Determination of Multiple Steady States in an Active Membrane Transport Model

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A necessary and sufficient condition is applied to determine the possibility of multiple positive steady states in a complex, active membrane transport model with a cycle, which is performed by pump proteins coupled to a source of metabolic energy. A set of rate constants and two corresponding steady states are computed. Hysteresis phenomena are observed. A signature of multiplicity is derived, which can be applied in mechanism identifications if steady-state concentrations for some species are measured. The bifurcation of multiple steady states is also displayed.

Key words: Multiple Steady States; Bifurcation; Active Membrane Transport Models.

1. Introduction

Biological systems can sometimes give rise to complex behavior, such as oscillations, multiple steady states and unstable steady states. Active transport, an important path to self-regulation and self-control in living cells, is performed by pump proteins coupled to a source of metabolic energy, usually ATP hydrolysis. These pumps contain specific binding sites for the transported solute and for the molecule involved in the energy supply. The transport is accomplished by a conformational change of the pump in transferring the solute across the membrane. This process can be modeled by specific kinetic steps for the binding and conformational changes, which are similar to those occurring in standard chemical kinetics. Some theoretical and experimental studies on this field have been done [1–4]. Based on some experimental results [5–9], Vieira and Bisch [10] have recently proposed a model (model 5) with monomers as pump units. They have applied stoichiometric network analysis [11–13] to study the stability of steady states and have found a set of rate constants showing steady-state multiplicity. Recently, a necessary and sufficient condition for the determination of multiple positive steady states in isothermal chemical reaction networks was developed [14]. (A positive steady state is one for which all species have positive concentrations.) In this work, the condition is applied to show the possibility of multiple steady states in the Models of [10]. Moreover, the results indicate that there exist some "signatures" of steady-state multiplicities which are useful for mechanism identifications in experimental studies. A set of positive rate constants and two corresponding positive steady states are constructed. Hysteresis phenomena and bifurcation of multiplicity are demonstrated.

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2. Theoretical Background

The example of the Model 5 of [10] is

$$
\begin{array}{lll}
A_1 \rightleftarrows A_2 & & \\
\uparrow\downarrow \quad\;\; \uparrow\downarrow & & A_3 + A_4 \rightleftarrows 2A_4\,, \\
A_6 \qquad A_3 & & \\
\uparrow\downarrow \quad\;\; \uparrow\downarrow & & A_6 + A_7 \rightleftarrows A_8 \rightleftarrows A_1 + A_7\,. \\
A_5 \rightleftarrows A_4\,, & &
\end{array}
\tag{1a}
$$

Species A_1 through A_8 denote respectively the monomers A, B, C, D, E, F, W, and Z, used in the model. Network (1a) contains one cycle $A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \rightarrow A_5 \rightarrow A_6 \rightarrow A_1$. During the cycle one ligand molecule is transported from the external to the internal medium. Each pump transports only one ion per cycle. The ligand concentration and the concentrations of ATP, ADP, and Pi (adenosine tri- and di-phosphate and inorganic phosphate, respectively) are considered as externally controlled parameters. Phosphorylation-dephosphorylation reactions and conformational transitions are treated as

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elementary steps. The autocatalytic reaction $A_3+A_4 \rightleftarrows 2A_4$ is introduced to simulate dynamic cooperation in active membrane transport. It comes from the fact that membranes of specialized cells of multicellular organisms and subcellular compartments of all eukaryotic cells have a restricted number of different proteins and usually a high concentration of each type, facilitating the interactions between them. Finally, the reactions $A_6+A_7 \rightleftarrows A_8 \rightleftarrows A_1+A_7$ model the formation of molecular complexes with other molecules, which should lead to activated monomers. From the kinetic point of view, the formation of an intermediate complex (A_8, pump-molecule) generates a new chemical pathway. A more detailed relation of network (1) with reality is described in [10].

