_{n-1}}{d_n}r_{n-1}x_{n-1} \\ -\frac{d_n}{d_1}mr_n & 0 & \cdots & -\frac{d_n}{d_{n-1}}r_{n-1}x_n & -r_{n-1}x_{n-1} - r_{2n} \end{bmatrix}.$$

We denote by a_{ij} the (i,j)-entry in $\tilde{J}$. Clearly, for any $2 < i < n-2$, $|a_{ii}|= \sum_{j\neq i}|a_{ij}|$. By (38), for $i = 2, n-1, n$, we have $|a_{ii}|= \sum_{j\neq i}|a_{ij}|$. By Definition 3.3, in order to make $\tilde{J}$ to be column diagonally dominant, we only need to make sure $|a_{ii}|\leq \sum_{j\neq i}|a_{ij}|$ for $i = 1$ and $i = n-2$. That means that it is sufficient to show that for $x = (\hat{\xi}_1, \ldots, \hat{\xi}_{n-2}, \frac{\epsilon\hat{\xi}_{n-1}}{\hat{\xi}_n}, \frac{\hat{\xi}_n}{\epsilon})$, and for $r_{n+1} = r_{n+3} = \cdots = r_{2n} = \epsilon$, we have the inequalities below if $\epsilon > 0$ is sufficiently small:

$$\begin{cases} \frac{1}{d_1}r_1x_2 + \frac{d_n}{d_1}mr_n \ \leq \ r_1x_2 + r_n + r_{n+1} \\ r_{n-3}x_{n-3} + d_{n-1}r_{n-2}x_{n-1} \ \leq \ r_{n-3}x_{n-3} + r_{n-2}x_{n-1} + r_{2n-2} \end{cases}. \tag{40}$$

By (39), $d_{n-1} < 1$. So, the second inequality in (40) holds for any positive r and x. We substitute (39) into the first inequality in (40). Then we have

$$\begin{aligned} mr_1r_{n-1}r_{n-2}r_nx_1x_{n-2}x_{n-1} \ \leq\ & (r_1r_nx_1 + r_1r_{n+1}x_1 + r_1r_{n+2}x_2 + r_nr_{n+2} \\ & + r_{n+1}r_{n+2})(r_{n-2}r_{n-1}x_{n-2}x_{n-1} + r_{n-1}r_{2n-1}x_{n-1} \\ & + r_{2n}(r_{n-2}x_{n-2} + r_{n-1}x_n + r_{2n-1})). \end{aligned} \tag{41}$$

For $x = (\hat{\xi}_1, \ldots, \hat{\xi}_{n-2}, \frac{\epsilon\hat{\xi}_{n-1}}{\hat{\xi}_n}, \frac{\hat{\xi}_n}{\epsilon})$, and for $r_{n+1} = r_{n+3} = \cdots = r_{2n} = \epsilon$, the inequality (41) is

$$\begin{aligned} (mr_1r_{n-1}r_{n-2}r_n\hat{\xi}_1\hat{\xi}_{n-2}\frac{\hat{\xi}_{n-1}}{\hat{\xi}_n})\epsilon \ \leq\ & (r_1r_n\hat{\xi}_1 + r_1r_{n+2}\hat{\xi}_2 + r_nr_{n+2} + (r_1\hat{\xi}_1 + r_{n+2})\epsilon) \\ & (r_{n-1}\hat{\xi}_n + (r_{n-1}\frac{\hat{\xi}_{n-1}}{\hat{\xi}_n} + 1)(r_{n-2}\hat{\xi}_{n-2}\epsilon + \epsilon^2)). \end{aligned} \tag{42}$$

Note that when $\epsilon = 0$, the left-hand side of (42) is zero, and the right-hand side is positive. So, the inequality (42) clearly holds for sufficiently small $\epsilon > 0$. □

