Part II
Standard Models and Approaches in Systems Biology
5 Metabolism

Introduction

Living cells require energy and material for building membranes, storing molecules, replenishing enzymes, replication and repair of DNA, movement, and many other processes. Through metabolism cells acquire energy and use it to build new cells. Metabolism is the means by which cells survive and reproduce. Metabolism is the general term for two kinds of reactions: (1) catabolic reactions (breakdown of complex compounds to get energy and building blocks) and (2) anabolic reactions (construction of complex compounds used in cellular functioning). Metabolism is a highly organized process. It involves thousands of reactions that are catalyzed by enzymes.

Metabolic networks consist of reactions transforming molecules of one type into molecules of another type. In modeling terms, the concentrations of the molecules and their rates of change are of special interest. The basic concepts of reaction networks, which are outlined here, may also be applied for other types of cellular reaction networks, e.g., signal transduction pathways. In this chapter metabolism will be studied on three levels of abstraction:

1. Enzyme kinetics investigates the dynamic properties of the individual reactions in isolation.
2. The network character of metabolism is studied with stoichiometric analysis considering the balance of compound production and degradation.
3. Metabolic control analysis quantifies the effect of perturbations in the network employing the individual dynamics of concentration changes and their integration in the network.

Note that most modeling approaches for individual biochemical reactions or networks of such reactions that are presented in this chapter also apply for other types of networks, such as signaling cascades or binding of transcription factors to DNA. Since the modeling of metabolic networks is the most elaborate, it is subsumed here.

In order to illustrate the theoretical concepts, we will apply a running example throughout this chapter. This example comprises a subset of reactions of glycolysis.
in yeast as represented by Hynne and colleagues (2001). You can also find the complete model and many other models in modeling databases (Snoep and Olivier 2002).

**Example 1**

We will consider the first four reactions from the upper part of glycolysis as well as reactions balancing the energy currency ATP and ADP as represented in Fig. 5.1.

![Schematic representation of the upper part of glycolysis](image)

**Fig. 5.1** Schematic representation of the upper part of glycolysis, i.e., the degradation of glucose in order to yield energy and building blocks for cellular processes. Abbreviations: Gluc6P: glucose-6-phosphate; Fruc6P: fructose-6-phosphate; Fruc1,6P2: fructose-1,6-bisphosphate; ATP: adenosine-triphosphate; ADP: adenosine-diphosphate; AMP: adenosine-monophosphate. Reactions: v1: hexokinase; v2: consumption of glucose-6-phosphate in other pathways; v3: phosphoglucoisomerase; v4: phosphofructokinase; v5: aldolase; v6: ATP production in lower glycolysis; v7: ATP consumption in other pathways; v8: adenylate kinase.

The ODE system for this reaction system is given by

\[
\begin{align*}
\frac{d}{dt} \text{Gluc6P} &= v_1 - v_2 - v_3 \\
\frac{d}{dt} \text{Fruc6P} &= v_3 - v_4 \\
\frac{d}{dt} \text{Fruc1,6P2} &= v_4 - v_5 \\
\frac{d}{dt} \text{ATP} &= -v_1 - v_2 - v_3 + v_6 - v_7 - v_8 \\
\frac{d}{dt} \text{ADP} &= v_1 + v_2 + v_3 - v_6 + v_7 + 2v_8 \\
\frac{d}{dt} \text{AMP} &= -v_8.
\end{align*}
\]  

(5-1)

Abbreviations are explained in the legend of Fig. 5.1. The individual rate expressions read
\[ \nu_1 = \frac{V_{\text{max},1} \cdot \text{ATP}(t) \cdot \text{Glucose}}{1 + \frac{\text{ATP}(t)}{K_{\text{ATP},1}} + \frac{\text{Glucose}}{K_{\text{Glucose},1}}} \quad \text{or} \quad \nu_1 = \frac{V_{\text{max},1} \cdot \text{ATP}(t)}{K_{\text{ATP},1} + \text{ATP}(t)} \quad (5-2) \]

\[ \nu_2 = k_2 \cdot \text{ATP}(t) \cdot \text{Gluc6P}(t) \quad (5-3) \]

\[ \nu_3 = \frac{V_{\text{max},3}^f \cdot \text{Gluc6P}(t) - V_{\text{max},3}^r \cdot \text{Fruc6P}(t)}{1 + \frac{\text{Gluc6P}(t)}{K_{\text{Gluc6P},3}} + \frac{\text{Fruc6P}(t)}{K_{\text{Fruc6P},3}}} \quad (5-4) \]

\[ \nu_4 = \frac{V_{\text{max},4} \cdot (\text{Fruc6P}(t))^2}{K_{\text{Fruc6P},4} \left(1 + \kappa \left(\frac{\text{ATP}(t)}{\text{AMP}(t)}\right)^2\right) + (\text{Fruc6P}(t))^2} \quad (5-5) \]

\[ \nu_5 = k_5 \cdot \text{Fruc1,6P}_2(t) \quad (5-6) \]

\[ \nu_6 = k_6 \cdot \text{ADP}(t) \quad (5-7) \]

\[ \nu_7 = k_7 \cdot \text{ATP}(t) \quad (5-8) \]

\[ \nu_8 = k_{8f} \cdot \text{ATP}(t) \cdot \text{AMP}(t) - k_{8r} \cdot (\text{ADP}(t))^2, \quad (5-9) \]

with the following parameters:

\[ \text{Glucose} = 12.8174 \text{ mM}, \quad V_{\text{max},1} = 1398.00 \text{ mM \cdot min}^{-1}, \quad K_{\text{ATP},1} = 0.10 \text{ mM}, \]

\[ K_{\text{Glucose},1} = 0.37 \text{ mM}, \quad V_{\text{max},1} = 50.2747 \text{ mM \cdot min}^{-1} \]

\[ k_2 = 2.26 \text{ mM}^{-1} \cdot \text{min}^{-1} \]

\[ V_{\text{max},3}^f = 140.282 \text{ mM \cdot min}^{-1}, \quad V_{\text{max},3}^r = 140.282 \text{ mM \cdot min}^{-1}, \quad K_{\text{Gluc6P},3} = 0.80 \text{ mM}, \]

\[ K_{\text{Fruc6P},3} = 0.15 \text{ mM} \]

\[ V_{\text{max},4} = 44.7287 \text{ mM \cdot min}^{-1}, \quad K_{\text{Fruc6P},4} = 0.021 \text{ mM}^2, \quad \kappa = 0.15 \]

\[ k_5 = 6.04662 \text{ min}^{-1} \]

\[ k_6 = 68.48 \text{ min}^{-1} \]

\[ k_7 = 3.21 \text{ min}^{-1} \]

\[ k_{8f} = 432.9 \text{ mM}^{-1} \cdot \text{min}^{-1}, \quad k_{8r} = 133.33 \text{ mM}^{-1} \cdot \text{min}^{-1} \]

The temporal evolution of the concentrations starting from arbitrarily given values is shown in Fig. 5.2.
In this chapter it will be illustrated how metabolic models such as this model of upper glycolysis can be described and analyzed. We will present possible choices for rate equations and give reasoning for these choices. The information contained in the network structure, i.e., in the stoichiometry, shall be extracted: Are there main routes through the network? Which fluxes are possible in steady state? Will substances be produced or consumed, or are there conservation relations for metabolite concentrations? Metabolic control analysis will enable us to assess the influence of parameter changes in one part of the network on changes of variables (here, steady-state fluxes or concentrations) at any place of the network.

5.1 Enzyme Kinetics and Thermodynamics

This chapter deals with the deterministic kinetic modeling of individual biochemical reactions. The basic quantities are the concentration $S$ of a substance $S$ (i.e., the number $n$ of molecules of this substance per volume $V$) and the rate $v$ of a reaction (i.e., the change of concentration $S$ per time $t$). This type of modeling is macroscopic or phenomenological compared to the microscopic approach, where single molecules and their interactions are considered.

