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Multi-equilibrium property of metabolic networks: MMN module

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SUMMARY

This paper studies the multi-equilibrium property of the multiple substrates and multiple products with no inhibition (MMN) module. On the basis of the topological structure, a model for such module is established in the form of a set of nonlinear ordinary differential equations. It is shown that the injectivity of the MMN module is equivalent to the nonsingularity of Jacobian matrix of its rate function, and a necessary and sufficient condition for the injectivity is obtained by using the Hadamard product. For non-injective MMN module, a sufficient condition for existence of multiple positive equilibria is provided by introducing the concept of input-matrix. For a type of commonly encountered MMN module—$\mathscr{A}$-MMN module—a structure-oriented criterion for judging its injectivity is given. For $\mathscr{A}$-MMN modules with some special structure, it is shown that there does not exist multiply equilibria and the equilibrium (if exists) is asymptotically stable. Examples and simulations are given to illustrate the results obtained. Copyright © 2012 John Wiley & Sons, Ltd.

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1. INTRODUCTION

Multi-equilibrium of metabolic networks is a fundamental and significant property in systems biology [1–7], including the capacity for admitting multiple equilibria and the dynamic behaviors of the equilibria. The former considers the existence of multiple equilibria and the latter focuses mainly on their stabilities. This problem has very important realistic meaning. For example, the multi-equilibrium property of the photosynthetic carbon metabolic network in the mesophyll cells is closely related to the productivity of food crops [8–10]. It is not only expensive but also difficult, even not possible, to explore this problem via biological experiments. Hence, many scientists tried to investigate this issue by using mathematical models from the theoretical point of view [11–13]. These works depended on not only the structure but also parameter information of the mathematical models. On the one hand, obtaining such information is very difficult. On the other hand, the parameter information varies greatly with individuals and is seriously influenced by environmental factors (temperature, PH value and so forth). For these two main reasons, the theoretical analysis is usually difficult, and the generalization and application of the theoretical results are limited. Compared with both the structure and parameter information of a metabolic network, the structure information is relatively more stable and easier to be obtained. As a result, structure-oriented analysis methods on the multi-equilibrium property of general metabolic networks are eagerly required.

On the basis of the topological structure of networks, some works related to the multi-equilibrium property have been carried out in [14–21], and a summary on these works can be found in [3].

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For chemical reaction networks, Craciun and Feinberg [17] developed a very rich theory to injectivity tests by means of the Jacobian matrix of the rate function based on the law of mass action. For metabolic networks, to make full use of the topological structure and reduce the difficulty and complexity of the theoretical analysis and practical application, Lei *et al*. [3] proposed a 'modularization' idea: regarding a metabolic network as an assembly of some basic building blocks (called metabolic modules) with specific structures and functions, then to investigate the entire network by analyzing the functional characteristic and interactions of these basic modules. According to the numbers of the substrates and products and the existence of inhibition, [3] decomposed a metabolic network into four classes of basic modules, called the Single substrate and Single product with No inhibition (SSN), the Single substrate and Single product with Inhibition (SSI), the Multiple substrate and Multiple product with No inhibition (MMN) and the Multiple substrate and Multiple product with Inhibition (MMI), respectively. Here, a feasible method of realizing such decomposition may be to take initially each metabolic reaction as a module and then extend it to the maximum through adding its neighbor nodes according to the definition of basic module. Furthermore, Lei *et al*. [3] gave an equivalent condition for the injectivity of SSN module, and proved that SSN module with output nodes cannot have multi-equilibrium, the equilibrium (if existed) was shown to be asymptotically stable in [4]. Lei *et al*. [5] studied the SSI module and provided a necessary condition for the existence of multi-equilibrium of the SSI module, in which each reaction had no more than one inhibitor.

This paper focuses on the multiple substrates and multiple products with no inhibition (MMN) module, which commonly exists in metabolic networks. In fact, a metabolic network where every reaction has no inhibitor can be seen as one or more MMN modules. Thus, the study on the multi-equilibrium property of the MMN module not only is a key step to realize the 'modularization' idea proposed by [3] but also can be directly applied to investigate some specific metabolic networks, and provides a valuable basis to the research on general MMI module.

Compared with the SSN modules, the MMN ones are more complex in topological structure. Firstly, the metabolite in the MMN module can be associated with each other by a lot of reactions, and the transformation relationships among metabolites are versatile and flexible, which may cause high complexity of the topological structure. Secondly, the reaction mechanism in the MMN module is also more complicated than the one in SSN module. For example, the rate of a given metabolic reaction in SSN module depends mainly on the concentration of one kind of metabolite, but the one in MMN module may be related to the concentrations of several or even all kinds of metabolites. Thus, the complexity of the MMN module is mainly derived from the strong coupling of substrates and products, which is different from the one in SSI module caused by the negative feedback inhibition.

Because of the special structure of the MMN module, at least three difficulties need to be overcome for the theoretical analysis of the equilibrium property. The first is to set up an unified model, to which the difficulty comes from the complex topology and coupling among metabolic reactions. The second is to deal with the nonlinearity. The rate of a reaction with multiple substrates is multi-variable, which makes the nonlinearity of the model of MMN module stronger than the one of SSN module, or even than the one of SSI module. The methods in [3, 5] do not work here, then we have to develop new techniques to cope with this nonlinearity. The third is to remove parameter information and extract structure information. The starting point of 'modularization' idea is oriented towards the topological structure, and our aim is also to study the effect of the network structure on the multi-equilibrium property. Thus, it is necessary, although difficult because of the increase of model parameters and complexity of network structure, to separate the structure and parameter information effectively and appropriately.

As mentioned in [3], to describe the rate of a metabolic reaction, the Michaelis–Menten and Hill kinetics [22, 23] require less information about the intermediate reactions compared with the law of mass action, and the parameters in the Michaelis–Menten and Hill kinetics have clear biological meaning and can be regulated by experimental techniques. On the other hand, an enzyme-catalyzed metabolic reaction usually contains several intermediate reactions, and they are unknown in many cases. Thus, we take the Hill-form kinetics as the modeling mechanism in this paper, which makes the theoretical analysis more difficult than the one in [17] because the model of a network based on the Hill-form kinetics is much more complex than the one based on the law of mass action.

To establish a unified model and conduct theoretical analysis for the MMN module, we construct a special vector space and use a nonlinear ordinary differential equations to formulate the model by means of the projection operator. It is shown that the injectivity of the MMN module is equivalent to the nonsingularity of Jacobian matrix of its rate function; and then, an easy-to-verify, necessary and sufficient condition for the injectivity is provided by using the Hadamard product. Furthermore, a sufficient condition on the existence of multiple positive equilibria is given via introducing the concept of input-matrix. For a class of commonly encountered MMN module—$\mathscr{A}$-MMN module—a criterion for judging the injectivity is obtained, which is completely based on the network structure. Especially, for a special class of $\mathscr{A}$-MMN module, the Jacobian matrix of their rate functions is shown to be diagonally dominated with a chain of nonzero elements, which implies that such module cannot have multiple equilibria, and the equilibrium (if exists) is asymptotically stable.

The rest of this paper is organized as follows. Section 2 describes the formulation of the MMN module. Section 3 gives the multi-equilibrium property of both the MMN module and the $\mathscr{A}$-MMN module, including the judging criterion of the injectivity and sufficient condition on existence of multiple positive equilibria. Section 4 uses some numerical examples and simulations to demonstrate the effectiveness of the methods and results developed in this paper. Section 5 provides concluding remarks and related future works. Some detailed mathematical results and proofs are put into the appendix section.

2. MODELING OF MMN MODULE

In this section, the modeling method of the MMN module is provided, and some symbols used in this paper are also given. We use a digraph to reveal the inter-relationship among the metabolites, and Hill-form kinetics to describe the reaction rate. On the basis of the network structure, a model of the MMN module is given by a set of ordinary differential equations.

2.1. Definitions and symbols

To clarify what an MMN module is, we need several concepts. Firstly, the metabolic reactions are classified into four classes according to the numbers of substrates and products and the existence of inhibitions.

Definition 2.1 ([3]*)*
A metabolic reaction is called a single substrate and single product (SS) reaction, if it contains only one substrate and one product; otherwise, called a multiple substrate or multiple product (MM) reaction. An SS (or MM) metabolic reaction is called an SS (or MM) reaction with inhibition, SSI (or MMI) for short, if there exist some inhibitors of the reaction; otherwise, called an SS (or MM) reaction with no inhibition, SSN (or MMN) for short.

Then, the metabolites in a group of reactions with no inhibition are classified into three classes.

Definition 2.2
For a group of metabolic reactions with no inhibition, its interaction graph is a digraph by viewing the metabolite and reaction as its node and edge, whose direction is from the substrate to product. A node is called an input node, if the direction of each edge connecting it points to other nodes; a node is called an output node, if the direction of each edge connecting it points to itself; a node is called a state node if it is neither an input node nor an output node.

Remark 2.1
A reversible reaction will be viewed as two reactions of a forward one and a reverse one. For example, regard $A + B \overset{E}{\rightleftharpoons} C + D$ as a combination of the forward reaction $A + B \overset{E}{\rightarrow} C + D$ and the reverse reaction $C + D \overset{E}{\rightarrow} A + B$.

Now, we can give the definition of the MMN module.

Definition 2.3
(MMN Module)
For a given metabolic network, the set of all the metabolites is denoted by $\mathscr{M}_{\mathcal{N}}$, and the set of all the reactions is denoted by $\mathscr{R}_{\mathcal{N}}$. $(\mathscr{M}, \mathscr{R})$ is called an MMN metabolic module of this network, if the following conditions are satisfied:

(i) $\mathscr{M}$ is a nonempty subset of $\mathscr{M}_{\mathcal{N}}$;
(ii) $\mathscr{R} \subseteq \mathscr{R}_{\mathcal{N}}$ is nonempty and consists of all the reactions that are relevant to the metabolites in $\mathscr{M}$. Here, a reaction is said to be relevant to a metabolite S, if S is a substrate, product or inhibitor of this reaction;
(iii) all the reactions in $\mathscr{R}$ have no inhibitor;
(iv) if there exist input nodes and output nodes, then for any state node $S \in \mathscr{M}$, there exists a directed path from some input node to S and a directed path from S to some output node in the interaction graph of $\mathscr{R}$; and
(v) the undirected graph constructed as follows is connected: remove all the input nodes and output nodes (if any) and edges connected them, and replace each directed edge by an undirected edge in the interaction graph of $\mathscr{R}$.

Remark 2.2
In the definition earlier, (iv) and (v) proceed from the practical metabolic networks and the 'modularization' idea. As Lei *et al*. [3] mentioned, (iv) is from biological systems, mainly because in a living organism any metabolite must be synthesized from other metabolites and be converted into an output; (v) is essential for the modularization decomposition.

Remark 2.3
(i) If all the reactions in $(\mathscr{M}, \mathscr{R})$ are SSN reactions, then $(\mathscr{M}, \mathscr{R})$ is an SSN module [3].
(ii) Denote the set of input nodes, the set of output nodes and the set of state nodes by $\mathscr{I}$, $\mathscr{O}$ and $\mathscr{S}$, respectively. By Definition 2.2, it can be seen that

$$\mathscr{I} \cap \mathscr{O} = \emptyset, \quad (\mathscr{I} \cup \mathscr{O}) \cap \mathscr{S} = \emptyset, \quad \mathscr{I} \cup \mathscr{O} \cup \mathscr{S} = \mathscr{M}.$$

(iii) A metabolic network without any inhibitor can always be divided into one or more MMN modules. Especially, if (iv) and (v) of Definition 2.3 are satisfied, then the network is an MMN module.

Here, we use an example to explain the definitions and symbols given earlier.

Example 2.1
The following is a group of metabolic reactions without inhibition:

$$H_1 \to S_1, S_1 \to S_2, S_1 \to S_3, S_1 + H_2 \to S_4,$$

$$S_2 \to S_5, S_2 + S_3 \to S_6 + P, S_3 + S_4 \to S_5 + S_6, S_5 \to S_2, S_6 \to S_4,$$

whose interaction graph (Figure 1) can be drawn according to Definition 2.2. The set containing all these reactions is denoted by $\mathscr{R}_0$, and $\mathscr{M}_0 \triangleq \{H_1, H_2, S_1, S_2, S_3, S_4, S_5, S_6, P\}$. By Definition 2.3, $(\mathscr{M}_0, \mathscr{R}_0)$ is an MMN module, the set of state nodes $\mathscr{S}_0 = \{S_1, S_2, S_3, S_4, S_5, S_6\}$, the set of input nodes $\mathscr{I}_0 = \{H_1, H_2\}$ and the set of output nodes $\mathscr{O}_0 = \{P\}$.

