3.1 Structural Analysis of Biochemical Systems

Summary

We discuss basic structural and dynamic properties of biochemical reaction networks. We introduce a stoichiometric description of networks and use it to formulate the system (or balance) equations. This will be demonstrated for a number of typical examples. The analysis of the mathematical properties of the stoichiometric matrix can reveal important properties of the reaction system: we can learn how moieties are conserved, even over dynamic changes of the whole system, and how steady-state fluxes are balanced within networks. The search for identifiable pathways in a complex network is also based on the stoichiometric matrix and leads to the concepts of flux cone, elementary flux modes, and extreme pathways.

3.1.1 System Equations

Stoichiometric coefficients denote the proportion of substrate and product molecules involved in a reaction. For example, for the reaction

$$S_1 + S_2 \rightleftharpoons 2P, \tag{3.1}$$

the stoichiometric coefficients of S_1, S_2, and P are -1, -1, and 2, respectively. The assignment of stoichiometric coefficients is not unique. We could also argue that for the production of one mole P, half a mole of each S_1 and S_2 have to be used and, therefore, choose $-1/2$, $-1/2$, and 1. Or, if we change the direction of the reaction, then we may choose 1, 1, and -2.

The change of concentrations in time can be described using ordinary differential equations (ODEs). For the reaction depicted in Eq. (3.1) and the first choice of stoichiometric coefficients, we obtain

$$\frac{dS_1}{dt} = -v, \quad \frac{dS_2}{dt} = -v, \quad \text{and} \quad \frac{dP}{dt} = 2v. \tag{3.2}$$

This means that the decay of S_1 with rate v is accompanied by the decay of S_2 with the same rate and by the production of P with the double rate.

For a metabolic network consisting of m substances and r reactions, the system dynamics is described by the *system equations* (or *balance equations*, since the balance of substrate production and degradation is considered) [1,2]:

$$\frac{dS_i}{dt} = \sum_{j=1}^{r} n_{ij} v_j \quad \text{for} \quad i = 1, \ldots, m. \tag{3.3}$$

The quantities n_{ij} are the stoichiometric coefficients of the ith metabolite in the jth reaction. Here, we assume that the reactions are the only cause for concentration changes and that no mass flow occurs due to convection or diffusion. External metabolites are not included in the balance equations. These metabolites are not described in the model because they are not considered relevant or their concentrations are kept constant by processes out of the scope of the model. The balance equations (Eq. (3.3)) can also be applied if the system consists of several compartments. In this case, every compound in different compartments has to be considered as an individual compound and transport steps are formally considered as reactions transferring the compound belonging to one compartment into the same compound belonging to the other compartment. Volume differences must be taken into account, since a certain number of molecules moving from one compartment to another change the concentrations in these compartments differently in case of different volumes (see Section 7.3).

The stoichiometric coefficients n_{ij} assigned to the compounds S_i and the reactions v_j can be combined into the *stoichiometric matrix* $\mathbf{N}$ with

$$\mathbf{N} = \{n_{ij}\} \quad \text{for} \quad i = 1, \ldots, m \quad \text{and} \quad j = 1, \ldots, r,$$

or

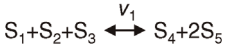
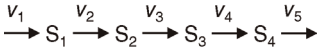
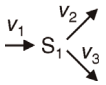
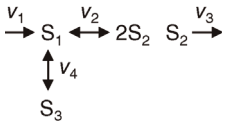
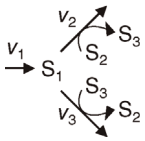
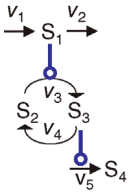
$$\mathbf{N} = \begin{array}{c} \begin{matrix} v_1 & v_2 & \cdots & v_r \end{matrix} \\ \begin{pmatrix} n_{11} & n_{12} & \cdots & n_{1r} \\ n_{21} & n_{22} & \cdots & n_{2r} \\ \vdots & \vdots & \ddots & \vdots \\ n_{m1} & n_{m2} & \cdots & n_{mr} \end{pmatrix} \end{array} \begin{matrix} S_1 \\ S_2 \\ \vdots \\ S_m \end{matrix}, \tag{3.4}$$

where each column belongs to a reaction and each row to a compound. Table 3.1 provides some examples for reaction networks and their respective stoichiometric matrices.