Chemical and biochemical kinetics rely on the assumption that the reaction rate $v$ at a certain point in time and space can be expressed as a unique function of the concentrations of all substances at this point in time and space. Classical enzyme kinetics assumes for simplicity’s sake a spatial homogeneity (the “well-stirred” test tube) and no direct dependency of the rate on time:

$$v(t) = v(S(t)).$$  \hspace{1cm} (5-10)

In more advanced modeling approaches moving towards whole-cell modeling, spatial inhomogeneities are taken into account, paying tribute to the fact that many components are membrane-bound or that cellular structures hinder the free movement of molecules. However, in most cases one can assume that diffusion is rapid enough to allow for an even distribution of all substances in space.
Enzymes catalyze biochemical reactions. Enzymes are proteins, often in complex with cofactors (Chapter 2, Section 2.1). They have a catalytic center, are usually highly specific, and remain unchanged by the reaction. One enzyme molecule catalyzes about a thousand reactions per second (the so-called turnover number ranges from $10^2$ s$^{-1}$ to $10^7$ s$^{-1}$). This leads to a rate acceleration of about $10^6$- to $10^{12}$-fold compared to the uncatalyzed, spontaneous reaction.

5.1.1
The Law of Mass Action

Biochemical kinetics is based on the mass action law, introduced by Guldberg and Waage in the 19th century (Waage and Guldberg 1864; Guldberg and Waage 1867, 1879). It states that the reaction rate is proportional to the probability of a collision of the reactants. This probability is in turn proportional to the concentration of reactants to the power of the molecularity, i.e., the number in which they enter the specific reaction. For a simple reaction like

$$ S_1 + S_2 \leftrightarrow 2P, \quad (5-11) $$

the reaction rate reads

$$ v = v_+ - v_- = k_+ S_1 \cdot S_2 - k_- P^2. \quad (5-12) $$

$v$ is the net rate, $v_+$ the rate of the forward reaction, $v_-$ the rate of the backward reaction, and $k_+$ and $k_-$ are the respective proportionality factors, the so-called kinetic or rate constants. The molecularity is 1 for each substrate of the forward reaction and 2 for the backward reaction. If we measure the concentration in moles per liter (mol $\cdot$ L$^{-1}$ or M) and the time in seconds (s), then the rates have the unit M $\cdot$ s$^{-1}$. Accordingly, the rate constants for bimolecular reactions have the unit M $\cdot$ s$^{-1}$. Rate constants of monomolecular reactions have the dimension s$^{-1}$. The general mass action rate law for a reaction with substrate concentrations $S_i$ and product concentrations $P_j$ reads

$$ v = v_+ - v_- = k_+ \prod_i S_i^{m_i} - k_- \prod_j P_j^{m_j}, \quad (5-13) $$

where $m_i$ and $m_j$ denote the respective molecularities of $S_i$ and $P_j$ in this reaction (Heinrich and Schuster 1996).

The equilibrium constant $K_{eq}$ (we will also use the simpler symbol $q$) characterizes the ratio of substrate and product concentrations in equilibrium ($S_{eq}$ and $P_{eq}$), i.e., the state with equal forward and backward rates. The rate constants are related to $K_{eq}$ in the following way:

$$ K_{eq} = \frac{k_+}{k_-} = \frac{\prod_j P_{eq}^{m_j}}{\prod_i S_{eq}^{m_i}}. \quad (5-14) $$
The relation between the thermodynamic description and the kinetic description of biochemical reactions will be outlined in Section 5.1.2.

The dynamics of the concentrations for Eq. (5-11) is described by the ODEs

\[ \frac{d}{dt} S_1 = \frac{d}{dt} S_2 = -v \]
\[ \frac{d}{dt} P = 2v. \]

(5-15)

The time course of \( S_1, S_2 \) and \( P \) is obtained by integration of these ODEs.

Example 5-2

The kinetics of a simple decay such as

\[ S \rightarrow \]

(5-16)

is described by \( v = kS \) and \( \frac{d}{dt} S = -kS \). Integration of this ODE from time \( t = 0 \) with the initial concentration \( S_0 \) to an arbitrary time \( t \) with concentration \( S(t) \),

\[ \int_{S_0}^{S} \frac{dS}{S} = -\int_{t=0}^{t} kdt, \]

yields the temporal expression \( S(t) = S_0 e^{-kt}. \)

5.1.2

Reaction Kinetics and Thermodynamics

An important purpose of metabolism is to extract energy from nutrients, which is necessary for the synthesis of molecules, for growth, and for proliferation. We distinguish between energy-supplying reactions, energy-demanding reactions, and energetically neutral reactions. The principles of reversible thermodynamics and their application to chemical reactions allow understanding of energy circulation in the cell. This is eased by the assumption that biological reactions usually occur in hydrous solution at constant pressure and constant temperature with negligible volume changes.

Whether a reaction occurs spontaneously or not, in which direction a reaction proceeds, and the position of the equilibrium are important characteristics of a biochemical process. The first law of thermodynamics, i.e., the law of energy conservation, tells us only that the total energy of a system remains constant during any process. The second law of thermodynamics declares that a process occurs spontaneously only if it increases the total entropy of the system. Unfortunately, entropy is usually not directly measurable. A more suitable measure is the Gibbs free energy \( G \), which is the energy capable of carrying out work under isotherm-isobar conditions, i.e., at constant temperature and constant pressure. The change of the free energy is given as

\[ \Delta G = \Delta H - T \Delta S, \]

(5-17)
where $\Delta H$ is the change in enthalpy, $\Delta S$ is the change in entropy, and $T$ is the absolute temperature in Kelvin. $\Delta G$ is a measure for the driving force, the spontaneity of a chemical reaction. If $\Delta G < 0$ then the reaction proceeds spontaneously under release of energy (exergonic process). If $\Delta G > 0$ then the reaction is energetically not favorable and will not occur spontaneously (endergonic process). $\Delta G = 0$ means that the system has reached its equilibrium. Endergonic reactions may proceed if they obtain energy from a strictly exergonic reaction by energetic coupling. Free energy is usually given for standard conditions ($\Delta G^0$), i.e., for a concentration of the reaction partners of 1 M, temperature $T = 298$ K, and, for gaseous reactions, a pressure of $p = 98.1$ kPa = 1 atm. The unit is kJ mol$^{-1}$. For the free energy difference, a set of relations holds as follows. The free energy difference is related to redox potential $E_{\text{red/ox}}$:

$$\Delta G = -nF \cdot E_{\text{red/ox}},$$

(5-18)

where $n$ is the number of transferred charges and $F$ is the Faraday constant (96,500 coulomb). The free energy difference for a reaction can be calculated from the difference of the sums of free energies of its products $P$ and its substrates $S$:

$$\Delta G = \sum G_P - \sum G_S.$$

(5-19)

The enzyme cannot change the free energies of the substrates and products of a reaction, nor their differences, but it changes the so-called reaction path, thereby lowering the activation energy for the reaction. The transition state theory explains this (Haynie 2001). It has been observed that many substances or mixtures are thermodynamically unstable, since $\Delta G \ll 0$ (see Tab. 5.1). Nevertheless, they can be stored under normal conditions for a long time. The reason is that during the course of a reaction, the metabolites must pass one or more transition states of maximal free energy, in which bonds are solved or newly formed. The transition state is unstable; the respective molecule configuration is called an activated complex. It has a lifetime of around one molecule vibration, $10^{-14} \ldots 10^{-13}$ s, and it can hardly be experimentally verified. The difference $\Delta G^\neq$ of free energy between the reactants and

<table>
<thead>
<tr>
<th>Tab. 5.1 Values of $\Delta G^0$ for some important reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reaction</strong></td>
</tr>
<tr>
<td>$2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$</td>
</tr>
<tr>
<td>$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$</td>
</tr>
<tr>
<td>$\text{Pi} + \text{H}_2\text{O} \rightarrow 2\text{Pi}$</td>
</tr>
<tr>
<td>$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{Pi}$</td>
</tr>
<tr>
<td>Glucose-6-phosphate + $\text{H}_2\text{O} \rightarrow$ Glucose + Pi</td>
</tr>
<tr>
<td>Glucose + Pi $\rightarrow$ Glucose-6-phosphate + $\text{H}_2\text{O}$</td>
</tr>
<tr>
<td>Glucose-1-phosphate $\rightarrow$ Glucose-6-phosphate</td>
</tr>
<tr>
<td>Glucose-6-phosphate $\rightarrow$ Fructose-6-phosphate</td>
</tr>
<tr>
<td>Glucose + 6 $\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$</td>
</tr>
</tbody>
</table>

Source: Lehninger 1975
the activated complex determines the dynamics of a reaction: the higher this differ-
ence, the lower the probability that the molecules may pass this barrier and the lower
the rate of the reaction. The value of $\Delta G^\neq$ depends on the type of altered bonds, on
steric, electronic, or hydrophobic demands, and on temperature.