If $S_1 \to S_2$, $S_1 \to S_3$, $S_3 + S_4 \to S_5 + S_6$ and $S_6 \to S_4$ were removed from $\mathscr{R}_0$, then the rest will be divided into two MMN modules because (iv) and (v) in Definition 2.3 do not hold.

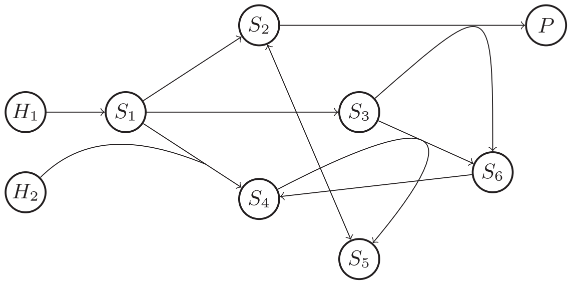

Figure 1. Interaction graph of a group of metabolic reactions with no inhibition.

For a finite set I and number field $\mathbb{K}$, the vector space over $\mathbb{K}$ generated by I is denoted by $\mathbb{K}^I = \left\{\sum_{i\in I} \alpha_i i : \alpha_i \in \mathbb{K}\right\}$; for $J \subseteq I$, the projection operator $\Omega_J(\cdot)$ from $\mathbb{K}^I$ to $\mathbb{K}^J$ is given by

$$\Omega_J : \mathbb{K}^I \to \mathbb{K}^J$$
$$\sum\nolimits_{i\in I} \alpha_i i \mapsto \sum\nolimits_{i\in J} \alpha_i i.$$

In addition, $\mathbb{Z}_+$, $\overline{\mathbb{Z}}_+$, $\mathbb{R}$, $\mathbb{R}_+$ and $\overline{\mathbb{R}}_+$ are the sets of positive integers, non-negative integers, real numbers, positive real numbers and non-negative real numbers, respectively; '$0^0 = 1$' is appointed in this paper.

2.2. Model

Before modeling the MMN module, we firstly give an appropriate expression to describe the rate of the reaction with no inhibition and consider a metabolic reaction with N_s kinds of substrates and N_p kinds of products:

$$a_1 A_1 + \cdots + a_{N_s} A_{N_s} \to b_1 B_1 + \cdots + b_{N_P} B_{N_p}, \tag{1}$$

where $a_i \in \overline{\mathbb{Z}}_+$ and $b_j \in \overline{\mathbb{Z}}_+$ are the stoichiometric coefficients with respect to the substrate A_i and product B_j, respectively, $1 \leqslant i \leqslant N_s$, $1 \leqslant j \leqslant N_p$. We adopt the following Hill-form kinetics to describe the rate of (1):

$$v = V \cdot \prod_{i=1}^{N_s} \left(\frac{[A_i]^{n_i}}{k_i + [A_i]^{n_i}}\right)^{a_i}, \tag{2}$$

where $[\cdot]$ denotes the concentration of '$\cdot$'; the parameters $V \in \mathbb{R}_+$, $k_i \in \mathbb{R}_+$ and $n_i \in \mathbb{Z}_+$ are the maximum reaction rate, Michaelis–Menten constant and Hill coefficient, respectively. The reaction (1) generates A_i with the rate of $a_i v$ and consumes B_j with the rate of $b_j v$, $1 \leqslant i \leqslant N_s$ and $1 \leqslant j \leqslant N_p$.

Remark 2.4
Here, the main reasons of using (2) to describe the rate of (1) are as follows:

(i) if $N_s = 1$, then (2) is the Hill kinetics [22];
(ii) if (1) is a bi-substrate reaction, then the Michaelis-Menten kinetics [13, 23] is a special form of (2); and
(iii) in practice, the metabolic reaction with three or more substrates is not so common, but its real rate is very complex. In this case, (2) is just an approximate rate of (1), whose advantage is that (2) still obeys the Hill or Michaelis–Menten mechanism when the concentrations of $N_s - 1$ or $N_s - 2$ kinds of substrates are constant.

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Remark 2.5
From (2), it can be seen that the reaction rate of (1) described by the Hill-form kinetics is a rational fractional function with $2N_s + 1$ parameters, but the one described by the law of mass action in [17] is a multilinear function with only one parameter.

Now, we set up the model of the MMN module $(\mathscr{M}, \mathscr{R})$. Let $\mathscr{I}$, $\mathscr{O}$ and $\mathscr{S}$ be the set of input nodes, the set of output nodes and the set of state nodes, respectively. Then, a reaction in $\mathscr{R}$ can be represented as

$$\mathcal{A} \to \mathcal{B}, \quad \mathcal{A} \in \overline{\mathbb{R}}_+^{\mathscr{I}\cup\mathscr{S}}, \quad \mathcal{B} \in \overline{\mathbb{R}}_+^{\mathscr{S}\cup\mathscr{O}}.$$

Assume that there are n kinds of metabolites in $\mathscr{S}$ and m reactions in $\mathscr{R}$ and denote

$$\mathscr{S} = \{S_1, \ldots, S_n\},$$

$$\mathscr{R} = \{\mathcal{A}_1 \to \mathcal{B}_1, \ldots, \mathcal{A}_m \to \mathcal{B}_m\}.$$

The coordinate vector of $\Omega_{\mathscr{S}}(\mathcal{A}_j)$, which is the projection of $\mathcal{A}_j$ from $\overline{\mathbb{R}}_+^{\mathscr{I}\cup\mathscr{S}}$ to $\overline{\mathbb{R}}_+^{\mathscr{S}}$, with respect to the basis $\{S_1, \ldots, S_n\}$ is denoted by α_j. Similarly, the coordinate vector of $\Omega_{\mathscr{S}}(\mathcal{B}_j)$, which is the projection of $\mathcal{B}_j$ from $\overline{\mathbb{R}}_+^{\mathscr{S}\cup\mathscr{O}}$ to $\overline{\mathbb{R}}_+^{\mathscr{S}}$, with respect to the basis $\{S_1, \ldots, S_n\}$ is denoted by β_j. $\alpha_{i,j}$ and $\beta_{i,j}$ are the i-th component of α_j and β_j, respectively, $1 \leqslant i \leqslant n$, $1 \leqslant j \leqslant m$. Represent the set of input nodes as $\mathscr{I} = \left\{I_{\bar{1}}, \ldots, I_{\bar{l}}\right\}$, denote the coordinate vector of $\Omega_{\mathscr{I}}(\mathcal{A}_j)$ with respect to the basis $\left\{I_{\bar{1}}, \ldots, I_{\bar{l}}\right\}$ by γ_j, and let $\gamma_{\bar{\tau},j}$ be the coordinate component of γ_j with respect to $I_{\bar{\tau}}$, $1 \leqslant \tau \leqslant l$, $1 \leqslant j \leqslant m$.

The rate of $\mathcal{A}_j \to \mathcal{B}_j$ is denoted by v_j, which is given by (2), that is,

$$v_j = \mu_j V_j \prod_{i=1}^{n} \left(\frac{x_i^{n_{i,j}}}{k_{i,j} + x_i^{n_{i,j}}} \right)^{\alpha_{i,j}}, \tag{3}$$

where $\mu_j = \prod_{\tau=1}^{l} \left(\frac{[I_\tau]^{n_{\bar{\tau},j}}}{k_{\bar{\tau},j} + [I_\tau]^{n_{\bar{\tau},j}}} \right)^{\gamma_{\bar{\tau},j}}$, $x_i = [S_i]$, $1 \leqslant j \leqslant m$, $1 \leqslant i \leqslant n$. Let $\mu = \{\mu_1, \ldots, \mu_m\}^T$, $\nu = \{V_1, \ldots, V_m\}^T$, $\kappa_j = (k_{1,j}, \ldots, k_{n,j})^T$, $\eta_j = (n_{1,j}, \ldots, n_{n,j})^T$, $\kappa = (\kappa_1, \ldots, \kappa_m)$ and $\eta = (\eta_1, \ldots, \eta_m)$. Then, $v_j = v_j(x) = v_j\left(x; \mu_j, V_j, \kappa_j, \eta_j\right)$ with $x = (x_1, \ldots, x_n)^T$, $j = 1, \ldots, m$.

Given $S_i \in \mathscr{S}$, the change rate of its concentration is equal to the rate of generating it minus the one of consuming it, or mathematically,

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = \sum_{j=1}^{m} (\beta_{i,j} - \alpha_{i,j}) v_j, \quad 1 \leqslant i \leqslant n. \tag{4}$$

Then, taking the concentrations of state nodes x as the variables, one can obtain a model of $(\mathscr{M}, \mathscr{R})$ with respect to the rate form (2):

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \begin{pmatrix} \frac{\mathrm{d}x_1}{\mathrm{d}t} \\ \vdots \\ \frac{\mathrm{d}x_n}{\mathrm{d}t} \end{pmatrix} = \begin{pmatrix} r_1(x; \mu, \nu, \kappa, \eta) \\ \vdots \\ r_n(x; \mu, \nu, \kappa, \eta) \end{pmatrix} \triangleq r(x; \mu, \nu, \kappa, \eta) = \sum_{j=1}^{m} (\beta_j - \alpha_j) v_j, \tag{5}$$

where $r_i(x; \mu, \nu, \kappa, \eta)$ is given by the right of (4), $i = 1, \ldots, n$. Let $P = (\alpha_1, \ldots, \alpha_m)$, $Q = (\beta_1, \ldots, \beta_m)$, $W = Q - P$ and $v = (v_1, \ldots, v_m)^T$. By (5), we have

$$\frac{\mathrm{d}x}{\mathrm{d}t} = r(x; \mu, \nu, \kappa, \eta) = Wv. \tag{6}$$

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Remark 2.6

(i) Only the concentrations of the state nodes are viewed as the variables of model (5), the ones of the input nodes are viewed as the model parameters.

(ii) The matrices P and Q represent the structure information of the MMN module, which are not related to the exact rate form of the metabolic reaction and parameters in the rate.

Definition 2.4

$r(x; \mu, \nu, \kappa, \eta)$ is called the rate function of $(\mathscr{M}, \mathscr{R})$ with respect to the rate form (2), or the rate function for short; accordingly, $R = \left\{ r(x; \mu, \nu, \kappa, \eta) : \mu \in \mathbb{R}_+^m, \nu \in \mathbb{R}_+^m, \kappa \in \mathbb{R}_+^n \times \mathbb{R}_+^m, \eta \in \mathbb{Z}_+^n \times \mathbb{Z}_+^m \right\}$ is called the set of rate functions of $(\mathscr{M}, \mathscr{R})$. If, for any given $r \in R$, r is injective on $\mathbb{R}_+^n$, then we say $(\mathscr{M}, \mathscr{R})$ is injective. Otherwise, $(\mathscr{M}, \mathscr{R})$ is non-injective.

Definition 2.5

$\bar{x} \in \mathbb{R}_+^n$ is called an equilibrium of $(\mathscr{M}, \mathscr{R})$, if there exists $r \in R$ such that $r(\bar{x}) = 0$. If there exists $r \in R$ such that the algebraic equation $r = 0$ has at least two roots in $\mathbb{R}_+^n$, then we say $(\mathscr{M}, \mathscr{R})$ has the capacity for admitting multiple positive equilibria; otherwise, $(\mathscr{M}, \mathscr{R})$ has not the capacity for admitting multiple positive equilibria.

3. MULTI-EQUILIBRIUM PROPERTY OF MMN MODULE

This section gives two criteria for judging the injectivity of MMN module. For the non-injective MMN module, a sufficient condition for existence of multiple positive equilibria is provided. Then, the results are used to analyze the multi-equilibrium property of a special type of the MMN module—$\mathscr{A}$-MMN module.