Note that the stoichiometric matrix $\mathbf{N}$ does not contain information about whether reactions are reversible or irreversible. In order to determine the signs in $\mathbf{N}$, the direction of the arrows must be assigned, for example, as positive "from left to right" and "from top to bottom." If the net flow of a reaction proceeds in the opposite direction as the arrow indicates, the value of rate v is negative.

Altogether, the mathematical description of the metabolic system consists of a vector $\mathbf{S} = (S_1, S_2, \ldots, S_n)^T$ of concentration values, a vector $\mathbf{v} = (v_1, v_2, \ldots, v_r)^T$ of reaction rates, a parameter vector $\mathbf{p} = (p_1, p_2, \ldots, p_m)^T$, and the stoichiometric matrix $\mathbf{N}$. If the system is in steady

Table 3.1 Different reaction networks, their stoichiometric matrices, and the respective system of ODEs.

	Network	*Stoichiometric matrix*	*ODE system*
N1		$\mathbf{N} = \begin{pmatrix} -1 \\ -1 \\ -1 \\ 1 \\ 2 \end{pmatrix}$	$\frac{dS_1}{dt} = \frac{dS_2}{dt} = \frac{dS_3}{dt} = -v_1$ $\frac{dS_4}{dt} = v_1$ $\frac{dS_5}{dt} = 2v_1$
N2		$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix}$	$\frac{dS_1}{dt} = v_1 - v_2$ $\frac{dS_2}{dt} = v_2 - v_3$ $\frac{dS_3}{dt} = v_3 - v_4$ $\frac{dS_4}{dt} = v_4 - v_5$
N3		$\mathbf{N} = \begin{pmatrix} 1 & -1 & -1 \end{pmatrix}$	$\frac{dS_1}{dt} = v_1 - v_2 - v_3$
N4		$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & -1 \\ 0 & 2 & -1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}$	$\frac{dS_1}{dt} = v_1 - v_2 - v_4$ $\frac{dS_2}{dt} = 2v_2 - v_3$ $\frac{dS_3}{dt} = v_4$
N5		$\mathbf{N} = \begin{pmatrix} 1 & -1 & -1 \\ 0 & -1 & 1 \\ 0 & 1 & -1 \end{pmatrix}$	$\frac{dS_1}{dt} = v_1 - v_2 - v_4$ $\frac{dS_2}{dt} = -v_2 + v_3$ $\frac{dS_3}{dt} = v_2 - v_3$
N6		$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}$	$\frac{dS_1}{dt} = v_1 - v_2$ $\frac{dS_2}{dt} = v_4 - v_3$ $\frac{dS_3}{dt} = -v_4 + v_3$ $\frac{dS_4}{dt} = v_5$

Note that external metabolites are neither drawn in the network nor included in the stoichiometric matrix. Thin arrows denote reactions and bold arrows denote activation.

state, we can also consider the vector $\mathbf{J} = (J_1, J_2, \ldots, J_r)^{\mathrm{T}}$ containing the steady-state fluxes. With these notions, the balance equation reads

$$\frac{d\mathbf{S}}{dt} = \mathbf{N}\mathbf{v}, \tag{3.5}$$

a compact form that is suited for various types of analyses.

3.1.2 Information Encoded in the Stoichiometric Matrix N

The stoichiometric matrix contains important information about the structure of the metabolic network. Using the stoichiometric matrix, we may calculate which combinations of individual fluxes are possible in steady state (i.e., calculate the admissible steady-state flux space).

We may easily find out dead ends and unbranched reaction pathways. In addition, we may discover the conservation relations for the included reactants.