Figure 5.3 presents a simplified view of the reaction course. The substrate and the
product are situated in local minima of the free energy; the active complex is as-
signed to the local maximum. The free energy difference $\Delta G$ is proportional to the
logarithm of the equilibrium constant of the respective reaction:

$$\Delta G = -RT \ln K_{eq},$$

(R = gas constant, $8.314 \text{ J mol}^{-1} \text{K}^{-1}$). The value of $\Delta G^\neq$ corresponds to the kinetic
constant $k_+$ of the forward reaction (Eqs. (12)–(14)) by $\Delta G^\neq = -RT \ln k_+$, while $\Delta G^\neq + \Delta G$ is related to the rate constant $k_-$ of the backward reaction.

The interaction of the reactants with an enzyme may alter the reaction path and
thereby lead to lower values of $\Delta G^\neq$. Furthermore, the free energy may assume
more local minima and maxima along the path of reaction. They are related to un-
stable intermediary complexes. Values for the difference of free energy for some bio-
logically important reactions are given in Tab. 5.1.

The detailed consideration of enzyme mechanisms by applying the mass action
law for single events has led to a number of standard kinetic descriptions, which will
be explained in the following sections.

5.1.3

**Michaelis-Menten Kinetics**

Brown (1902) proposed the first enzymatic mechanism for the reaction of invertase,
which holds for all one-substrate reactions without backward reaction and without
effectors in general:

$$E+S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow[k_3]{k_2} E+P.$$  (5-21)
It comprises a reversible formation of an enzyme-substrate complex ES from the free enzyme E and the substrate S and an irreversible release of the product P from the enzyme E. The respective system of ODEs for the dynamics of this reaction reads as follows:

\[
\frac{dS}{dt} = -k_1 E \cdot S + k_{-1} ES \tag{5-22}
\]

\[
\frac{dES}{dt} = k_1 E \cdot S - (k_{-1} + k_2) ES \tag{5-23}
\]

\[
\frac{dE}{dt} = -k_1 E \cdot S + (k_{-1} + k_2) ES \tag{5-24}
\]

\[
\frac{dP}{dt} = k_2 ES \tag{5-25}
\]

The rate of the reaction is equal to the negative rate of decay of the substrate as well as to the rate of product formation:

\[
v = -\frac{dS}{dt} = \frac{dP}{dt} \tag{5-26}
\]

This ODE system (Eqs. (5-22)–(5-26)) cannot be solved analytically. Assumptions have been used to simplify this system in a satisfactory way. Michaelis and Menten (1913) assumed that the conversion of E and S to ES and vice versa is much faster than the decomposition of ES into E and P (so-called quasi-equilibrium between the free enzyme and the enzyme-substrate complex), or in terms of the constants

\[
k_1, k_{-1} \gg k_2 \tag{5-27}
\]

Briggs and Haldane (1925) assumed that during the course of reaction a state is reached where the concentration of the ES complex remains constant. This assumption is justified only if the initial concentration of the substrate is much larger than the concentration of the enzyme, \(S(t = 0) \gg E\); otherwise, this steady state will never be reached. They suggested the more general assumption of a quasi-steady state of the ES complex:

\[
\frac{dES}{dt} = 0 \tag{5-28}
\]

An expression for the reaction rate will be derived using the ODE system in Eqs. (5-22)–(5-25) and the assumption of a quasi-steady state for ES. Adding Eqs. (5-23) and (5-24) results in

\[
\frac{dES}{dt} + \frac{dE}{dt} = 0 \text{ or } E_{\text{total}} = E + ES \tag{5-29}
\]
In this reaction, enzyme is neither produced nor consumed; it may be free or involved in the complex, but its total concentration remains constant.

Introducing Eq. (5-29) into Eq. (5-23) under the steady-state assumption (Eq. (5-28)) yields

\[
ES = \frac{k_1 E_{\text{total}} S}{k_1 S + k_{-1} + k_2} = \frac{E_{\text{total}} S}{S + \frac{k_{-1} + k_2}{k_1}}. \tag{5-30}
\]

For the reaction rate, this yields

\[
v = \frac{k_2 E_{\text{total}} S}{S + \frac{k_{-1} + k_2}{k_1}}. \tag{5-31}
\]

In enzyme kinetics it is convention to present Eq. (5-31) in a simpler form, which is important in both theory and practice:

\[
v = \frac{V_{\text{max}} S}{S + K_m}. \tag{5-32}
\]

Equation (5-32) is the expression for Michaelis-Menten kinetics. The parameters have the following meaning: the maximal velocity,

\[
V_{\text{max}} = k_2 E_{\text{total}}, \tag{5-33}
\]

is the maximal rate that can be attained when the enzyme is completely saturated with substrate. The Michaelis constant,

\[
K_m = \frac{k_{-1} + k_2}{k_1}, \tag{5-34}
\]

is equal to the substrate concentration that yields the half-maximal reaction rate. For the quasi-equilibrium assumption (Eq. (5-27)), it holds that \(K_m \approx k_{-1}/k_1\). The meaning of the parameters can be seen from the plot of rate versus substrate concentration (Fig. 5.4). The plot has a hyperbolic shape.

Reaction \(v_1\), Eq. (5-2), is described with Michaelis-Menten kinetics.

5.1.3.1 How to Derive a Rate Equation

Below we will present some enzyme kinetic standard examples. Individual mechanisms for your specific enzyme of interest may be more complicated or merely differ from these standards. Therefore, we summarize here the general way of deriving a rate equation.

1. Draw an illustration of all steps to consider (e.g., Eq. (5-21)). It contains all substrates and products (S and P) and \(n\) free or bound enzyme species (E and ES).
2. The right sites of the ODEs for the concentrations changes sum up the rates of all steps leading to or away from a certain substance (e.g., Eqs. (5-22)–(5-25)). The rates follow mass action kinetics (Eq. (5-12)).

3. The sum of all enzyme-containing species is equal to the total enzyme concentration \( E_{total} \) (the right side of all differential equations for enzyme species sum up to zero). This constitutes one equation.

4. The assumption of a quasi-steady state for \( n - 1 \) enzyme species (i.e., setting the right sides of the respective ODEs equal to zero) together with step 3 results in \( n \) algebraic equations for the concentrations of the \( n \) enzyme species.

5. The reaction rate is equal to the rate of product formation (e.g., Eq. (5-26)). Introduce the respective concentrations of enzyme species resulting from step 4.

5.1.3.2 Parameter Estimation and Linearization of the Michaelis-Menten Equation

To assess the values of the parameters \( V_{max} \) and \( K_m \) for an isolated enzyme, one measures the initial rates for different initial concentrations of the substrate. Since the rate is a nonlinear function of the substrate concentration, one has to determine the parameters by nonlinear regression. Another way is to transform Eq. (5-32) to a linear relation between variables and then apply linear regression.