The corresponding mass action differential equations for network (1a) are

$$
\begin{aligned}
\frac{dc_1}{dt} &= -k_{A_1\to A_2}c_1 + k_{A_2\to A_1}c_2 - k_{A_1\to A_6}c_1 \\
&\quad + k_{A_6\to A_1}c_6 + k_{A_8\to A_1+A_7}c_8 - k_{A_1+A_7\to A_8}c_1c_7 ,\\
\frac{dc_2}{dt} &= k_{A_1\to A_2}c_1 - k_{A_2\to A_1}c_2 - k_{A_2\to A_3}c_2 + k_{A_3\to A_2}c_3 ,\\
\frac{dc_3}{dt} &= k_{A_2\to A_3}c_2 - k_{A_3\to A_2}c_3 - k_{A_3\to A_4}c_3 + k_{A_4\to A_3}c_4 \\
&\quad - k_{A_3+A_4\to 2A_4}c_3c_4 + k_{2A_4\to A_3+A_4}c_4^2 ,\\
\frac{dc_4}{dt} &= k_{A_3\to A_4}c_3 - k_{A_4\to A_3}c_4 - k_{A_4\to A_5}c_4 + k_{A_5\to A_4}c_5 \\
&\quad + k_{A_3+A_4\to 2A_4}c_3c_4 - k_{2A_4\to A_3+A_4}c_4^2 ,\\
\frac{dc_5}{dt} &= k_{A_4\to A_5}c_4 - k_{A_5\to A_4}c_5 - k_{A_5\to A_6}c_5 + k_{A_6\to A_5}c_6 ,\\
\frac{dc_6}{dt} &= k_{A_5\to A_6}c_5 - k_{A_6\to A_5}c_6 + k_{A_1\to A_6}c_1 - k_{A_6\to A_1}c_6 \\
&\quad - k_{A_6+A_7\to A_8}c_6c_7 + k_{A_8\to A_6+A_7}c_8 ,\\
\frac{dc_7}{dt} &= -k_{A_6+A_7\to A_8}c_6c_7 + k_{A_8\to A_6+A_7}c_8 \\
&\quad + k_{A_8\to A_1+A_7}c_8 - k_{A_1+A_7\to A_8}c_1c_7 ,\\
\frac{dc_8}{dt} &= k_{A_6+A_7\to A_8}c_6c_7 - k_{A_8\to A_6+A_7}c_8 \\
&\quad - k_{A_8\to A_1+A_7}c_8 + k_{A_1+A_7\to A_8}c_1c_7 , \qquad (1b)
\end{aligned}
$$

where c_i, $i=1, \ldots, 8$ denote respectively the concentrations of species $A_1, A_2, \ldots, A_8$, and $k_{i\to j}$ denotes the rate constant for the reaction $i\to j$. From (1b), two mass conservation conditions must be satisfied:

$$
\begin{aligned}
&\frac{d(c_7+c_8)}{dt} = 0 ,\\
&\frac{d(c_1+c_2+c_3+c_4+c_5+c_6+c_8)}{dt} = 0 . \qquad (1c)
\end{aligned}
$$

In steady states, all species concentrations do not change with time (t), i.e. $dc_i/dt=0$, $i=1, \ldots, 8$. We are interested to know whether there exists a set of positive rate constants for network (1a) such that its (isothermal) mass action differential equations (1b) admit more than one positive steady state under the constraint of mass conservation (1c).

Based upon a parameter transformation and linear algebra, a necessary and sufficient condition for the determination of multiple positive steady states in general reaction networks has been proved in [14]. The assumptions are that the systems are isothermal, the density is constant, the reactions are mass actions, and the steady states are positive. They can be applied to networks with or without cycles. According to the method, the necessary and sufficient condition forms a system of inequalities and equations of $\boldsymbol{\mu}(=[\mu_1, \ldots, \mu_8]=\ln(\boldsymbol{c}'/\boldsymbol{c}'')=[\ln(c_1'/c_1''), \ldots, \ln(c_8'/c_8'')])$ with $\boldsymbol{c}'=[c_1', \ldots, c_8']$ and $\boldsymbol{c}''=[c_1'', \ldots, c_8'']$ denoting two positive steady states. If solutions of the system exist and if they satisfy some requirements from mass conservation, then the network under study can exhibit multiple steady states for some rate constants. Otherwise, the network can admit at most one positive steady state, no matter what rate constants the system might have. The method is presented below and the terminology is already defined in [14].