3.1. Injectivity criterion

By Definition 2.4–2.5, it can be seen that the injectivity of the MMN module is a sufficient condition for the absence of multiple equilibria or the non-injectivity is a necessary condition for the existence of multiple equilibria. But verifying whether a vector-valued function is injective is not easy, and even quite difficult when some parameters are unknown. This subsection will give two equivalent conditions for the injectivity of the MMN module, with which verifying injectivity becomes easier or even can be realized by the use of computer softwares.

To make the thought concise, we will begin with the case of scalar functions. Let f be with one variable and continuously differentiable. Then, f is injective if and only if its derivative is nonzero everywhere because of Differential Mean-Value Theorem. This naturally arises a question whether Jacobian matrix of the vector-valued function can be used to determine its injectivity. It is regrettable that the injectivity of the vector-valued function is not equivalent to the everywhere nonsingularity of its Jacobian matrix. For example, given the function $f_1(s,t) = \left(\sqrt{2}e^{s/2}\cos(te^{-s}), \sqrt{2}e^{s/2}\sin(te^{-s})\right)^T$ [24], the determinant of its Jacobian matrix is $\det\left[\left(\frac{\partial f_1}{\partial s}, \frac{\partial f_1}{\partial t}\right)\right] \equiv 1$ and $f_1(0, t + 2k\pi) = f_1(0,t)$. This tells us that f_1 is non-injective, although its Jacobian matrix is nonsingular. For function $f_2(s,t) = \left(\frac{1}{3}(s-1)^3, t\right)^T$ [5], it can be seen that $f_2(s,t)$ is injective on $\mathbb{R}^2$, although the determinant of its Jacobian matrix is zero on $\{(s,t) \in \mathbb{R}^2 : s = 1\}$ because $\det\left[\left(\frac{\partial f_2}{\partial s}, \frac{\partial f_2}{\partial t}\right)\right] = (s-1)^2$. Fortunately, the rate function of the MMN module has a property similar to the Differential Mean-Value Theorem. On the basis of such property, we can prove that the injectivity of the MMN module is equivalent to the everywhere nonsingularity of Jacobian matrix of its rate function.

For convenience, denote the Jacobian matrix $\frac{\mathrm{d}r}{\mathrm{d}x}$ by $\mathscr{D}_r(x)$.

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Lemma 3.1
For any $r \in R$, $b = (b_1, \ldots, b_n)^T \in \mathbb{R}_+^n$ and $a = (a_1, \ldots, a_n)^T \in \mathbb{R}_+^n$, there exist $\tilde{r} \in R$ and $c = (c_1, \ldots, c_n)^T \in \mathbb{R}_+^n$ such that

$$r(b) - r(a) = \mathscr{D}_{\tilde{r}}(c)(b-a) \tag{7}$$

and

$$\min\{a_i, b_i\} \leqslant c_i \leqslant \max\{a_i, b_i\}, \quad 1 \leqslant i \leqslant n. \tag{8}$$

Proof
Choose $\tilde{r}$ and c by the way of Lemma A.5, and c_i as follows

$$c_i = \begin{cases} \frac{b_i - a_i}{\ln b_i - \ln a_i}, & a_i \neq b_i; \\ a_i, & a_i = b_i. \end{cases}$$

Then, $c_i > 0$ and $\xi_i = b_i - a_i$, $1 \leqslant i \leqslant n$. By Lemma A.5, (7) is true.

Without loss of generality, for any given i, let $b_i \geqslant a_i$. If $b_i = a_i$, then $a_i \leqslant c_i \leqslant b_i$; otherwise, consider the following functions:

$$g_1(t) = b_i \ln b_i - b_i + t - b_i \ln t, \quad 0 < t \leqslant b_i,$$

$$g_2(t) = a_i \ln a_i - a_i + t - a_i \ln t, \quad a_i \leqslant t.$$

Noticing $g_1'(t) = 1 - b_i/t$, $g_1(b_i) = 0$ and $g_2'(t) = 1 - a_i/t$, $g_2(a_i) = 0$, we have $g_1(t) \geqslant 0$, $0 < t \leqslant b_i$ and $g_2(t) \geqslant 0$, $a_i \leqslant t$. Thus,

$$b_i - c_i = b_i - \frac{b_i - a_i}{\ln b_i - \ln a_i} = \frac{g_1(a_i)}{\ln b_i - \ln a_i} > 0,$$

$$c_i - a_i = \frac{b_i - a_i}{\ln b_i - \ln a_i} - a_i = \frac{g_2(b_i)}{\ln b_i - \ln a_i} > 0,$$

which implies $a_i \leqslant c_i \leqslant b_i$. Therefore, one can obtain (8). □

Remark 3.1
At first glance, this lemma is very similar to the well-known Differential Mean Value Theorem. But, actually there are some differences between them. Firstly, r and $\tilde{r}$ are often not equal. Secondly, $\tilde{r}$ may depend on a and b.

Lemma 3.2
For any $r \in R$, $c \in \mathbb{R}_+^n$ and $\xi \in \mathbb{R}^n$, there exist $\tilde{r} \in R$, $b \in \mathbb{R}_+^n$ and $a \in \mathbb{R}_+^n$ such that

$$\mathscr{D}_r(c)\xi = \tilde{r}(b) - \tilde{r}(a)$$

and

$$b - a = \xi.$$

Proof
Choose $\tilde{r}$, b and a by the way of Lemma A.6, and a_i as follows

$$a_i = \begin{cases} \frac{\xi_i}{e^{\xi_i/c_i} - 1}, & \xi_i \neq 0; \\ \text{any positive real number}, & \xi_i = 0. \end{cases}$$

Then, we have

$$a_i > 0, \quad b_i = a_i + \xi_i = \frac{\xi_i e^{\xi_i/c_i}}{e^{\xi_i/c_i} - 1} > 0, \quad 1 \leqslant i \leqslant n,$$

which implies the lemma by Lemma A.6. □

Theorem 3.1
The MMN module $(\mathscr{M}, \mathscr{R})$ is injective if and only if

$$\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) \neq 0, \quad \forall x \in \mathbb{R}_+^n, \quad \forall r \in R. \tag{9}$$

Proof
Firstly, prove the sufficiency. Supposing (9) is true, we prove that $(\mathscr{M}, \mathscr{R})$ is injective. If $(\mathscr{M}, \mathscr{R})$ was non-injective, then there would exist $r \in R$ such that r is non-injective on $\mathbb{R}_+^n$, that is to say, there would exist $a \in \mathbb{R}_+^n$ and $b \in \mathbb{R}_+^n$ such that

$$b - a \neq 0, \quad r(b) - r(a) = 0. \tag{10}$$

For these r, a and b, by Lemma 3.1, there would exist $\tilde{r} \in R$ and $c \in \mathbb{R}_+^n$ such that

$$r(b) - r(a) = \mathscr{D}_{\tilde{r}}(c)(b - a),$$

which together with (10) implies $\det(\mathscr{D}_{\tilde{r}}(c)) = 0$. This contradicts (9). Thus, $(\mathscr{M}, \mathscr{R})$ is injective, and the sufficiency is proved.

Now, we prove the necessity. Supposing $(\mathscr{M}, \mathscr{R})$ is injective, we prove (9). If (9) was not true, then there would exist $r \in R$ and $c \in \mathbb{R}_+^n$ such that $\det(\mathscr{D}_r(c)) = 0$. Therefore, there would exist $0 \neq \xi \in \mathbb{R}^n$ such that

$$\mathscr{D}_r(c)\xi = 0. \tag{11}$$

For these r, c and ξ, by Lemma 3.2, there would exist $\tilde{r} \in R$, $a \in \mathbb{R}_+^n$ and $b \in \mathbb{R}_+^n$ such that

$$b - a = \xi, \quad \mathscr{D}_r(c)\xi = \tilde{r}(b) - \tilde{r}(a). \tag{12}$$

Noticing $a \neq b$ because of $\xi \neq 0$, by (11) and (12), we know that $\tilde{r}$ is non-injective on $\mathbb{R}_+^n$, which contradicts the injectivity of $(\mathscr{M}, \mathscr{R})$. Thus, (9) is true, which implies the necessity. □

Corollary 3.1
If W in (6) satisfies

$$\mathrm{Rank}(W) < n, \tag{13}$$

then $(\mathscr{M}, \mathscr{R})$ is non-injective.

Proof
By (6), we have $\frac{\mathrm{d}r}{\mathrm{d}x} = W\frac{\mathrm{d}v}{\mathrm{d}x}$, which together with (13) implies

$$\mathrm{Rank}\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) \leqslant \min\left\{\mathrm{Rank(W)}, \mathrm{Rank}\left(\frac{\mathrm{d}v}{\mathrm{d}x}\right)\right\} < n.$$

Thus, $\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) \equiv 0$. By Theorem 3.1, the corollary is obtained. □

Corollary 3.2
If $m < n$, then $(\mathscr{M}, \mathscr{R})$ is non-injective.

Remark 3.2
If the number of metabolic reactions is less than the one of state nodes, then the MMN module is non-injective by Corollary 3.2.

Theorem 3.1 plays an important role in this paper and supplies a method of judging injectivity. More importantly, it is also a bridge between structure-oriented method and the multi-equilibrium study on the MMN module. On the basis of this theorem, we can obtain an easy-to-verify equivalent condition on injectivity.

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Definition 3.1 ([25])
Let $A = (a_{ij})_{m\times n}$ and $B = (b_{ij})_{m\times n}$. The Hadamard or Schur product of A and B is the matrix $A \circ B = (a_{ij}b_{ij})_{m\times n}$.

Definition 3.2
For the n-by-m matrix U with $n \leqslant m$, putting its j_1-th, ..., j_n-th column together in sequence forms a new square matrix denoted by $U(j_1,\dots,j_n)$, where $j_i \in M \triangleq \{1,\dots,m\}$, $1 \leqslant i \leqslant n$.

Lemma 3.3
Let $E = E(x;\kappa,\eta) = (\zeta_1,\dots,\zeta_m)$, where ζ_j is given by (A.1), $j = 1,\dots,m$. If $m < n$, then

$$\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) = 0. \tag{14}$$

Otherwise,

$$\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) = \sum_{\{j_1,\dots,j_n\}\subseteq M}\prod_{i=1}^{n} x_i^{-1}v_{j_i}\det(W(j_1,\dots,j_n))\det(E(j_1,\dots,j_n)\circ P(j_1,\dots,j_n)). \tag{15}$$

Proof
If $m < n$, (14) is obtained by Corollary 3.1. Otherwise, by (A.3) we have

$$\begin{aligned}
&\left(\prod_{i=1}^{n} x_i\right)\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right)\\
&=\left(\prod_{i=1}^{n} x_i\right)\det\left(\left[\frac{\partial r}{\partial x_1},\dots,\frac{\partial r}{\partial x_n}\right]\right)\\
&=\left(\prod_{i=1}^{n} x_i\right)\det\left(\left[\sum_{j=1}^{m}(\beta_j-\alpha_j)v_j\frac{k_{1,j}n_{1,j}}{k_{1,j}+x_1^{n_{1,j}}}\frac{\alpha_{1,j}}{x_1},\dots,\sum_{j=1}^{m}(\beta_j-\alpha_j)v_j\frac{k_{n,j}n_{n,j}}{k_{n,j}+x_n^{n_{n,j}}}\frac{\alpha_{n,j}}{x_n}\right]\right)\\
&=\det\left(\left[\sum_{j=1}^{m}(\beta_j-\alpha_j)v_j\frac{k_{1,j}n_{1,j}}{k_{1,j}+x_1^{n_{1,j}}}\alpha_{1,j},\dots,\sum_{j=1}^{m}(\beta_j-\alpha_j)v_j\frac{k_{n,j}n_{n,j}}{k_{n,j}+x_n^{n_{n,j}}}\alpha_{n,j}\right]\right)\\
&=\sum_{j_1\in M}\cdots\sum_{j_n\in M}\det\left(\left[\frac{(\beta_{j_1}-\alpha_{j_1})v_{j_1}k_{1,j_1}n_{1,j_1}\alpha_{1,j_1}}{k_{1,j_1}+x_1^{n_{1,j_1}}},\dots,\frac{(\beta_{j_n}-\alpha_{j_n})v_{j_n}k_{n,j_n}n_{n,j_n}\alpha_{n,j_n}}{k_{n,j_n}+x_n^{n_{n,j_n}}}\right]\right)\\
&=\sum_{j_1\in M}\cdots\sum_{j_n\in M}\prod_{i=1}^{n}v_{j_i}\det\left(\left[\frac{(\beta_{j_1}-\alpha_{j_1})k_{1,j_1}n_{1,j_1}\alpha_{1,j_1}}{k_{1,j_1}+x_1^{n_{1,j_1}}},\dots,\frac{(\beta_{j_n}-\alpha_{j_n})k_{n,j_n}n_{n,j_n}\alpha_{n,j_n}}{k_{n,j_n}+x_n^{n_{n,j_n}}}\right]\right).
\end{aligned} \tag{16}$$