The advantage of the transformed equations is that one may read the parameter values more or less directly from the graph obtained by linear regression of the measurement data. In the Lineweaver-Burk plot (Lineweaver and Burk 1934) (Tab. 5.2), the values for \( V_{max} \) and \( K_m \) can be obtained from the intersections of the graph with the ordinate and the abscissa, respectively. The Lineweaver-Burk plot is also helpful for discrimination of different types of inhibitions (see below). The drawback of the transformed equations is that they may be sensitive to errors for low or high substrate concentrations or rates. Eadie and Hofstee (Eadie 1942) and Hanes and Woolf (Hanes 1932) have introduced other types of linearization to overcome this limitation.
5.1.3.3 The Michaelis-Menten Equation for Reversible Reactions

In practice, many reactions are reversible. The enzyme may catalyze the reaction in both directions. Consider the following mechanism:

$$E+S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow[k_2]{k_{-2}} E+P.$$  \hspace{1cm} (5-35)

The product formation is given by

$$\frac{dP}{dt} = k_2 ES - k_{-2}P = \nu, \hspace{1cm} (5-36)$$

and the respective rate equation reads

$$\nu = E_{\text{total}} \frac{Sq - P}{Sk_1 \frac{1}{k_{-1}} + \frac{1}{k_{-2}} + \frac{k_2}{k_{-1} k_{-2}}} = \frac{\nu_{\text{for max}}}{K_mS} S - \frac{\nu_{\text{back max}}}{K_mP} P. \hspace{1cm} (5-37)$$

While the parameters $k_{\pm 1}$ and $k_{\pm 2}$ are the kinetic constants of the individual reaction steps, the phenomenological parameters $\nu_{\text{for max}}$ and $\nu_{\text{back max}}$ denote the maximal velocity in forward or backward direction, respectively, under zero product or substrate concentration, and the phenomenological parameters $K_{mS}$ and $K_{mP}$ denote the substrate or product concentration causing half maximal forward or backward rate. They are related in the following way:

$$K_{eq} = \frac{\nu_{\text{for max}}}{\nu_{\text{back max}}} \frac{K_mP}{K_mS} \hspace{1cm} (5-38)$$

(Haldane 1930). Reaction $\nu_3$, Eq. (5-4), is of the reversible Michaelis-Menten type.

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### Tab. 5.2 Different approaches for the linearization of Michaelis-Menten enzyme kinetics.

<table>
<thead>
<tr>
<th>Lineweaver-Burk</th>
<th>Eadie-Hofstee</th>
<th>Hanes-Woolf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformed equation</td>
<td>$\frac{1}{\nu} = \frac{K_m}{V_{\text{max}}} \frac{1}{S} + \frac{1}{V_{\text{max}}}$</td>
<td>$\nu = V_{\text{max}} - K_m \frac{\nu}{S}$</td>
</tr>
<tr>
<td>New variables</td>
<td>$\frac{1}{\nu} \cdot \frac{1}{S}$</td>
<td>$\nu$, $\frac{\nu}{S}$</td>
</tr>
<tr>
<td>Graphical representation</td>
<td><img src="image" alt="Graphical Representation" /></td>
<td><img src="image" alt="Graphical Representation" /></td>
</tr>
</tbody>
</table>

Tab. 5.2 Different approaches for the linearization of Michaelis-Menten enzyme kinetics.
5.1.4

Regulation of Enzyme Activity by Protein Interaction

Enzymes can immensely increase the rate of a reaction, but this is not their only function. Enzymes are involved in metabolic regulation in various ways. Their production and degradation are often adapted to the current requirements of the cell. Furthermore, they may be targets of effectors, both inhibitors and activators.

The effectors are proteins or other molecules that influence the performance of the enzymatic reaction. The interaction of effector and enzyme changes the reaction rate. Such regulatory interactions that are crucial for the fine-tuning of metabolism will be considered here (Schellenberger 1989).

Basic types of inhibition are distinguished by the enzyme's state, in which the enzyme may bind the effector (i.e., the free enzyme E, the enzyme-substrate complex ES, or both), and by the ability of different complexes to release the product. The general pattern of inhibition is schematically represented in Fig. 5.5. The different types result if some of the reactions cannot occur.

\[
\begin{align*}
E + S & \xrightleftharpoons[k_1]{k_i} ES \xrightarrow{k_2} E + P \\
& + + \\
& + \\
& + \\
& + \\
& + \\
& + \\
& + \\
& + \\
& + \\
& + \\
& + \\
& + \\
& + \\
\end{align*}
\]

Fig. 5.5 General scheme of inhibition in Michaelis-Menten kinetics. Reactions 1 and 2 belong to the standard scheme of Michaelis-Menten kinetics. Competitive inhibition is given if in addition reaction 3 (and not reactions 4, 5, or 6) occurs. Uncompetitive inhibition involves reactions 1, 2, and 4, and noncompetitive inhibition comprises reactions 1, 2, 3, 4, and 5. Appearance of reaction 6 indicates partial inhibition.

The rate equations are derived according to the following scheme:

1. Consider binding equilibriums between compounds and their complexes:

\[
K_m \equiv \frac{k_{-1}}{k_1} = \frac{E \cdot S}{ES}, \quad K_{1,3} = \frac{k_{-3}}{k_3} = \frac{E \cdot I}{EI}, \quad K_{1,4} = \frac{k_{-4}}{k_4} = \frac{ES \cdot I}{ESI}, \quad K_{1,5} = \frac{k_{-5}}{k_5} = \frac{EI \cdot S}{ESI}
\]

Note that if all reactions can occur, the Wegscheider condition (Wegscheider 1902) holds in the form

\[
\frac{k_1 k_4}{k_{-1} k_{-4}} = \frac{k_3 k_5}{k_{-3} k_{-5}},
\]

which means that the difference in the free energies between E and ESI is independent of the choice of the reaction path (via ES or EI).

2. Take into account the moiety conservation for the total enzyme (include only those complexes that occur in the course of reaction):

\[
E_{total} = E + ES + EI + ESI.
\]
3. The reaction rate is equal to the rate of product formation:

\[ v = \frac{dP}{dt} = k_2 ES + k_6 ESI. \] (5-42)

Equations (5-39)–(5-41) comprise four independent equations for the four unknown concentrations of E, ES, EI, and ESI. Their solution can be inserted into Eq. (5-42). The effect of the inhibitor depends on the concentrations of substrate and inhibitor and on the relative affinities to the enzyme. Table 5.3 lists the different types of inhibition for irreversible and reversible Michaelis-Menten kinetics together with the respective rate equations.

In the case of competitive inhibition, the inhibitor competes with the substrate for the binding site (or inhibits substrate binding by binding elsewhere to the enzyme) without being transformed itself. An example of this type is the inhibition of succinate dehydrogenase by malonate. The enzyme converts succinate to fumarate, forming a double bond. Malonate has two carboxyl groups, like the proper substrates, and may bind to the enzyme, but the formation of a double bond cannot take place. Since substrates and the inhibitor compete for the binding sites, a high concentration of one of them may displace the other one. For very high substrate concentrations, the same maximal velocity as without inhibitor is reached, but the effective \( K_m \) value is increased.

In the case of uncompetitive inhibition, the inhibitor I binds only to the ES complex. The reason may be that the substrate binding causes a conformational change, which opens a new binding site. Since S and I do not compete for binding sites, an increase in the concentration of S cannot displace the inhibitor. In the presence of inhibitor, the original maximal rate cannot be reached (lower \( V_{\text{max}} \)). For example, an inhibitor concentration of \( I = K_{i,4} \) halves the \( K_m \) value as well as \( V_{\text{max}} \). Uncompetitive inhibition occurs rarely for one-substrate reactions, but more frequently in the case of two substrates. One example is inhibition of arylsulfatase by hydrazine.

Noncompetitive inhibition is present if substrate binding to the enzyme does not alter the binding of the inhibitor. There must be different binding sites for substrate and inhibitor. In the classical case, the inhibitor has the same affinity to the enzyme with or without bound substrate. If the affinity changes, this is called mixed inhibition. A standard example is inhibition of chymotrypsin by \( \text{H}^+ \) ions.

If the product can also be formed from the enzyme-substrate-inhibitor complex, the inhibition is only partial. For high rates of product release (high values of \( k_6 \)), this can even present an activating instead of an inhibiting effect.

Competitive, uncompetitive, and noncompetitive inhibition also apply for the reversible Michaelis-Menten mechanism. The respective rate equations are also listed in Tab. 5.3.
Tab. 5-3  Types of inhibition for irreversible and reversible Michaelis-Menten kinetics.