In steady state, it holds that

$$\frac{\mathrm{d}\mathbf{S}}{\mathrm{d}t} = \mathbf{N}\mathbf{v} = \mathbf{0}. \tag{3.6}$$

Note that $\mathbf{0}$ is a vector with length n, that is, $\mathbf{0} = (0, 0, \ldots, 0)^{\mathrm{T}}$. The right equality sign in Eq. (3.6) denotes a linear equation system for determination of the rates $\mathbf{v}$. From linear algebra, it is known that this equation has nontrivial solutions only for $\mathrm{Rank}(\mathbf{N}) < r$ (see Section 15.1 for an introduction to linear algebra). A kernel matrix $\mathbf{K}$ fulfilling

$$\mathbf{N}\mathbf{K} = \mathbf{0} \tag{3.7}$$

expresses the respective linear dependencies between the columns of the stoichiometric matrix [3]. $\mathbf{K}$ consists of $r - \mathrm{Rank}(\mathbf{N})$ basis vectors as columns and can be determined using the Gauss algorithm (see mathematical textbooks). The kernel is not uniquely defined. Multiplication of $\mathbf{K}$ with a regular matrix $\mathbf{Q}$ of appropriate size ($\mathbf{K}' = \mathbf{K} \cdot \mathbf{Q}$, equivalently to linear combination of the columns of $\mathbf{K}$) yields another valid kernel $\mathbf{K}'$ of $\mathbf{N}$.

Every possible set $\mathbf{J}$ of steady-state fluxes can be expressed as linear combination of the columns $\mathbf{k}_i$ of $\mathbf{K}$:

$$\mathbf{J} = \sum_{i=1}^{r-\mathrm{Rank}(\mathbf{N})} \alpha_i \cdot \mathbf{k}_i. \tag{3.8}$$

The coefficients must have units corresponding to the units of reaction rates (e.g., mM s^{-1}).

If the entries in a certain row are zero in all basis vectors, we have found an equilibrium reaction. In any steady state, the net rate of this reaction must be zero. For the reaction system N4 in Table 3.1, it holds that $r = 4$ and $\mathrm{Rank}(\mathbf{N}) = 3$. Its kernel consists of only one column $\mathbf{K} = \begin{pmatrix} 1 & 1 & 1 & 0 \end{pmatrix}^{\mathrm{T}}$. Hence, $v_4 = \sum_{i=1}^{1} \alpha \cdot 0 = 0$. In any steady state, the rates of production and degradation of S_3 must be equal, thereby leading to zero net change.

If all basis vectors contain the same entries for a set of rows, this indicates an unbranched reaction path. In each steady state, the net rate of all respective reactions is equal.

Up to now, we have not been concerned about (ir) reversibility of reactions in the network. The irreversibility of a reaction does not affect the stoichiometric matrix. However, it has consequences for the choice of basis vectors $\mathbf{k}_i$ for the kernel $\mathbf{K}$. A set of basis vectors must be chosen to satisfy the signs of fluxes when calculated by Eq. (3.8).

Example 3.1

For the network N2 in Table 3.1, we have $r = 5$ reactions and $\mathrm{Rank}(\mathbf{N}) = 4$. The kernel matrix contains just $1 = 5 - 4$ basis vectors, which are multiples of $\mathbf{k}_1 = \begin{pmatrix} 1 & 1 & 1 & 1 & 1 \end{pmatrix}^{\mathrm{T}}$. This means that in steady state the flux through all reactions must be equal.

Network N3 comprises $r = 3$ reactions and has $\mathrm{Rank}(\mathbf{N}) = 1$. Each representation of the kernel matrix contains $3 - 1 = 2$ basis vectors, for example,

$$\mathbf{K} = \begin{pmatrix} \mathbf{k}_1 & \mathbf{k}_2 \end{pmatrix} \quad \text{with} \quad \mathbf{k}_1 = \begin{pmatrix} 1 \\ 1 \\ 0 \end{pmatrix}, \quad \mathbf{k}_2 = \begin{pmatrix} 1 \\ 0 \\ 1 \end{pmatrix}, \tag{3.9}$$

and for the steady-state flux holds

$$\mathbf{J} = \alpha_1 \cdot \mathbf{k}_1 + \alpha_2 \cdot \mathbf{k}_2. \tag{3.10}$$

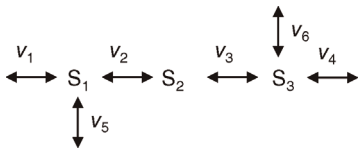
Network N6 can present a small signaling cascade. It has five reactions and $\mathrm{Rank}(\mathbf{N}) = 3$. Two basis vectors of the kernel are

$$\mathbf{k}_1 = \begin{pmatrix} 1 & 1 & 0 & 0 & 0 \end{pmatrix}^{\mathrm{T}},$$
$$\mathbf{k}_2 = \begin{pmatrix} 0 & 0 & 1 & 1 & 0 \end{pmatrix}^{\mathrm{T}}. \tag{3.11}$$

If we calculate the possible steady-state fluxes according to Eq. (3.10), we can easily see that in every steady state it holds that production and degradation of S_1 are balanced ($J_1 = J_2$) and that the fluxes through the cycle are equal ($J_3 = J_4$). In addition, J_5 must be equal to zero, otherwise S_4 would accumulate. One could prevent the last effect by also including the degradation of S_4 into the network.