A necessary and sufficient condition: *Consider an N-species reaction network with a reaction set R and stoichiometric subspace S_t. Suppose the network has rank s and r reactions with p reversible reaction pairs. Let the reaction set for an arbitrary spanning subnetwork be F and let $\{\boldsymbol{d}^{(1)}, \boldsymbol{d}^{(2)}, \ldots, \boldsymbol{d}^{(r-p-s)}\}$ be a set of corresponding spanning-subnetwork vectors. Then the corresponding isothermal mass action differential equations for the given network have the capacity to admit (stoichiometrically compatible) multiple positive steady states if and only if there exists a nonzero vector $\boldsymbol{\mu}\in \mathcal{R}^N$ which is sign compatible with S_t, and also the numbers $\xi_1, \xi_2, \ldots, \xi_{r-p-s}, \alpha_1, \alpha_2, \ldots, \alpha_{r-p-s}$ satisfy the following two conditions:*

(i) For all reversible reactions $y_i \rightleftarrows y_j \in R$ with $y_i \to y_j \in F$,

$$
\sum_{L=1}^{r-p-s} [\xi_L \exp(\boldsymbol{y}_i\cdot\boldsymbol{\mu}) + \alpha_L]\, d_{i\to j}^{(L)} \quad \textit{and}
$$

$$
\sum_{L=1}^{r-p-s} [\xi_L \exp(\boldsymbol{y}_j\cdot\boldsymbol{\mu}) + \alpha_L]\, d_{i\to j}^{(L)}
$$

are sign compatible with $(\boldsymbol{y}_j-\boldsymbol{y}_i)\cdot\boldsymbol{\mu}$. (The symbol "·" means the standard dot product.)

(ii) For all irreversible reactions $y_i \to y_j \in R$,

$$\sum_{L=1}^{r-p-s} \xi_L\, d^{(L)}_{i\to j} > 0 \quad \textit{and}$$

$$\sum_{L=1}^{r-p-s} [\xi_L \exp(\boldsymbol{y}_i \cdot \boldsymbol{\mu}) + \alpha_L]\, d^{(L)}_{i\to j} = 0\,.$$

If the answer is yes, the set of solutions $\{\boldsymbol{\mu}, \xi_L, \alpha_L, L=1, \ldots, r-p-s\}$ to the conditions (i) and (ii) can be used to construct a set of positive rate constants and two corresponding positive steady states $\boldsymbol{c}'$ and $\boldsymbol{c}''$. They are presented in the Appendix.

3. Results and Discussion

A spanning subnetwork of a network under consideration consists of all the irreversible reactions and one (and only one) reaction of each reversible reaction pair. A spanning subnetwork for network (1a) can be constructed in the following way:

$$\begin{array}{cccl} A_1 & \to & A_2 & \\ \uparrow & & \downarrow & A_3 + A_4 \to 2A_4\,, \\ A_6 & & A_3 & \\ \uparrow & & \downarrow & A_6 + A_7 \to A_8 \to A_1 + A_7\,. \\ A_5 & \leftarrow & A_4\,, & \end{array} \tag{2}$$

The spanning-subnetwork vectors $\{\boldsymbol{d}^{(1)}, \boldsymbol{d}^{(2)}, \ldots, \boldsymbol{d}^{(r-p-s)}\}$ are the linearly independent (nonzero) solutions to the vector equation

$$\sum_{i\to j\in F} d^{(L)}_{i\to j}(\boldsymbol{y}_j - \boldsymbol{y}_i) = \boldsymbol{0}\,, \quad L = 1, 2, \ldots, r-p-s\,. \tag{3}$$

For the spanning subnetwork (2), from (3) we have $L=1, 2, 3$ $(=r-p-s=18-9-6)$,

$$\begin{aligned} & d^{(L)}_{A_1\to A_2}(\mathbf{A}_2 - \mathbf{A}_1) + d^{(L)}_{A_2\to A_3}(\mathbf{A}_3 - \mathbf{A}_2) \\ & + d^{(L)}_{A_3\to A_4}(\mathbf{A}_4 - \mathbf{A}_3) + d^{(L)}_{A_4\to A_5}(\mathbf{A}_5 - \mathbf{A}_4) \\ & + d^{(L)}_{A_5\to A_6}(\mathbf{A}_6 - \mathbf{A}_5) + d^{(L)}_{A_6\to A_1}(\mathbf{A}_1 - \mathbf{A}_6) \\ & + d^{(L)}_{A_3+A_4\to 2A_4}(2\mathbf{A}_4 - \mathbf{A}_3 - \mathbf{A}_4) \\ & + d^{(L)}_{A_6+A_7\to A_8}(\mathbf{A}_8 - \mathbf{A}_6 - \mathbf{A}_7) \\ & + d^{(L)}_{A_8\to A_1+A_7}(\mathbf{A}_1 + \mathbf{A}_7 - \mathbf{A}_8) = 0\,. \end{aligned} \tag{4}$$