<table>
<thead>
<tr>
<th>Name</th>
<th>Implementation</th>
<th>Equation – irreversible</th>
<th>Equation – reversible case</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive</td>
<td>I binds only to free E; P-release only from ES-complex</td>
<td>( v = \frac{V_{\text{max}} S}{K_m \cdot i_3 + S} )</td>
<td>( v = \frac{V_{\text{fmax}} S}{K_{\text{mS}}} - \frac{V_{\text{rmax}} P}{K_{\text{mP}}} )</td>
<td>( K_m ) changes, ( V_{\text{max}} ) remains; S and I compete for the binding place; high S may out compete I</td>
</tr>
<tr>
<td></td>
<td>( k_{-3} = k_{+3} = k_6 = 0 )</td>
<td></td>
<td>( v = \frac{V_{\text{fmax}} S}{K_{\text{mS}}} - \frac{V_{\text{rmax}} P}{K_{\text{mP}}} ) ( \frac{P}{K_{\text{mP}} + i_3} )</td>
<td></td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>I binds only to the ES-complex; P-release only from ES-complex</td>
<td>( v = \frac{V_{\text{max}} S}{K_m + S \cdot i_4} )</td>
<td>( v = \frac{V_{\text{fmax}} S}{K_{\text{mS}}} - \frac{V_{\text{rmax}} P}{K_{\text{mP}}} ) ( \frac{S}{K_{\text{mS}} + \frac{P}{K_{\text{mP}}} \cdot i_4} )</td>
<td>( K_m ) and ( V_{\text{max}} ) change, but their ratio remains; S may not out compete I</td>
</tr>
<tr>
<td></td>
<td>( k_{-3} = k_{+3} = k_6 = 0 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncompetitive</td>
<td>I binds to E and ES; P-release only from ES</td>
<td>( v = \frac{V_{\text{max}} S}{(K_m + S) \cdot i_3} )</td>
<td>( v = \frac{V_{\text{fmax}} S}{K_{\text{mS}}} - \frac{V_{\text{rmax}} P}{K_{\text{mP}}} ) ( \frac{S}{K_{\text{mS}} + \frac{P}{K_{\text{mP}}} \cdot i_4} )</td>
<td>( K_m ) remains, ( V_{\text{max}} ) changes; S may not out compete I</td>
</tr>
<tr>
<td></td>
<td>( K_{i_3} = K_{i_4}, k_6 = 0 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed inhibition</td>
<td>I binds to E and ES; P-release only from ES</td>
<td>( v = \frac{V_{\text{max}} S}{K_m \cdot i_4 + S \cdot i_3} )</td>
<td></td>
<td>( K_m ) and ( V_{\text{max}} ) change; ( K_{i_3} &gt; K_{i_4} ): competitive- noncompetitive inhibition; ( K_{i_3} &lt; K_{i_4} ): noncompetitive-uncompetitive inhibition</td>
</tr>
<tr>
<td></td>
<td>( K_{i_3} \neq K_{i_4}, k_6 = 0 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial inhibition</td>
<td>I may bind to E and ES; P-release from ES and ESI</td>
<td>( v = \frac{V_{\text{max}} S \left( 1 + \frac{k_6 \cdot I}{k_2 K_{-3}} \right)}{K_m \cdot i_4 + S \cdot i_3} )</td>
<td></td>
<td>( K_m ) and ( V_{\text{max}} ) change; if ( k_6 &gt; k_2 ), activation instead of inhibition.</td>
</tr>
<tr>
<td></td>
<td>( K_{i_3} \neq K_{i_4}, k_6 \neq 0 )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: \( \frac{k_{-3}}{k_3}, K_{i_3} = \frac{k_{-4}}{k_4}, i_3 = 1 + \frac{I}{K_{i_3}}, i_4 = 1 + \frac{I}{K_{i_4}} \)
5.1.5

**Inhibition by Irreversible Binding of Inhibitor to Enzyme**

An irreversible inhibitor binds irreversibly to the active site of the enzyme:

\[
E + I \rightarrow EI .
\]  

(5-43)

This prevents binding of the appropriate substrate and may destroy the catalytic center and lead to a denaturation of the enzymes. In any case, an initial inhibitor concentration of \( I_0 \) decreases the effective concentration of the enzyme from the initial concentration \( E_0 \) to the value \( E_0 - I_0 \). A molar excess of inhibitor leads to complete loss of catalytic activity. At a molar excess of enzyme, this yields a reduction of maximal velocity to \( V'_{\text{max}} = k_2 (E_0 - I_0) \).

**Example 5-3**

Covalent binding of iodacetate to SH groups of proteins:

\[
E - SH + I \cdot CH_2 \cdot CO_2 \rightarrow E - S \cdot CH_2 \cdot CO_2 + HI
\]

5.1.6

**Substrate Inhibition**

A common characteristic of enzymatic reactions is the increase of the reaction rate with increasing substrate concentration \( S \) up to the maximal velocity \( V_{\text{max}} \). But in some cases, a decrease of the rate above a certain value of \( S \) is recorded. A possible reason for this is the binding of a further substrate molecule to the enzyme-substrate complex, yielding the complex \( ESS \), which cannot form a product. This kind of inhibition is reversible if the second substrate can be released. The rate equation can be derived using the scheme of uncompetitive inhibition by replacing the inhibitor by another substrate. It reads,

\[
v = k_2 \frac{V_{\text{max}} S}{K_m + S \left( 1 + \frac{S}{K_I} \right)} .
\]

(5-44)

This expression has a maximum at

\[
S_{\text{opt}} = \sqrt{K_m K_I} \quad \text{with} \quad v_{\text{opt}} = \frac{V_{\text{max}}}{1 + 2 \sqrt{K_m / K_I}} .
\]

(5-45)

The dependence of \( v \) on \( S \) is shown in Fig. 5.6. A typical example for substrate inhibition is the binding of two succinate molecules to malonate dehydrogenase, which possesses two binding pockets for the carboxyl group. This is schematically represented in Fig. 5.6.
Inhibition by Binding of Inhibitor to Substrate

The reaction rate is also decreased if an inhibitor forms a tight complex with the substrate:

\[
S + I \rightarrow SI .
\]  

(5.46)

The effective substrate concentration is decreased to \( S_{\text{eff}} = S_0 - SI \). According to the mass action law, for the actual concentrations it holds that

\[
\frac{(I_0 - SI)(S_0 - SI)}{SI} = K_I .
\]  

(5.47)

This allows calculating of the effective enzyme concentration:

\[
S_{\text{eff}} = S_0 - \frac{I_0 + S_0 + K_I}{2} + \sqrt{\left(\frac{I_0 + S_0 + K_I}{2}\right)^2 - S_0 \cdot I_0} .
\]  

(5.48)

At high substrate concentrations, the reaction rate in the presence of an inhibitor reaches the maximal velocity of the non-inhibited reaction. The Lineweaver-Burk plot is nonlinear.

5.1.8 Binding of Ligands to Proteins

Every molecule that binds to a protein is a ligand, irrespective of whether it is the subject of a reaction or not. Below we consider binding to monomer and oligomer proteins. In oligomers, there may be interactions between the binding sites on the subunits.
Consider binding of one ligand (S) to a protein (E) with only one binding site:

\[ E + S \leftrightarrow ES. \]  \hspace{1cm} (5-49)

The binding constant \( K_B \) is given by

\[ K_B = \frac{ES}{E \cdot S}. \]  \hspace{1cm} (5-50)

The reciprocal of \( K_B \) is the dissociation constant \( K_D \). The fractional saturation \( Y \) of the protein is determined by the number of subunits that have bound ligands, divided by the total number of subunits. The fractional saturation for one subunit is

\[ Y = \frac{ES}{E_{total}} = \frac{ES}{ES + E} = \frac{K_B \cdot S}{K_B \cdot S + 1}. \]  \hspace{1cm} (5-51)

The plot of \( Y \) versus \( S \) at a constant total enzyme concentration is a hyperbola, like the plot of \( v \) versus \( S \) in the Michaelis-Menten kinetics. In a process where the binding of \( S \) to \( E \) is the first step, followed by product release, and where the initial concentration of \( S \) is much higher than the initial concentration of \( E \), the rate is proportional to the concentration of \( ES \) and it holds that

\[ \frac{v}{V_{max}} = \frac{ES}{E_{total}} = Y. \]  \hspace{1cm} (5-52)

If the protein has several binding sites, then interactions may occur between these sites, i.e., the affinity to further ligands may change after binding of one or more ligands. This phenomenon is called cooperativity. Positive or negative cooperativity denotes an increase or decrease, respectively, in the affinity of the protein to a further ligand. Homotropic or heterotropic cooperativity denotes that the binding to a certain ligand influences the affinity of the protein to a further ligand of the same or another type, respectively.

