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MATHEMATICAL DESCRIPTIONS OF THE STEADY-STATE FLUX CONE

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Abstract. The increasing amount of available molecular data has enabled the reconstruction of genome-scale metabolic networks of numerous living organisms. A thorough understanding of these complex networks requires the use of efficient computational and mathematical approaches. In this review, we present the key methods largely used to model and analyze metabolic networks. We make focus on constraint-based modeling which describes the solution space containing all the feasible metabolic behaviors of a living organism under steady-state conditions. The properties of this flux space can mainly be investigated either by optimization-based approaches or by pathway-based network analysis.

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1 Introduction

The impressive advances in molecular biology stimulate growing interest in the understanding of living organisms at the system level, complementing efforts of the reductionist approach that has predominated in molecular biology for the last century. The main objective of such an integrative paradigm is to link the behavior of living matter to the structure and dynamics of the system, resulting in a much better understanding of biological systems and efficient engineering and design of strains that are more suitable for chemical production (Bro et al., 2006).

The operation of a living organism depends on its ability to acquire nutrients from the environment and transform them to the necessary molecules. Such transformations are performed by metabolic reactions. A *metabolic reaction*, generally speaking, refers to a chemical process that takes place in living organisms, which allows them to feed, evolve, and reproduce (Fell, 2000). Metabolic reactions contribute to many biological functions, including the degradation of chemical compounds for the creation of energy or the assembly of cellular constituents. During a metabolic reaction, the compounds that will react are called *substrates*. The latter will be converted into different molecules called *products* as a result of the reaction. The substrates and the products of the considered metabolic reactions are called *metabolites*. The *stoichiometric* coefficient of a metabolite in a reaction is the number of molecules of that metabolite used in the reaction. Metabolites are in general transformed step-by-step to other molecules by a sequence of reactions. This set of interconnected reactions is commonly called a *metabolic network*.

The enormous amount of molecular data has allowed a rapid reconstruction of a growing number of genome-scale metabolic networks. These large networks have been known to be highly complex. Accordingly, the need for rigorous mathematical and computational methods which focus on the systemic properties of these complex metabolic networks is becoming increasingly pressing. Diverse methods have been proposed, including *metabolic control, stochastic, cybernetic, kinetic* and *constraint-based analysis* (Fell, 1992; Gillespie, 2007; Heinrich and Schuster, 1996; Patnaik, 2001; Price et al., 2004).

Thanks to its ability to analyze genome-scale metabolic networks while using very little information, constraint-based modeling has recently attracted considerable attention in the lack of accurate kinetic information. This approach is based on considering a series of constraints, including stoichiometric and thermodynamic constraints, that controls the activities of a metabolic network under steady-state conditions. These constraints limit the range of allowable behaviors of the metabolic network, each corresponding to a possible metabolic phenotype. The space of these attainable metabolic behaviors satisfying these constraints is called the *steady-state flux cone*.

This review is structured as follows. We start in Section 2 with some mathematical preliminaries about polyhedral cones and linear programming. In Section 3, we give an overview of the main methods used to model and analyze metabolic networks. We make focus on constraint-based approaches that use linear constraints such as stoichiometric and thermodynamic constraints to define the space of all possible behaviors of a metabolic network at steady state. After explaining the optimization-based approaches which aim to identify single metabolic behaviors that optimize a predefined criterion of optimality, we shall address the well-known concept of elementary modes.

2 Mathematical Preliminaries

In this section, we will give a brief overview of the mathematical concepts that we will be using throughout this review. We especially recall fundamental concepts of linear algebra and polyhedral theory. We also present linear programming which provides an efficient way of searching for optimum solutions of linear programs.

