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On the relation between reactions and complexes of (bio)chemical reaction networks

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HIGHLIGHTS

- Connection between two methodologies, flux coupling analysis and chemical reaction network theory, is established.
- Flux-focused approaches and concentration-centric approaches are bridged.
- Two mappings are introduced, of which one is a homomorphism, and which provide an interface between the two approaches.
- The mathematical formulation relies on the simple concepts of equivalence classes, partitions and lattices.
- The approach can reveal a significant reduction of complexity of the considered chemical reaction network.

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ABSTRACT

Robustness of biochemical systems has become one of the central questions in systems biology although it is notoriously difficult to formally capture its multifaceted nature. Maintenance of normal system function depends not only on the stoichiometry of the underlying interrelated components, but also on the multitude of kinetic parameters. Invariant flux ratios, obtained within flux coupling analysis, as well as invariant complex ratios, derived within chemical reaction network theory, can characterize robust properties of a system at steady state. However, the existing formalisms for the description of these invariants do not provide full characterization as they either only focus on the flux-centric or the concentration-centric view. Here we develop a novel mathematical framework which combines both views and thereby overcomes the limitations of the classical methodologies. Our unified framework will be helpful in analyzing biologically important system properties.

1. Introduction

Biochemical networks have evolved to operate in the face of internal and external perturbations (Kitano, 2004). The response to these perturbations has shaped the systemic architectural blueprint comprising multiple layered and interrelated components (e.g., genes, proteins, metabolites). The dynamic processes involving network-related biochemical components depend on a multitude of kinetic parameters, which remain elusive even for medium-size systems. Therefore, methods establishing a connection between structure and dynamics of biochemical systems hold the promise to enable the rigorous study of processes taking place on the underlying biochemical networks both at steady-state as well as dynamic setting.

Two different classes of approaches have been developed to facilitate parameter-independent analysis of biochemical networks: (i) flux-focused approaches, including: flux balance analysis (FBA) (Varma and Palsson, 1994) and its derivatives–flux variability analysis (FVA) (Mahadevan and Schilling, 2003) and flux coupling analysis (FCA) (Burgard et al., 2004; Marashi and Bockmayr, 2011), elementary flux modes (EFMs) (Schuster et al., 2000), and extreme pathways (Schilling et al., 1999); and (ii) concentration-centric approaches, rooted in chemical reaction network theory (CRNT) (Horn and Jackson, 1972; Feinberg, 1979, 1995) and stoichiometric network analysis (Clarke, 1988).

Given a biochemical network, FBA relies on a linear programming formulation to calculate the steady-state fluxes under the assumption that the investigated organism operates toward optimizing an objective function (e.g., optimizing yield for metabolic networks (Varma and Palsson, 1994)). FVA also has a linear programming formulation, with the aim of calculating the minimum and maximum values of individual steady-state fluxes for a particular value of the objective. FCA can be used to determine pairs of reactions whose flux ratio is the same in each steady state under the same environmental conditions. Like FBA and FVA, this approach can also be cast as a linear program. On the other hand, approaches based on EFMs allow decomposition of a given network into its smallest functional units operating in a steady state (Schuster et al., 2000; Schilling et al., 1999). Although the problem

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of determining the set of all EFMs for a given biochemical network is computationally demanding, recent parallelized implementations of algorithms for EFM computation facilitate EFM-based analysis of genome-scale metabolic networks (Terzer and Stelling, 2008). Essential to both flux-based approaches is the usage of the underlying stoichiometric matrix which, without a specified kinetics, cannot be employed to make statements about steady-state metabolite concentrations.

In contrast, CRNT uses mass-action formulation to study the qualitative behavior of the steady-state concentrations of the components regardless of the parameter values, i.e., for all steady-state reaction fluxes of the mass-action system satisfying the constraints imposed by the stoichiometry. The results of this framework answer questions related to the possibility for existence of multiple steady states, and rely on a structural index determined by interleaving the graph-theoretic and stoichiometric descriptions of the investigated network (Horn and Jackson, 1972; Feinberg, 1979, 1995; Gunawardena, 2003; Conradi et al., 2007).

Biochemical network invariants are of particular interest specifically because they relate to the principle of homeostasis. For instance, under the steady-state assumption, the concentrations of components do not change and, thus, are invariant. However, invariants in biochemical networks can be defined not only with respect to changes over time, but also changes with respect to different steady states that the system may assume under same environmental conditions (i.e., initial conditions and/or constraints). Note that the latter excludes the analysis of trivial invariants which are imposed in the form of conservation relations (Schilling et al., 1999; Heinrich and Schuster, 1996).

In other words, invoking the steady-state assumption may induce additional invariants with respect to individual components or their combinations, which can ultimately reveal possible reduction in complexity of the system. As already stated, FCA provides the means for determining pairs of reactions whose ratio of fluxes is the same in each steady state the system may assume.

In general, changes in fluxes and concentrations, as key descriptors of the transitional behavior in biochemical networks, depend on each other. This stems from the fact that the reaction rate, i.e., flux, is cast as a function of the concentrations of the considered components. Therefore, the question arises whether there exist invariants on the level of concentrations and, if so, whether there is a connection between flux- and concentration-invariants. The answer to this question of course depends on the choice of kinetic law providing the relation between reaction fluxes and concentrations.