For given $\{j_1,\dots,j_n\}\subseteq M$, denote Δ be the permutation group generated by $\{j_1,\dots,j_n\}$. Then, the coefficient of $\prod_{i=1}^{n} v_{j_i}$ in (16) is

$$\begin{aligned}
&\sum_{\delta\in\Delta}\det\left(\left[\frac{(\beta_{\delta(1)}-\alpha_{\delta(1)})k_{1,\delta(1)}n_{1,\delta(1)}\alpha_{1,\delta(1)}}{k_{1,\delta(1)}+x_1^{n_{1,\delta(1)}}},\dots,\frac{(\beta_{\delta(n)}-\alpha_{\delta(n)})k_{n,\delta(n)}n_{n,\delta(n)}\alpha_{n,\delta(n)}}{k_{n,\delta(n)}+x_n^{n_{n,\delta(n)}}}\right]\right)\\
&=\sum_{\delta\in\Delta}\prod_{i=1}^{n}\frac{k_{i,\delta(i)}n_{i,\delta(i)}}{k_{i,\delta(i)}+x_i^{n_{i,\delta(i)}}}\alpha_{i,\delta(i)}\det(W(\delta(1),\dots,\delta(n)))\\
&=\sum_{\delta\in\Delta}\prod_{i=1}^{n}\frac{k_{i,\delta(i)}n_{i,\delta(i)}}{k_{i,\delta(i)}+x_i^{n_{i,\delta(i)}}}\alpha_{i,\delta(i)}\mathrm{sign}(\delta)\det(W(j_1,\dots,j_n)).
\end{aligned}$$

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By (A.1) and Definition 3.1, we have

$$\sum_{\delta\in\Delta}\prod_{i=1}^{n}\frac{k_{i,\delta(i)}n_{i,\delta(i)}}{k_{i,\delta(i)}+x_i^{n_{i,\delta(i)}}}\alpha_{i,\delta(i)}\mathrm{sign}(\delta)=\det(E(j_1,\ldots,j_n)\circ P(j_1,\ldots,j_n)).$$

Thus, the lemma is proved. □

Remark 3.3
$\prod_{i=1}^{n} x_i^{-1} v_{j_i} \det(W(j_1,\ldots,j_n))\det(E(j_1,\ldots,j_n)\circ P(j_1,\ldots,j_n))$ is called a term in the determinant expansion of Jacobian matrix $\frac{\mathrm{d}r}{\mathrm{d}x}$.

Definition 3.3
Given a group of functions $f_1(\cdot),\ldots,f_\lambda(\cdot)$, we say the functions have the same sign on the set X if

$$\mathrm{sign}(f_1(x))=\mathrm{sign}(f_2(x))=\cdots=\mathrm{sign}(f_\lambda(x)),\quad \forall x\in X.$$

Theorem 3.2
The MMN module $(\mathscr{M},\mathscr{R})$ is injective if and only if $m\geqslant n$ and there exist nonzero terms in the determinant expansion of $\frac{\mathrm{d}r}{\mathrm{d}x}$, and all these nonzero terms have the same sign on $\mathbb{R}_+^n$ for any $r\in R$.

Proof
By Theorem 3.1 and Lemma 3.3, the sufficiency is true.

The following is the proof of the necessity. Suppose $(\mathscr{M},\mathscr{R})$ is injective. Then, by Corollary 3.2, we have $m\geqslant n$. By Theorem 3.1 and Lemma 3.3, there exist nonzero terms in the determinant expansion of $\frac{\mathrm{d}r}{\mathrm{d}x}$. If these nonzero terms do not have the same sign on $\mathbb{R}_+^n$, then there exist $\hat{x}\in\mathbb{R}_+^n$, $\{j_1,\ldots,j_n\}\subseteq M$ and $\{i_1,\ldots,i_n\}\subseteq M$ such that

$$\det(W(j_1,\ldots,j_n))\det\left(E\left(\hat{x};\kappa,\eta\right)(j_1,\ldots,j_n)\circ P(j_1,\ldots,j_n)\right)>0,$$

$$\det(W(i_1,\ldots,i_n))\det\left(E\left(\hat{x};\kappa,\eta\right)(i_1,\ldots,i_n)\circ P(i_1,\ldots,i_n)\right)<0.$$

Hence,

$$D_r\left(\hat{x}\right)\xrightarrow{V_{j_1}\to\infty,\ldots,V_{j_n}\to\infty}\infty,\quad D_r\left(\hat{x}\right)\xrightarrow{V_{i_1}\to\infty,\ldots,V_{i_n}\to\infty}-\infty.$$

Because $\det\left(\frac{\mathrm{d}}{\mathrm{d}x}r(x;\mu,\nu,\kappa,\eta)\right)$ is continuous with respect to ν, there exists $\bar{\nu}$ such that

$$r\left(x;\mu,\bar{\nu},\kappa,\eta\right)\in R,\quad D_{r(x;\mu,\bar{\nu},\kappa,\eta)}\left(\hat{x}\right)=0,$$

which contradicts the injectivity of $(\mathscr{M},\mathscr{R})$. Thus, the necessity is proved. □

Theorem 3.1–3.2 present sufficient and necessary conditions for not only the injectivity but also the non-injectivity of the $(\mathscr{M},\mathscr{R})$. On the basis of these conditions, we can conveniently verify the non-injectivity of the MMN module. But the non-injectivity is only a necessary condition on the capacity for admitting multiple positive equilibria (Example 4.1), because $(\mathscr{M},\mathscr{R})$ has the capacity for admitting multiple positive equilibria implies that there exist $r\in R$, $a\in\mathbb{R}_+^n$ and $b\in\mathbb{R}_+^n$ with $a\neq b$ such that not only $r(a)=r(b)$ but also $r(a)=r(b)=0$.

3.2. Sufficient existence condition of multiple positive equilibria

As mentioned earlier, the non-injective MMN module may not have the capacity for admitting multiple positive equilibria. In this subsection, a sufficient condition for existence of multiple positive equilibria will be provided.

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Definition 3.4
Let $M_0 = \{j \in M : \alpha_j = 0\} \triangleq \{\tau_1, \ldots, \tau_h\}$. $\Phi = (\beta_{\tau_1}, \ldots, \beta_{\tau_h})$ is called the input-matrix of the MMN module $(\mathscr{M}, \mathscr{R})$. Furthermore, if the linear equations

$$\Phi(x_1, x_2, \ldots, x_h)^T = \sum_{i=1}^{h} x_i \beta_{\tau_i} = b \tag{17}$$

had a solution in $\mathbb{R}_+^h$ for any $b \in \mathbb{R}_+^n$, then Φ is said to be positive nonsingular.

Lemma 3.4
If the input-matrix Φ of $(\mathscr{M}, \mathscr{R})$ is positive nonsingular and there exist $r = r(x; \mu, \nu, \kappa, \eta) \in R$, $a \in \mathbb{R}_+^n$ and $b \in \mathbb{R}_+^n$ with $a \neq b$ such that

$$\sum_{j \in M/M_0} (\alpha_j - \beta_j) v_j(a) = \sum_{j \in M/M_0} (\alpha_j - \beta_j) v_j(b) \in \mathbb{R}_+^n, \tag{18}$$

then $(\mathscr{M}, \mathscr{R})$ has the capacity for admitting multiple positive equilibria.

Proof
Because Φ is positive nonsingular, by (18) and Definition 3.4, we know

$$\Phi(x_1, x_2, \ldots, x_h)^T = \sum_{j \in M/M_0} (\alpha_j - \beta_j) v_j(a)$$

has a solution in $\mathbb{R}_+^h$ denoted by $\left(x_1^*, \ldots, x_h^*\right)^T$. By (17), we have

$$\sum_{i=1}^{h} x_i^* \beta_{\tau_i} = \sum_{j \in M/M_0} (\alpha_j - \beta_j) v_j(a). \tag{19}$$

For $j \in M/M_0$, let $\widetilde{V}_j = V_j$. Without loss of generality, let $j = \tau_i$ for $j \in M_0$ and $\widetilde{V}_j = x_i^*/\mu_j$, which implies $\tilde{v}_j = x_i^*$. Thus, $\tilde{r}(x; \mu, \tilde{\nu}, \kappa, \eta) \in R$. By (5), it can be seen that

$$\begin{aligned}\tilde{r}(a; \mu, \tilde{\nu}, \kappa, \eta) &= \sum_{j \in M/M_0} (\beta_j - \alpha_j) \tilde{v}_j(a) + \sum_{j \in M_0} (\beta_j - \alpha_j) \tilde{v}_j(a) \\ &= \sum_{j \in M/M_0} (\beta_j - \alpha_j) v_j(a) + \sum_{\tau_i \in M_0} x_i^* \beta_{\tau_i},\end{aligned}$$

which together with (19) indicates $\tilde{r}(a) = 0$. Similarly, we have $\tilde{r}(b) = 0$. Thus, the lemma is obtained by Definition 2.5. □

Remark 3.4
(18) implies $r(a) = r(b)$, but the converse is not true. In fact, $r(a) = r(b)$ if and only if

$$\sum_{j \in M/M_0} (\alpha_j - \beta_j) v_j(a) = \sum_{j \in M/M_0} (\alpha_j - \beta_j) v_j(b).$$

Theorem 3.3
If the input-matrix Φ of the MMN module $(\mathscr{M}, \mathscr{R})$ is positive nonsingular and there exist $r = r(x; \mu, \nu, \kappa, \eta) \in R$ and $c \in \mathbb{R}_+^n$ such that

$$\det(\mathscr{D}_r(c)) = 0 \tag{20}$$

and

$$\sum_{j \in M/M_0} (\alpha_j - \beta_j) v_j(c) \in \mathbb{R}_+^n, \tag{21}$$

then $(\mathscr{M}, \mathscr{R})$ has the capacity for admitting multiple positive equilibria.

Proof
To prove the theorem, by Lemma 3.4, it is sufficient to show that there exist $\tilde{r} = \tilde{r}\,(x;\mu,\tilde{\nu},\tilde{\kappa},\eta) \in R$, $a \in \mathbb{R}^n_+$ and $b \in \mathbb{R}^n_+$ with $a \neq b$ such that

$$\sum_{j\in M/M_0} (\alpha_j - \beta_j) v_j (a;\mu_j,\tilde{\nu}_j,\tilde{\kappa}_j,\eta_j) = \sum_{j\in M/M_0} (\alpha_j - \beta_j) v_j (b;\mu_j,\tilde{\nu}_j,\tilde{\kappa}_j,\eta_j) \in \mathbb{R}^n_+.$$

The following gives such $\tilde{r}, a$ and b.

By (20), we know that there exists nonzero $\xi \in \mathbb{R}^n$ such that $\mathscr{D}_r(c)\xi = 0$. Let $\xi_N = \xi/N$ for any given $N \in \mathbb{Z}_+$. Then,

$$\mathscr{D}_r(c)\xi_N = 0. \tag{22}$$

For these $r(x;\mu,\nu,\kappa,\eta) \in R$, $c \in \mathbb{R}^n_+$ and $\xi_N \in \mathbb{R}^n$, by Lemma A.6, there exist $r_N = r_N(x;\mu,\nu^N,\kappa^N,\eta) \in R$, $b_N \in \mathbb{R}^n_+$ and $a_N \in \mathbb{R}^n_+$ such that

$$\mathscr{D}_r(c)\xi_N = r_N\left(b_N;\mu,\nu^N,\kappa^N,\eta\right) - r_N\left(a_N;\mu,\nu^N,\kappa^N,\eta\right), \tag{23}$$

r_N, b_N and a_N are selected in the way of (A.9), (A.10) and (A.11) with $b_i = c_i$ and the 'any positive real number' in (A.10) and (A.11) are chosen as $k_{i,j}$ and V_j, respectively.