2.1 Polyhedral cones

In the following, we denote by $\mathbb{R}^n$ the n-dimensional vector space over $\mathbb{R}$. The superscript "T" denotes *transposition*. Given two vectors $a, b \in \mathbb{R}^n$, $a^T b$ stands for the inner product of a and b. We denote by $Supp(a)$ the *support* of a vector $a \in \mathbb{R}^n$, i.e., $Supp(a) = \{i \in \{1, \ldots, n\} \mid a_i \neq 0\}$. A vector $a \in \mathbb{R}^n$ is a *conic combination* of the vectors $x^1, \ldots, x^p \in \mathbb{R}^n$ if

$$a = \sum_{i=1}^{p} \lambda_i x^i, \text{ for some } \lambda_1, \ldots, \lambda_p \geq 0.$$

For a set $X \subseteq \mathbb{R}^n$, $X \neq \emptyset$, the *conic hull* of X, denoted by cone(X), is the set of all conic combinations of finitely many vectors of X.

A non-empty subset $C \subseteq \mathbb{R}^n$ is called a *(convex) cone* if $\lambda x + \mu y \in C$, for all $x, y \in C$ and $\lambda, \mu \geq 0$. A cone C is *polyhedral*, if C is the set of solutions of a finite system of linear homogeneous inequalities, i.e., $C = \{x \in \mathbb{R}^n \mid Ax \geq 0\}$, for some real matrix $A \in \mathbb{R}^{m \times n}$. If this is the case, the *lineality space* of C, denoted by lin.space(C), is defined by

$$\text{lin.space}(C) = \{x \in \mathbb{R}^n \mid Ax = 0\}.$$

For any $a \in \mathbb{R}^n \setminus \{0\}$, the vector subspace $H = \{x \in \mathbb{R}^n \mid a^T x = 0\}$ is called a *hyperplane*. H divides the vector space $\mathbb{R}^n$ into two *halfspaces*: $H^+ = \{x \in \mathbb{R}^n \mid a^T x \geq 0\}$ and $H^- = \{x \in$

$\mathbb{R}^n \mid a^T x \leq 0\}$. Therefore, a cone $C = \{x \in \mathbb{R}^n \mid Ax \geq 0\}$ can be considered as the intersection of finitely many halfspaces.

A cone C is *finitely generated* if there exist $g^1, \ldots, g^s \in \mathbb{R}^n$ such that $C = \text{cone}\{g^1, \ldots, g^s\} = \{\lambda_1 g^1 + \ldots + \lambda_s g^s \mid \lambda_1, \ldots, \lambda_s \geq 0\}$. If this is the case, the vectors g^i for $i = 1, \ldots, s$ are called *generating vectors* of the cone C. The set $B = \{g^1, \ldots, g^s\}$ is called a *minimal set of generating vectors* if no proper subset of B generates the cone C.

A famous theorem of Farkas-Minkowski-Weyl (see e.g., (Schrijver, 1986)) states that a convex cone is polyhedral if and only if it is finitely generated. This theorem asserts that every cone admits two types of representations, either as the solution set of a finite system of linear homogeneous inequalities or as the conic hull of a finite set of generators. These are commonly called *external* and *internal representation*, respectively. For the rest of this review, we will consider only polyhedral cones and simply use the term cone.

Any non-zero element $r \in C$ is called a *ray* of C. Two rays r and r' are equivalent if $r = \lambda r'$, for some $\lambda > 0$. If this is the case, we write $r \cong r'$. A ray r is *extreme* if there do not exist rays $r^1, r^2 \in C, r^1 \ncong r^2$, such that $r = r^1 + r^2$. A cone C is called *pointed* if $C = \{x \in \mathbb{R}^n \mid Ax \geq 0\}$ for some $A \in \mathbb{R}^{m \times n}$ with $rank(A) = n$, or equivalently, lin.space$(C) = \{0\}$.