The three sets of linearly independent solutions of (4) are

$$\begin{aligned} & d^{(1)}_{A_1\to A_2} = 1,\; d^{(1)}_{A_2\to A_3} = 1,\; d^{(1)}_{A_3\to A_4} = 1, \\ & d^{(1)}_{A_4\to A_5} = 1,\; d^{(1)}_{A_5\to A_6} = 1,\; d^{(1)}_{A_6\to A_1} = 1, \end{aligned} \tag{5a}$$

and $d^{(1)}_{i\to j} = 0$ for all other reactions $i \to j$ in set F;

$$d^{(2)}_{A_3\to A_4} = -1,\; d^{(2)}_{A_3+A_4\to 2A_4} = 1, \tag{5b}$$

and $d^{(2)}_{i\to j} = 0$ for all other reactions $i \to j$ in set F;

$$\begin{aligned} & d^{(3)}_{A_6\to A_1} = -1,\; d^{(3)}_{A_6+A_7\to A_8} = 1, \\ & d^{(3)}_{A_8\to A_1+A_7} = 1, \end{aligned} \tag{5c}$$

and $d^{(3)}_{i\to j} = 0$ for all other reactions $i \to j$ in set F.

From the spanning subnetwork (2) and its corresponding vectors in (5), the conditions (i) of the necessary and sufficient conditions become

$$\begin{aligned} & \xi_1 \exp(\mu_1) + \alpha_1 \text{ and } \xi_1 \exp(\mu_2) + \alpha_1 \\ & \text{are sign compatible with (s.c.w.) } \mu_2 - \mu_1, \end{aligned} \tag{6a}$$

$$\begin{aligned} & \xi_1 \exp(\mu_2) + \alpha_1 \text{ and } \xi_1 \exp(\mu_3) + \alpha_1 \\ & \text{are s.c.w. } \mu_3 - \mu_2, \end{aligned} \tag{6b}$$

$$\begin{aligned} & \xi_1 \exp(\mu_3) + \alpha_1 - \xi_2 \exp(\mu_3) - \alpha_2 \text{ and} \\ & \xi_1 \exp(\mu_4) + \alpha_1 - \xi_2 \exp(\mu_4) - \alpha_2 \\ & \text{are s.c.w. } \mu_4 - \mu_3, \end{aligned} \tag{6c}$$

$$\begin{aligned} & \xi_1 \exp(\mu_4) + \alpha_1 \text{ and } \xi_1 \exp(\mu_5) + \alpha_1 \\ & \text{are s.c.w. } \mu_5 - \mu_4, \end{aligned} \tag{6d}$$

$$\begin{aligned} & \xi_1 \exp(\mu_5) + \alpha_1 \text{ and } \xi_1 \exp(\mu_6) + \alpha_1 \\ & \text{are s.c.w. } \mu_6 - \mu_5, \end{aligned} \tag{6e}$$

$$\begin{aligned} & \xi_1 \exp(\mu_6) + \alpha_1 - \xi_3 \exp(\mu_6) - \alpha_3 \text{ and} \\ & \xi_1 \exp(\mu_1) + \alpha_1 - \xi_3 \exp(\mu_1) - \alpha_3 \\ & \text{are s.c.w. } \mu_1 - \mu_6, \end{aligned} \tag{6f}$$

$$\begin{aligned} & \xi_2 \exp(\mu_3 + \mu_4) + \alpha_2 \text{ and} \\ & \xi_2 \exp(2\mu_4) + \alpha_2 \text{ are s.c.w. } \mu_4 - \mu_3, \end{aligned} \tag{6g}$$

$$\begin{aligned} & \xi_3 \exp(\mu_6 + \mu_7) + \alpha_3 \text{ and} \\ & \xi_3 \exp(\mu_8) + \alpha_3 \text{ are s.c.w. } \mu_8 - \mu_6 - \mu_7, \end{aligned} \tag{6h}$$

$$\begin{aligned} & \xi_3 \exp(\mu_8) + \alpha_3 \text{ and} \\ & \xi_3 \exp(\mu_1 + \mu_7) + \alpha_3 \text{ are s.c.w. } \mu_1 + \mu_7 - \mu_8. \end{aligned} \tag{6i}$$

(A real number a is said to be s.c.w. a real number b if a and b have the same sign, i.e. a is positive if b is positive, a is negative if b is negative, and a is zero if b is zero.)

