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Steady states and stability in metabolic networks without regulation

Oleksandr Ivanov [a,b,*], Arjan van der Schaft [b], Franz J. Weissing [a]

[a] Theoretical Research in Evolutionary Life Sciences, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747AG Groningen, The Netherlands

[b] Systems, Control and Applied Analysis Group, Johan Bernoulli Institute for Mathematics and Computer Science, University of Groningen, Nijenborgh 9, 9747AG Groningen, The Netherlands

HIGHLIGHTS

- Properties of steady states in metabolic networks with monotonic kinetics are considered.
- Stoichiometry and network structure determine uniqueness and stability of steady states.
- In single-substrate–single-product network with no cycles the steady state is unique and stable.
- In multiple-substrate–multiple-product networks the set of steady states can form a manifold.
- In metabolic networks with simple stoichiometry steady states are locally asymptotically stable.

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ABSTRACT

Metabolic networks are often extremely complex. Despite intensive efforts many details of these networks, e.g., exact kinetic rates and parameters of metabolic reactions, are not known, making it difficult to derive their properties. Considerable effort has been made to develop theory about properties of steady states in metabolic networks that are valid for any values of parameters. General results on uniqueness of steady states and their stability have been derived with specific assumptions on reaction kinetics, stoichiometry and network topology. For example, deep results have been obtained under the assumptions of mass–action reaction kinetics, continuous flow stirred tank reactors (CFSTR), concordant reaction networks and others. Nevertheless, a general theory about properties of steady states in metabolic networks is still missing. Here we make a step further in the quest for such a theory. Specifically, we study properties of steady states in metabolic networks with monotonic kinetics in relation to their stoichiometry (simple and general) and the number of metabolites participating in every reaction (single or many).

Our approach is based on the investigation of properties of the Jacobian matrix. We show that stoichiometry, network topology, and the number of metabolites that participate in every reaction have a large influence on the number of steady states and their stability in metabolic networks. Specifically, metabolic networks with single-substrate–single-product reactions have disconnected steady states, whereas in metabolic networks with multiple-substrates–multiple-product reactions manifolds of steady states arise. Metabolic networks with simple stoichiometry have either a unique globally asymptotically stable steady state or asymptotically stable manifolds of steady states. In metabolic networks with general stoichiometry the steady states are not always stable and we provide conditions for their stability. In order to demonstrate the biological relevance we illustrate the results on the examples of the TCA cycle, the mevalonate pathway and the Calvin cycle.

* Corresponding author at: Theoretical Research in Evolutionary Life Sciences, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747AG Groningen, The Netherlands. Tel.: +31 503638086.
E-mail address: o.ivanov@rug.nl (O. Ivanov).

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1. Introduction

Metabolism is a key characteristic of life. Understanding of metabolism, in particular the number of steady states in metabolic networks and their stability, is crucial for various disciplines in the life sciences. This is a difficult task, since even in the simplest organisms metabolic networks are very complex (Ma and Zeng, 2003). The complexity arises due to the large number of metabolites and reactions, and an intricate topology of the network.

The availability of high-throughput omics data and the development of statistical and structural methods for the analysis of these data allowed the reconstruction of many metabolic networks (Jamshidi and Palsson, 2008). However, even in networks where the structure has been unraveled, the exact form of the reaction kinetics typically is not known. If the reaction kinetics can be inferred, many kinetic parameters are often not known. In view of this complexity and the lack of detailed quantitative information there is a need for developing more qualitative techniques to investigate the properties of metabolic networks.

Two aspects are crucial for the understanding of a metabolic network. The first one is the network structure, determined by network topology and stoichiometry. Network topology is defined by interconnections between metabolites via reactions they participate in. Stoichiometry is specified by the number of molecules by which the metabolites participate in different reactions. The second is reaction kinetics. The solution to the problem of lack of quantitative knowledge about metabolic networks is to investigate their properties based on general assumptions on their structure and kinetics. This direction of research is known as chemical reaction network theory (Feinberg, 1979). The main results of chemical reaction network theory are reviewed in several papers (Feinberg, 1979; Gunawardena, 2003; Angeli, 2009). Chemical reaction network theory can be structured by different approaches for studying the number of possible steady states and their stability. The first approach is based on concepts of balancing and related Deficiency theory (Horn and Jackson, 1972; Feinberg, 1987, 1988, 1995; van der Schaft et al., 2013, 2015). The second approach is based on construction of the so called species-reaction graph (Craciun and Feinberg, 2006). The third approach is based on the properties of the stoichiometric and Jacobian matrices. The present paper is based on the third approach. We briefly review results on the third approach below. For CFSTR networks, i.e., with inflow and outflow of all metabolites, and with mass action kinetics Craciun and Feinberg (2005) showed that the network does not have the capacity for multiple steady states if the determinant of the Jacobian matrix is not equal to zero, and it does have such capacity if the determinant of the Jacobian matrix is equal to zero. For CFSTR networks with non-autocatalytic (NAC) kinetics Banaji et al. (2007) showed that if the stoichiometric matrix has the 'SSD property' (strongly sign determined), i.e., the property that all of its submatrices are either singular or else 'sign nonsingular' (i.e., sign of its determinant is nonzero and can be determined from the signs of its entries), then the network cannot admit multiple steady states. Another line of research concentrates on the number of metabolites participating in each reaction as substrates and products. In particular it was shown that in metabolic networks with a single substrate and single product in each reaction (SSSP) and with Hill kinetics multiple steady states are precluded (Lei et al., 2010), while in the case when multiple substrates and products (MSMP) may participate in a single reaction, then multiple steady states are precluded if and only if the Jacobian matrix is nonsingular (Guo et al., 2012). Banaji and Baigent (2008) proved uniqueness and global asymptotic stability of a steady state for SSSP metabolic with monotonic kinetics and with simple stoichiometry (stoichiometric coefficients are ± 1 or 0) for any network topology. Independently, Flach and Schnell (2010) showed that in SSSP metabolic networks with monotonic kinetics with simple stoichiometry and with linear and branched topologies a steady state is locally asymptotically stable. Reznik and Segré (2010) conjectured local asymptotic stability of steady states for SSSP metabolic networks with simple stoichiometry, monotonic kinetics and with irreversible reactions. Later Reznik et al. (2013) proved this analytically.

In metabolic networks in the majority of pathways it is the case that in each reaction only one molecule of every reactant metabolite participates (this is referred to as *simple stoichiometry*) (Palsson, 2011). An example of SSSP pathways with simple stoichiometry is the metabolism of xylose. The most common type of metabolic pathways is MSMP with simple stoichiometry. Examples are glycolysis, the TCA cycle, galactolysis, pentose phosphate pathway, and many others.

However, simple stoichiometry is *not always* the case in metabolic networks. For example, in such SSSP pathways as the Calvin cycle, the mevalonate pathway, and thiolysis reactions, several molecules participate as substrates or products in a single metabolic reaction (general stoichiometry). Finally, the urea cycle is an example of an MSMP network with general stoichiometry.

Together there are four combinations of stoichiometry types (simple, general) and number of metabolites as substrates or products in a reaction (SSSP, MSMP), and they all have biological relevance. The aim of this paper is to investigate the number of steady states (single or multiple) and their stability in each of these network types under the general assumption of *monotonic kinetics*. Since the case of SSSP with simple stoichiometry has been investigated in detail in previous work, as we described above, we concentrate our efforts on the three remaining cases. We study these network properties by investigating the properties of the Jacobian matrix.

This paper is structured as follows. We start in Section 2 with an introduction to metabolic networks and important definitions. Next, we give general results on steady states of metabolic networks without any assumptions on reaction kinetics and network structure (Section 3). Then, in Section 4 we consider properties of steady states and their stability in metabolic networks with an assumption that all reactions are of single-substrate–single-product (SSSP) type with simple and general kinetics. In Section 5 we give results on properties of steady states and their stability in multiple-substrate–multiple-product (MSMP) metabolic networks with simple and general kinetics. We give an overview of our results and their implications in the Section 6.

2. Metabolic networks

A *metabolic network* is a set of chemical species, also called metabolites, together with metabolic reactions in which these metabolites participate.

We denote the metabolite number i with the capital letter X_i and its concentration by x_i. The dynamics of metabolite concentrations in a metabolic network consisting of m metabolites and n chemical reactions is described by a system of ordinary differential equations (Palsson, 2011):

$$\dot{x} = Sv(x). \tag{1}$$

Here x is the m-dimensional vector of metabolite concentrations, S is the $m \times n$ stoichiometric matrix and $v(x)$ is an n-dimensional vector of reaction rates, which are functions of the metabolite concentrations. In the stoichiometric matrix the rows correspond to different metabolites and the columns correspond to different reactions. The entry of the stoichiometric matrix s_{ij} represents the number of molecules of metabolite X_i used in reaction j. If X_i is a *substrate* of a reaction j then $S_{i,j} = -s_{i,j} < 0$, if X_i is a *product* then $S_{i,j} = s_{i,j} > 0$. If X_i does not participate in reaction j then $S_{i,j} = s_{i,j} = 0$.

We also assume that the rates of inflow reactions are constant. On contrary, outflow reaction rates depend on metabolite concentrations.

Importantly, the Eq. (1) allows to decompose the dynamics of metabolite concentrations in a structural part (topology and stoichiometry), represented by the stoichiometric matrix S, and a kinetics part, represented by the vector $v(x)$.

2.1. Topology and stoichiometry

The nonzero entries of the stoichiometric matrix specify interconnections of metabolites by metabolic reactions, i.e., topology of the network (in the sense of graph topology). The values of nonzero entries specify the stoichiometry, i.e., the number of molecules with which each metabolite participates in reaction.

In case of simple stoichiometry of metabolic networks only one molecule of every reactant metabolite participates in reactions. This leads to the fact that the entries of the stoichiometric matrix are ± 1 or 0. In the case of *general stoichiometry* metabolites may participate in reactions with arbitrary number of molecules, and therefore corresponding stoichiometric coefficients may admit arbitrary integer values.