5.1.9 Positive Homotropic Cooperativity and the Hill Equation

Consider a dimeric protein with two identical binding sites. The binding to the first ligand facilitates the binding to the second ligand:

\[ \begin{align*}
E_2 + S & \overset{\text{slow}}{\rightarrow} E_2S \\
E_2S + S & \overset{\text{fast}}{\rightarrow} E_2S_2,
\end{align*} \]  \hspace{1cm} (5-53)

where \( E \) is a monomer and \( E_2 \) a dimer. The fractional saturation is given by

\[ Y = \frac{E_2S + 2E_2S_2}{2E_{2,\text{total}}} = \frac{E_2S + E_2S_2}{2E_2 + 2E_2S + 2E_2S_2}. \]  \hspace{1cm} (5-54)
If the affinity to the second ligand is strongly increased by binding to the first ligand, then $E_2S$ will react with $S$ as soon as it is formed, and the concentration of $E_2S$ can be neglected. In the case of complete cooperativity, i.e., every protein is either empty or fully bound, Eq. (5-53) reduces to

$$E_2 + 2S \rightarrow E_2S_2.$$  \hspace{1cm} (5-55)

The binding constant reads

$$K_B = \frac{E_2S_2}{E_2 \cdot S^2}.$$  \hspace{1cm} (5-56)

and the fractional saturation is

$$Y = \frac{2 \cdot E_2S_2}{2 \cdot E_{2 \text{,total}}} = \frac{E_2S_2}{E_2 + E_2S_2} = \frac{K_BS^2}{1 + K_BS^2}.$$  \hspace{1cm} (5-57)

Generally, for a protein with $n$ subunits it holds that

$$v = V_{max}Y = \frac{V_{max}K_BS^n}{1 + K_BS^n}.$$  \hspace{1cm} (5-58)

This is the general form of the Hill equation. It implies complete homotropic cooperativity. Plotting the fractional saturation $Y$ versus substrate concentration $S$ yields a sigmoid curve with the inflection point at $1/K_B$. The quantity $n$ (often “$h$” is used instead) is termed the Hill coefficient.

The derivation of this expression was based on experimental findings concerning the binding of oxygen to hemoglobin (Hb) (Hill 1910, 1913). In 1904 Bohr and co-workers found that the plot of the fractional saturation of Hb with oxygen against the oxygen partial pressure had a sigmoid shape. Hill (1909) explained this with interactions between the binding sites located at the hem subunits. At this time it was already known that every subunit hem binds one molecule of oxygen. Hill assumed complete cooperativity and predicted an experimental Hill coefficient of 2.8. Today it is known that hemoglobin has four binding sites but that the cooperativity is not complete. The sigmoidal binding characteristic has the advantage that Hb binds strongly to oxygen in the lung with a high oxygen partial pressure, while it can release $O_2$ easily in the body with low oxygen partial pressure.

5.1.10 The Monod-Wyman-Changeux Rate Expression for Enzymes with Sigmoid Kinetics

In 1965 Monod and colleagues presented a model explaining sigmoidal enzyme kinetics taking into account the interaction of subunits of an enzyme (Monod et al. 1965). A more comprehensive model has been presented by Koshland et al. (1966). The model of Monod et al. uses the following assumptions: (1) the enzyme consists...
of \( n \) identical subunits, (2) each subunit can assume an active (R) or an inactive (T) conformation, (3) all subunits change their conformations at the same time (concerted change), and (4) the equilibrium between the R and the T conformations is given by an allosteric constant:

\[
L = \frac{T_0}{R_0}.
\]  

(5-59)

The index \( i \) for \( T_i \) and \( R_i \) denotes the number of bound substrate molecules. The binding constants for the active and inactive conformations are given by \( K_R \) and \( K_T \), respectively. If substrate molecules can bind only to the active form, i.e., if \( K_T = 0 \), then the rate can be given as

\[
V = \frac{V_{\text{max}} K_R S}{(1 + K_R S)} \left(1 + \frac{L}{(1 + K_R S)^n}\right),
\]  

(5-60)

where the factor \( \frac{V_{\text{max}} K_R S}{(1 + K_R S)} \) corresponds to the Michaelis-Menten rate expression, while the factor \( \left(1 + \frac{L}{(1 + K_R S)^n}\right)^{-1} \) is a regulatory factor.

For \( L = 0 \) the plot \( v \) versus \( S \) is a hyperbola as in Michaelis-Menten kinetics. For \( L > 0 \) one gets a sigmoid curve shifted to the right. A typical value for the allosteric constant is \( L \approx 10^8 \).

In the case that the substrate can also bind to the inactive state \( (K_T \neq 0) \), one gets

\[
V = \frac{V_{\text{max}} S}{(1 + K_R S)} \left(\frac{K_R + K_T L \left(\frac{1 + K_T S}{1 + K_R S}\right)^n}{1 + L \left(\frac{1 + K_T S}{1 + K_R S}\right)^n}\right).
\]  

(5-61)

Up to now we have considered only homotropic and positive effects in the model of Monod, Wyman, and Changeux. But this model is also well suited to explain the dependence of the reaction rate on activators and inhibitors. Activators \( A \) bind only to the active conformation, and inhibitors \( I \) bind only to the inactive conformation. This shifts the equilibrium to the respective conformation. Effectively, the binding to effectors changes \( L \):

\[
L' = \frac{(1 + K_I I)^n}{(1 + K_A A)^n}.
\]  

(5-62)

\( K_I \) and \( K_A \) denote binding constants. The interaction with effectors is a heterotropic effect. An activator weakens the sigmoidity, while an inhibitor strengthens it as shown in Figure 5.7.

As an example, the kinetics of the enzyme phosphofructokinase, which catalyzes the transformation of fructose-6-phosphate and ATP to fructose-1,6-bisphosphate,
can be described by the model of Monod, Wyman, and Changeux. AMP, NH₄, and K⁺ are activators, while ATP is an inhibitor (see Example 5-1).

### 5.2 Metabolic Networks

In this section we will discuss basic structural and dynamic properties of metabolic networks. We will introduce a stoichiometric description of networks and learn how moieties and fluxes are balanced within networks.

The basic elements of a metabolic network model are (1) the substances with their concentrations and (2) the reactions or transport processes changing the concentrations of the substances. In biological environments, reactions are usually catalyzed by enzymes, and transport steps are carried out by transport proteins or by pores. Thus they can be assigned to identifiable biochemical compounds.

**Stoichiometric coefficients** denote the proportion of substrate and product molecules involved in a reaction. For example, for the reaction depicted in Eq. (5-11), the stoichiometric coefficients of S₁, S₂, and P are −1, −1, and 2. 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 S₁ and S₂ 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 ODEs. For the reaction depicted in Eq. (5-11) and the first choice of stoichiometric coefficients, we have

$$\frac{dS_1}{dt} = -v, \quad \frac{dS_2}{dt} = -v, \quad \text{and} \quad \frac{dP}{dt} = 2v. \quad (5-63)$$

This means that the degradation of S₁ with rate v is accompanied by the degradation of S₂ with the same rate and by the production of P with the double rate.
5.2.1 Systems Equations

For a metabolic network consisting of $m$ substances and $r$ reactions, the systems dynamics is described by systems equations (or balance equations, since the balance of substrate production and degradation is considered):

$$\frac{dS_i}{dt} = \sum_{j=1}^{r} n_{ij} v_j \quad \text{for} \quad i = 1, \ldots, m \quad (5-64)$$

(Glansdorff and Prigogine 1971; Reder 1988). The quantities $n_{ij}$ are the stoichiometric coefficients of metabolite $i$ in reaction $j$. Here, we assume that the reactions are the only reason for concentration changes and that no mass flow occurs due to convection or to diffusion. The balance equations (Eq. (5-64)) 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.

The stoichiometric coefficients $n_{ij}$ assigned to the substances $S_i$ and the reactions $v_j$ can be combined into the so-called stoichiometric matrix

$$N = \{n_{ij}\} \quad \text{for} \quad i = 1, \ldots, m \quad \text{and} \quad j = 1, \ldots, r \quad (5-65)$$

where each column belongs to a reaction and each row to a substance.