Example 3.2

Consider the reaction scheme



(3.12)

The system comprises $r = 6$ reactions. The stoichiometric matrix reads

$$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 1 \end{pmatrix}$$

with $\mathrm{Rank}(\mathbf{N}) = 3$. Thus, the kernel matrix is spanned by three basis vectors, for example, $\mathbf{k}_1 = \begin{pmatrix} 1 & 1 & 1 & 0 & 0 & -1 \end{pmatrix}^{\mathrm{T}}$, $\mathbf{k}_2 = \begin{pmatrix} 1 & 0 & 0 & 0 & 1 & 0 \end{pmatrix}^{\mathrm{T}}$, and $\mathbf{k}_3 = \begin{pmatrix} -1 & -1 & -1 & -1 & 0 & 0 \end{pmatrix}^{\mathrm{T}}$. The entries for the second and third reactions are always equal; thus, in any steady state, the fluxes through reactions 2 and 3 must be equal.

3.1.3 The Flux Cone

The stoichiometric analysis of biochemical network analysis can be modified by considering only irreversible reactions (e.g., by splitting reversible reactions into two irreversible ones). Based on such a unidirectional representation, the basis vectors (Eq. (3.8)) form a convex cone in the flux space. This mapping relates stoichiometric analysis to the concepts of convex geometry as follows. The steady-state assumption requires that a flux vector is an element of the null space of the stoichiometric matrix **N** spanned by matrix **K**. A row of **K** can be interpreted as a hyperplane in flux space. The intersection of all these hyperplanes forms the null space. Provided that all reactions are unidirectional or irreversible, the intersection of the null space with the semipositive orthant of the flux space forms a polyhedral cone, the flux cone. The intersection procedure results in a set of rays or edges starting at 0, which fully describe the cone. The edges are represented by vectors and any admissible steady state of the system is a positive combination of these vectors. An illustration is presented in Figure 3.1.

3.1.4 Elementary Flux Modes and Extreme Pathways

A stringent definition of the term "pathway" in a metabolic network is not straightforward. A descriptive definition of a pathway is a set of reactions that are linked by common metabolites. Typical examples include glycolysis or different amino acid synthesis pathways. More detailed inspection of metabolic maps such as the *Boehringer chart* [4] shows that metabolism is highly interconnected and better addressed as a network. Pathways that are known for a long time from biochemical experience are already hard to recognize, and it is even harder to find out new pathways, for example, in metabolic maps that have been reconstructed from sequenced genomes of bacteria.

The problem of clearly identifying functional pathways has been elaborated in the concepts of *elementary flux modes* [3,5–10] and *extreme pathways* [11–14]. In both cases, the stoichiometry of a metabolic network is investigated to discover which direct routes are possible that lead from one external metabolite to another external metabolite. Both approaches use the steady-state assumption and take into account that some reactions are reversible, while others are irreversible. Despite these two constraints, we still obtain too many solutions, especially for larger networks. For elementary flux modes, this problem is solved by the requirement that they cannot be further decomposed, while extreme fluxes are bound to the generating vectors of the flux cone, as explained below.

We start with defining a *flux mode* **M**. It is the set of flux vectors that represent direct routes through the network between external metabolites. In mathematical terms, it is defined as the set

$$\mathbf{M} = \left\{\mathbf{v} \in \mathbf{R}^r | \mathbf{v} = \lambda \mathbf{v}^*, \lambda > 0\right\}, \tag{3.13}$$

where $\mathbf{v}^*$ is an r-dimensional vector (unequal to the null vector) fulfilling two conditions:

1) the steady-state condition $\mathbf{Nv} = \mathbf{0}$, that is, Eq. (3.6), and
2) sign restriction, that is, the flux directions in $\mathbf{v}^*$ fulfill the prescribed irreversibility relations. $\mathbf{v}^{\text{irr}}$ denotes the subvector of $\mathbf{v}^*$ that contains only nonnegative fluxes.