The transformation of the first mass conservation condition of (1c) requires that the set $[\mu_7, \mu_8]$ contains both a positive and negative number, or else consists entirely of zeros. Similarly, from the second condition of (1c), the same requirement must be satisfied by the set $[\mu_1, \ldots, \mu_6, \mu_8]$.

We will show that there exists a set of $\boldsymbol{\mu}=[\mu_1, \ldots, \mu_8]$ satisfying (6a)–(6i) and the requirement of mass conservation mentioned above. Equations (6a) and (6b) indicate that $\mu_2-\mu_1$ and $\mu_3-\mu_2$ must have the same sign. Equations (6d) and (6e) indicate that $\mu_5-\mu_4$ and $\mu_6-\mu_5$ must have the same sign. Equations (6h) and (6i) indicate that $\mu_8-\mu_6-\mu_7$ and $\mu_1+\mu_7-\mu_8$ must have the same sign, and the sign must be the same as $\mu_1-\mu_6$. Thus, the terms whose signs are allowed to be changed independently are $\mu_2-\mu_1$, $\mu_5-\mu_4$, $\mu_1-\mu_6$ and $\mu_4-\mu_3$. Several cases are listed in Table 1. The results of the possibility and impossibility of multiple positive steady states for some cases are discussed below.

Case No. 1: Suppose $\mu_2<\mu_1$, $\mu_4>\mu_3$, $\mu_5>\mu_4$ and $\mu_1>\mu_6$. The assumptions $\mu_2<\mu_1$ and $\mu_5>\mu_4$ indicate $\mu_1>\mu_2>\mu_3$, $\mu_6>\mu_5>\mu_4$ and $\xi_1\neq 0$. Equations (6a), (6b), (6d), and (6e) indicate

$$\begin{Bmatrix} \xi_1 \exp(\mu_1) \\ \xi_1 \exp(\mu_2) \\ \xi_1 \exp(\mu_3) \end{Bmatrix} < -\alpha_1 < \begin{Bmatrix} \xi_1 \exp(\mu_4) \\ \xi_1 \exp(\mu_5) \\ \xi_1 \exp(\mu_6) \end{Bmatrix}. \qquad (7)$$

If $\xi_1>0$, (7) leads to $\mu_6>\mu_5>\mu_4>\mu_1>\mu_2>\mu_3$, which contradicts the assumption $\mu_1>\mu_6$. If $\xi_1<0$, (7) leads to $\mu_1>\mu_2>\mu_3>\mu_6>\mu_5>\mu_4$, which contradicts the assumption $\mu_4>\mu_3$. Thus, there doesn't exist any solution of (6a)–(6i). According to the necessary and sufficient condition, network (1) cannot admit multiple positive steady states for this case. The cases No. 2 and 3 in Table 1 are similar to No. 1.

Case No. 4: Suppose $\mu_2>\mu_1$, $\mu_4<\mu_3$, $\mu_5>\mu_4$ and $\mu_1>\mu_6$. The assumptions indicate

$$\mu_1+\mu_7>\mu_8>\mu_6+\mu_7, \qquad (8a)$$

$$\mu_3>\mu_2>\mu_1>\mu_6>\mu_5>\mu_4. \qquad (8b)$$

Equations (6b)–(6d) indicate $-\xi_2\exp(\mu_3)-\alpha_2<0$ and $-\xi_2\exp(\mu_4)-\alpha_2<0$. Equation (6g) indicates $\xi_2\exp(\mu_3+\mu_4)+\alpha_2<0$ and $\xi_2\exp(2\mu_4)+\alpha_2<0$. The combination gives

$$\begin{Bmatrix} \xi_2 \exp(\mu_3+\mu_4) \\ \xi_2 \exp(2\mu_4) \end{Bmatrix} < -\alpha_2 < \begin{Bmatrix} \xi_2 \exp(\mu_3) \\ \xi_2 \exp(\mu_4) \end{Bmatrix}. \qquad (8c)$$