Metabolic reactions may involve single metabolite as substrate and single metabolite as a product (*single-substrate–single-product reactions or SSSP*), and may involve multiple metabolites as substrates and products (*multiple-substrate–multiple-product reactions or MSMP*). The majority of metabolic networks have metabolic reactions with multiple substrates and products (Steuer and Junker, 2008).

Metabolic networks can have different topology. Below we give several examples.

Linear network:

$$\xrightarrow{v_1} X_1 \xleftrightarrow{v_2} X_2 \xleftrightarrow{v_3} X_3 \xrightarrow{v_4} \tag{2}$$

The dynamics of the linear network (2) can be represented by the following system of differential equations (left), and in matrix form $\dot{x} = Sv(x)$ (right):

$$\begin{aligned} \dot{x}_1 &= v_1 - v_2(x) \\ \dot{x}_2 &= v_2(x) - v_3(x) \\ \dot{x}_3 &= v_3(x) - v_4(x) \end{aligned} \qquad \begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \end{pmatrix} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2(x) \\ v_3(x) \\ v_4(x) \end{pmatrix}. \tag{3}$$

Branched network:

$$\begin{array}{r} \xrightarrow{v_1} X_1 \xleftrightarrow{v_2} X_2 \xleftrightarrow{v_3} X_3 \xrightarrow{v_5} \\ X_2 \xleftrightarrow{v_4} X_4 \xrightarrow{v_6} \end{array} \begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \\ \dot{x}_4 \end{pmatrix} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 & 0 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2(x) \\ v_3(x) \\ v_4(x) \\ v_5(x) \\ v_6(x) \end{pmatrix} \tag{4}$$

Cyclic network:

$$\begin{array}{r} \xrightarrow{v_1} X_1 \xleftrightarrow{v_2} X_2 \xleftrightarrow{v_3} X_3 \xleftrightarrow{v_4} X_1 \\ X_3 \xrightarrow{v_5} \end{array} \begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \end{pmatrix} = \begin{pmatrix} 1 & -1 & 0 & 1 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2(x) \\ v_3(x) \\ v_4(x) \\ v_5(x) \end{pmatrix} \tag{5}$$

Networks that consist of combinations of linear and branched parts and contain no cycles are called networks with *tree topology*.

A *reaction complex* is a set of substrates or products of a reaction. That is a set of substrates of a metabolic reaction is one complex and set of products is another complex. For example, in the following reaction $X_1 + X_2 \leftrightarrow X_3$ the reaction complexes are $C_1 = \{X_1 + X_2\}$ and $C_2 = \{X_3\}$.

A *path* between nodes i and j in a network is a sequence of links that lead from the node i to node j.

A complex C_i said to be connected to complex C_j if there exists a path between them. Note, that path and connectedness does not imply directionality.

A *linkage class* is a maximal set of connected reaction complexes. Reaction set

$$X_1 + X_2 \leftrightarrow X_3$$

$$X_5 \leftrightarrow X_6$$

has two linkage classes $X_1 + X_2 \leftrightarrow X_3$ and $X_5 \leftrightarrow X_6$.

2.2. Reaction kinetics

Topology and stoichiometry of metabolic networks are often known, while the specific dependencies of reaction rates on metabolite concentrations are typically not well determined in practice. In theoretical works the following reaction rates are often assumed:

- Mass–action kinetics: $v(x) = k_+ \prod_s x_s - k_- \prod_p x_p$, where $v(x)$ is a scalar function, k_+ and k_- are constants, s is an index of reaction substrate, p is an index of reaction product, x_s is the concentration of reaction substrate X_s and x_p is the concentration of reaction product X_p.
- Michaelis–Menten kinetics: $v(x) = \frac{v_{max} x}{K + x}$, where x is the concentration of reaction substrate, v_{max} is the maximal reaction rate and K_m is the Michaelis constant.
- Hill kinetics: $v(x_s) = \frac{v_{max} x^n}{K^n + x^n}$, where x is the concentration of reaction substrate, and K and n are a positive constants.
- Convenience kinetics (Liebermeister and Klipp, 2006): $v(x_s, x_p) = \frac{v_f \frac{x_s}{K_s} - v_r \frac{x_p}{K_p}}{\frac{x_s}{K_s} + \frac{x_p}{K_p} + 1}$, where v_f and v_r are maximal forward and reversed reaction rates respectively, x_s and x_p are concentrations of reaction substrate and product respectively, K_s and K_p are constants of equilibrium.

All these types of kinetics have in common is that the reaction rate is faster at higher substrate concentrations, slower at higher product concentrations, and not affected by other metabolites than substrates and products. We define *monotonic* reaction kinetics as follows:

$$\frac{\partial v_j(x)}{\partial x_i} = \begin{cases} g_{j,i}(x) > 0, & \text{if } X_i \text{ is a substrate of reaction } j, \quad \text{i.e. } S_{i,j} = -s_{i,j} < 0 \\ -g_{j,i}(x) \le 0, & \text{if } X_i \text{ is a product of reaction } j, \quad \text{i.e. } S_{i,j} = s_{i,j} > 0 \\ g_{j,i}(x) = 0, & \text{otherwise}, \quad \text{i.e. } S_{ij} = 0 \end{cases} \tag{6}$$

Note that $s_{i,j}$ and $g_{j,i}(x)$ are always nonnegative numbers. As indicated by the notation $g_{j,i}(x)$ depend on metabolite concentrations x. For each $g_{j,i}(x)$ this dependence will be suppressed from now on, and we will write $g_{j,i}$ instead. This assumption of monotonic kinetics is very natural and general. For example, mass–action, Michaelis–Menten kinetics, Hill kinetics and convenience kinetics are monotonic. The monotonicity property (6) excludes the possibility of regulation. For example, substrate inhibition of reaction and the influence of metabolites that are not substrates or products are not possible. Monotonicity assumption (6) also excludes autocatalytic reactions.

We define *irreversible* reactions as reactions for which reaction rate does not depend on the concentration of a product. That is, if X_i is the product in an irreversible reaction j then $\partial v_j(x)/\partial x_i = 0$. For irreversible reactions the set of inequalities that describe monotonic kinetics (6) simplifies to

$$\frac{\partial v_j(x)}{\partial x_i} = \begin{cases} g_{j,i}(x) > 0, & \text{if } X_i \text{ is a substrate of reaction } j, \quad \text{i.e. } S_{i,j} = -s_{i,j} < 0 \\ g_{j,i}(x) = 0, & \text{otherwise}, \quad \text{i.e. } S_{i,j} \ge 0 \end{cases} \tag{7}$$

Although in principle all chemical reactions are reversible due to the laws of thermodynamics, many reactions in metabolic networks can be considered irreversible in practice (Cornish-Bowden and Cárdenas, 2000). In fact, in many organisms there are more irreversible reactions than reversible ones. For example, in *Saccharomyces cerevisiae* 719 out of 1149 reactions are irreversible, in *Helicobacter pylori* 314 reactions out of 479 are irreversible (Nishikawa et al., 2008). Under some natural conditions more than 92% of metabolic reactions are 'effectively' irreversible (Nishikawa et al., 2008).

2.3. Linear approximation

We linearize dynamical system that represents behavior of a metabolic network by the means of the *Jacobian matrix*. For a dynamical system $\dot{x} = f(x)$ the Jacobian is denoted as $J(x) = \frac{\partial f(x)}{\partial x}$. In our case

$$J(x) = \frac{\partial (Sv(x))}{\partial x} = S \frac{\partial (v(x))}{\partial x} = S \cdot Dv(x). \tag{8}$$

The matrix $Dv(x) \equiv (\partial v(x)/\partial x)$ is the *gradient* matrix (Palsson, 2011). For each x $Dv(x)$ is $n \times m$ matrix, where n is the number of reactions and m is the number of metabolites. The matrix $Dv(x)$ describes the dependencies of the reaction rates on the concentrations of metabolites. A row j of the matrix $Dv(x)$ represents derivatives of the rate of a reaction j with respect to the concentration of different metabolites, while each column i represents the derivatives of different reaction rates with respect to the concentration of a metabolite X_i.

We define the matrix S_0 as the stoichiometric matrix in which all the entries S_{km} that correspond to inflows of all metabolites k in reactions m are replaced by zeroes. It follows that the Jacobian matrix (8) can be presented as

$$J(x) = S_0 Dv(x). \tag{9}$$

This is because the entries of the gradient matrix that correspond to the inflow entries (recall that the inflow reaction rates are assumed to be constant) of the stoichiometric matrix are zero. Therefore, the inflow entries of the stoichiometric matrix will always be multiplied by zero entries of the gradient matrix, and inflow entries of the stoichiometric matrix will not affect the entries of the Jacobian matrix.

Due to the monotonicity assumption (6) we have

$$\operatorname{sign}(g_{j,i}(x)) = \operatorname{sign}(-S_{0i,j}). \tag{10}$$

The expression (10) shows an important relation between the structure of metabolic network, represented by the matrix S_0, and reaction kinetics, represented by the matrix $Dv(x)$.

Example 1.

The gradient matrix and S_0 matrix for the network (2) obtained from the structure of the stoichiometric matrix, based on the assumptions listed above are

$$S_0 = \begin{pmatrix} 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix}, \quad Dv(x) = \begin{pmatrix} 0 & 0 & 0 \\ g_{2,1} & -g_{2,2} & 0 \\ 0 & g_{3,2} & -g_{3,3} \\ 0 & 0 & g_{4,3} \end{pmatrix}. \tag{11}$$

The Jacobian matrix is

$$J(x) = S_0 Dv(x) = \begin{pmatrix} -g_{2,1} & g_{2,2} & 0 \\ g_{2,1} & -g_{3,2} - g_{2,2} & g_{3,3} \\ 0 & g_{3,2} & -g_{4,3} - g_{3,3} \end{pmatrix}. \tag{12}$$

3. Steady states

At steady state concentrations of all metabolites remain constant, i.e. $\dot{x} = f(x^*) = 0$. The Eq. (1) at steady state is

$$Sv(x^*) = 0. \tag{13}$$

Below we first investigate general properties of steady states in metabolic networks without applying any assumptions. The key question is there a single steady state or multiple? In case there are multiple steady states then how are they organized? To answer these questions we investigate the Jacobian matrix (9).