2.2 Linear programming

Linear programming (LP) refers to maximizing or minimizing any linear function, known as the *objective function*, subject to linear inequalities. Given a matrix $A \in \mathbb{R}^{m \times n}$, a vector $b \in \mathbb{R}^m$ and a vector $c \in \mathbb{R}^n$, the corresponding LP problem, is denoted by

$$\max\{c^T x : Ax \leq b\}.$$

Each of the linear inequalities $A_{i*}x \leq b_i$ for $i = 1, \ldots, m$ is called a *linear constraint*. A vector $x^* \in \mathbb{R}^n$ is a *feasible solution* if x^* satisfies all the linear constraints, i.e., $Ax^* \leq b$. If in addition, $c^T x^* \geq c^T x$ for all feasible solutions x, x^* is called an *optimal solution*. The *feasible region* is the set of all feasible solutions. A linear program is *feasible* if its feasible region is not empty, otherwise it is called *infeasible*. If some variables are required to be integers but others can be real, the considered linear program is referred to as a *mixed integer linear programming (MILP) problem*. One can use the *simplex method* to solve a linear program. This approach lists adjacent vertices of the feasible region, such that the objective function improves or remains unchanged at each new vertex. Another effective polynomial time algorithm is the method *interior point*. The reader concerned could refer to (Schrijver, 1986).

3 Metabolic network analysis

3.1 Steady-state flux cone

The difference between the rate of formation and consumption of a given metabolite is, according to the kinetic theory, equal to the change over time in its concentration. The behavior of a metabolic network can then be captured mathematically as a system of ordinary differential equations (Heinrich and Schuster, 1996). A compact expression of this system of equations is

$$\frac{dx}{dt} = Sv, \tag{1}$$

where S is the *stoichiometric matrix*, x denotes the $m-$dimensional vector of internal metabolite concentrations and v stands for the *flux distribution* with elements which correspond to the n fluxes through reactions. The flux vector v is actually a nonlinear functions of metabolite concentrations x as well as of certain kinetic parameters. Except for very simple cases, using

suitable nonlinear solvers, the constraint system (1) can be solved numerically but not analytically. Nevertheless, if we do not consider metabolite concentrations, then the constraint system (1) is linear in the fluxes through reactions.

Determination of steady states plays a key role in metabolic networks analysis (Schuster and Hilgetag, 1994). Under steady-state conditions, the change in the concentration of a compound over time across all reactions within the system becomes zero. This hypothesis holds for most metabolic reactions since they are usually much faster than environmental variations (Gagneur and Klamt, 2004; Varma and Palsson, 1994). The steady-state assumption is expressed by the following *flux balance equation*

$$Sv = 0.$$

This equation describes the *stoichiometric constraints* which state that, for any metabolite in the considered network, the total consumption rate must be equal to the total formation rate.

Stoichiometric constraints are required for characterizing metabolic network behavior, but far from sufficient. These constraints allow for a wide variety of potential steady-state flux distributions, namely all flux vectors that form the null space of the stoichiometric matrix. To reduce the range of possible flux distributions, further constraints imposed by *thermodynamic* considerations are used. Indeed, since each irreversible reaction can operate only in the forward direction, fluxes through irreversible reactions must be bigger than or equal to zero. This is expressed by the following linear inequalities

$$v_i \geq 0, \text{ for all } i \in Irr, \tag{2}$$

where Irr is the set of irreversible reactions in the considered metabolic network.

According to (Pfeiffer et al., 1999), a series of reactions forms a *functionally coherent set* in metabolism if the flux vector v carried out by these reactions fulfills the stoichiometric and thermodynamic constraints, i.e., v satisfies the following system of linear constraints

$$Sv = 0, \;\; v_i \geq 0, \text{ for all } i \in Irr, \tag{3}$$

where the number of constraints $(m + |Irr|)$ is always significantly smaller than the number n of unknown rates. This set of linear constraints is, therefore, usually undetermined. Furthermore, due to the linear inequalities (2), the constraint system (3) can not be solved using standard linear algebra. It is shown in polyhedral theory that the solutions of the mathematical problem (3) define in the flux space a polyhedral cone. Accordingly, the set of all possible flux distributions over the network at steady state, defines a polyhedral cone

$$C = \{v \in \mathbb{R}^n \mid Sv = 0, \; v_i \geq 0, \text{for all } i \in Irr\}, \tag{4}$$

which is called the *steady-state flux cone* (Clarke, 1980).