Equation (8c) says $\xi_2\neq 0$. If $\xi_2>0$, (8c) leads to $\mu_3<0$ and $\mu_4<0$. From (8b) we have $\mu_i<0$, $\forall i=1, \ldots, 6$. Satisfaction of the mass conservation condition requires that $\mu_8>0$ and $\mu_7<0$, which contradicts (8a). If $\xi_2<0$, we get $\mu_i>0$, $\forall i=1, \ldots, 6$, $\mu_8<0$ and $\mu_7>0$, which again contradicts (8a). Thus, there is no solution of (6a)–(6i) such that $\boldsymbol{\mu}$ is sign compatible with the stoichiometric subspace for network (1). According to the necessary and sufficient condition, network (1) cannot admit multiple positive steady states for this case. The cases No. 5, 6, and 7 in Table 1 are similar to No. 4.

Case No. 8: Suppose $\mu_2>\mu_1$, $\mu_4<\mu_3$, $\mu_5<\mu_4$ and $\mu_1>\mu_6$. Some analyses give a set of inequalities shown in (9a) to satisfy (6a)–(6i) and to be sign compatible with the stoichiometric subspace for network (1):

$$\begin{aligned} &\mu_3>\mu_2>\mu_1>\mu_4>\mu_5>\mu_6, \\ &\mu_1+\mu_7>\mu_8>\mu_6+\mu_7, \\ &\mu_3>0,\ \mu_4<0,\ \mu_7>0,\ \mu_8<0. \end{aligned} \qquad (9a)$$

Based on (9a), we find the set of solutions

$$\begin{aligned} &\mu_1=0.1,\ \mu_2=0.12,\ \mu_3=0.15, \\ &\mu_4=-0.4,\ \mu_5=-0.5,\ \mu_6=-0.6, \\ &\mu_7=0.4,\ \mu_8=-0.1,\ \xi_1=\xi_2=\xi_3=1, \\ &\alpha_1=-e^{-0.23},\ \alpha_2=-e^{-0.24},\ \alpha_3=-e^{-0.22}. \end{aligned} \qquad (9b)$$

According to (9b) and the formula shown in the Appendix, a set of rate constants and two corresponding positive steady state $\boldsymbol{c}'$ and $\boldsymbol{c}''$ are constructed in (10). The steady states in (10b) satisfy the mass conservation conditions mentioned in (1c).

Table 1. The sign pattern of the possibility of multiple positive steady states for eight cases.

Signs	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
$\mu_2-\mu_1$	–	+	+	+	–	–	–	+
$\mu_4-\mu_3$	+	+	+	–	+	–	–	–
$\mu_5-\mu_4$	+	–	+	+	–	+	–	–
$\mu_1-\mu_6$	+	+	+	+	+	+	+	+
poss. of m.p.s.s.	no	no	no	no	no	no	no	yes

$$A_1 \underset{1.773959}{\overset{2.614154}{\rightleftarrows}} A_2 ,$$

$$A_2 \underset{1.046184}{\overset{1.363810}{\rightleftarrows}} A_3 ,$$

$$A_3 \underset{0.005303}{\overset{0.001735}{\rightleftarrows}} A_4 , \quad A_3 + A_4 \underset{0.002582}{\overset{0.036414}{\rightleftarrows}} 2A_4 ,$$

$$A_4 \underset{0.766181}{\overset{0.971648}{\rightleftarrows}} A_5 , \quad A_6 + A_7 \underset{0.179170}{\overset{2.636854}{\rightleftarrows}} A_8 \underset{0.118578}{\overset{1.082519}{\rightleftarrows}} A_1 + A_7 .$$

$$A_5 \underset{1.469615}{\overset{1.675081}{\rightleftarrows}} A_6 ,$$

$$A_6 \underset{0.002516}{\overset{0.006476}{\rightleftarrows}} A_1 , \tag{10a}$$

$$\begin{aligned}
c_1' &\approx 6.30500 & c_1'' &\approx 5.70500 ,\\
c_2' &\approx 8.84333 & c_2'' &\approx 7.84333 ,\\
c_3' &\approx 10.76874 & c_3'' &\approx 9.26874 ,\\
c_4' &\approx 2.03324 & c_4'' &\approx 3.03324 ,\\
c_5' &\approx 1.54149 & c_5'' &\approx 2.54149 ,\\
c_6' &\approx 1.21637 & c_6'' &\approx 2.21637 ,\\
c_7' &\approx 0.30332 & c_7'' &\approx 0.20332 ,\\
c_8' &\approx 0.95083 & c_8'' &\approx 1.05083 .
\end{aligned} \tag{10b}$$