Noticing $\xi_N \neq 0$ and (A.9), we have

$$a_N \neq b_N \tag{24}$$

and

$$b_N \equiv c, \quad \lim_{N\to\infty} a_N = c. \tag{25}$$

Let $y_0 = b_i^{n_{i,j}}$, $y = a_i^{n_{i,j}}$, $p = k_{i,j}/\left(k_{i,j} + c_i^{n_{i,j}}\right)$ in Lemma A.3. Then, $\lim_{N\to\infty} k^N_{i,j} = k_{i,j}$ by (A.10) and (25). Therefore,

$$\lim_{N\to\infty} \kappa^N = \kappa. \tag{26}$$

By (A.2), we have $\lim_{N\to\infty} \alpha_j * \zeta_j(c;\kappa_j,\eta_j) * \xi_N = 0$, which implies $\lim_{N\to\infty} \frac{\alpha_j * \zeta_j(c;\kappa_j,\eta_j)*\xi_N}{e^{\alpha_j*\zeta_j(c;\kappa_j,\eta_j)*\xi_N}-1} = 1$. Thus, $\lim_{N\to\infty} V_j^N = V_j$ by (25)–(26) and (A.11). Furthermore,

$$\lim_{N\to\infty} \nu^N = \nu. \tag{27}$$

By (25)–(27), one can obtain

$$\lim_{N\to\infty} \sum_{j\in M/M_0} (\alpha_j - \beta_j) v_j\left(b_N;\mu_j,V_j^N,\kappa_j^N,\eta_j\right) = \sum_{j\in M/M_0} (\alpha_j - \beta_j) v_j (c;\mu_j,V_j,\kappa_j,\eta_j). \tag{28}$$

This together with (21) implies that there exist positive integer N_0 such that

$$\sum_{j\in M/M_0} (\alpha_j - \beta_j) v_j\left(b_{N_0};\mu_j,V_j^{N_0},\kappa_j^{N_0},\eta_j\right) \in \mathbb{R}^n_+. \tag{29}$$

By (22)–(23) and Remark 3.4, we have

$$\sum_{j\in M/M_0} (\alpha_j - \beta_j) v_j\left(a_{N_0};\mu_j,V_j^{N_0},\kappa_j^{N_0},\eta_j\right) = \sum_{j\in M/M_0} (\alpha_j - \beta_j) v_j\left(b_{N_0};\mu_j,V_j^{N_0},\kappa_j^{N_0},\eta_j\right). \tag{30}$$

Let $\tilde{r} = r_{N_0}, a = a_{N_0}$ and $b = b_{N_0}$. Then, by (24) and (29)–(30), it can be seen that such $\tilde{r}, a$ and b are the very ones we are looking for. The theorem is proved. □

Remark 3.5

(i) Theorem 3.3 not only gives a sufficient condition for existence of multiple positive equilibria but also supplies a method of finding the equilibrium.
(ii) The positive nonsingularity of the input matrix Φ of the MMN module is not necessary for the existence of multiple positive equilibria (Example 4.2).

3.3. $\mathscr{A}$-MMN module

$\mathscr{A}$-MMN metabolic module (to be given in the succeeding text) is an important type of the MMN module. Because of its special characteristic, we can give an injectivity criterion completely based on the topological structure.

Definition 3.5
For any $j \in M/M_0$, if there exists $\varepsilon_j \in \{1, \dots, n\}$ such that

$$\alpha_{i,j} \neq 0 \quad \Leftrightarrow \quad i = \varepsilon_j, \tag{31}$$

then $(\mathscr{M}, \mathscr{R})$ is called an $\mathscr{A}$-MMN module.

Remark 3.6

(i) SSN module [3] is an $\mathscr{A}$-MMN one.
(ii) By (31), we have

$$v_j = \begin{cases} \mu_j, & j \in M_0; \\ v_j(x_{\varepsilon_j}), & j \in M/M_0, \end{cases} \tag{32}$$

which implies that the metabolic reaction rate in the $\mathscr{A}$-MMN module is either a constant or with only one variable.

Theorem 3.4
$\mathscr{A}$-MMN module $(\mathscr{M}, \mathscr{R})$ is injective if and only if $m \geqslant n$ and there exists nonzero element in the set

$$\{\det(P(j_1, \dots, j_n)) \det(W(j_1, \dots, j_n)) : \{j_1, \dots, j_n\} \subseteq M\},$$

and all these nonzero elements have the same sign.

Proof
Because $(\mathscr{M}, \mathscr{R})$ is an $\mathscr{A}$-MMN module, by (31), (A.1) and Definition 3.1, we have

$$\det(E(j_1, \dots, j_n) \circ P(j_1, \dots, j_n)) = \prod_{i=1}^{n} \frac{k_{\varepsilon_{j_i}, j_i} n_{\varepsilon_{j_i}, j_i}}{k_{\varepsilon_{j_i}, j_i} + x_i^{n_{\varepsilon_{j_i}, j_i}}} \det(P(j_1, \dots, j_n)),$$

which together with

$$\prod_{i=1}^{n} k_{\varepsilon_{j_i}, j_i} n_{\varepsilon_{j_i}, j_i} \Big/ \left(k_{\varepsilon_{j_i}, j_i} + x_i^{n_{\varepsilon_{j_i}, j_i}}\right) > 0$$

implies

$$\det(E(j_1, \dots, j_n) \circ P(j_1, \dots, j_n)) = 0 \quad \Leftrightarrow \quad \det(P(j_1, \dots, j_n)) = 0$$

and

$$\text{sign}(\det(E(j_1, \dots, j_n) \circ P(j_1, \dots, j_n))) = \text{sign}(\det(P(j_1, \dots, j_n))).$$

By Lemma 3.3 and Theorem 3.2, the lemma is proved. □

Remark 3.7
The method given by Theorem 3.4 does not depend on the model parameter. Even though the model parameters are unknown, the injectivity still can be judged according to the topological structure of the $\mathscr{A}$-MMN module.

The results given earlier tell us how to judge the injectivity according to the network structure of the $\mathscr{A}$-MMN module. Then, one may ask: what kinds of structures can result in injectivity or non-injectivity? This problem is very difficult. As an initial result, we now give a structure that implies injectivity.

Definition 3.6 ([26]*)*
A matrix $D = (d_{ij})_{m\times m}$ is called to be diagonally dominated with a chain of nonzero elements if it satisfies the following conditions:

(A) $|d_{ii}| \geqslant \sum_{j\neq i} |d_{ji}|, \quad i = 1, 2, \ldots, m;$
(B) $M^+ = \left\{ i \in M : |d_{ii}| > \sum_{j\neq i} |d_{ji}| \right\} \neq \emptyset;$
(C) if $i \notin M^+$, then there exists a sequence:

$$d_{i_1 i},\ d_{i_2 i_1},\ \ldots,\ d_{i_l i_{l-1}},\ d_{j i_l},$$

where every element is nonzero and $j \in M^+$.

Lemma 3.5 ([26]*)*
Let $A = (a_{ij})_{m\times m}$ be diagonally dominated with a chain of nonzero elements. If $a_{ii} < 0$, $i = 1, 2, \ldots, m$, then

$$\det A \neq 0, \quad \text{and} \quad Re(\lambda_i(A)) < 0,\ i = 1, 2, \ldots, m,$$

where $Re(\cdot)$ denotes the real part of the complex number '$\cdot$' and $\lambda_i(A)$ is the i-th eigenvalue of the matrix A.

Theorem 3.5
If an $\mathscr{A}$-MMN module $(\mathscr{M}, \mathscr{R})$ has an output node and

$$\alpha_{\varepsilon_j, j} - \beta_{\varepsilon_j, j} \geqslant I_{[\Omega_{\mathscr{O}}(\beta_j)\neq 0]} + \sum_{i\neq\varepsilon_j} \beta_{i,j}, \quad j \in M/M_0, \tag{33}$$

then

(i) $(\mathscr{M}, \mathscr{R})$ is injective, and has no capacity for admitting multiple positive equilibria;
(ii) if $(\mathscr{M}, \mathscr{R})$ has an equilibrium, then the equilibrium is asymptotically stable.

Proof
Firstly, prove that Jacobian matrix $\frac{\mathrm{d}r}{\mathrm{d}x}$ is diagonally dominated with a chain of nonzero elements.

By (4), we have

$$\frac{\partial r_j}{\partial x_i} = \sum_{k\in M/M_0} (\beta_{j,k} - \alpha_{j,k}) \frac{\partial v_k}{\partial x_i} + \sum_{k\in M_0} (\beta_{j,k} - \alpha_{j,k}) \frac{\partial v_k}{\partial x_i},$$

which together with (32) implies

$$\frac{\partial r_j}{\partial x_i} = \sum_{k\in M/M_0} (\beta_{j,k} - \alpha_{j,k}) \frac{\partial v_k}{\partial x_i} = \sum_{k\in M/M_0, \varepsilon_k = i} (\beta_{j,k} - \alpha_{j,k}) \frac{\partial v_k}{\partial x_i}.$$

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By (31), it can be seen that

$$\frac{\partial r_j}{\partial x_i} = \begin{cases} -\sum\limits_{k\in M/M_0,\varepsilon_k=i} (\alpha_{\varepsilon_k,k} - \beta_{\varepsilon_k,k}) \frac{\partial v_k}{\partial x_i}, & j = i; \\ \sum\limits_{k\in M/M_0,\varepsilon_k=i} \beta_{j,k} \frac{\partial v_k}{\partial x_i}, & j \neq i, \end{cases} \tag{34}$$

and for $j \neq i$,

$$\frac{\partial r_j}{\partial x_i} \neq 0 \quad \Leftrightarrow \quad \exists k_0 \in M,\ s.t.\ \alpha_{i,k_0}\beta_{j,k_0} \neq 0. \tag{35}$$

By (33), we know

$$\alpha_{\varepsilon_k,k} - \beta_{\varepsilon_k,k} \geqslant 0, \quad k \in M/M_0, \tag{36}$$

which together with (34) indicates

$$\left|\frac{\partial r_i}{\partial x_i}\right| = \sum_{k\in M/M_0,\varepsilon_k=i} (\alpha_{\varepsilon_k,k} - \beta_{\varepsilon_k,k}) \frac{\partial v_k}{\partial x_i},$$

$$\sum_{j\neq i}\left|\frac{\partial r_j}{\partial x_i}\right| = \sum_{j\neq i}\frac{\partial r_j}{\partial x_i} = \sum_{j\neq i}\sum_{k\in M/M_0,\varepsilon_k=i} \beta_{j,k}\frac{\partial v_k}{\partial x_i} = \sum_{k\in M/M_0,\varepsilon_k=i}\sum_{j\neq\varepsilon_k} \beta_{j,k}\frac{\partial v_k}{\partial x_i}.$$

Thus, by (33) we have

$$\left|\frac{\partial r_i}{\partial x_i}\right| \geqslant \sum_{j\neq i}\left|\frac{\partial r_j}{\partial x_i}\right| \tag{37}$$

and

$$\left|\frac{\partial r_i}{\partial x_i}\right| > \sum_{j\neq i}\left|\frac{\partial r_j}{\partial x_i}\right| \quad \Leftrightarrow \quad \exists \bar{k}_0,\ s.t.\ \alpha_{i,\bar{k}_0} \neq 0, \Omega_{\mathscr{O}}\left(\beta_{\bar{k}_0}\right) \neq 0, 1 \leqslant i \leqslant n. \tag{38}$$

The inequality (37) implies that $\frac{\mathrm{d}r}{\mathrm{d}x}$ satisfies the condition (A) of Definition 3.6. Because $(\mathscr{M},\mathscr{R})$ has an output node, by (38) we know

$$\left\{ i \in \{1,2,\dots,n\} : \left|\frac{\partial r_i}{\partial x_i}\right| > \sum_{j\neq i}\left|\frac{\partial r_j}{\partial x_i}\right| \right\} \neq \emptyset.$$

Hence, $\frac{\mathrm{d}r}{\mathrm{d}x}$ also satisfies the condition (B) of Definition 3.6.