Given that the flux cone typically contains infinitely many potential steady-state flux distributions, it is important to figure out which of these feasible flux distributions the metabolic network under consideration currently displays. Constraint-based methods have attempted to analyze metabolic networks using several mathematical and computational tools (linear algebra, polyhedral theory, and linear programming, to name but a few). There are two main paradigms to analyze a metabolic network: searching for optimal metabolic behaviors using *optimization-based approaches*, or assessing the properties of the whole steady-state flux cone by means of *pathway-based network analysis*. We will give in the following an overview of these two types of constraint-based modeling.

3.2 Optimization-based approaches

Optimization-based approaches consider that metabolic networks function optimally, driven by an objective. To apply such methods, we must first define a most probable physiologically

relevant objective of a living system. This is interesting because it may enable us to identify rules regulating the activity of a metabolic network under various environmental conditions. These governing rules are important not only for improving our understanding of the living strains, but also for engineering and designing strains that are more suitable for the production of chemicals (Bro et al., 2006; Burgard and Maranas, 2001).

A widely used approach defines an objective function and seeks for its maximal value within the feasible region to determine an optimal flux distribution (Kauffman et al., 2004; Lee et al., 2006). This method, called *flux balance analysis (FBA)*, employs an optimization strategy which uses, in addition to the stoichiometric and thermodynamic constraints, flux capacity constraints that impose bounds on reactions fluxes. Other physicochemical constraints can also be considered to further shrink the space of all feasible flux distributions. All these additional constraints are important for the use of optimization techniques because the feasible domain must be bounded in the direction of the objective function. More formally, given that the objective function is linear, FBA uses the following linear programming problem:

$$\begin{array}{l} \max\ c^T v \\ \text{subject to:} \\ Sv = 0, \\ l_i \leq v_i \leq u_i \text{ for all } i \in \{1, \ldots, n\}, \end{array} \tag{5}$$

where c denotes the vector defining the objective function which is linear in the fluxes of reactions (Ramakrishna et al., 2001). The bounds l_i and u_i are the minimum and maximum flux capacities of reactions $i \in \{1, \ldots, n\}$. Particularly, $l_i = 0$ for every irreversible reaction i. By modifying the vector c in the linear problem (5), various objective functions may be evaluated, each capturing specific details regarding the laws controlling metabolic networks. Most optimization-based methods presume that the maximization of biomass production (growth) is a well-suitable objective function for optimal operation of a metabolic network (Varma and Palsson, 1994; Edwards and Palsson, 2000). Indeed, it is commonly assumed that microorganisms behave in such a way that their metabolic networks allow the most effective resource transfer to generate more cells. This basic theory of optimization has been largely used in many experiments, such as predicting the optimum performance of a metabolic network under a variety of growth conditions, investigating gene essentiality, and determining targets for metabolic engineering (Bro et al., 2006; Edwards et al., 2001; Pál et al., 2006).

Certain studies pointed out that some living organisms may be driven by different objectives (ATP production, nutrient uptake, and overall flux, to name but a few) depending on the environmental conditions (Edward and Palsson, 1998). Accordingly, no particular objective function could completely capture the optimal functioning of metabolic networks under all environmental circumstances. Checking whether a hypothesized objective function is consistent with experimental flux data is consequently a mandatory task. A recent work systematically assessed the relevance of eleven chosen objective functions to predict rates in *E. coli* in six different growth mediums (Schuetz et al., 2007). Certain methods, namely *ObjFind* and *invFBA*, propose to infer the objective functions that fit better with observed experimental data (Burgard and Maranas, 2003; Zhao et al., 2016).