The change of the steady state concentration c_1 with the rate constant $k_{A_1 \to A_2}$ for network (10a) has been simulated numerically by the Runge-Kutta method. In Fig. 1, the steady states and bistability occurring in network (10a) are illustrated as hysteresis with variation of the rate constant $k_{A_1 \to A_2}$. The steady states c_1' and c_1'' in Fig. 1 are those given in (10b). The steady state $\boldsymbol{c}'$ in (10b) is established at a smaller $k_{A_1 \to A_2}$ (less than about 2.59), while the steady state $\boldsymbol{c}''$ in (10b) is established at a larger $k_{A_1 \to A_2}$ (larger than about 2.617). In between, a hysteresis loop occurs and the steady state depends on the initial concentrations. Figure 2 shows a two-parameter ($k_{A_3 + A_4 \to 2A_4}$, $k_{A_1 \to A_2}$) bifurcation diagram of network (10) for different values of $k_{A_6 + A_7 \to A_8}$. Inside the cusp regions, there are three steady states, two stable ones and one unstable one. Only a single steady state exists outside.

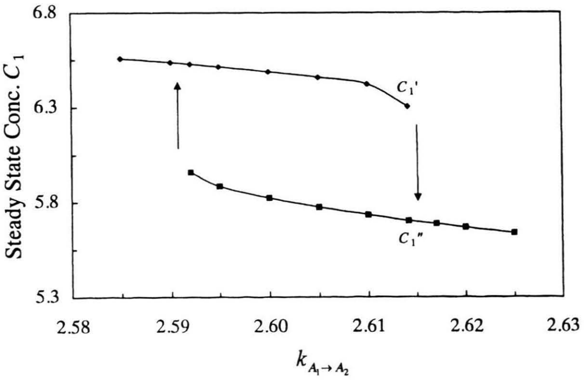

Fig. 1. The change of the steady state concentration c_1 with the rate constant $k_{A_1 \to A_2}$ for network (10).

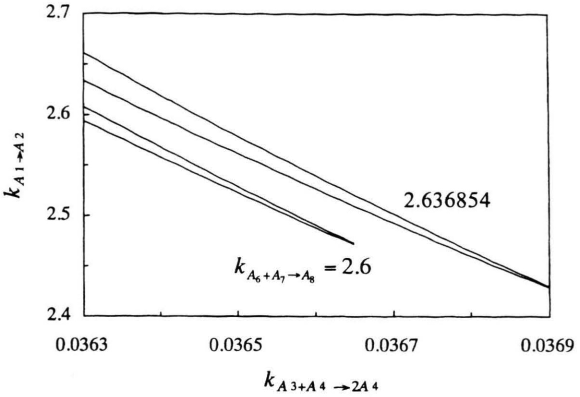

Fig. 2. The locus of multiple-steady-state bifurcation for network (10) in the ($k_{A_3 + A_4 \to 2A_4}$, $k_{A_1 \to A_2}$) plane for different values of the rate constant $k_{A_6 + A_7 \to A_8}$.

4. Concluding Remarks

The necessary and sufficient condition is applied to show that network (1) has the capacity to admit multiple steady states. The vector $\boldsymbol{\mu} \in \mathcal{R}^N$ in the condition is defined by two mass-conservation positive steady states $\boldsymbol{c}'$ and $\boldsymbol{c}''$ [14]:

$$\begin{aligned}
\boldsymbol{\mu} &= [\mu_1, \ldots, \mu_N] = \ln(\boldsymbol{c}'/\boldsymbol{c}'') \\
&= [\ln(c_1'/c_1''), \ldots, \ln(c_N'/c_N'')] .
\end{aligned} \tag{11}$$

From (11), the $\boldsymbol{\mu}$ can be viewed as "signatures" of steady-state multiplicities. For network (1), the results in Table 1 show one set of signatures (No. 8) of multiple positive steady states and deny other seven signatures (No. 1–7). Note that if a pair of steady states, $\boldsymbol{c}'$ and $\boldsymbol{c}''$, is consistent with one of these systems in Table 1, the same pair will be consistent with the inversion, the system obtained by reversing all the inequality signs. This is true simply by interchanging the roles of the two steady states in calculating μ in (11). Thus, the results of Table 1 actually include all of the sixteen cases with no equality signs. For the signatures involving equality signs, the analysis is easier and one can follow the similar procedures discussed above. The inequality (9a) can be applied to identify the reaction mechanism if steady-state concentrations for some species are measured.