Disregarding any assumptions on kinetics, stoichiometry and topology we can state the following theorem.

Theorem 1. *Let at least one steady state x^* exist. Let* $\mathrm{rank}(S_0) = m - k$. *Then the set of steady states is a manifold of dimension $d \geq k$.*

Proof. By definition $Sv(x^*) = 0$. By the Implicit Function Theorem the set of steady states $\{x^* \mid Sv(x^*) = 0\}$ is a manifold with dimension corresponding to the rank deficiency of the Jacobian matrix $J(x^*)$. For arbitrary matrices A and B $\mathrm{rank}(AB) \leq \min(\mathrm{rank}\ (A), \mathrm{rank}\ (B))$ (Datta, 2006). Since $J(x) = S_0 Dv(x)$ then $\mathrm{rank}(J(x^*)) \leq \mathrm{rank}(S_0)$. Suppose we have $\mathrm{rank}(S_0) = m - k$ then the rank deficiency of $J(x^*)$ is at least k and therefore the set of steady states $\{x^* \mid Sv(x^*) = 0\}$ is a manifold with dimension $d \geq k$.□

Remark. It is possible that the manifold of steady states consists of a number of disconnected components. In case $d = 0$ the steady states are disconnected points in the space of metabolite concentrations. As a result of the Theorem 1 to get disconnected steady states full rank of the Jacobian matrix is required.

Now, the question is under which conditions on stoichiometry a metabolic network can be expected to have manifold of steady states? The easiest case occurs when several metabolites participate in only one reaction complex (Example 2). In this case the corresponding rows of the stoichiometric matrix will be equal, increasing the rank deficiency of the stoichiometric matrix, and thereby increasing the rank deficiency of the Jacobian matrix. When several reaction complexes share a metabolite then it is possible that the sum of several rows of S_0 is equal to zero, which also increases the rank deficiency of the stoichiometric and Jacobian matrices.

Example 2.

Consider the following metabolic network

$$\xrightarrow{v_1} X_1 \xrightarrow{v_2} X_2$$

$$X_1 + X_2 \xleftrightarrow{v_3} X_3 + X_4 \xleftrightarrow{v_4} X_5 \xrightarrow{v_5} \tag{14}$$

The dynamic behavior of this system $\dot{x} = Sv(x)$ is represented by

$$\begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \end{pmatrix} = \begin{pmatrix} 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3(x) \\ v_4(x) \\ v_5(x) \end{pmatrix}. \tag{15}$$

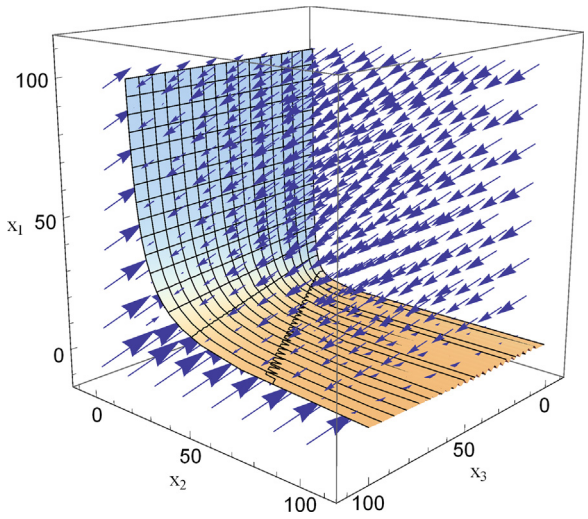

Fig. 1. The set steady states of the metabolic network (14) is two-dimensional manifold. The two-dimensional manifold is depicted in the space of concentrations x_1, x_2 and x_3. The vector field (blue arrows) represents the convergence of solutions to the two-dimensional manifold of steady states.

The matrix S_0 is

$$S_0 = \begin{pmatrix} 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix}. \tag{16}$$

The rank deficiency of S_0 is equal to two. Accordingly, the set of steady states comprises manifold of dimension larger of equal to two. By making specific assumptions on reaction kinetics, this manifold can be calculated explicitly (Supplementary Appendix A), as illustrated in Fig. 1.

Example 3.

The stoichiometric matrix for the TCA cycle (Supplementary Appendix B) has rank deficiency equal to five. Hence, irrespectively of specific reaction kinetics and parameters, we can already conclude that a steady state, if it exists, is part of a steady state manifold of dimension larger or equal to five.

The rest of the paper is focused on stability of steady states. Here again the Jacobian matrix plays a very important role. A steady state is *locally asymptotically stable* if and only if all eigenvalues of the Jacobian matrix (9) have negative real part (Arrowsmith and Place, 1992). Since stability is easier to judge in single-substrate–single-product networks we will treat this case first and after that we generalize to multiple-substrate–multiple-product networks.

4. Single-substrate–single-product metabolic networks

First, we consider the case of simple stoichiometry. Banaji and Baigent (2008) proved the following theorem.

Theorem 2. *(Banaji and Baigent, 2008) Consider single-substrate–single-product metabolic network. Assume the stoichiometry is simple and reaction rates are monotonic. Then the steady state, if it exists, is unique and globally asymptotically stable for any network topology.*

As we mentioned above, majority of metabolic pathways have simple stoichiometry. However, there are pathways with general stoichiometry which Theorem 2 does not cover. Consider for example mevalonate pathway in Eq. (17).

$$\longrightarrow 2\ \mathrm{A-CoA} \longrightarrow \mathrm{AA-CoA} \longrightarrow \mathrm{HMG-CoA} \longrightarrow \mathrm{MA} \longrightarrow \mathrm{M-5-PP} \longrightarrow \mathrm{IPP} \longrightarrow \mathrm{DMAPP} \longrightarrow. \tag{17}$$

Here, A-CoA is acetyl-CoA, AA-CoA is acetoacetyl-CoA, HMG-CoA is 3-hydroxy-3-methylglutaryl-CoA, MA is mevalonic acid, M-5-P is mevalonate-5-phosphate, M-5-PP is mevalonate-5-pyrophosphate, IPP is isopentenyl-5-pyrophosphate, DMAPP is dimethylallylpyrophosphate.

Mevalonate pathway produces molecules IPP and DMAPP which are used to make isoprenoids, a rich class of over 30,000 molecules, such as steroid hormones, cholesterol, coenzyme Q10, vitamin K and others. In the first reaction of mevalonate pathway two molecules of acetyl-CoA (A-CoA) are combined producing acetoacetyl-CoA (AA-CoA) and CoA-SH. The concentration of cofactor CoA-SH can be assumed to be constant, so this pathway can be considered as SSSP, as it is depicted in Eq. (17).

Theorem 3 below generalize Theorem 2 to SSSP networks with general stoichiometry, and with linear, branched and tree topology.

We will prove the results below using the theory of M-matrices. By definition an M-matrix is a matrix with nonpositive off-diagonal entries, $J_{i,j}(x) \leq 0$ for all $i \neq j$, and all leading principal minors positive (Horn and Johnson, 1991).

A *leading principal minor* f_r of a matrix is a square upper-left submatrix that consists of entries in rows and columns from 1 to r.

M-matrices is an important class of matrices with many equivalent properties and important relationship to stability and injectivity (Plemmons, 1977).

Lemma 1. *If $-J(x)$ is an M-matrix then the steady state in a corresponding metabolic network, if it exists, is unique.*□

Proof. The class of nonsingular M-matrices is a subset of class of P-matrices. There is a result stating that if the Jacobian of a function is a P-matrix (or equivalently a negative P-matrix), this guarantees injectivity of the set of a function on any rectangular region of $\mathbb{R}^n$ (Gale and Nikaido, 1965).

Lemma 2. *If $-J(x)$ is an M-matrix then the steady state, if it exists, in a corresponding metabolic network is locally asymptotically stable.*

Proof. The eigenvalues of M-matrices always have a positive real part (Plemmons, 1977). Therefore, if $-J(x)$ is an M-matrix then $J(x)$ have eigenvalues with negative real part. As a consequence in systems with Jacobian being negative M-matrix if steady state exists, then it is unique and locally asymptotically stable.□

Theorem 3. *Consider single-substrate–single-product metabolic network with general stoichiometry. Assume reaction rates are monotonic and network has tree topology. Then the steady state, if it exists, is unique and locally asymptotically stable.*

Proof. According to Lemma 1 and Lemma 2 to prove that in SSSP metabolic network with monotonic reaction rates, general stoichiometry and tree topology the steady state, if it exist, is always unique and locally asymptotically stable we only need to prove that $-J(x)$ is always an M-matrix. We first show that this theorem holds for linear networks, then for branched networks and finally for networks with tree topology.

4.1. Linear networks

A general representation of linear metabolic network with single-substrate–single-product reactions is of the form

$$\xrightarrow{v_1} X_1 \overset{v_2}{\longleftrightarrow} X_2 \overset{v_3}{\longleftrightarrow} \dots \overset{v_m}{\longleftrightarrow} X_m \xrightarrow{v_{m+1}} \tag{18}$$

Stoichiometric coefficients in (18) are omitted for the illustration purpose.