Here, we focus on mass action kinetics, representing the simplest and most fundamental law of kinetics, to establish a connection between flux and concentration-invariants. By interleaving the flux- and concentration-invariants, we provide a fundamentally new theoretical approach which can be used to uncover dependencies between fluxes and between concentrations, ultimately leading to a better understanding of system complexity.

Therefore, our study establishes a connection between the two different views of computational systems biology—the flux-centric and the concentration-centric view. Since the theories and methods pertaining to the two views use different notations, a brief overview is provided to describe the used notation.

2. Methods

In chemistry, the law of mass action was established by Guldberg and Waage in the nineteenth century (Guldberg and Waage, 1899; Abrash, 1986). It assumes a mixture of large numbers of components which are homogeneously distributed, allowing approximation of the components' behavior with continuous variables. A reversible reaction, i.e., a reaction which can proceed in the forward and backward direction, is split into two reactions—the irreversible forward reaction and the irreversible backward reaction. The components consumed by an irreversible reaction are called *substrates*, while those produced are referred to as *products*. A reaction's rate is then modeled to be proportional to the product of the concentrations of the participating substrates, especially in the case of an elementary reaction which cannot be further divided into intermediate steps (Moore, 1986, p. 385). Under realistic chemical conditions, it is often the case that a given reaction almost certainly proceeds in one direction. In this situation, with the assumption that the reaction rate in one of the directions can be neglected, the reaction is treated as irreversible. Therefore, most models of biochemical networks consist of a mixture of reversible and irreversible reactions.

Here, for the application of specific theoretical methodology, each biochemical network must be transformed to an equivalent one that consists only of irreversible reactions. Such a transformation is performed as follows (Gagneur and Klamt, 2004): Let the complete set of reactions be denoted by $\mathcal{R}=\mathcal{R}_{irr}\cup\mathcal{R}_{rev}$, where $\mathcal{R}_{irr}$ denotes the subset of irreversible reactions and $\mathcal{R}_{rev}$ the subset of reversible reactions. The set of reactions $\mathcal{R}'_{irr}$ is derived by splitting each reversible reaction from $\mathcal{R}_{rev}$ into two irreversible reactions, one in each direction. The original network can then be described by a new set of reactions $\mathcal{R}'=\mathcal{R}'_{irr}\cup\mathcal{R}_{irr}$ with $|\mathcal{R}'|=2|\mathcal{R}_{rev}|+|\mathcal{R}_{irr}|$. The starting point for our methodologies derived here is always a biochemical network which is of this form, i.e., we assume that $\mathcal{R}$ denotes a set of irreversible reactions (see Example 1).

Example 1. The eight irreversible reactions in the set $\mathcal{R}=\{R_1,R_2,R_3,R_4,R_5,R_6,R_7,R_8\}$, given by

$R_1 \coloneqq \mathrm{A}\to\mathrm{X}$ $\quad$ $R_5 \coloneqq \mathrm{A}+\mathrm{C}\to\mathrm{D}$

$R_2 \coloneqq \mathrm{X}\to\mathrm{A}$ $\quad$ $R_6 \coloneqq \mathrm{D}\to\mathrm{A}+\mathrm{C}$

$R_3 \coloneqq \mathrm{B}\to\mathrm{X}$ $\quad$ $R_7 \coloneqq \mathrm{B}+\mathrm{C}\to\mathrm{E}$

$R_4 \coloneqq \mathrm{X}\to\mathrm{B}$ $\quad$ $R_8 \coloneqq \mathrm{E}\to\mathrm{B}+\mathrm{C}$,

can in fact be regarded as four reversible reactions. The reversible reactions are formed by R_1 and R_2, R_3 and R_4, R_5 and R_6 as well as R_7 and R_8.

The results from flux-centric approaches rely on investigating vector spaces associated with the stoichiometric matrix N (see Example 2). The principal object in the flux-centric approaches is given by the reactions and their fluxes in which the flux is defined as the turnover rate of molecules in a metabolic network. Here the term "flux of reaction R_i" is used synonymously to "reaction rate of R_i". A crucial vector space is that of the kernel of the stoichiometric matrix N, which is represented by the set of flux vectors v that fulfill $Nv=\mathbf{0}$ and which describe the possible steady-state fluxes (positive and negative) of the considered biochemical system. Thus, the kernel of N describes all possible steady-state fluxes of the considered biochemical system.

Example 2. The set of reactions from Example 1 give rise to the following stoichiometric matrix:

$$N=\begin{array}{c} \begin{matrix} R_1 & R_2 & R_3 & R_4 & R_5 & R_6 & R_7 & R_8 \end{matrix} \\ \begin{bmatrix} -1 & 1 & 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & -1 & 1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ 1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \end{bmatrix} \end{array} \begin{matrix} \\ \mathrm{A} \\ \mathrm{B} \\ \mathrm{C} \\ \mathrm{D} \\ \mathrm{E} \\ \mathrm{X}. \end{matrix}$$

The concentration-centric approaches, represented by CRNT, use a notation which combines linear algebra and set theory (Gunawardena, 2003). For a given set of reactions, the set of complexes $\mathcal{C}$ is composed of the left- and right-hand sides of each reaction arrow. Any reaction $y\to y'\in\mathcal{R}$ can then easily be defined in terms of its complexes $y,y'\in\mathcal{C}$.