Example 5-4

For the simple network

$$\begin{align*}
\text{\large \text{\textsection}} & S_1 \quad \text{\large \text{\textsection}} \quad 2S_2 \quad \text{\large \text{\textsection}} \\
\uparrow & \quad \downarrow \quad \uparrow \\
S_3 & & v_4
\end{align*} \quad (5-66)$$

the stoichiometric matrix reads

$$N = \begin{pmatrix}
1 & -1 & 0 & -1 \\
0 & 2 & -1 & 0 \\
0 & 0 & 0 & 1
\end{pmatrix} \quad (5-67)$$

Note that in Eq. (5-66) all reactions may be reversible. In order to determine the signs of $N$, the direction of the arrows is artificially assigned as positive “from left to right” and “from the top down.” If, for example, the net flow proceeds from $S_3$ to $S_1$, the value of rate $v_4$ is negative.
Altogether, the mathematical description of the metabolic system consists of a vector \( \mathbf{S} = (S_1, S_2, ..., S_n)^T \) of concentration values, a vector \( \mathbf{v} = (v_1, v_2, ..., v_r)^T \) of reaction rates, a parameter vector \( \mathbf{p} = (p_1, p_2, ..., p_m)^T \), and the stoichiometric matrix \( \mathbf{N} \). If the system is in steady state, we can also consider the vector \( \mathbf{J} = (J_1, J_2, ..., J_r)^T \) containing the steady state fluxes. With these notions, the balance equation reads

\[
\frac{d\mathbf{S}}{dt} = \mathbf{Nv}.
\] (5-68)

### Example 5-5

For our running example (Example 5-1) of the upper glycolysis model, the concentration vector is

\[
\mathbf{S} = \begin{pmatrix}
 Gluc6P \\
 Fruc6P \\
 Fruc1,6P_2 \\
 ATP \\
 ADP \\
 AMP
\end{pmatrix},
\] (5-69)

the vector of reaction rates is \( \mathbf{v} = (v_1, v_2, ..., v_8)^T \), the parameter vector is given by

\[
\mathbf{p} = \begin{pmatrix}
 Glucose, V_{\text{max},1}, K_{\text{ATP},1}, K_{\text{Glucose},1}, k_2, V_{\text{max},3}^f, V_{\text{max},3}^r, K_{\text{Gluc6P},3}, K_{\text{Fruc6P},3}, \\
 V_{\text{max},4}, K_{\text{F6P},4}, \kappa_4, k_5, k_7, k_{8f}, k_{8r}
\end{pmatrix}^T,
\] (5-70)

and the stoichiometric matrix reads

\[
\mathbf{N} = \begin{pmatrix}
 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\
 -1 & -1 & 0 & -1 & 0 & 1 & -1 & -1 \\
 1 & 1 & 0 & 1 & 0 & -1 & 1 & 2 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1
\end{pmatrix}.
\] (5-71)

### 5.2.2 Information Contained in the Stoichiometric Matrix \( \mathbf{N} \)

The stoichiometric matrix contains important information about the structure of the metabolic network. Using the stoichiometric matrix, we can calculate which combinations of individual fluxes are possible in steady state (i.e., calculate the admissible steady-state flux space). We may easily discover dead ends and unbranched reaction pathways. In addition, we may find out the conservation relations for the included reactants.
In steady state it holds that

\[
\frac{dS}{dt} = Nv = 0 \quad (5-72)
\]

(Reeder 1988). The right equality sign denotes a linear equation system for determination of the rates \(v\). This equation has nontrivial solutions only for \(\text{Rank } N < r\) (Chapter 3, Section 3.1). The kernel matrix \(K\) fulfilling

\[
NK = 0 \quad (5-73)
\]

can express the respective linear dependencies (Heinrich and Schuster 1996). The choice of the kernel is not unique. It can be determined using the Gauss algorithm described in Chapter 3 (Section 3.1). It contains as columns \(r – \text{Rank} N\) basis vectors. Every possible set of steady-state fluxes can be expressed as a linear combination of the columns \(k_i\) of \(K\)

\[
J = \sum_{i=1}^{r-\text{Rank} N} \alpha_i \cdot k_i . \quad (5-74)
\]

The coefficients must have respective units (\(M \cdot s^{-1}\) or \(\text{mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}\)).

**Example 5-6**

For the system

\[
P_1 \xrightarrow{v_1} S \xleftarrow{v_2} P_2 \xrightarrow{v_3} P_3 \quad (5-75)
\]

the stoichiometric matrix is \(N = (1 \ 1 \ 1)\). We have \(r = 3\) reactions and \(\text{Rank } N = 1\). Each representation of the kernel matrix contains \(3 - 1 = 2\) basis vectors, e.g.,

\[
K = (k_1 \ k_2) \quad \text{with} \quad k_1 = \begin{pmatrix} 1 \\ -1 \\ 0 \end{pmatrix}, \quad k_2 = \begin{pmatrix} 1 \\ 0 \\ 1 \end{pmatrix}, \quad (5-76)
\]

and for the steady state flux it holds that

\[
J = \alpha_1 \cdot k_1 + \alpha_2 \cdot k_2 . \quad (5-77)
\]
Example 5-7

The stoichiometric matrix for the running example is given in Eq. (5-71). It comprises \( r = 8 \) reactions and has \( \text{Rank} = 5 \). Thus the kernel matrix has three linearly independent columns. A possible solution of Eq. (5-73) is

\[
K = (k_1 \ k_2 \ k_3) \quad \text{with} \quad k_1 = \begin{pmatrix} -1 \\ -1 \\ 0 \\ 0 \\ 0 \\ 2 \\ 0 \\ 0 \end{pmatrix}, \quad k_2 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 2 \\ 0 \\ 0 \end{pmatrix}, \quad k_3 = \begin{pmatrix} 0 \\ -1 \\ 1 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix}.
\] (5-78)

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 the respective reaction must be zero.

Example 5-8

For the reaction system in Eq. (5-66), the stoichiometric matrix reads

\[
N = \begin{pmatrix} 1 & -1 & 0 & -1 \\ 0 & 2 & -2 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}
\]

with \( r = 4 \) and \( \text{Rank} N = 3 \). Its kernel consists only of one column \( K = (1 \ 1 \ 1 \ 0)^T \). Hence it yields \( v_4 = \sum_{i=1}^{4} x_i \cdot 0 = 0 \). In any steady state, the rates of production and degradation of \( S_3 \) must equal.

Example 5-9

For the running example, the entry in the last row of the kernel matrix, Eq. (5-78), is always zero. Hence, in steady state the rate of reaction \( v_8 \) must always vanish.

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.
Example 5-10
Consider the reaction scheme
\[ v_1 \rightarrow S_1 \rightarrow v_2 \rightarrow S_2 \rightarrow v_3 \rightarrow S_3 \rightarrow v_4 \rightarrow v_5 \rightarrow v_6. \] (5-79)

The system comprises \( r = 6 \) reactions. The stoichiometric matrix reads
\[
N = \begin{pmatrix}
1 & -1 & 0 & 0 & -1 & 0 \\
0 & 1 & -1 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 0 & 1
\end{pmatrix}
\]
with \( \text{Rank} \, N = 3 \). Thus the kernel matrix is spanned by three basis vectors, e.g.,
\( k_1 = (1 \, 1 \, 1 \, 0 \, 0 \, -1) \), \( k_2 = (1 \, 0 \, 0 \, 1 \, 0 \, 0) \), and \( k_3 = (-1 \, -1 \, -1 \, 0 \, 0 \, 0) \). 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.

Example 5-11
In the glycolysis model, the entries for the third, fourth, and fifth reactions are equal for each column of the kernel matrix (Eq. (5-78)). Therefore, reactions 3–5 constitute an unbranched pathway. In steady state, they must have equal rates.

Up to now, we have not been concerned about (ir)reversibility of reactions in the network. If a certain reaction is considered irreversible, this has no consequences for the stoichiometric matrix \( N \) but rather for the kernel \( K \). The set of vectors belonging to \( K \) is restricted by the condition that some values may not become negative (or positive, depending on the definition of flux direction). If in Example 5-9 reaction 4 is considered irreversible, then the columns of \( K \) must not contain a negative entry in the fourth row. Choosing \( k_3 = (a \, a \, a \, a \, 0 \, 0) \) with \( a > 0 \) fulfills this condition.