The Jacobian matrix for linear SSSP networks with general stoichiometry is tridiagonal with negative diagonal entries and positive off-diagonal entries:

$$J(x) = \begin{pmatrix} -s_{1,2}g_{2,1} & s_{1,2}g_{2,2} & & & & \\ s_{2,2}g_{2,1} & -s_{2,2}g_{2,2} - s_{2,3}g_{3,2} & s_{2,3}g_{3,3} & & & \\ & s_{3,3}g_{3,2} & \ddots & \ddots & & \\ & & \ddots & \ddots & s_{m-1,m}g_{m,m} & \\ & & & s_{m,m}g_{m,m-1} & -s_{m,m}g_{m,m} - s_{m,m+1}g_{m+1,m} \end{pmatrix}. \tag{19}$$

We prove by induction that the leading principal minor of the Jacobian matrix (19) is of the form

$$f_k = (-1)^k \prod_{i=1}^{k} s_{i,i+1}g_{i+1,i}. \tag{20}$$

The first leading principal minor of the Jacobian matrix (19) is $f_1 = -s_{1,2}g_{2,1}$. The second leading principal minor is $f_2 = s_{1,2}s_{2,3}g_{2,1}g_{3,2}$. Both f_1 and f_2 satisfy the Eq. (20). Let us assume that the Eq. (20) is true and find the next leading principal minor f_{k+1}. Using the recursion equation for a determinant of a tridiagonal matrix (El-Mikkawy, 2004) we get

$$\begin{aligned} f_{k+1} &= (-s_{k+1,k+1}g_{k+1,k+1} - s_{k+1,k+2}g_{k+2,k+1})f_k \\ &\quad - s_{k+1,k+1}g_{k+1,k}s_{k,k+1}g_{k+1,k+1}f_{k-1} \\ &= (-s_{k+1,k+1}g_{k+1,k+1} - s_{k+1,k+2}g_{k+2,k+1})(-1)^k \prod_{i=1}^{k} s_{i,i+1}g_{i+1,i} \\ &\quad - s_{k+1,k+1}g_{k+1,k}s_{k,k+1}g_{k+1,k+1}(-1)^{k-1} \prod_{i=1}^{k-1} s_{i,i+1}g_{i+1,i} \\ &= -s_{k+1,k+2}g_{k+2,k+1}(-1)^k \prod_{i=1}^{k} s_{i,i+1}g_{i+1,i} \\ &= (-1)^{k+1} \prod_{i=1}^{k+1} s_{i,i+1}g_{i+1,i}, \end{aligned}$$

which is according to Eq. (20).

From the Eq. (20) we conclude that all leading principal minors of the matrix (19) alternate in sign starting from negative. Combining this with positive off-diagonal entries results in that the Jacobian matrix (18) is negative M-matrix. According to Lemma 1 and Lemma 2 this means that if the steady state of a general linear metabolic network (18) exists then it is always unique and locally asymptotically stable.

4.2. Branched networks

A general representation of branched metabolic network with single-substrate–single-product reactions is of the form

$$\begin{aligned} &\xrightarrow{v_1} X_1 \overset{v_2}{\longleftrightarrow} \dots \overset{v_p}{\longleftrightarrow} X_p \overset{v_{p+1}}{\longleftrightarrow} \dots \overset{v_y}{\longleftrightarrow} X_y \xrightarrow{v_{m+1}} \\ &\qquad X_p \overset{v_{y+1}}{\longleftrightarrow} \dots \overset{v_m}{\longleftrightarrow} X_m \xrightarrow{v_{m+2}} \end{aligned} \tag{21}$$

Without loss of generality we assume that there is a linear chain of y metabolites, in which branching occurs at the metabolite X_p. We numerate all reactions sequentially in the first branch from 1 to y, and then continue numerating in the second branch from $y+1$ to m. Outflow reactions ($m+1$ and $m+2$) we numerate as the last ones. In Appendix C in Supplementary materials we prove that the corresponding Jacobian matrix for the network (21) is negative M-matrix. Therefore, in metabolic network (21) if the steady state exists it is always unique and locally asymptotically stable steady state.

4.3. Networks with tree topology

Networks with tree topology consist of combinations of linear and branched networks, and do not contain cycles. In Appendix D in Supplementary materials we prove that the Jacobian matrix for metabolic network with tree topology is an M-matrix. Therefore in SSSP metabolic networks with general stoichiometry and tree topology if the steady state exists it is always unique and locally asymptotically stable.□

Example 4.

In this example we apply Theorem 3 to mevalonate pathway. We assume all reaction rates in this pathway to be monotonic. Since Mevalonate pathway is linear and SSSP then irrespectively of specific form of reaction rates and reaction parameters the steady state, if it exists, is unique and locally asymptotically stable.

4.4. Cyclic networks

In contrast to networks with tree topology cyclic networks do not always have unique and locally asymptotically stable steady state. For simplicity we show the condition for the uniqueness of a steady state (if it exists) and local asymptotic stability for cyclic network with irreversible reactions. An example of such network is Calvin cycle (Eq. (22)). In Calvin cycle one molecule of ribulose 5-phosphate breaks into two molecules of 3-phosphoglycerate, one of which is used in Calvin cycle, and the other one is used in other metabolic pathways.

$$\begin{aligned}
&\xrightarrow{v_1} R-1,5-\mathrm{bP} \xrightarrow{v_2} 2\ \mathrm{PG}\\
&\mathrm{PG} \xrightarrow{v_3} 1,3-\mathrm{bPG} \xrightarrow{v_4} G-3-P \xrightarrow{v_5} R-5-P \xrightarrow{v_6} R-1,5-\mathrm{bP}\\
&\mathrm{PG} \xrightarrow{v_7}
\end{aligned} \tag{22}$$

Here, R-1,5-bP is ribulose 1,5-biphosphate, PG is 3-phosphoglycerate, 1,3-bPG is 1,3-biphoshoglycerate, G-3-P is glyceraldehyde-3-phosphate, R-5-P is ribulose 5-phosphate.

A general representation of cyclic metabolic network with single-substrate–single-product irreversible reactions is of the form

$$\begin{aligned}
&\xrightarrow{v_1} X_1 \xrightarrow{v_2} X_2 \xrightarrow{v_3} \ldots \xrightarrow{v_m} X_m \xrightarrow{v_{m+1}} X_1\\
&X_k \xrightarrow{v_{m+2}}.
\end{aligned} \tag{23}$$

Here the last metabolite in a cycle X_m is transformed into the first metabolite X_1 and some metabolite X_k outflows from the system. The Jacobian matrix in this case is the following:

$$J(x)=\begin{pmatrix}
-s_{1,2}g_{2,1} & & & & & & & & & s_{1,m+1}g_{m+1,m}\\
s_{2,2}g_{2,1} & \ddots & & & & & & & & \\
 & \ddots & -s_{k-1,k}g_{k,k-1} & & & & & & & \\
 & & s_{k,k}g_{k,k-1} & -s_{k,k+1}g_{k+1,k}-s_{k,m+2}g_{m+2,k} & & & & & & \\
 & & & s_{k+1,k+1}g_{k+1,k} & -s_{k+1,k+2}g_{k+2,k+1} & & & & & \\
 & & & & -s_{k+1,k+2}g_{k+2,k+1} & \ddots & & & & \\
 & & & & & \ddots & -s_{m-1,m}g_{m,m-1} & & & \\
 & & & & & & -s_{m,m}g_{m,m-1} & -s_{m,m+1}g_{m+1,m}
\end{pmatrix}. \tag{24}$$

The Jacobian matrix (24) is lower triangular except for the positive entry $s_{1,m+1}g_{m+1,m}$ in the upper right corner of the matrix.

Theorem 4. *Consider single-substrate–single-product cyclic metabolic network (23) with general stoichiometry. Assume reactions are irreversible and reaction rates are monotonic. Then the steady state, if it exists, is unique and locally asymptotically stable if*

$$(s_{k,k+1}g_{k+1,k}+s_{k,m+2}g_{m+2,k})\prod_{i=2,i\neq k}^{m+1}s_{i-1,i}>s_{1,m+1}g_{k+1,k}\prod_{i=2}^{m}s_{i,i}. \tag{25}$$

Here the term $g_{k+1,k}=\partial v_{k+1}/\partial x_k$ corresponds to the derivative of the reaction rate of reaction k with respect to the concentration of the metabolite X_k. The term $g_{m+2,k}=\partial v_{m+2}/\partial x_k$ corresponds to the derivative of the reaction rate of the outflow reaction with respect to the metabolite X_k. The stoichiometric coefficient $s_{k,k+1}$ corresponds to the number of molecules of the metabolite X_k which are used in the reaction k: $s_{k,k+1}X_k \longrightarrow s_{k+1,k+1}X_{k+1}$. The stoichiometric coefficient $s_{k,m+2}$ corresponds to the number of molecules of the metabolite X_k which outflow as the result of the reaction $m+2$: $s_{k,m+2}X_k \longrightarrow$. The stoichiometric coefficient $s_{1,m+1}$ corresponds to the number of molecules of the metabolite X_1 which are produced in the reaction $m+1$ that closes the cycle: $s_{m,m+1}X_m \longrightarrow s_{1,m+1}X_1$. The term $\prod_{i=2}^{m}s_{i-1,i}$ corresponds to the product of stoichiometric coefficients that appear on the diagonal of the Jacobian matrix and correspond to the consumption of reaction substrates. The term $\prod_{i=2}^{m}s_{i,i}$ corresponds to the product of the stoichiometric coefficients that appear on the off-diagonal entries of the Jacobian matrix and correspond to creation of reaction products.

We give the proof of Theorem 4 in Appendix E in Supplementary materials.

Example 5.