If the inequality (37) cannot be strictly established, then by the condition (iv) of Definition 2.3, there exist $i_1,\dots,i_{j_0}$ such that $\alpha_{i,i_1} \neq 0$, $\Omega_{\mathscr{O}}(\beta_{i_{j_0}}) \neq 0$, and there exists t_j such that $\beta_{t_j,i_j}\alpha_{t_j,i_{j+1}} \neq 0$, $j = 1,\dots,j_0-1$. Thus, by (35), we know the elements in the following sequence:

$$\frac{\partial r_{t_1}}{\partial x_i}, \frac{\partial r_{t_2}}{\partial x_{t_1}}, \dots, \frac{\partial r_{t_{j_0-1}}}{\partial x_{t_{j_0-2}}},$$

are nonzero; and by (38) we have

$$\left|\frac{\partial r_{t_{j_0-1}}}{\partial x_{t_{j_0-1}}}\right| > \sum_{j\neq t_{j_0-1}}\left|\frac{\partial r_j}{\partial x_{t_{j_0-1}}}\right|,$$

which implies that $\frac{\mathrm{d}r}{\mathrm{d}x}$ satisfies the condition (C) of Definition 3.6. Thus, $\frac{\mathrm{d}r}{\mathrm{d}x}$ is diagonally dominated with a chain of nonzero elements.

Table I. Parameter values.

Parameter	Value	Parameter	Value	Parameter	Value
V_1	1	$n_{1,1}$	1	$k_{2,2}$	2
V_2	1	$n_{2,2}$	1		
μ_1	1	$k_{1,1}$	1		

Therefore, by (34) and (36), we have $\frac{\partial r_i}{\partial x_i} < 0$, $i = 1, \ldots, n$, which implies that the diagonal elements of $\frac{\mathrm{d}r}{\mathrm{d}x}$ are negative. By Lemma 3.5, one can obtain

$$\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) \neq 0, \quad Re\left(\lambda_i\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right)\right) < 0,\ i = 1, \ldots, n.$$

Thus, (i) is true by Theorem 3.1, and (ii) is proved by [27, Theorem 2.4.18]. □

Corollary 3.3
SSN module with an output node is injective and has at most one equilibrium [3, Theorem 3.1]; and the equilibrium, if any, is asymptotically stable [4, Theorem 2].

Remark 3.8
(i) (33) is only related to the network structure, not to the model parameters.
(ii) Generally speaking, the asymptotical stability in Theorem 3.5 is local. But if the set of state nodes of $(\mathscr{M}, \mathscr{R})$ has only two kinds of metabolites, then the stability is global [28].

4. EXAMPLES AND SIMULATIONS

Example 4.1
Consider MMN module $(\mathscr{M}_1, \mathscr{R}_1)$:

$$H_1 \to S_1, \quad S_1 \rightleftharpoons S_2.$$

Let $\mathscr{S}_1 = \{S_1, S_2\}$, $\mathscr{R}_1 = \{\mathcal{A}_i \to \mathcal{B}_i : i = 1, 2, 3\}$, where $\mathcal{A}_1 \to \mathcal{B}_1 \triangleq H_1 \to S_1$, $\mathcal{A}_2 \to \mathcal{B}_2 \triangleq S_1 \to S_2$, $\mathcal{A}_3 \to \mathcal{B}_3 \triangleq S_2 \to S_1$.

By (5), a model of $(\mathscr{M}_1, \mathscr{R}_1)$ is given by

$$\frac{\mathrm{d}x}{\mathrm{d}t} = r(x; \mu, \nu, \kappa, \eta) = \begin{pmatrix} v_1 - v_2 + v_3 \\ v_2 - v_3 \end{pmatrix}.$$

It can be seen that $r = 0$ is no solution due to $v_1 = \mu_1 > 0$. Thus, $(\mathscr{M}_1, \mathscr{R}_1)$ has no capacity for admitting multiple positive equilibria. Because

$$\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) = \det\left[\begin{pmatrix} -\frac{dv_2}{dx_1} & \frac{dv_3}{dx_2} \\ \frac{dv_2}{dx_1} & -\frac{dv_3}{dx_2} \end{pmatrix}\right] = \frac{dv_2}{dx_1}\frac{dv_3}{dx_2} \det\left[\begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix}\right] = 0,$$

$(\mathscr{M}_1, \mathscr{R}_1)$ is non-injective by Theorem 3.1. In fact, if the model parameters take values from Table I, then we have

$$r\begin{pmatrix} 1 \\ 2 \end{pmatrix} = r\begin{pmatrix} 2 \\ 4 \end{pmatrix} = \begin{pmatrix} 1 \\ 0 \end{pmatrix},$$

which implies $(\mathscr{M}_1, \mathscr{R}_1)$ is non-injective again. To sum up, although it is non-injective, $(\mathscr{M}_1, \mathscr{R}_1)$ has no capacity for admitting multiple positive equilibria.

Example 4.2
MMN module $(\mathscr{M}_2, \mathscr{R}_2)$ is given by

$$H_1 \to S_1, \quad H_2 \to S_3, \quad S_1 \rightleftharpoons S_2, \quad S_2 + S_3 \to P_1 + P_2.$$

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Table II. Parameter values.

Parameter	Value	Parameter	Value	Parameter	Value
V_3	3	$k_{1,3}$	2	$n_{1,3}$	2
V_4	1	$k_{2,4}$	1	$n_{2,4}$	1
V_5	6	$k_{2,5}$	1	$k_{3,5}$	1
$n_{3,5}$	1	$n_{2,5}$	1	μ_1	1
μ_2	1				

Let $\mathscr{S}_2 = \{S_1, S_2, S_3\}$, $\mathscr{R}_2 = \{\mathcal{A}_1 \to \mathcal{B}_1, \dots, \mathcal{A}_5 \to \mathcal{B}_5\}$, where $\mathcal{A}_1 \to \mathcal{B}_1 \triangleq H_1 \to S_1$, $\mathcal{A}_2 \to \mathcal{B}_2 \triangleq H_2 \to S_3$, $\mathcal{A}_3 \to \mathcal{B}_3 \triangleq S_1 \to S_2$, $\mathcal{A}_4 \to \mathcal{B}_4 \triangleq S_2 \to S_1$, $\mathcal{A}_5 \to \mathcal{B}_5 \triangleq S_2 + S_3 \to P_1 + P_2$.

Then, by Definition 3.4, we have

$$\Phi = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 1 \end{pmatrix}.$$

Thus, the input-matrix Φ of $(\mathscr{M}_2, \mathscr{R}_2)$ is singular. If the model parameters take values from Table II, then a model of $(\mathscr{M}_2, \mathscr{R}_2)$ is

$$\frac{\mathrm{d}x}{\mathrm{d}t} = r(x; \mu, \nu, \kappa, \eta) = \begin{pmatrix} 1 + \frac{x_2}{1+x_2} - \frac{3x_1}{2+x_1} \\ \frac{3x_1}{2+x_1} - \frac{x_2}{1+x_2} - \frac{6x_2x_3}{(1+x_2)(1+x_3)} \\ 1 - \frac{6x_2x_3}{(1+x_2)(1+x_3)} \end{pmatrix}.$$

In this case, $r\left(\left(2, 1, \frac{1}{2}\right)^T\right) = r\left(\left(3, 4, \frac{5}{19}\right)^T\right) = 0$, which implies that $(\mathscr{M}_2, \mathscr{R}_2)$ has multiple positive equilibria though the input-matrix Φ is singular.

Example 4.3

$(\mathscr{M}_3, \mathscr{R}_3)$ is an MMN module as follows:

$$H_1 + H_2 \to S_1, \quad H_1 + 3S_1 \to 2S_2 + S_3,$$

$$H_1 + H_2 + 2S_2 \to S_4 + P_1 + P_2, \quad S_3 \to P_1 + P_2, \quad 2S_4 \to S_1 + P_2.$$

The set of its state nodes has four kinds of metabolites: S_1, S_2, S_3 and S_4, and the aforementioned five reactions are denoted by $\mathcal{A}_1 \to \mathcal{B}_1, \dots, \mathcal{A}_5 \to \mathcal{B}_5$, respectively. Then, we obtain a model of $(\mathscr{M}_3, \mathscr{R}_3)$ as follows:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = r(x; \mu, \nu, \kappa, \eta) = \begin{pmatrix} v_1 + v_5 - 3v_2 \\ 2v_2 - 2v_3 \\ v_2 - v_4 \\ v_3 - 2v_5 \end{pmatrix}. \tag{39}$$

By Definition 3.5, we know that $(\mathscr{M}_3, \mathscr{R}_3)$ is an $\mathscr{A}$-MMN module. Noticing $\det([\alpha_2, \alpha_3, \alpha_4, \alpha_5])$ $\det([\beta_2 - \alpha_3, \beta_3 - \alpha_3, \beta_4 - \alpha_4, \beta_5 - \alpha_5]) = 120$, by Theorem 3.4, $(\mathscr{M}_3, \mathscr{R}_3)$ is injective.

Furthermore, $(\mathscr{M}_3, \mathscr{R}_3)$ also satisfies (33). Thus, by (39) we have

$$\frac{\mathrm{d}r}{\mathrm{d}x} = \begin{pmatrix} -3v_2' & 0 & 0 & v_5' \\ 2v_2' & -2v_3' & 0 & 0 \\ v_2' & 0 & -v_4' & 0 \\ 0 & v_3' & 0 & -2v_5' \end{pmatrix}.$$

By Definition 3.6, $\frac{\mathrm{d}r}{\mathrm{d}x}$ is diagonally dominated with a chain of nonzero elements and $\det\left(\frac{\mathrm{d}r}{\mathrm{d}x}\right) = 10v_2'v_3'v_4'v_5' > 0$. This also implies $(\mathscr{M}_3, \mathscr{R}_3)$ is injective.

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On the other hand,

$$\det\left(\lambda I - \frac{\mathrm{d}r}{\mathrm{d}x}\right) = (\lambda + v_4')\left[\lambda^3 + (3v_2' + 2v_3' + 2v_5')\lambda^2 + (6v_2'v_3' + 6v_2'v_5' + 4v_3'v_5')\lambda + 10v_2'v_3'v_5'\right].$$

By [27, theorem 2.4.3], we know that the real parts of the eigenvalues of Jacobian matrix $\frac{\mathrm{d}r}{\mathrm{d}x}$ are all negative. Thus, the equilibrium, if any, of $(\mathscr{M}_3, \mathscr{R}_3)$ is asymptotically stable. If the model parameters take values from Table III, then $(\mathscr{M}_3, \mathscr{R}_3)$ has only the following positive equilibrium

$$\bar{x} = \begin{pmatrix} \frac{3}{40}\left(1 + \sqrt[3]{5} + \sqrt[3]{25}\right) \\ \frac{7}{40}\left(1 + \sqrt{5}\right) \\ \frac{2}{13} \\ \frac{2}{33}\left(2 + \sqrt{70}\right) \end{pmatrix}.$$

Table III. Parameter values.