On the other hand, when studying the consequences of gene deletion on the metabolic capabilities of a mutant strain, the objectives used for wild-type systems may not be accurate to capture the metabolic behavior of a knocked-out living organism. The later is assumed by a recent approach, called *minimization of metabolic adjustment* (MOMA), to display a flux distribution closest to the optimal flux distribution prior to the gene deletion (Segrè et al., 2002). To achieve this, MOMA determines a flux vector with the smallest euclidean distance to the

optimal wild-type flux vector by solving the following quadratic program:

$$\begin{aligned}
&\min\ (v-w)^T(v-w)\\
&\text{subject to:}\\
&Sv = 0,\\
&l_i \le v_i \le u_i \text{ for all } i \in \{1,\ldots,n\},\\
&v_j = 0,\ \text{for all } j \in A,
\end{aligned}$$

where w denotes the wild-type flux distribution and A stands for the set of reactions corresponding to the deleted genes. Alternatively, *regulatory on-off minimization (ROOM)* assumes that the metabolic behavior of a mutant strain corresponds to a flux distribution with a mixed integer linear programming (MILP) problem:

$$\begin{aligned}
&\min\ \textstyle\sum_{i=1}^{n} c_i y_i\\
&\text{subject to:}\\
&Sv = 0,\\
&v_i - y_i(u_i - w_i^u) \le w_i^u \text{ for all } i \in \{1,\ldots,n\},\\
&v_i - y_i(l_i - w_i^l) \ge w_i^l \text{ for all } i \in \{1,\ldots,n\},\\
&v_j = 0,\ \text{for all } j \in A,\\
&y_i \in \{0,1\},
\end{aligned}$$

where c_i is the cost for a change in the flux through reaction i, and for each reaction $i \in \{1,\ldots,n\}$, $y_i = 1$ if there is a significant flux change in v_i, i.e., $v_i \notin [w_i^l, w_i^u]$ with $[w^l, w^u]$ being an interval around the wild-type flux distribution w, and $y_i = 0$ otherwise.

Several other optimization-based methods have been proposed to analyze metabolic networks including automated curation of metabolic reconstructions (Kumar et al., 2007), recovering metabolic pathways via optimization (Beasley and Planes, 2007), analysis of gene essentiality (Burgard et al., 2001; Almaas et al., 2005; Pál et al., 2006) and metabolic engineering (Burgard and Maranas, 2001; Bro et al., 2006). Although these methods have proven effective in evaluating metabolic capabilities for many microorganisms, their findings strongly depend on the definition of the objective function. In addition, these approaches assume that metabolic systems operate in accordance with a single optimization rule. It has been shown, however, that a microorganism could be driven by different optimization objectives depending on the growth conditions (Schuetz et al., 2007). Furthermore, since an optimal solution with respect to a suitable objective function need not be unique, these optimization-based techniques often return a randomly selected flux distribution from the optimal flux space. This space is, in general, an infinite convex set and requires an adequate description. For the above reasons, a good alternative is to apply pathway-based network analysis to assess the properties of the whole steady-state flux cone.

3.3 Pathway-based Network Analysis

Pathway-based network analysis (Klamt and Stelling, 2003; Papin et al., 2003; Schilling et al., 2000b) has been become an indispensable approach in computational biology. This analysis makes a focus on describing the infinite flux cone C (defined in equation (4)) using a finite set of generators. It is important to distinguish whether the flux cone is pointed or not. The flux cone is by definition pointed if its lineality space

$$\text{lin.space}(C) = \{v \in C \mid v_i = 0, \text{for all } i \in Irr\} \tag{6}$$

is reduced to the origin, i.e., no trivial steady-state flux distribution can use only reversible reactions. Specifically, if all reactions are irreversible, i.e., $Irr = \{1,\ldots,n\}$, then $\text{lin.space}(C) = \{0\}$ and so the flux cone is pointed. In this case, the flux cone is generated by the unique and minimal set of its extreme rays. The case becomes more complex in the presence of reversible

reactions. In this case, the flux cone may be non-pointed and has no longer a unique and minimal description by its extreme rays. To deal with this situation, a constraint- based approach has been proposed to investigate the properties of the steady-state flux cone by means of sets of irreversible reactions (Larhlimi and Bockmayr, 2009). Other methods suggest re-configuring the metabolic network in order to render the flux cone pointed (Clarke, 1980; Schilling et al., 2000a; Larhlimi and Bockmayr, 2008).