Proof of Theorem 4.5. Let $r_{n+1} = r_{n+3} = \ldots = r_{2n} = \epsilon$. For $n = 3$, choose the rate constants r_1, r_2, r_3, r_5 as in (30). For $n > 3$, choose the rate constants $r_1, \ldots, r_n, r_{n+2}$ as in (31). By the proof of Theorem 4.4, for these rate constants, $\widetilde{K}_{m,n}$ has three nondegenerate positive steady states $\hat{x}^{(i)}$ $(i = 1, 2, 3)$ if ϵ is a sufficiently small positive number, where $\hat{x}^{(3)}$ has the form $(\hat{\xi}_1, \ldots, \hat{\xi}_{n-2}, \frac{\epsilon\hat{\xi}_{n-1}}{\hat{\xi}_n}, \frac{\hat{\xi}_n}{\epsilon})$, and $\hat{x}^{(1)} = (1, 1, \ldots, 1)$. By Lemmas 5.8–5.9 and Theorem 3.6, all non-zero eigenvalues of $J|_{x=\hat{x}^{(3)}}$ have negative real parts. Note our choice of rate constants also satisfies the inequality (12). So, by Theorem 3.6 and Lemma 5.7, when $\epsilon = 0$, all non-zero eigenvalues of $J|_{x=\hat{x}^{(1)}}$ have negative real parts. Note that the eigenvalues of a matrix vary continuously under continuous perturbations of entries. So, if ϵ is a sufficiently small positive number, all non-zero eigenvalues of $J|_{x=\hat{x}^{(1)}}$ also have negative real parts. By the proof of Theorem 4.4, $\det J|_{x=\hat{x}^{(i)}} \neq 0$, for $i = 1, 3$. So both $\hat{x}^{(1)}$ and $\hat{x}^{(3)}$ are locally asymptotically stable.

5.3. **Non-empty open region for bistability. Proof of Theorem 4.6.** The case for $n = 3$ is obvious. For any odd integer $n > 3$, by the inequality (12), the inequality (9) holds if and only if

$$(m-1)r_1r_{i-1} \;>\; m((r_1+r_n)r_{n+2}-(m-1)r_1r_n), \;\; i = 4, \ldots, n-1. \tag{43}$$

And by the inequalities (11), the inequality (43) holds if and only if

$$(m-1)r_1r_{n-2} \;>\; m((r_1+r_n)r_{n+2}-(m-1)r_1r_n).$$

Then, it is easy to see that the open set in $\mathbb{R}^{n+1}_{>0}$ determined by the inequalities (7)–(12) is equivalent to the set given in (14).

6. **Summary.** In this paper, we prove that the fully open extension of a sequestration network admits three nondegenerate positive steady states, two of which are locally asymptotically stable (bistability). We also give an open region in the parameter space as well as explicit choices of rate constants to ensure bistability.

In the future, it would be interesting to investigate the configuration of the positive steady states and study how the stability of them changes as the rate constants vary. Moreover, based on numerical experiments, we propose the following conjecture (Conjecture 1) for the future study:

Conjecture. For any integer $m \geq 2$, and for any odd integer $n \geq 3$, the maximum number of nondegerate steady states of $\widetilde{K}_{m,n}$ is three, and the network $\widetilde{K}_{m,n}$ is multistationary if and only if it is bistable.

We hope that the techniques exploited to prove stability in this paper, e.g. the Gershgorin circle theorem and analyzing steady states at infinity, can be applied to more general chemical reaction networks.

Acknowledgments. We would like to acknowledge Anne Shiu for her generous support and valuable advice. We also thank Badal Joshi for his comments on the first draft of our work.

REFERENCES

[1] C. Bagowski and J. Ferrell, Bistability in the JNK cascade, *Curr. Biol.*, **11** (2001), 1176–1182.
[2] M. Banaji and C. Pantea, Some results on injectivity and multistationarity in chemical reaction networks, *SIAM J. Appl. Dyn. Syst.*, **15** (2016), 807–869.
[3] C. Conradi, E. Feliu, M. Mincheva and C. Wiuf, Identifying parameter regions for multistationarity, *PLoS Comput. Biol.*, **13** (2017).
[4] C. Conradi and A. Shiu, Dynamics of post-translational modification systems: Recent progress and future challenges, *Biophys. J.*, **114** (2018), 507–515.
[5] G. Craciun and M. Feinberg, Multiple equilibria in complex chemical reaction networks. I. The injectivity property, *SIAM J. Appl. Math.*, **65** (2005), 1526–1546.
[6] G. Craciun, Y. Z. Tang and M. Feinberg, Understanding bistability in complex enzyme-driven reaction networks, *Proc. Natl. Acad. Sci. USA*, **103** (2006), 8697–8702.
[7] G. Craciun and M. Feinberg, Multiple equilibria in complex chemical reaction networks: Semiopen mass action systems, *SIAM J. Appl. Math.*, **70** (2010), 1859–1877.
[8] B. N. Datta, An elementary proof of the stability criterion of Liénard and Chipart, *Linear Algebra Appl.*, **22** (1978), 89–96.
[9] A. Dickenstein, M. P. Millan, A. Shiu and X. X. Tang, Multistationarity in structured reaction networks, *Bull. Math. Biol.*, **81** (2019), 1527–1581.