Parameter	Value	Parameter	Value	Parameter	Value
V_2	4	$k_{1,2}$	0.3	$n_{1,2}$	1
V_3	8	$k_{2,3}$	0.7	$n_{2,3}$	1
V_4	6	$k_{3,4}$	1	$n_{3,4}$	1
V_5	3.5	$k_{4,5}$	2	$n_{4,5}$	1
μ_1	1	μ_2	0.5	μ_3	0.25

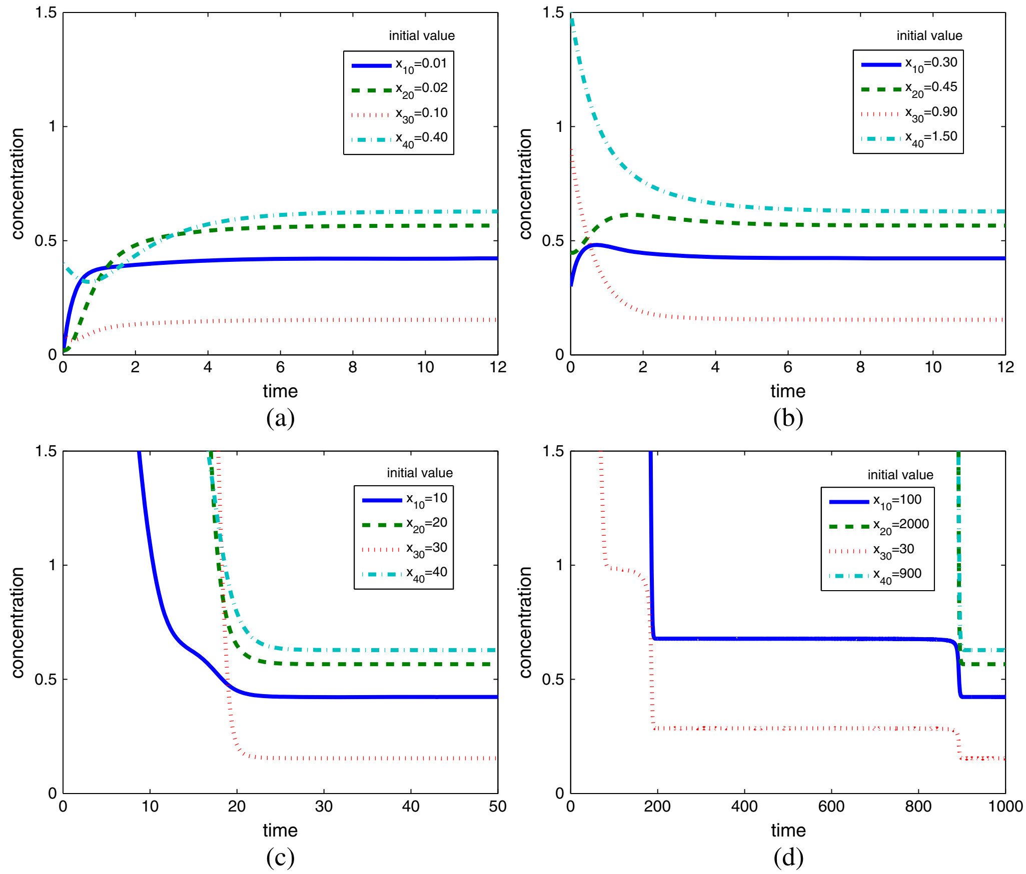

Figure 2. Trajectories of $(\mathscr{M}_3, \mathscr{R}_3)$ starting from four different initial value sets $(x_{10}, x_{20}, x_{30}, x_{40})^T$: (a) $(0.01, 0.02, 0.10, 0.40)^T$, (b) $(0.30, 0.45, 0.90, 1.50)^T$, (c) $(10, 20, 30, 40)^T$ and (d) $(100, 2000, 30, 900)^T$.

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Figure 2 describes the dynamic behaviors of $(\mathscr{M}_3, \mathscr{R}_3)$ starting from four different initial values $(x_{10}, x_{20}, x_{30}, x_{40})^T$. In the subfigures (a) and (b), the initial values are in a small neighborhood of $\bar{x}$, and the trajectories converge to $\bar{x}$, which is consistent with Theorem 3.5. In the subfigures (c) and (d), the trajectories also converge to $\bar{x}$, although the distances between the initials value and $\bar{x}$ are large.

5. CONCLUDING REMARK

It is fundamental and of practical significance to regulate the multi-steady-sate performance of metabolic networks by changing the external conditions or readjusting the network structure. To do so, two questions may have to be solved: (i) how to judge whether or not a metabolic network can admit multiple equilibria; (ii) what kinds of topological structures can result in multiple equilibria. From the theoretical point of view, the paper provided a partial answer to them. For the MMN module, two necessary and sufficient injectivity conditions and a sufficient non-injective condition for the existence of multiple positive equilibrium were provided. For a type of important MMN module—$\mathscr{A}$-MMN module—a structure-oriented criterion for injectivity judgement was given. Especially, for a kind of $\mathscr{A}$-MMN module with some special structure, the module was shown to have no capacity for admitting multi-equilibria, and the equilibrium (if exists) was asymptotically stable.

As future works, many challenging and meaningful problems are worth considering, such as the dynamic characteristic of the MMN module with more general structures, the multi-equilibrium property of MMI module, how to realize switchings among the different equilibria, and so on.

APPENDICES

Lemma A.1

If $y_i > 0$, $i = 1, \ldots, 4$, and

$$y_5 = \ln\left(\frac{y_2}{y_1}\right), \quad y_6 = \begin{cases} -y_3 \cdot \frac{\ln\left(\frac{y_2}{y_1}\right) + \ln\left(\frac{y_4+y_1}{y_4+y_2}\right)}{\ln\left(\frac{y_4+y_1}{y_4+y_2}\right)}, & y_1 \neq y_2; \\ \text{any positive real number}, & y_1 = y_2, \end{cases}$$

then

$$y_6 > 0, e^{\frac{y_6}{y_6+y_3} y_5} = \frac{y_2}{y_4+y_2} \frac{y_4+y_1}{y_1}.$$

Proof

If $y_1 = y_2$, then the lemma is true. Otherwise, let $g(t) = \log_{\frac{y_2}{y_1}}\left(\frac{t+y_1}{t+y_2}\right)$, $t \in (0, \infty)$. Noticing

$$\frac{\mathrm{d}}{\mathrm{d}t} g(t) = \frac{1}{(t+y_1)(t+y_2)} \frac{y_2 - y_1}{\ln(y_2/y_1)} > 0, \; g(0) = -1, \; \lim_{t \to +\infty} g(t) = 0,$$

we have $g(t) \in (-1, 0)$. Thus, by $y_6 = -y_3 \frac{1+g(y_4)}{g(y_4)}$, we have $y_6 > 0$. Substituting y_6 into $e^{\frac{y_6}{y_6+y_3} y_5}$ results in $e^{\frac{y_6}{y_6+y_3} y_5} = \frac{y_2}{y_4+y_2} \frac{y_4+y_1}{y_1}$, which implies the lemma. □

Lemma A.2

If $z_1 > 0$, $z_2 > 0$, $z_3 \neq 1$ and

$$z_4 = \begin{cases} \frac{z_2^{z_3} - z_1^{z_3}}{z_1^{z_3-1} - z_2^{z_3-1}}, & z_1 \neq z_2; \\ \text{any positive real number}, & z_1 = z_2, \end{cases}$$

then

$$\frac{z_2}{z_4+z_2} \frac{z_4+z_1}{z_1} = \left(\frac{z_2}{z_1}\right)^{z_3}, \quad z_4 > 0 \qquad \Leftrightarrow \qquad 0 < z_3 < 1.$$

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Proof

If $z_1 = z_2$, the lemma is true. Otherwise, substituting $z_4 = \frac{z_2^{z_3} - z_1^{z_3}}{z_1^{z_3-1} - z_2^{z_3-1}}$ into $\frac{z_2}{z_4+z_2}\frac{z_4+z_1}{z_1}$, we have $\frac{z_2}{z_4+z_2}\frac{z_4+z_1}{z_1} = \left(\frac{z_2}{z_1}\right)^{z_3}$. Let $\epsilon = z_2/z_1$. Then,

$$
\begin{aligned}
z_4 > 0 &\Leftrightarrow \frac{z_2^{z_3} - z_1^{z_3}}{z_1^{z_3-1} - z_2^{z_3-1}} > 0 \\
&\Leftrightarrow \frac{\epsilon^{z_3} - 1}{\frac{1}{z_1}\left(1 - \epsilon^{z_3-1}\right)} > 0 \\
&\Leftrightarrow \left(\epsilon^{z_3} - 1\right)\left(\epsilon^{z_3} - \epsilon\right) < 0 \\
&\Leftrightarrow \min(1,\epsilon) < \epsilon^{z_3} < \max(1,\epsilon) \\
&\Leftrightarrow \min\left\{\log_\epsilon\left(\min(1,\epsilon)\right), \log_\epsilon\left(\max(1,\epsilon)\right)\right\} \\
&\quad < z_3 < \max\left\{\log_\epsilon\left(\min(1,\epsilon)\right), \log_\epsilon\left(\max(1,\epsilon)\right)\right\} \\
&\Leftrightarrow 0 < z_3 < 1.
\end{aligned}
$$

Thus, the lemma is proved. □

Lemma A.3

If $y_0 > 0$ and $p \neq 1$, then

$$\lim_{0<y\to y_0} \frac{y_0^p - y^p}{y^{p-1} - y_0^{p-1}} = \frac{y_0 p}{1-p}.$$

Proof

By L' Hospital's Rule [29], we have

$$\lim_{0<y\to y_0} \frac{y_0^p - y^p}{y^{p-1} - y_0^{p-1}} = \lim_{0<y\to y_0} \frac{-py^{p-1}}{(p-1)y^{p-2}} = \lim_{0<y\to y_0} \frac{py}{(1-p)} = \frac{y_0 p}{1-p},$$

which implies the lemma. □

Lemma A.4

For any $\xi = (\xi_1, \ldots, \xi_n)^T \in \mathbb{R}^n$, we have

$$\frac{\mathrm{d}r}{\mathrm{d}x}(\xi) = \sum_{j=1}^{m} (\beta_j - \alpha_j)(\alpha_j * \zeta_j * \xi)v_j,$$

where ζ_j is given by

$$\zeta_j = \zeta_j(x; \kappa_j, \eta_j) \triangleq \left(\frac{k_{1,j}n_{1,j}}{k_{1,j} + x_1^{n_{1,j}}}, \ldots, \frac{k_{n,j}n_{n,j}}{k_{n,j} + x_n^{n_{n,j}}}\right)^T, \tag{A.1}$$

and the scalar product '$*$' is defined as

$$\alpha_j * \zeta_j(x; \kappa_j, \eta_j) * \xi \triangleq \sum_{i=1}^{n} \frac{k_{i,j}n_{i,j}}{k_{i,j} + x_i^{n_{i,j}}} \frac{\alpha_{i,j}}{x_i}\xi_i, \quad 1 \leqslant j \leqslant m. \tag{A.2}$$

Proof

By (5), we have

$$\frac{\partial r}{\partial x_i} = \frac{\partial}{\partial x_i}\left[\sum_{j=1}^{m} (\beta_j - \alpha_j)v_j\right] = \sum_{j=1}^{m} (\beta_j - \alpha_j)\frac{\partial v_j}{\partial x_i}.$$

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This together with

$$\frac{\partial v_j}{\partial x_i} = v_j \frac{k_{i,j} n_{i,j}}{k_{i,j} + x_i^{n_{i,j}}} \frac{\alpha_{i,j}}{x_i}$$

indicates

$$\frac{\partial r}{\partial x_i} = \sum_{j=1}^{m} (\beta_j - \alpha_j) v_j \frac{k_{i,j} n_{i,j}}{k_{i,j} + x_i^{n_{i,j}}} \frac{\alpha_{i,j}}{x_i}, \quad 1 \leqslant i \leqslant n. \tag{A.3}$$