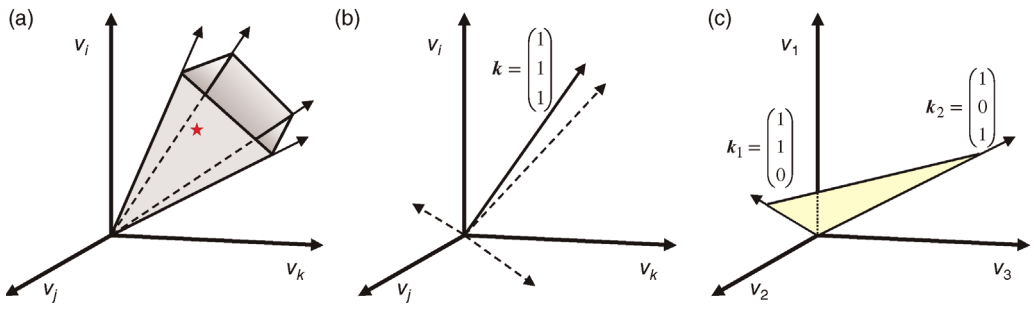


Figure 3.1 Flux cone: schematic representation of the subspace of feasible steady states within the space spanned by all positive-valued vectors for rates of irreversible reactions, v_i, $i = 1, \ldots, r$. Only three dimensions are shown. Feasible solutions are linear combinations of basis vectors of matrix **K** (see text). (a) Illustrative representation of the flux cone for a higher dimensional system (with $r - \text{Rank}(\mathbf{N}) = 4$). The basis vectors of **K** are rays starting at the origin. The line connecting the four rays indicates possible limits for real flux distributions set by constraints. The little asterisk indicates one special feasible solution for the fluxes. (b) The flux cone for an unbranched reaction chain of arbitrary length, such as the network N2 in Table 3.1, is just a ray since **K** is represented by a single basis vector containing only 1s. (c) The flux cone for network N3 in Table 3.1 is the plane spanned by the basis vectors $\mathbf{k}_1 = \begin{pmatrix}1 & 1 & 0\end{pmatrix}^T$ and $\mathbf{k}_2 = \begin{pmatrix}1 & 0 & 1\end{pmatrix}^T$.

A flux mode $\mathbf{M}$ comprising $\mathbf{v}$ is called reversible if the set $\mathbf{M}'$ comprising $-\mathbf{v}$ is also a flux mode.

A flux mode is an *elementary flux mode* if it uses a minimal set of reactions and cannot be further decomposed, that is, the vector $\mathbf{v}$ cannot be represented as nonnegative linear combination of two vectors that fulfill conditions (1) and (2) but contain more zero entries than $\mathbf{v}$. An elementary flux mode is a minimal set of enzymes that could operate at steady state, with all the irreversible reactions used in the appropriate direction. The number of elementary flux modes is at least as high as the number of basis vectors of the null space. The set of elementary flux modes is uniquely defined. Pfeiffer *et al.* [6] developed a software ("Metatool") to calculate the elementary flux modes for metabolic networks.

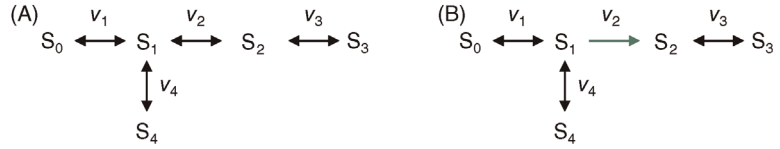
A flux mode is an *extreme pathway* if

1) all reactions are nonnegative, that is, $\mathbf{v} = \mathbf{v}^{\mathrm{irr}}$;
2) it belongs to the edges of the flux cone, which also means that it represents a basis vector of $\mathbf{K}$.

To achieve the first, reversible reactions are broken down into their forward and backward components and exchange fluxes have to be defined in the appropriate direction. This way, the set of extreme pathways is a

Example 3.3

The systems (A) and (B) differ by the fact that reaction 2 is either reversible or irreversible.