In this example we apply Theorem 4 to Calvin cycle (Eq. (22)). The Jacobian matrix in this case is

$$J(x) = SDv(x)$$

$$= \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 2 & -1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 \end{pmatrix} \begin{pmatrix} 0 & 0 & 0 & 0 & 0 \\ g_{2,1} & 0 & 0 & 0 & 0 \\ 0 & g_{3,2} & 0 & 0 & 0 \\ 0 & 0 & g_{4,3} & 0 & 0 \\ 0 & 0 & 0 & g_{5,4} & 0 \\ 0 & 0 & 0 & 0 & g_{6,5} \\ 0 & g_{7,2} & 0 & 0 & 0 \end{pmatrix}$$

$$= \begin{pmatrix} -g_{2,1} & 0 & 0 & 0 & g_{6,5} \\ 2g_{2,1} & -g_{3,2}-g_{7,2} & 0 & 0 & 0 \\ 0 & g_{3,2} & -g_{4,3} & 0 & 0 \\ 0 & 0 & g_{4,3} & -g_{5,4} & 0 \\ 0 & 0 & 0 & g_{5,4} & -g_{6,5} \end{pmatrix}.$$

In Calvin cycle there are $m = 5$ metabolites, and second metabolite outflows from the pathway, that is $k = 2$. According to Eq. (25) the condition for Calvin cycle to have unique and locally asymptotically stable steady state is $(g_{3,2}+g_{7,2}) > 2g_{3,2}$, which finally gives $g_{7,2} > g_{3,2}$, or alternatively $\frac{\partial v_7(x_2)}{\partial x_2} > \frac{\partial v_3(x_2)}{\partial x_2}$.

Example 6.

Consider the following cyclic network:

$$\begin{aligned} &\xrightarrow{v_1} X_1 \xrightarrow{v_2} X_2 \\ &2X_2 \xrightarrow{v_3} X_3 \xrightarrow{v_4} X_1 \\ &X_3 \xrightarrow{v_5}. \end{aligned} \tag{26}$$

The stoichiometric matrix for this network is

$$S = \begin{pmatrix} 1 & -1 & 0 & 1 & 0 \\ 0 & 1 & -2 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 \end{pmatrix} \tag{27}$$

The inflow reaction rate v_1 is constant. We assume that the other reaction rates obey Michaelis–Menten kinetics:

$$v_i = \frac{v_{\max,i} x_i}{x_i + K_i}, i = 2, 3, 4, 5. \tag{28}$$

The locally asymptotically stable steady state for this system and the vector field in the phase space of metabolite concentrations are depicted in Fig. 2.

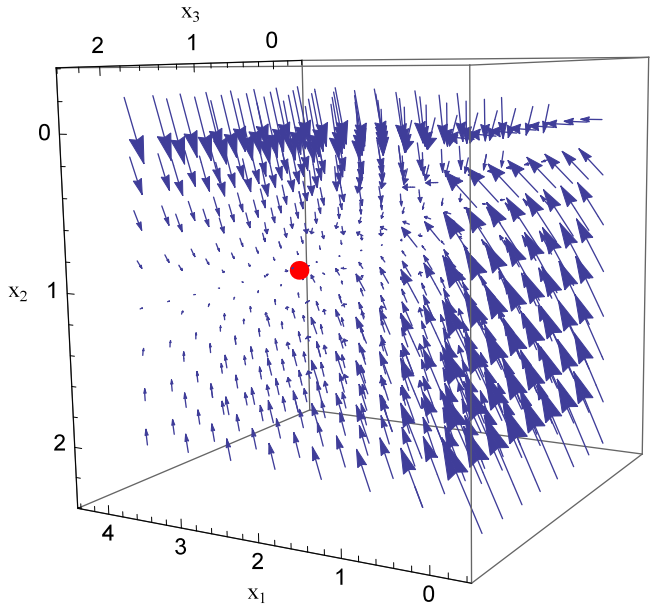

Fig. 2. Locally asymptotically stable steady state (red point) and vector field (blue arrows) for the cyclic metabolic network (24). Parameters are presented in Appendix A in Supplementary materials.

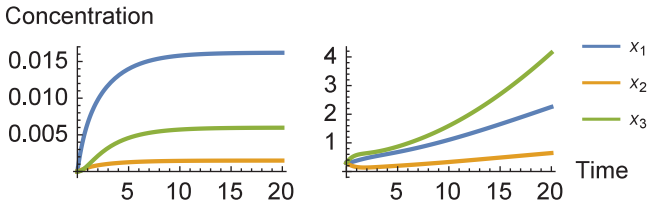

Fig. 3. Dynamics of metabolite concentrations of the metabolic network (27) near stable (left plot) and unstable (right plot) steady states. Initial conditions are: {0.0, 0.0, 0.0} (left plot) and {0.3, 0.3, 0.3} (right plot). The steady states are: {0.0162, 0.0015, 0.006} and {0.1728, 0.0369, 0.1592}.

Example 7.

Now we consider the cycle with an unstable steady state. Consider the following cyclic network

$$\begin{aligned} &\xrightarrow{v_1} X_1 \xrightarrow{v_2} X_2 \xrightarrow{v_3} 2X_3 \\ &X_3 \xrightarrow{v_4} X_1 \\ &X_2 \xrightarrow{v_5}. \end{aligned} \tag{29}$$

The stoichiometric matrix for this network is

$$S = \begin{pmatrix} 1 & -1 & 0 & 1 & 0 \\ 0 & 1 & -1 & 0 & -1 \\ 0 & 0 & 2 & -1 & 0 \end{pmatrix} \tag{30}$$

The inflow reaction rate v_1 is constant. The rest of the reaction rates are assumed to be of Michaelis–Menten type of kinetics (Supplementary Appendix A). In contrast to the previous example, there are two steady states in this metabolic network (Fig. 3). The first steady state is locally asymptotically stable and the second one is unstable.

Example 8.

Interestingly, if for the network (29) we assume mass action kinetics, i.e. $v_2 = k_1x_1$, $v_3 = k_2x_2$, $v_4 = k_3x_3$, $v_5 = k_4x_2$, then in this case the steady state is unique: $x_1^* = (v_1(k_2+k_4))/(k_1(k_4-k_2))$, $x_2^* = v_1/(k_4-k_2)$, $x_3^* = 2k_2v_1/(k_3(k_4-k_2))$. The steady state is stable if $k_4 > k_2$.

To summarize, in single-substrate–single-product metabolic networks with general stoichiometry and with monotonic reaction rates the steady state, if it exists, is unique and locally asymptotically stable in case there is no cycle in the network. Moreover, for cyclic networks multiple steady states are possible (both stable and unstable). The inequality (25) is a sufficient condition for uniqueness and local asymptotic stability of a steady state for cyclic networks with irreversible reactions.

5. Multiple-substrates–multiple-product metabolic networks

5.1. General considerations

In *multiple-substrates–multiple-product (MSMP) metabolic networks* the reaction complexes may contain more than one metabolite. According to Theorem 1 steady states in MSMP metabolic networks may form manifolds. The question is under which conditions manifolds of steady states are locally asymptotically stable? As in the case of single-substrate–single-product metabolic networks we will consider stability properties of manifolds of steady states for the cases of simple and general stoichiometry.

5.2. Simple stoichiometry

We first show local asymptotically stability of manifolds of steady states for linear and branched MSMP networks with irreversible reactions. Then we show that there is direct relationship between SSSP and MSMP networks with reversible reactions in case in the MSMP network every metabolite participates in only one reaction, and not in other reaction complexes. That is linkage classes do not share metabolites, e.g. metabolites X_3 and X_4 in metabolic network (14).

Theorem 5. *Consider multiple-substrate–multiple-product metabolic network. Assume the stoichiometry is simple. Assume all reactions are irreversible and reaction rates are monotonic. Assume also that the network has tree topology and every metabolite participates in only one reaction complex. Then the manifold of steady states of the network is locally asymptotically stable.*

Proof. We first show that this theorem holds for metabolic networks with linear topology, then for networks with branched topology and finally for tree topology.□

5.2.1. Linear networks

A general representation of linear metabolic network with multiple-substrate–multiple-product reactions is of the form

$$\xrightarrow{v_1} C_1 \xrightarrow{v_2} C_2 \xrightarrow{v_3} \dots \xrightarrow{v_m} C_q \xrightarrow{v_{m+1}} \tag{31}$$

Here $C_i = \sum s_{ji}X_k$ represent different reaction complexes.

The Jacobian matrix is in this case is a block lower triangular matrix.

$$J(x)=\begin{pmatrix} B_{1,1} & & & \\ B_{2,1} & B_{2,2} & & \\ & \ddots & \ddots & \\ & & B_{q,q-1} & B_{q,q} \end{pmatrix} \tag{32}$$

The diagonal block $B_{j,j}$ that corresponds to reaction j with d substrates is

$$B_{j,j}=\begin{pmatrix} -g_{j,i} & -g_{j,i+1} & \cdots & -g_{j,i+d} \\ -g_{j,i} & -g_{j,i+1} & \cdots & -g_{j,i+d} \\ \vdots & \vdots & \ddots & \vdots \\ -g_{j,i} & -g_{j,i+1} & \cdots & -g_{j,i+d} \end{pmatrix}. \tag{33}$$

All the entries in diagonal block $B_{j,j}$ are negative, moreover the rows in every diagonal block are the same, resulting in $rank(B_{j,j})=1$. Hence, the only nonzero eigenvalue in each diagonal block $B_{j,j}$ is equal to the sum of its diagonal entries, i.e. to the trace of the block, $\lambda_1=Tr(B_{j,j})$. Since the trace of each diagonal block is always negative, the only nonzero eigenvalue λ_1 of $B_{j,j}$ is negative. The last diagonal blocks correspond to outflow reactions and consist of only one nonzero negative entry in each block, so the corresponding eigenvalues are all negative. The eigenvalue spectrum of the lower block triangular matrix consists of the union of eigenvalues of each diagonal block. As a result, the spectrum of the Jacobian matrix in this case consists of negative and zero eigenvalues.

In this case the number of zero eigenvalues will correspond to the dimension of manifold of steady states. The rest of the eigenvalues are real and negative, so manifolds of steady states for linear networks are locally asymptotically stable.