Thus,

$$\begin{aligned} \frac{\mathrm{d}r}{\mathrm{d}x}(\xi) &= \left(\frac{\partial r}{\partial x_1}, \ldots, \frac{\partial r}{\partial x_n}\right)(\xi) = \sum_{i=1}^{n} \left(\frac{\partial r}{\partial x_i}\right) \xi_i \\ &= \sum_{i=1}^{n} \sum_{j=1}^{m} (\beta_j - \alpha_j) v_j \frac{k_{i,j} n_{i,j}}{k_{i,j} + x_i^{n_{i,j}}} \frac{\alpha_{i,j}}{x_i} \xi_i \\ &= \sum_{j=1}^{m} (\beta_j - \alpha_j) v_j \sum_{i=1}^{n} \frac{k_{i,j} n_{i,j}}{k_{i,j} + x_i^{n_{i,j}}} \frac{\alpha_{i,j}}{x_i} \xi_i, \end{aligned}$$

which together with (A.1) and (A.2) implies the lemma. □

Lemma A.5

For any $r = r(x; \mu, \nu, \kappa, \eta) \in R$, $b = (b_1, \ldots, b_n)^T \in \mathbb{R}_+^n$ and $a = (a_1, \ldots, a_n)^T \in \mathbb{R}_+^n$, there exist $\tilde{r} = \tilde{r}\,(x; \mu, \tilde{\nu}, \tilde{\kappa}, \eta) \in R$, $c = (c_1, \ldots, c_n)^T \in \mathbb{R}_+^n$ and $\xi \in \mathbb{R}^n$ such that

$$r(b) - r(a) = \mathscr{D}_{\tilde{r}}(c)\xi,$$

where ξ and c satisfy

$$\frac{\xi_i}{c_i} = \ln\left(\frac{b_i}{a_i}\right), c_i > 0; \tag{A.4}$$

$\tilde{\nu}, \tilde{\kappa}$ can be selected as

$$\tilde{k}_{i,j} = \begin{cases} -c_i^{n_{i,j}} \cdot \frac{1+t}{t}, \quad t = \frac{1}{n_{i,j}} \log_{\left(\frac{b_i}{a_i}\right)} \left(\frac{k_{i,j} + a_i^{n_{i,j}}}{k_{i,j} + b_i^{n_{i,j}}}\right), & \text{if } a_i \neq b_i; \\ \text{any positive real number}, & \text{if } a_i = b_i; \end{cases} \tag{A.5}$$

$$\widetilde{V}_j = \begin{cases} V_j \prod_{i=1}^{n} \left(\frac{a_i^{n_{i,j}}}{k_{i,j} + a_i^{n_{i,j}}} \frac{\tilde{k}_{i,j} + c_i^{n_{i,j}}}{c_i^{n_{i,j}}}\right)^{\alpha_{i,j}} \frac{e^{\alpha_j * \zeta_j(c; \tilde{\kappa}_j, \eta_j) * \xi} - 1}{\alpha_j * \zeta_j(c; \tilde{\kappa}_j, \eta_j) * \xi}, & \text{if } \alpha_j * \zeta_j(c; \tilde{\kappa}_j, \eta_j) * \xi \neq 0; \\ \text{any positive real number}, & \text{if } \alpha_j * \zeta_j(c; \tilde{\kappa}_j, \eta_j) * \xi = 0. \end{cases} \tag{A.6}$$

Proof

From (A.4), we know $c \in \mathbb{R}_+^n$. Noticing (A.6) and $x/(e^x - 1) > 0$, $\forall x \neq 0$, one can get $\widetilde{V}_j > 0$, $1 \leqslant j \leqslant m$. Let $y_1 = a_i^{n_{i,j}}$, $y_2 = b_i^{n_{i,j}}$, $y_3 = c_i^{n_{i,j}}$ and $y_4 = k_{i,j}$ in Lemma A.1. Then, by (A.4) and (A.5) we have

$$\exp\left\{\frac{n_{i,j} \tilde{k}_{i,j}}{\tilde{k}_{i,j} + c_i^{n_{i,j}}} \cdot \frac{\xi_i}{c_i}\right\} = \frac{b_i^{n_{i,j}}}{k_{i,j} + b_i^{n_{i,j}}} \frac{k_{i,j} + a_i^{n_{i,j}}}{a_i^{n_{i,j}}} \tag{A.7}$$

and

$$\tilde{k}_{i,j} > 0, \quad 1 \leqslant i \leqslant n, \; 1 \leqslant j \leqslant m.$$

Hence, $\tilde{r}(x; \mu, \tilde{\nu}, \tilde{\kappa}, \eta) \in R$.

By (A.2) and (A.7), we know

$$e^{\alpha_j * \zeta_j(c;\tilde{\kappa}_j,\eta_j)*\xi} = \prod_{i=1}^{n} \exp\left\{ \frac{n_{i,j}\tilde{k}_{i,j}}{\tilde{k}_{i,j} + c_i^{n_{i,j}}} \cdot \frac{\alpha_{i,j}\xi_i}{c_i} \right\} = \prod_{i=1}^{n} \left(\frac{b_i^{n_{i,j}}}{k_{i,j} + b_i^{n_{i,j}}} \frac{k_{i,j} + a_i^{n_{i,j}}}{a_i^{n_{i,j}}} \right)^{\alpha_{i,j}}, \quad 1 \leqslant j \leqslant m. \tag{A.8}$$

This together with Lemma (A.4) and (A.6) implies

$$\begin{aligned}
\mathscr{D}_{\tilde{r}}(c)\xi &= \sum_{j=1}^{m} \mu_j \widetilde{V}_j \prod_{i=1}^{n} \left(\frac{c_i^{n_{i,j}}}{\tilde{k}_{i,j} + c_i^{n_{i,j}}} \right)^{\alpha_{i,j}} \left[\alpha_j * \zeta_j(c;\tilde{\kappa}_j,\eta_j) * \xi \right] (\beta_j - \alpha_j) \\
&= \sum_{j=1}^{m} \mu_j V_j \prod_{i=1}^{n} \left(\frac{a_i^{n_{i,j}}}{k_{i,j} + a_i^{n_{i,j}}} \right)^{\alpha_{i,j}} \left(e^{\alpha_j * \zeta_j(c;\tilde{\kappa}_j,\eta_j)*\xi} - 1 \right) (\beta_j - \alpha_j) \\
&= \sum_{j=1}^{m} \mu_j V_j \left[\prod_{i=1}^{n} \left(\frac{b_i^{n_{i,j}}}{k_{i,j} + b_i^{n_{i,j}}} \right)^{\alpha_{i,j}} - \prod_{i=1}^{n} \left(\frac{a_i^{n_{i,j}}}{k_{i,j} + a_i^{n_{i,j}}} \right)^{\alpha_{i,j}} \right] (\beta_j - \alpha_j).
\end{aligned}$$

Hence, by (5) we have $r(b) - r(a) = \mathscr{D}_{\tilde{r}}(c)\xi$, which gives the lemma. □

Lemma A.6

For any $r = r(x;\mu,\nu,\kappa,\eta) \in R$, $c \in \mathbb{R}_+^n$ and $\xi \in \mathbb{R}^n$, there exist $\tilde{r} = \tilde{r}(x;\mu,\tilde{\nu},\tilde{\kappa},\eta) \in R$, $b \in \mathbb{R}_+^n$ and $a \in \mathbb{R}_+^n$ such that

$$\mathscr{D}_r(c)\xi = \tilde{r}(b) - \tilde{r}(a)$$

with

$$\frac{b_i}{a_i} = e^{\frac{\xi_i}{c_i}}, b_i > 0, a_i > 0, \tag{A.9}$$

$$\tilde{k}_{i,j} = \begin{cases} \dfrac{\left(b_i^{n_{i,j}}\right)^t - \left(a_i^{n_{i,j}}\right)^t}{\left(a_i^{n_{i,j}}\right)^{t-1} - \left(b_i^{n_{i,j}}\right)^{t-1}}, \quad t = \dfrac{k_{i,j}}{k_{i,j} + c_i^{n_{i,j}}}, & \text{if } a_i \neq b_i; \\ \text{any positive real number}, & \text{if } a_i = b_i, \end{cases} \tag{A.10}$$

$$\widetilde{V}_j = \begin{cases} V_j \prod_{i=1}^{n} \left(\dfrac{\tilde{k}_{i,j} + a_i^{n_{i,j}}}{a_i^{n_{i,j}}} \dfrac{c_i^{n_{i,j}}}{k_{i,j} + c_i^{n_{i,j}}} \right)^{\alpha_{i,j}} \dfrac{\alpha_j * \zeta_j(c;\kappa_j,\eta_j) * \xi}{e^{\alpha_j * \zeta_j(c;\kappa_j,\eta_j)*\xi} - 1}, & \text{if } \alpha_j * \zeta_j(c;\kappa_j,\eta_j) * \xi \neq 0; \\ \text{any positive real number}, & \text{if } \alpha_j * \zeta_j(c;\kappa_j,\eta_j) * \xi = 0. \end{cases} \tag{A.11}$$

Proof

By (A.9) and (A.11), we have $a \in \mathbb{R}_+^n$, $b \in \mathbb{R}_+^n$ and $\widetilde{V}_j > 0$, $1 \leqslant j \leqslant m$. Let $z_1 = a_i^{n_{i,j}}$, $z_2 = b_i^{n_{i,j}}$ and $z_3 = k_{i,j} / \left(k_{i,j} + c_i^{n_{i,j}}\right)$ in Lemma A.2. Then, noticing $0 < z_3 < 1$ and (A.10), we have

$$\frac{b_i^{n_{i,j}}}{\tilde{k}_{i,j} + b_i^{n_{i,j}}} \frac{\tilde{k}_{i,j} + a_i^{n_{i,j}}}{a_i^{n_{i,j}}} = \left(\frac{b_i}{a_i} \right)^{\frac{n_{i,j}k_{i,j}}{k_{i,j} + c_i^{n_{i,j}}}} \tag{A.12}$$

and

$$\tilde{k}_{i,j} > 0, \quad 1 \leqslant i \leqslant n,\ 1 \leqslant j \leqslant m.$$

Thus, $\tilde{r}(x;\mu,\tilde{\nu},\tilde{\kappa},\eta) \in R$.

From (A.2) and (A.9), it follows that

$$\prod_{i=1}^{n}\left(\frac{b_i}{a_i}\right)^{\frac{n_{i,j}k_{i,j}}{k_{i,j}+c_i^{n_{i,j}}}\cdot\alpha_{i,j}} = \prod_{i=1}^{n}\left(e^{\frac{\xi_i}{c_i}}\right)^{\frac{n_{i,j}k_{i,j}}{k_{i,j}+c_i^{n_{i,j}}}\cdot\alpha_{i,j}} = \exp\left\{\sum_{i=1}^{n}\frac{n_{i,j}k_{i,j}}{k_{i,j}+c_i^{n_{i,j}}}\frac{\alpha_{i,j}\xi_i}{c_i}\right\} = e^{\alpha_j*\zeta_j(c;\kappa_j,\eta_j)*\xi}.$$

Then, by (A.12) we have

$$\prod_{i=1}^{n}\left(\frac{b_i^{n_{i,j}}}{\tilde{k}_{i,j}+b_i^{n_{i,j}}}\frac{\tilde{k}_{i,j}+a_i^{n_{i,j}}}{a_i^{n_{i,j}}}\right)^{\alpha_{i,j}} = \prod_{i=1}^{n}\left(\frac{b_i}{a_i}\right)^{\frac{n_{i,j}k_{i,j}}{k_{i,j}+c_i^{n_{i,j}}}\cdot\alpha_{i,j}} = e^{\alpha_j*\zeta_j(c;\kappa_j,\eta_j)*\xi},\ 1\leqslant j\leqslant m.$$

This together with (5) and (A.11) gives

$$\begin{aligned}
&\tilde{r}(b)-\tilde{r}(a)\\
&=\sum_{j=1}^{m}\mu_j\widetilde{V}_j\left[\prod_{i=1}^{n}\left(\frac{b_i^{n_{i,j}}}{\tilde{k}_{i,j}+b_i^{n_{i,j}}}\right)^{\alpha_{i,j}}-\prod_{i=1}^{n}\left(\frac{a_i^{n_{i,j}}}{\tilde{k}_{i,j}+a_i^{n_{i,j}}}\right)^{\alpha_{i,j}}\right](\beta_j-\alpha_j)\\
&=\sum_{j=1}^{m}\mu_j\widetilde{V}_j\prod_{i=1}^{n}\left(\frac{a_i^{n_{i,j}}}{\tilde{k}_{i,j}+a_i^{n_{i,j}}}\right)^{\alpha_{i,j}}\left[\prod_{i=1}^{n}\left(\frac{b_i^{n_{i,j}}}{\tilde{k}_{i,j}+b_i^{n_{i,j}}}\frac{\tilde{k}_{i,j}+a_i^{n_{i,j}}}{a_i^{n_{i,j}}}\right)^{\alpha_{i,j}}-1\right](\beta_j-\alpha_j)\\
&=\sum_{j=1}^{m}\mu_jV_j\prod_{i=1}^{n}\left(\frac{c_i^{n_{i,j}}}{k_{i,j}+c_i^{n_{i,j}}}\right)^{\alpha_{i,j}}[\alpha_j*\zeta_j(c;\kappa_j,\eta_j)*\xi](\beta_j-\alpha_j).
\end{aligned}$$

Hence, from Lemma A.4, we have $\mathscr{D}_r(c)\xi=\tilde{r}(b)-\tilde{r}(a)$. Thus, the lemma is true. □

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