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Steady states and stability in metabolic networks without regulation

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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.

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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):

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