5.2.2. Branched networks

A general representation of branched metabolic network with multiple-substrate–multiple-product reactions is of the form

$$\begin{aligned} &\xrightarrow{v_1}C_1\xrightarrow{v_2}\dots\xrightarrow{v_p}C_p\xrightarrow{v_{p+1}}\dots\xrightarrow{v_y}C_y\xrightarrow{v_{m+1}} \\ &\quad C_p\xrightarrow{v_{y+1}}\dots\xrightarrow{v_m}C_q\xrightarrow{v_{m+2}}. \end{aligned} \tag{34}$$

The Jacobian matrix for branched networks with multiple-substrates–multiple-products, with simple stoichiometry and irreversible reactions has lower block triangular structure, as in the case for linear networks (32). The only different diagonal block $B_{p,p}$ is the one that corresponds to the reaction complex C_p at the branching point. Suppose there are d metabolites in the branching complex C_p, and the branching reactions are $p+1$ and $y+1$. Then the general structure of the corresponding $d\times d$ diagonal block is the following:

$$B_{p,p}=\begin{pmatrix} -g_{p+1,i}-g_{y+1,i} & -g_{p+1,i+1}-g_{y+1,i+1} & \cdots & -g_{p+1,i+d}-g_{y+1,i+d} \\ -g_{p+1,i}-g_{y+1,i} & -g_{p+1,i+1}-g_{y+1,i+1} & \cdots & -g_{p+1,i+d}-g_{y+1,i+d} \\ \vdots & \vdots & \ddots & \vdots \\ -g_{p+1,i}-g_{y+1,i} & -g_{p+1,i+1}-g_{y+1,i+1} & \cdots & -g_{p+1,i+d}-g_{y+1,i+d} \end{pmatrix}. \tag{35}$$

As in the case of other diagonal blocks all entries of B_{pp} are negative and all rows are equal. So the only nonzero eigenvalue of this block is negative. Overall, as in the case of linear networks (31) the number of zero eigenvalues will correspond to the dimension of manifold of steady states and the rest of eigenvalues are negative. Therefore, manifolds of steady states in case of branched networks (34) are locally asymptotically stable.

5.2.3. Networks with tree topology

Metabolic networks with tree topology also have the Jacobian matrix with block lower triangular structure (32). Diagonal blocks corresponds have the same structure as in the case of linear networks (33) (for complexes that are not at the branching point) and branched networks (35) (for complexes that are the branching point). Therefore, manifolds of steady states in case of metabolic networks with tree graph topology are locally asymptotically stable.□

5.2.4. Coordinate transformations in MSMP networks with simple stoichiometry

Now we drop the assumption that reactions are irreversible and we show that in some cases with an appropriate coordinate transformation manifolds of steady states may be considered with respect to stability as a single steady state in the space of reaction complexes.

Theorem 6. *Consider multiple-substrate–multiple-product metabolic network. Assume the stoichiometry is simple. Assume reaction rates are monotonic. Assume every metabolite participates in only one reaction complex. Then the manifold of steady states is locally asymptotically stable.*

Proof. In case multiple metabolites participate in one reaction complex only then the differential equations that describe their dynamics are the same. In this case it is possible to make coordinate transformation and reduce this system. Suppose metabolites $X_1,\dots,X_k$ participate in the same reaction complex and do not participate in other reaction complexes. Then $\dot{x}_1=\dots=\dot{x}_k$. Then there is a manifold of steady state of dimension $k-1$. We make the following coordinate transformation:

$$\begin{aligned} \dot{z}_1&=\dot{x}_1 \\ \dot{z}_2&=\dot{x}_2-\dot{x}_1=0 \\ &\vdots \\ \dot{z}_k&=\dot{x}_k-\dot{x}_1=0. \end{aligned} \tag{36}$$

Suppose in metabolic network with multiple-substrate-multiple-product reactions in every complex with more than one metabolite metabolites do not participate in other reaction complexes. Then we can apply the above coordinate transformation in such a way that the

network reduces to a metabolic network with single-substrate–single-product reactions. The Jacobian matrix for such network is a negative M-matrix, so all its eigenvalues have negative real part. This implies local asymptotic stability of manifold of steady states for such network.

Note that Theorem 6 may be applied to networks with arbitrary topology.□

5.2.5. Numerical simulations of local stability

It is also possible that metabolites from the same reaction complex participate in other reactions or, in other words, different linkage classes share some metabolites. In this case the coordinate transformation above does not reduce the system with respect to shared metabolites. For such cases we studied local stability properties of manifolds of steady states using the structural kinetic modeling method, introduced by Steuer et al. (2006). In the structural kinetic modeling normalized parameters are used instead of standard parameters, i.e. v_{max} or K_m. Specifically, in the structural kinetic modeling a change of variables is performed in order to simplify the Jacobian matrix. Assuming that a positive steady state x^* exists, we can redefine the system (1) in the following way:

$$y_i(t)=\frac{x_i(t)}{x_i^*(t)},\quad \Lambda_{ij}=s_{ij}\frac{v_j(x^*)}{x_i^*}\quad\text{and}\quad \mu_j(x)=\frac{v_j(x)}{v_j(x^*)}. \tag{37}$$

Now the system of Eq. (1) can be rewritten as

$$\dot{y}=\Lambda\mu(y) \tag{38}$$

The Jacobian of the normalized system (36) evaluated at $y^*=1$ is

$$J_y=\Lambda\frac{\partial\mu(y)}{\partial y}\bigg|_{y^*=1}=\Lambda\theta_y^{\mu}. \tag{39}$$

Note that J_y can be easily transformed into the original Jacobian. The matrix Λ consists of stoichiometric matrix S, vector of steady state concentrations y^*, and the steady state fluxes $v(y^*)$. Steuer et al. (2006) proved that for metabolic reactions with no inhibition or cooperative behavior θ_y^{μ} is defined in the range [0, 1]. This well-defined range allows for an effective random sampling of parameters of Jacobian matrix in order to investigate dynamical properties of a given metabolic network.

We systematically investigated random networks of 10–100 metabolites in total with one or two metabolites in each reaction complex. We constructed the structure of Jacobian matrix for every such network. For every Jacobian matrix we evaluated its eigenvalues for 10^6 realizations of random sampling of parameters. For each of these realizations we counted the number of eigenvalues with strictly negative real part, positive real part, with imaginary part only and the number of zero eigenvalues. It turned out that for all the network and realizations of the Jacobian matrices we considered no eigenvalues were found with positive real part and eigenvalues with only imaginary part. The number of zero eigenvalues was always equal to the predicted dimension of steady states manifold from the rank deficiency of the stoichiometric matrix. The rest of eigenvalues had negative real part. Based on this result we make the following conjecture.

Conjecture 1. *Consider multiple-substrate–multiple-product metabolic network. Assume the stoichiometry is simple and reaction rates are monotonic. Then the manifold of steady states is locally asymptotically stable.*

To summarize, in multiple-substrate–multiple-product metabolic networks with simple stoichiometry and with monotonic reaction rates steady states form locally asymptotically stable manifolds of steady states in case every metabolite participates in only one reaction complex. We conjectured local asymptotically stability of manifolds of steady states.

5.3. General stoichiometry

In multiple-substrate–multiple-products metabolic networks steady states may form manifolds, according to the Theorem 1. In this section we investigate stability properties of steady states for linear and branched networks with irreversible reactions.

5.3.1. Linear networks

The Jacobian as in the case of MSMP with simple stoichiometry (32) is also a block lower triangular matrix.

The diagonal block $B_{j,j}$ that corresponds to the reaction j with d substrates is different from (33) and includes stoichiometric coefficients

$$B_{j,j}=\begin{pmatrix} -s_{i,j}g_{j,i} & -s_{i,j}g_{j,i+1} & \cdots & -s_{i,j}g_{j,i+d} \\ -s_{i+1,j}g_{j,i} & -s_{i+1,j}g_{j,i+1} & \cdots & -s_{i+1,j}g_{j,i+d} \\ \vdots & \vdots & \ddots & \vdots \\ -s_{i+d,j}g_{j,i} & -s_{i+d,j}g_{j,i+1} & \cdots & -s_{i+d,j}g_{j,i+d} \end{pmatrix}. \tag{40}$$

All the entries in diagonal block $B_{j,j}$ are negative, moreover $rank(B_{j,j})=1$ for all j. Hence, the only nonzero eigenvalue in each diagonal block $B_{j,j}$ is equal to the sum of its diagonal entries, i.e. to the trace of the block, $\lambda_1=Tr(B_{j,j})$. Since the trace of each diagonal block is always negative, the only nonzero eigenvalue λ_1 of $B_{j,j}$ is negative. So, as in the case of linear networks (31) the eigenvalue spectrum of the Jacobian matrix in this case consists of negative and zero eigenvalues, where the number of zero eigenvalues will correspond to the dimension of manifold of steady states. The rest of the eigenvalues are real and negative, so manifolds of steady states for linear networks are locally asymptotically stable.

5.3.2. Branched networks

The Jacobian matrix for branched networks with multiple-substrates–multiple-products irreversible reactions has a lower block triangular structure as in the case of linear networks (32). The diagonal block $B_{p,p}$ that corresponds to the reaction complex C_p at the branching point has a different structure than other diagonal blocks. Suppose there are d metabolites in the branching complex C_p and the

branching reactions are $p+1$ and $y+1$ then the general structure of the corresponding $d \times d$ diagonal block is the following:

$$B_{p,p} = \begin{pmatrix} -s_{i,p+1}g_{p+1,i} - s_{i,y+1}g_{y+1,i} & \cdots & -s_{i,p+1}g_{p+1,i+d} - s_{i,y+1}g_{y+1,i+d} \\ \vdots & \ddots & \vdots \\ -s_{i+d,p+1}g_{p+1,i} - s_{i+d,y+1}g_{y+1,i} & \cdots & -s_{i+d,p+1}g_{p+1,i+d} - s_{i,y+1}g_{y+1,i+d} \end{pmatrix}. \tag{41}$$

The eigenvalue spectrum of this block consist of $d-2$ zero eigenvalues and two nonzero eigenvalues

$$\lambda_1 = \frac{1}{2}\left[Tr(B_{p,p}) - \sqrt{Tr(B_{p,p})^2 - b_{p,p}}\right]$$

$$\lambda_2 = \frac{1}{2}\left[Tr(B_{p,p}) + \sqrt{Tr(B_{p,p})^2 - b_{p,p}}\right] \tag{42}$$

Here $Tr(B_{p,p})$ is the trace of the diagonal block $B_{p,p}$. The term $b_{p,p}$ consists of sum of products of entries of $B_{p,p}$. If $\lambda_1 < 0$ and $\lambda_2 < 0$ then branched metabolic network has a set of steady states in a form of locally asymptotically stable manifold. The number of zero eigenvalues corresponds to the dimension of the manifold. The eigenvalue $\lambda_2 = \frac{1}{2}\left[Tr(B_{p,p}) + \sqrt{Tr(B_{p,p})^2 - b_{p,p}}\right]$ has positive real part if and only if $b_{p,p} < 0$. In this case the set of steady states is locally repelling manifold. We did not obtain a general expression for the $b_{p,p}$ term for an arbitrary size of a block. In metabolic networks the number of metabolites in reaction complexes does not exceed three. By computing eigenvalues explicitly we obtained the expression of $b_{p,p}$ for the case when there are two, $d=2$, and three, $d=3$, metabolites in the branching complex.