(3.14)

The elementary flux modes connect the external metabolites S_0 and S_3, S_0 and S_4, or S_3 and S_4. The stoichiometric matrix and the flux modes for case (A) and case (B) are

$$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & -1 \\ 0 & 1 & -1 & 0 \end{pmatrix}, \quad \mathbf{v}^{\mathrm{A}} = \begin{pmatrix} 1 \\ 1 \\ 1 \\ 0 \end{pmatrix}, \begin{pmatrix} 1 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \begin{pmatrix} 0 \\ -1 \\ -1 \\ 1 \end{pmatrix}, \begin{pmatrix} -1 \\ -1 \\ -1 \\ 0 \end{pmatrix}, \begin{pmatrix} -1 \\ 0 \\ 0 \\ -1 \end{pmatrix}, \begin{pmatrix} 0 \\ 1 \\ 1 \\ -1 \end{pmatrix}, \quad \text{and}$$

$$\mathbf{v}^{\mathrm{B}} = \begin{pmatrix} 1 \\ 1 \\ 1 \\ 0 \end{pmatrix}, \begin{pmatrix} 1 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \begin{pmatrix} -1 \\ 0 \\ 0 \\ -1 \end{pmatrix}, \begin{pmatrix} 0 \\ 1 \\ 1 \\ -1 \end{pmatrix}. \tag{3.15}$$

The possible routes are illustrated in Figure 3.2.

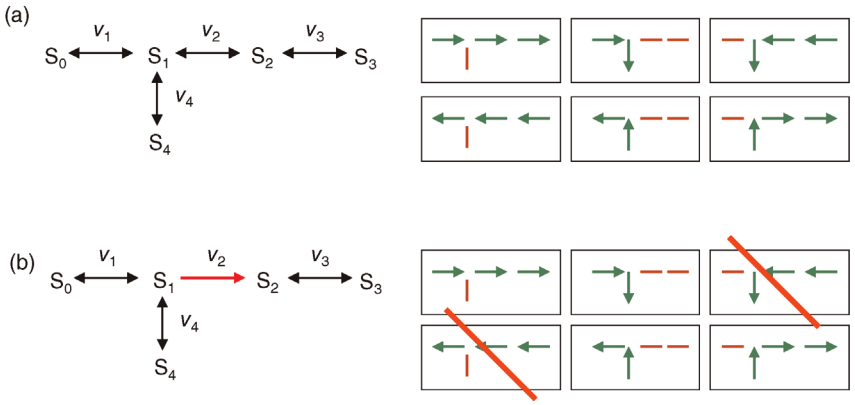


Figure 3.2 Schematic representation of elementary flux modes for the reaction network depicted in Eq. (3.14).

subset of the set of elementary flux modes and the extreme pathways are systemically independent.

Elementary flux modes and extreme pathways can be used to understand the range of metabolic pathways in a network, to test a set of enzymes for production of a desired product and detect nonredundant pathways, to reconstruct metabolism from annotated genome sequences and analyze the effect of enzyme deficiency, to reduce drug effects, and to identify drug targets. A specific application, the flux balance analysis will be explained in Section 3.2.1.

3.1.5 Conservation Relations – Null Space of $\mathbf{N}^T$

If a chemical entity is neither added to nor removed from the reaction system (neither produced nor degraded), its total concentration remains constant. This also holds if the substance interacts with other compounds by forming complexes.

For the mathematical derivation of the conservation relations [3], we consider a matrix $\mathbf{G}$ fulfilling

$$\mathbf{GN} = \mathbf{0}. \tag{3.16}$$

Due to Eq. (3.5), it follows

$$\mathbf{G}\dot{\mathbf{S}} = \mathbf{GNv} = \mathbf{0}. \tag{3.17}$$

Integrating this equation leads directly to the conservation relations

$$\mathbf{GS} = \text{constant}. \tag{3.18}$$

The number of independent rows of $\mathbf{G}$ is equal to $n - \text{Rank}(\mathbf{N})$, where n is the number of metabolites in the system. $\mathbf{G}^T$ is the kernel matrix of $\mathbf{N}^T$; hence, it has similar properties to $\mathbf{K}$. Matrix $\mathbf{G}$ can also be found using the Gauss algorithm. It is not unique, but every linear combination of its rows is again a valid solution (equivalent to a premultiplication of $\mathbf{G}$ with a regular matrix of appropriate size, i.e., $\mathbf{PG} = \mathbf{G}'$). There exists a simplest representation $\mathbf{G} = \begin{pmatrix} \mathbf{G}_0 & \mathbf{I}_{n-\text{Rank}(\mathbf{N})} \end{pmatrix}$. Finding this representation may be helpful for a simple statement of conservation relations, but this may necessitate renumbering and reordering of metabolite concentrations (see below).