Alternatively, a description of the flux cone without any reconfiguration has been proposed, using elementary modes (Schuster and Hilgetag, 1994; Schuster et al., 2002). An *elementary mode (EM)* is a steady-state flux vector using a minimal set of reactions. From a biological point of view, each EM uses a minimum set of reactions to convert a set of substrates into products. Because reactions are catalyzed by enzymes, each EM corresponds to a minimum set of enzymes which must be expressed by genes. This property is important because a living organism's effort to carry out a metabolic pathway increases with the amount of enzymes expressed (Papin et al., 2002).

More formally, a flux vector $e \in C \setminus \{0\}$ is an elementary mode if, and only if, there do not exist vectors $e' \in C$ and $e'' \in C$ such that

$$e = \lambda_1 e' + \lambda_2 e'' \text{ for some } \lambda_1, \lambda_2 > 0,$$

and

$$Supp(e') \subsetneq Supp(e) \text{ and } Supp(e'') \subsetneq Supp(e).$$

In addition, elementary modes form a convex basis of the steady-state flux cone. Each steady-state flux distribution can be considered as a non-negative linear combination of elementary modes (Schuster et al., 2002). In other words, if $e^1, \ldots, e^p$ are the elementary modes of the flux cone C, each feasible flux vector $v \in C$ is a non-negative linear combination of $e^1, \ldots, e^p$

$$v = \sum_{k=1}^{p} \lambda_k e^k \text{ for some } \lambda_k \geq 0.$$

Tab. 1 lists the main practical applications of elementary modes. For instance, they are useful for studying reaction deletions, i.e., the removal of some reactions from the metabolic network. Indeed, if a set of reactions are removed from a metabolic network, all elementary modes not using these reactions form the complete set of elementary modes in the modified network. This property is of great interest in the calculation of minimal cut sets to identify reactions that must be removed to make a target reaction inactive (Klamt, 2006). Elementary modes can also be used to assess the robustness of a metabolic network against mutations and environmental perturbations (Wilhelm et al., 2004).

Table 1: Principal applications of elementary modes to analyze metabolic network properties.

Application	Reference
Correlated reactions	(Pfeiffer et al., 1999)
Mutant viability	(Cakir et al., 2004; Stelling et al., 2002)
Control-effective flux analysis	(Cakir et al., 2004, 2007; Stelling et al., 2002)
Pathways with maximal yields	(Krömer et al., 2006; Schuster et al., 2000; Schwender et al., 2004)
Thermodynamically infeasible cycles	(Gagneur and Klamt, 2004)
Minimal cut sets	(Klamt, 2006)
Dynamical capabilities of a metabolic system	(Steuer et al., 2007)
Network robustness	(Wilhelm et al., 2004)

4 Conclusion

In this review, we show the crucial role of mathematical and computational methods to achieve a deep understanding of living organisms. In particular, constraint-based modeling shows large applicability in the study of complex metabolic networks. However, several challenges still remain, notably in providing efficient methods that scale well for large genome-scale models of complex microorganisms. From a computational point of view, calculating a description of the flux cone corresponds to calculating a convex basis. This computation, which may be inefficient for large-scale metabolic networks, is still a challenging task. Further advancements in metabolic network modeling (e.g., dividing the network into simpler sub-networks, considering reaction dependencies) and in algorithm implementation may enhance the current methods.

Constraint-based metabolic network analysis assumes that living organisms operate under steady-state conditions and so no predictions about the dynamic behavior of the system can be performed. However, the insight gained about the structural properties of metabolism can serve as a basis for other studies. In particular, the incorporation of regulatory constraints and kinetic information would expand the scope of this approach and would provide useful modeling and efficient tools for genome-scale metabolic network analysis..

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