[10] M. Domijan and M. Kirkilionis, Bistability and oscillations in chemical reaction networks, *J. Math. Biol.*, **59** (2009), 467–501.

[11] E. Feliu, Injectivity, multiple zeros and multistationarity in reaction networks, *Proc. A*, **471** (2015), 20140530, 18 pp.

[12] B. Félix, A. Shiu and Z. Woodstock, Analyzing multistationarity in chemical reaction networks using the determinant optimization method, *Appl. Math. Comput.*, **287/288** (2016), 60–73.

[13] J. E. Ferrell, Jr. and E. M. Machleder, The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes, *Science*, **280** (1998), 895–898.

[14] S. A. Gerschgorin, Über die Abgrenzung der Eigenwerte einer Matrix, *Izv. Akad. Nauk. USSR Otd. Fiz.-Mat. Nauk (in German)*, **6** (1931), 749–754.

[15] H. Hong, X. X. Tang and B. C. Xia, Special algorithm for stability analysis of multistable biological regulatory systems, *J. Symbolic Comput.*, **70** (2015), 112–135.

[16] M. D. Johnston, S. Müller and C. Pantea, A deficiency-based approach to parametrizing positive equilibria of biochemical reaction systems, *Bull. Math. Biol.*, **81** (2019), 1143–1172.

[17] B. Joshi and A. Shiu, A survey of methods for deciding whether a reaction network is multistationary, *Math. Model. Nat. Phenom.*, **10** (2015), 47–67.

[18] B. Joshi and A. Shiu, Which small reaction networks are multistationary? *SIAM J. Appl. Dyn. Syst.*, **16** (2017), 802–833.

[19] R. K. Miller and A. N. Michel, *Ordinary Differential Equations*, Academic Press, New York-London, 1982.

[20] S. Müller, E. Feliu, G. Regensburger, C. Conradi, A. Shiu and A. Dickenstein, Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry, *Found. Comput. Math.*, **16** (2016), 69–97.

[21] N. Obatake, A. Shiu, X. X. Tang and A. Torres, Oscillations and bistability in a model of ERK regulation, *Journal of Mathematical Biology*, **79** (2019), 1515–1549.

[22] M. Pérez Millán and A. Dickenstein, The structure of MESSI biological systems, *SIAM J. Appl. Dyn. Syst.*, **17** (2018), 1650–1682.

[23] M. Pérez Millán, A. Dickenstein, A. Shiu and C. Conradi, Chemical reaction systems with toric steady states, *Bull. Math. Biol.*, **74** (2012), 1027–1065.

[24] A. Sadeghimanesh and E. Feliu, The multistationarity structure of networks with intermediates and a binomial core network, *Bulletin of Mathematical Biology*, **81** (2019), 2428–2462.

[25] P. M. Schlosser and M. Feinberg, A theory of multiple steady states in isothermal homogeneous CFSTRs with many reactions, *Chem. Eng. Sci.*, **49** (1994), 1749–1767.

[26] G. Shinar and M. Feinberg, Concordant chemical reaction networks, *Math. Biosci.*, **240** (2012), 92–113.

[27] A. Shiu and T. de Wolff, Nondegenerate multistationarity in small reaction networks, *Discrete Contin. Dyn. Syst. B*, **24** (2019), 2683–2700.

[28] M. Thomson and J. Gunawardena, The rational parameterisation theorem for multisite post-translational modification systems, *J. Theoret. Biol.*, **261** (2009), 626–636.

[29] C. Wiuf and E. Feliu, Power-law kinetics and determinant criteria for the preclusion of multistationarity in networks of interacting species, *SIAM J. Appl. Dyn. Syst.*, **12** (2013), 1685–1721.

[30] W. Xiong and J. E. Ferrell, A positive-feedback-based bistable 'memory module' that governs a cell fate decision, *Nature*, **426** (2003), 460–465.

Received June 2019; 1st revision January 2020; 2nd revision February 2020.

E-mail address: xiaoxian@buaa.edu.cn
E-mail address: jwang@laas.fr