If $d=2$ then

$$B_{p,p} = \begin{pmatrix} -s_{i,p+1}g_{p+1,i} - s_{i,y+1}g_{y+1,i} & -s_{i,p+1}g_{p+1,i+1} - s_{i,y+1}g_{y+1,i+1} \\ -s_{i+1,p+1}g_{p+1,i} - s_{i+1,y+1}g_{y+1,i} & -s_{i+1,p+1}g_{p+1,i+1} - s_{i+1,y+1}g_{y+1,i+1} \end{pmatrix} \tag{43}$$

In this case

$$b_{p,p} = \begin{vmatrix} s_{i,p+1} & s_{i,y+1} \\ s_{i+1,p+1} & s_{i+1,y+1} \end{vmatrix} \begin{vmatrix} g_{p+1,i} & g_{p+1,i+1} \\ g_{y+1,i} & g_{y+1,i+1} \end{vmatrix}$$

$$= (s_{i,p+1}s_{i+1,y+1} - s_{i+1,p+1}s_{i,y+1})(g_{p+1,i}g_{y+1,i+1} - g_{y+1,i}g_{p+1,i+1}) \tag{44}$$

If $d=3$ then

$$B_{p,p} = \begin{pmatrix} -s_{i,p+1}g_{p+1,i} - s_{i,y+1}g_{y+1,i} & -s_{i,p+1}g_{p+1,i+1} - s_{i,y+1}g_{y+1,i+1} & -s_{i,p+1}g_{p+1,i+2} - s_{i,y+1}g_{y+1,i+2} \\ -s_{i+1,p+1}g_{p+1,i} - s_{i+1,y+1}g_{y+1,i} & -s_{i+1,p+1}g_{p+1,i+1} - s_{i+1,y+1}g_{y+1,i+1} & -s_{i+1,p+1}g_{p+1,i+2} - s_{i+1,y+1}g_{y+1,i+2} \\ -s_{i+2,p+1}g_{p+1,i} - s_{i+2,y+1}g_{y+1,i} & -s_{i+2,p+1}g_{p+1,i+1} - s_{i+2,y+1}g_{y+1,i+1} & -s_{i+2,p+1}g_{p+1,i+2} - s_{i+2,y+1}g_{y+1,i+2} \end{pmatrix} \tag{45}$$

In this case

$$b_{p,p} = \begin{vmatrix} s_{i,p+1} & s_{i,y+1} \\ s_{i+1,p+1} & s_{i+1,y+1} \end{vmatrix} \begin{vmatrix} g_{p+1,i} & g_{p+1,i+1} \\ g_{y+1,i} & g_{y+1,i+1} \end{vmatrix} + \begin{vmatrix} s_{i,p+1} & s_{i,y+1} \\ s_{i+2,p+1} & s_{i+2,y+1} \end{vmatrix} \begin{vmatrix} g_{p+1,i} & g_{p+1,i+2} \\ g_{y+1,i} & g_{y+1,i+2} \end{vmatrix}$$

$$+ \begin{vmatrix} s_{i+1,p+1} & s_{i+1,y+1} \\ s_{i+2,p+1} & s_{i+2,y+1} \end{vmatrix} \begin{vmatrix} g_{p+1,i+1} & g_{p+1,i+2} \\ g_{y+1,i+1} & g_{y+1,i+2} \end{vmatrix} \tag{46}$$

It is possible that eigenvalues $\lambda_{1,2}$ (42) have imaginary parts in the case $b_{p,p} > Tr(B_{p,p})^2$.

We summarize the results above in the following theorem.

Theorem 7. *Consider multiple-substrate–multiple-product metabolic network. Assume the stoichiometry is general. Assume reactions are irreversible and reaction rates are monotonic. Assume the network has tree topology with each metabolite participating in one complex. Then the manifold of steady states is locally asymptotically stable if $b_{p,p} < 0$.*

Proof. Metabolic networks with tree topology and single linkage class also have lower block triangular structure of the Jacobian matrix (32). Diagonal blocks have the same structure as in the case of linear networks (33) (for complexes that are not at the branching point) or branched networks (41) (for complexes that are the branching point). In the second case reaction complexes contribute to instability. Therefore, manifolds of steady states in this case are locally asymptotically stable if the condition for eigenvalues of the diagonal block that corresponds to the branching point is satisfied.□

Example 9.

Consider the following branched network:

$$\xrightarrow{v_1} X_1$$

$$2X_1 \xrightarrow{v_2} X_2 + X_3 \xrightarrow{v_3} X_4 \xrightarrow{v_5}$$

$$X_2 + X_3 \xrightarrow{v_4} X_5 \xrightarrow{v_6} \tag{47}$$

The stoichiometric matrix for this network is

$$S=\begin{pmatrix}1&-2&0&0&0&0\\0&1&-1&-1&0&0\\0&1&-1&-1&0&0\\0&0&1&0&-1&0\\0&0&0&1&0&-1\end{pmatrix} \tag{48}$$

We assume that the reaction rate v_1 is constant. Reaction rates $v_2(x)$, $v_3(x)$, $v_4(x)$ obey convenience kinetics (Liebermeister and Klipp, 2006), and reaction rates $v_5(x)$, $v_6(x)$ obey Michaelis–Menten kinetics (Supplementary Appendix A). The manifold of steady states for this system and vector field in the phase space of metabolite concentrations x_1, x_2, x_3 are depicted in Fig. 4. The manifold of steady states (Fig. 4) is locally asymptotically stable.

Example 10.

Consider the following branched network:

$$\begin{aligned}&\xrightarrow{v_1}X_1\\&X_1\xrightarrow{v_2}2X_2+X_3\xrightarrow{v_3}X_4\xrightarrow{v_5}\\&X_2+2X_3\xrightarrow{v_4}X_5\xrightarrow{v_6}\end{aligned} \tag{49}$$

The stoichiometric matrix for this network is

$$S=\begin{pmatrix}1&-1&0&0&0&0\\0&1&-2&-1&0&0\\0&1&-1&-2&0&0\\0&0&1&0&-1&0\\0&0&0&1&0&-1\end{pmatrix} \tag{50}$$

We assume that the reaction rate v_1 is constant. Reaction rates $v_3(x)$, $v_4(x)$ obey convenience kinetics (Liebermeister and Klipp, 2006), and reaction rates $v_2(x)$, $v_5(x)$, $v_6(x)$ obey Michaelis–Menten kinetics (Supplementary Appendix A). The steady state in this case may become unstable (Fig. 5).

5.3.3. *Cyclic networks*

A general representation of cyclic metabolic network with multiple-substrate–multiple-product reactions is of the form

$$\begin{aligned}&\xrightarrow{v_1}C_1\xrightarrow{v_2}C_2\ldots\xrightarrow{v_{n-2}}C_m\xrightarrow{v_{n-1}}C_1\\&\qquad\qquad C_m\xrightarrow{v_n}\end{aligned} \tag{51}$$

Structural kinetic modeling (Steuer et al., 2006) demonstrates that stability is also not guaranteed under all conditions for metabolic cycles with multiple-substrate–multiple-product reactions. Conditions for stability of steady states in this case are to be found.

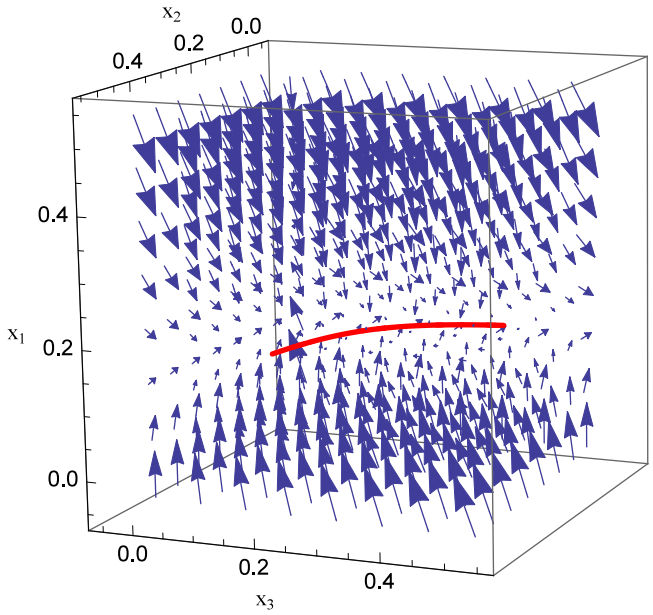

Fig. 4. Manifold of steady states (red line) and the vector field (blue arrows) for the branched metabolic network (45). The rate equations and parameters are presented in Appendix A in Supplementary materials. For the network (45) the steady state $x_3{}^*$ is a function of the steady state $x_2{}^*$: $x_3{}^*=\frac{x_2{}^*}{11x_2{}^*-1}$.