Importantly, conservation relations can be used to simplify the system of differential equations $\dot{\mathbf{S}} = \mathbf{Nv}$ describing the dynamics of our reaction system. The idea is to eliminate linear dependent differential equations and to replace them by appropriate algebraic equations. Below the procedure is explained systematically [2].

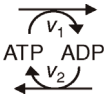
First we have to reorder the rows in the stoichiometric matrix $\mathbf{N}$ as well as in the concentration vector $\mathbf{S}$ such that a set of independent rows is on top and the dependent rows are at the bottom. Then the matrix $\mathbf{N}$ is split into the independent part $\mathbf{N}_{\text{indep}}$ and the dependent part $\mathbf{N}'$ and a *link matrix* $\mathbf{L}$ is introduced in the following way:

$$\mathbf{N} = \begin{pmatrix} \mathbf{N}_{\text{indep}} \\ \mathbf{N}' \end{pmatrix} = \mathbf{L}\mathbf{N}_{\text{indep}} = \begin{pmatrix} \mathbf{I}_{\text{Rank}(\mathbf{N})} \\ \mathbf{L}' \end{pmatrix} \mathbf{N}_{\text{indep}}. \tag{3.22}$$

$\mathbf{I}_{\text{Rank}(\mathbf{N})}$ is the identity matrix of size $\text{Rank}(\mathbf{N})$. The

Example 3.4

Consider a set of two reactions comprising a kinase and a phosphatase reaction

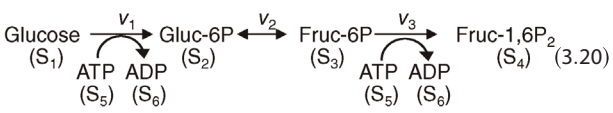


(3.19)

The metabolite concentration vector reads $\mathbf{S} = (ATP\ ADP)^T$, and the stoichiometric matrix is $\mathbf{N} = \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix}$ yielding $\mathbf{G} = (1\ 1)$. From the condition $\mathbf{GS}$=constant, it follows that $ATP + ADP$=constant. Thus, we have a conservation of adenine nucleotides in this system. The actual values of $ATP + ADP$ must be determined from the initial conditions.

Example 3.5

For the following model of the upper part of glycolysis



(3.20)

the stoichiometric matrix $\mathbf{N}$ (note the transpose!) and a possible representation of the conservation matrix $\mathbf{G}$ are given by

$$\mathbf{N}^T = \begin{pmatrix} -1 & 1 & 0 & 0 & -1 & 1 \\ 0 & -1 & 1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & -1 & 1 \end{pmatrix} \quad \text{and}$$

$$\mathbf{G} = \begin{pmatrix} 2 & 1 & 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 1 \\ 1 & 1 & 1 & 1 & 0 & 0 \end{pmatrix} = \begin{pmatrix} \mathbf{g}_1 \\ \mathbf{g}_2 \\ \mathbf{g}_3 \end{pmatrix}. \tag{3.21}$$

The interpretation of the second and third rows of $\mathbf{G}$ is straightforward, showing the conservation of adenine nucleotides ($\mathbf{g}_2$, $ADP + ATP$ = constant) and the conservation of sugars ($\mathbf{g}_3$), respectively. The interpretation of the first row is less intuitive. If we construct the linear combination $\mathbf{g}_4 = -\mathbf{g}_1 + 3 \cdot \mathbf{g}_2 + 2 \cdot \mathbf{g}_3 = (0\ \ 1\ \ 1\ \ 2\ \ 3\ \ 2)$, we find the conservation of phosphate groups.

differential equation system may be rewritten accordingly:

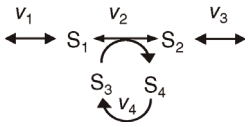
$$\dot{\mathbf{S}} = \begin{pmatrix} \dot{\mathbf{S}}_{\text{indep}} \dot{\mathbf{S}}_{\text{dep}} \end{pmatrix} = \begin{pmatrix} \mathbf{I}_{\text{Rank(N)}} \\ \mathbf{L}' \end{pmatrix} \mathbf{N}_{\text{indep}} \mathbf{v} \tag{3.23}$$

and the dependent concentrations fulfill

$$\dot{\mathbf{S}}_{\text{dep}} = \mathbf{L}' \cdot \dot{\mathbf{S}}_{\text{indep}}. \tag{3.24}$$

Example 3.6

For the reaction system



(3.27)

the stoichiometric matrix, the reduced stoichiometric matrix, and the link matrix read

$$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & -1 & 0 & 1 \\ 0 & 1 & 0 & -1 \end{pmatrix},$$

$$\mathbf{N}_{\text{indep}} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & -1 & 0 & 1 \end{pmatrix},$$

$$\mathbf{L} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -1 \end{pmatrix}, \quad \mathbf{L}' = \begin{pmatrix} 0 & 0 & -1 \end{pmatrix}.$$

The conservation relation $S_3 + S_4 = \text{constant}$ is expressed by $\mathbf{G} = \begin{pmatrix} 0 & 0 & 1 & 1 \end{pmatrix}$. The ODE system

$$\dot{S}_1 = v_1 - v_2,$$
$$\dot{S}_2 = v_2 - v_3,$$
$$\dot{S}_3 = v_4 - v_2,$$
$$\dot{S}_4 = v_2 - v_4$$

can be replaced by the algebro-differential equation system

$$\dot{S}_1 = v_1 - v_2,$$
$$\dot{S}_2 = v_2 - v_3,$$
$$\dot{S}_3 = v_4 - v_2,$$
$$S_3 + S_4 = \text{constant},$$

which has one differential equation less.

Integration leads to

$$\mathbf{S}_{\text{dep}} = \mathbf{L}' \cdot \mathbf{S}_{\text{indep}} + \text{constant}. \tag{3.25}$$

This relation is fulfilled during the entire time course. Thus, we may replace the original system by a reduced differential equation system

$$\dot{\mathbf{S}}_{\text{indep}} = \mathbf{N}_{\text{indep}} \mathbf{v} \tag{3.26}$$

supplemented with the set of algebraic equations (3.25).

Eukaryotic cells contain a variety of organelles such as the nucleus, mitochondria, or vacuoles, which are separated by membranes. Reaction pathways may cross these compartment boundaries. If a substance S occurs in two different compartments, for example, in the cytosol and in mitochondria, the respective concentrations can be assigned to two different variables, S^{C1} and S^{C2}. Formally, the transport across the membrane can be considered as a reaction with rate v. It is important to note that both compartments have different volumes V^{C1} and V^{C2}. Thus, transport of a certain amount of S with rate v from compartment C1 into the compartment C2 changes the concentrations differently:

$$V^{C1} \cdot \frac{\mathrm{d}}{\mathrm{d}t} S^{C1} = -v \quad \text{and} \quad V^{C2} \cdot \frac{\mathrm{d}}{\mathrm{d}t} S^{C2} = v, \tag{3.28}$$

where $V \cdot S$ denotes substance amount in moles. Compartment models are discussed in more detail in Section 7.3.

3.2 Constraint-Based Flux Optimization

Summary

Flux balance analysis, an optimality-based method for flux prediction, is one of the most popular modeling approaches for metabolic systems. Flux optimization methods do not describe *how* a certain flux distribution is realized (by kinetics or enzyme regulation), but *which* flux distribution is optimal for the cell – for example, providing the highest rate of biomass production at a limited inflow of external nutrients. This allows us to predict flux distributions without the need for a kinetic description. From a species' genome sequence, the metabolic network can be roughly predicted [15–17]. Even if we do not know anything about the enzyme kinetics, we can infer which metabolites the network can produce and which precursors are needed to produce biomass. Given a number of nutrients and a hypothesized optimality requirement, for example, for fast biomass production, we can try to predict an optimal flux distribution in the network.