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Appendix

Two steady states, $\boldsymbol{c}'$ and $\boldsymbol{c}''$, are computed by (A.1) and (A.2):

$$c'_L = c''_L + \sigma_L\,, \quad L = 1, 2, \ldots, N\,, \tag{A.1}$$

$$c''_L = \begin{cases} \sigma_L/[\exp(\mu_L) - 1]\,, & \text{for } \mu_L \neq 0\,, \\ \text{any positive real number}, & \text{for } \mu_L = 0\,, \end{cases} \tag{A.2}$$

where $\boldsymbol{\sigma}$ is a vector in the stoichiometric subspace ($\boldsymbol{\sigma} \in S_t$) and is sign compatible with $\boldsymbol{\mu}$, i.e., $\operatorname{sign} \sigma_L = \operatorname{sign} \mu_L$, $\forall L = 1, 2, \ldots, N$.

To calculate a rate constant $k_{i\to j}$, first the variable $\kappa_{i\to j}$ is computed according to (A.4)–(A.8). Then, by (A.3), the rate constant $k_{i\to j}$ is derived from variable $\kappa_{i\to j}$ and the steady state $\boldsymbol{c}''$ in (A.2).

$$k_{i\to j} = \frac{\kappa_{i\to j}}{\prod_{L=1}^{N} (c''_L)^{y_{iL}}}\,, \quad \forall\, y_i \to y_j \in R\,, \tag{A.3}$$

where y_{iL} is the stoichiometric coefficient of the species A_L complex y_i.

For all irreversible reactions $i \to j \in R$,

$$\kappa_{i\to j} = \sum_{L=1}^{r-p-s} \xi_L\, d^{(L)}_{i\to j}\,. \tag{A.4}$$

For all reversible reactions $i \rightleftarrows j \in R$ with $i \to j \in F$ and $\boldsymbol{y}_i \cdot \boldsymbol{\mu} \neq \boldsymbol{y}_j \cdot \boldsymbol{\mu}$,

$$\kappa_{i\to j} = \frac{\sum_{L=1}^{r-p-s} [\xi_L \exp(\boldsymbol{y}_j \cdot \boldsymbol{\mu}) + \alpha_L]\, d^{(L)}_{i\to j}}{\exp(\boldsymbol{y}_j \cdot \boldsymbol{\mu}) - \exp(\boldsymbol{y}_i \cdot \boldsymbol{\mu})}\,, \tag{A.5}$$

$$\kappa_{j\to i} = \frac{\sum_{L=1}^{r-p-s} [\xi_L \exp(\boldsymbol{y}_i \cdot \boldsymbol{\mu}) + \alpha_L]\, d^{(L)}_{i\to j}}{\exp(\boldsymbol{y}_j \cdot \boldsymbol{\mu}) - \exp(\boldsymbol{y}_i \cdot \boldsymbol{\mu})}\,. \tag{A.6}$$

For all reversible reaction $i \rightleftarrows j \in R$ with $i \to j \in F$ and $\boldsymbol{y}_i \cdot \boldsymbol{\mu} = \boldsymbol{y}_j \cdot \boldsymbol{\mu}$,

$$\kappa_{i\to j} > 0\,, \quad \kappa_{j\to i} > 0\,, \tag{A.7}$$

$$\kappa_{i\to j} - \kappa_{j\to i} = \sum_{L=1}^{r-p-s} \xi_L\, d^{(L)}_{i\to j}\,. \tag{A.8}$$

The qualified vector $\boldsymbol{\sigma} \in \mathscr{R}^8$ in (A.9) is used to compute the two steady states and the corresponding set of rate constants shown in (10).

$$\sigma_1 = 0.6\,,\ \sigma_2 = 1\,,\ \sigma_3 = 1.5\,,\ \sigma_4 = -1\,,$$
$$\sigma_5 = -1\,,\ \sigma_6 = -1\,,\ \sigma_7 = -0.1\,,\ \sigma_8 = -0.1\,. \tag{A.9}$$

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