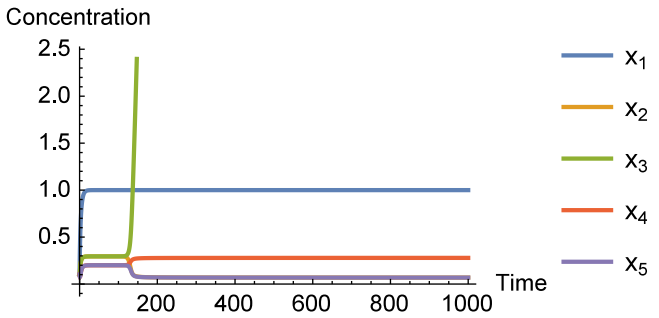

Fig. 5. Dynamics of metabolite concentrations of the metabolic network (47). Concentrations of metabolites x_1, x_3, x_4, x_5 quickly reach the steady state. Concentration of metabolite x_2 increases to infinity with time.

Table 1
Steady states in different types of metabolic networks.

Network type	Simple stoichiometry	General stoichiometry
Single-substrate–single-product (SSSP)	If a steady state exists it is always unique and globally asymptotically stable (Theorem 2).	If a steady state exists it is always unique and locally asymptotically in case there are no cycles in the network (tree topology) (Theorem 3). In case of cyclic network with irreversible reactions condition is provided for uniqueness and local asymptotic stability of a steady state (Theorem 4).
Multiple-substrate–multiple-product (MSMP)	Steady states are in form of manifolds (Theorem 1). Manifolds of steady states are locally asymptotically stable in the case every metabolite participates in only one reaction complex (Theorem 6). We conjecture local asymptotic stability of manifolds of steady states for any topology (Conjecture 1).	Steady states are in form of manifolds (Theorem 1). Manifolds are always locally asymptotically stable only in case of linear networks with irreversible reactions. For other topologies extra conditions are required for local stability (Theorem 7).

6. Discussion

We investigated steady states and their stability for various types of metabolic networks with monotonic reaction kinetics. Complementary to previous works, which mostly were concerned with specific stoichiometry, topology or kinetics, we made an attempt to characterize properties of steady for general types of metabolic networks. We classified metabolic networks based on stoichiometry (simple and general) and the number of metabolites that participate in each reaction complex (single or multiple). The results are summarized in Table 1. Our results are valid irrespectively of specific kinetic reaction rates, provided that they are monotonic, and irrespectively of specific values of parameters.

Metabolic networks with simple stoichiometry have either unique globally asymptotically stable steady state or asymptotically stable manifold of steady states. This is quite interesting result because most of metabolic networks have simple stoichiometry (Palsson, 2011). Therefore, in most metabolic pathways either steady states or manifolds of steady states are locally or globally asymptotically stable. This result is in agreement with latest insights from chemical reaction network theory (Shinar and Feinberg, 2013).

Moreover, local stability of steady states or manifolds of steady states is provided without any regulation. The role of metabolic regulation is to ensure biologically desirable location of a steady state, while simple stoichiometry and monotonic kinetics guarantee convergence of state space to this steady state or to manifold of steady states. The presence of regulation might lead to instability of steady states (Savageau, 1975; Heinrich et al., 1977). Moreover, in some cases there is a trade-off between the strength of regulation and stability of a steady state (Savageau, 1975).

For metabolic networks with general stoichiometry steady states are not always stable. In particular, in single-substrate–single-product metabolic networks steady state is unique and locally asymptotically stable if there is no cycle in the network. In contrast to metabolic networks with single-substrate–single-product reactions and simple stoichiometry, in case of general stoichiometry in cyclic networks multiple separated steady states are possible and stability of these steady states is not guaranteed under all conditions. For metabolic networks with multiple-substrate–multiple-product reactions asymptotic stability of manifolds of steady states is guaranteed only for linear networks with irreversible reactions.

As a conclusion, stoichiometry plays a very important role in stability of steady states in metabolic networks. In metabolic networks with multiple-substrate–multiple-product reactions steady states may form manifolds of steady states. Manifolds of steady states seem to be not biologically desirable. Small deviations from a steady state, due to external causes, may lead to a different position on the manifold of steady states, with physiologically undesirable values of metabolite concentrations. Metabolic networks can avoid the appearance of manifolds of steady states by participations of metabolites in different linkage classes, presence of single-substrate–single-product reactions and by means of regulation that drives a steady state to a desired position on a manifold. Inspecting the structure of metabolic networks in cells indeed indicates that most of them should have zero dimensional manifolds of steady states. That is metabolic networks have either a unique steady state or a set of separated steady states.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jtbi.2016.02.031.

References

Angeli, D., 2009. A tutorial on chemical reaction network dynamics. Eur. J. Control 15, 398–406.
Arrowsmith, D.K., Place, C.M., 1992. Dynamical Systems: Differential Equations, Maps, and Chaotic Behavior. Chapman Hall.
Banaji, M., Donell, P., Baigent, S., 2007. P-matrix properties, injectivity, and stability in chemical reaction systems. SIAM J. Appl. Math. 67, 1523–1547.
Banaji, M., Baigent, S., 2008. Electron transfer networks. J. Math. Chem. 43, 1355–1370.
Cornish-Bowden, A., Cárdenas, M.L., 2000. Information transfer in metabolic pathways. Effects of irreversible steps in computer models. Eur. J. Biochem. 268, 6616–6624.
Craciun, G., Feinberg, M., 2005. Multiple equilibria in complex chemical reaction networks: I. The injectivity property. SIAM J. Appl. Math. 65, 1526–1546.
Craciun, G., Feinberg, M., 2006. Multiple equilibria in complex chemical reaction networks: II. The species reaction graph. SIAM J. Appl. Math. 66, 1321–1338.
Datta, K.B., 2006. Matrix and Linear Algebra. Prentice Hall.
El-Mikkawy, M.E.A., 2004. On the inverse of a general tridiagonal matrix. Appl. Math. Comput. 150, 669–679.
Feinberg, M., 1979. Lectures on Chemical Reaction Networks. Unpublished written versions of lectures given at the Mathematics Research Center.
Feinberg, M., 1987. Chemical reaction network structure and the stability of complex isothermal reactors—I. The deficiency zero and deficiency one theorems. Chem. Eng. Sci. 42, 2229–2268.
Feinberg, M., 1988. Chemical reaction network structure and the stability of complex isothermal reactors—II. Multiple steady states for networks of deficiency one. Chem. Eng. Sci. 43, 1–25.
Feinberg, M., 1995. Multiple steady states for chemical reaction networks of deficiency one. Arch. Ration. Mech. Anal. 132, 371–406.
Flach, E.H., Schnell, S., 2010. Stability of open pathways. Math. Biosci. 228, 147–152.
Gale, D., Nikaido, H., 1965. The Jacobian matrix and global univalence of mappings. Math. Ann. 1569, 81–93.
Gunawardena, J., 2003. Chemical Reaction Network Theory for In-Silico Biologists. Technical Report. Bauer Center for Genomics Research, Harvard University, Cambridge, MA.
Guo, J., Zhang, J.F., Zhao, Y., 2012. Multi-equilibrium property of metabolic networks: MMN module. Int. J. Robust Nonlinear Control 24, 1505–1529.
Heinrich, R., Rapoport, S.M., Rapoport, T.A., 1977. Metabolic regulation and mathematical models. Prog. Biophys. Mol. Biol. 32.
Horn, F., Jackson, R., 1972. General mass action kinetics. Arch. Ration. Mech. Anal. 47, 81–116.
Horn, R.A., Johnson, C.R., 1991. Topics in Matrix Analysis. Cambridge University Press.
Jamshidi, N., Palsson, B.Ø., 2008. Formulating genome-scale models in the post-genome era. Mol. Syst. Biol. 4, 1–10.
Lei, H.B., Zhang, J.F., Chen, L., 2010. Multi-equilibrium property of metabolic networks: exclusion of multi-stability for SSN metabolic modules. Int. J. Robust Nonlinear Control 21, 1791–1806.
Liebermeister, W., Klipp, E., 2006. Bringing metabolic networks to life: convenience rate law and thermodynamic constraints. Theor. Biol. Med. Model., 41.
Ma, H., Zeng, A.P., 2003. Reconstruction of metabolic networks from genome data and analysis of their global structure for various organisms. Bioinform 19, 270–277.
Nishikawa, T., Gulbahce, N., Motter, A.E., 2008. Spontaneous reaction silencing in metabolic optimization. PLoS Comp. Biol., 4.
Palsson, B.Ø., 2011. Systems Biology: Simulation of Dynamic Network States. Cambridge University Press.
Plemmons, R.J., 1977. M-matrix characterizations. I – Nonsingular M-matrices. Linear Algebra Appl. 18, 175–188.
Reznik, E., Segré, D., 2010. On the stability of metabolic cycles. J. Theor. Biol. 266, 536–549.
Reznik, E., Watson, A., Chaudhary, O., 2013. The stubborn roots of metabolic cycles. J. R. Soc. Interface 10, 536–549.
Savageau, M.A., 1975. Optimal design of feedback control by inhibition: dynamic considerations. J. Mol. Evol. 5, 199–222.
Shinar, G., Feinberg. M., G., 2013. Concordant chemical reaction networks. Math. Biosci. 240, 92–113.
Steuer, R., Gross, T., Selbig, J., Blasius, B., 2006. Structural kinetic modelling of metabolic networks. Proc. Natl. Acad. Sci. 103, 11868–11873.
Steuer, R., Junker, B.H., 2008. Computational models of metabolism: stability and regulation in metabolic networks. Adv. Chem. Phys. 142, 105–251.
van der Schaft, A.J., Rao, S., Jayawardhana, B., 2013. On the mathematical structure of balanced chemical reaction networks governed by mass action kinetics. SIAM J. Appl. Math. 73, 953–973.
van der Schaft, A.J., Rao, S., Jayawardhana, B., 2015. A network dynamics approach to chemical reaction networks. Int. J. Control. http://dx.doi.org/10.1080/00207179.2015.1095353.