5.2.3 Elementary Flux Modes and Extreme Pathways

The definition of the term “pathway” in a metabolic network is not straightforward. A descriptive definition of a pathway is a set of subsequent reactions that are in each case linked by common metabolites. Typical examples include glycolysis or amino acid synthesis. More detailed inspection of metabolic maps such as the Boehringer map (Michal 1999) shows that metabolism is highly interconnected. Pathways that have been known for a long time from biochemical experience are hard to recognize. It is even harder to discover new pathways, e.g., in metabolic maps that have been reconstructed from sequence data for bacteria.
This problem has been elaborated in the concept of *elementary flux modes* (Heinrich and Schuster 1996; Pfeiffer et al. 1999; Schilling et al. 1999, 2000, 2002). Here, the stoichiometry of a metabolic network is investigated to find out which direct routes are possible that lead from one external metabolite to another external metabolite. The approach takes into account that some reactions are reversible, while others are irreversible.

A flux mode $M$ is a set of flux vectors that represent such direct routes through the metabolic networks. In mathematical terms, it is defined as the set

$$M = \{ v \in \mathbb{R}^r \mid v = \lambda v^*, \lambda > 0 \} , \quad (5-80)$$

where $v^*$ is an $r$-dimensional vector (unequal to the null vector) fulfilling two conditions: (1) steady state, i.e., Eq. (72), and (2) sign restriction, i.e., the flux directions in $v^*$ fulfill the prescribed irreversibility relations.

A flux mode $M$ comprising $v$ is called reversible if the set $M'$ comprising $-v$ 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, i.e., the vector $v$ cannot be represented as a nonnegative linear combination of two vectors that fulfill conditions (1) and (2) but contain more zero entries than $v$. The number of elementary flux modes is equal to or higher than the number of basis vectors of the null space.

### Example 5-12

The systems A and B differ by the (ir)reversibility of reaction 2.

$$\begin{align*}
A & : \quad S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} 2S_2 \xrightarrow{v_3} S_4 \\
& \quad \uparrow v_2 \downarrow v_2 \\
& \quad S_3 \\
B & : \quad S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} 2S_2 \xrightarrow{v_3} S_4 \\
& \quad \uparrow v_3 \downarrow v_3 \\
& \quad S_3
\end{align*} \quad (5-81)$$

The elementary flux modes connect the external metabolites $S_0$ and $S_3$, $S_0$ and $S_4$, or $S_3$ and $S_4$. For case A and case B, they read

$$v^A = \begin{pmatrix}
1 \\
1 \\
0 \\
0
\end{pmatrix}, \quad v^B = \begin{pmatrix}
1 \\
1 \\
0 \\
0
\end{pmatrix}$$

and

$$v^B = \begin{pmatrix}
1 \\
0 \\
0 \\
1
\end{pmatrix} \quad \text{and} \quad v^B = \begin{pmatrix}
1 \\
0 \\
0 \\
1
\end{pmatrix} \quad \text{and} \quad v^B = \begin{pmatrix}
1 \\
0 \\
0 \\
1
\end{pmatrix} \quad \text{and} \quad v^B = \begin{pmatrix}
1 \\
0 \\
0 \\
1
\end{pmatrix}$$

The possible routes are illustrated in Fig. 5.8.

The set of elementary flux modes is uniquely defined. Pfeiffer et al. (1999) have developed the software Metatool to calculate the elementary flux modes for metabolic networks.
The concept of extreme pathways (Schilling et al. 2000; Schilling and Palsson 2000; Wiback and Palsson 2002) is analogous to the concept of elementary flux modes, but here all reactions are constrained by flux directionality, while the concept of elementary flux modes allows for reversible reactions. To achieve this, reversible reactions are broken down into their forward and backward components. This way, the set of extreme pathways is a subset of the set of elementary flux modes and the extreme pathways are systemically independent.

Elementary flux modes 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 non-redundant pathways, to reconstruct metabolism from annotated genome sequences and analyze the effect of enzyme deficiency, to reduce drug effects, and to identify drug targets.

5.2.4 Flux Balance Analysis

Flux balance analysis (FBA) (Varma and Palsson 1994a, 1994b; Edwards and Palsson 2000a, 2000b; Ramakrishna et al. 2001) investigates the theoretical capabilities and operative modes of metabolism by involving further constraints in the stoichiometric analysis. The first constraint is set by the assumption of a steady state (Eqs. (5-72) and (5-73)). The second constraint is of a thermodynamic nature, respecting the irreversibility of reactions as considered in the concept of extreme pathways. The third constraint may result from the limited capacity of enzymes for metabolite conversion. For example, in the case of a Michaelis-Menten-type enzyme (Eq. (5-32)), the reaction rate is limited by the maximal rate, i.e., $0 \leq v \leq V_{max}$. In general, the constraints imposed on the magnitude of individual metabolic fluxes read

$$ a_i \leq v_i \leq \beta_i. $$

(5-83)
Further constraints may be imposed by biomass composition or other external conditions. The constraints confine the steady-state fluxes to a feasible set but usually do not yield a unique solution. The determination of a particular metabolic flux distribution has been formulated as a linear programming problem. The idea is to maximize an objective function $Z$ that is subject to the stoichiometric and capacity constraints:

$$Z = \sum_{i=1}^{r} c_i v_i \rightarrow \text{max}. \quad (5.84)$$

where $c_i$ represents weights for the individual rates. Examples of such objective functions are maximization of ATP production, minimization of nutrient uptake, maximal yield of a desired product, maximal growth rate, or a combination thereof.

**Example 5-13**

Maximization of ATP consumption in our running example (Example 5-1) necessitates

$$Z = v_1 + v_2 + v_4 - v_6 + v_7 + v_8 \rightarrow \text{max} \quad (5.85)$$

under the conditions of Eq. (5-78) and the maximal rates given in Example 5-1.

### 5.2.5 Conservation Relations: Null Space of $N^T$

If a substance is neither added to nor removed from the reaction system (neither produced nor degraded), its total concentration remains constant. This also holds if it interacts with other compounds by forming complexes. We have seen already as an example the constancy of the total enzyme concentration (Eq. (5-29)) when deriving the Michaelis-Menten rate equation. This was based on the assumption that enzyme production and degradation take place on a much larger timescale than the catalyzed reaction.

For the mathematical derivation of the conservation relations (Heinrich and Schuster 1996), we consider a matrix $G$ fulfilling

$$GN = 0. \quad (5.86)$$

Due to Eq. (5-68) it follows that

$$G\dot{S} = Gn = 0. \quad (5.87)$$

Integrating this equation leads directly to the conservation relations

$$GS = \text{const}. \quad (5.88)$$
The number of independent rows of $G$ is equal to $n - \text{Rank } N$, where $n$ is the number of metabolites in the system. $G^T$ is the kernel matrix of $N^T$; hence it has properties similar to those of $K$. Matrix $G$ can also be found using the Gauss algorithm. It is not unique, but every linear combination of its rows is again a valid solution. It exists a simplest representation $G = (G_0 \ I_{n-\text{Rank } N})$. Finding this representation may be helpful for simple statement of conservation relations, but this may necessitate renumbering and reordering of metabolite concentrations (see below).

Example 5-14

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

\[
\begin{align*}
\begin{array}{c}
\text{ATP} \\
\text{ADP}
\end{array} & \xrightarrow{v_1} \begin{array}{c}
\text{ATP} \\
\text{ADP}
\end{array} \\
\begin{array}{c}
\text{ATP} \\
\text{ADP}
\end{array} & \xrightarrow{v_2} \begin{array}{c}
\text{ATP} \\
\text{ADP}
\end{array}
\end{align*}
\]

(5-89)

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

Example 5-15

For the glycolysis model with the stoichiometric matrix given in Eq. (5-71), the only possible representation of the conservation matrix is given by multiples of $G = \begin{pmatrix} 0 & 0 & 0 & 1 & 1 \end{pmatrix}$. (5-90)

This means that again the sum of concentrations of adenine nucleotide-containing substances remains constant $(\text{AMP} + \text{ADP} + \text{ATP} = \text{const.})$.

Importantly, conservation relations can be used to simplify the system of differential equations $\dot{S} = Nv$ describing the dynamics of our reaction system. The idea is to eliminate linearly dependent differential equations and to replace them by appropriate algebraic equations. Below, the procedure is explained systematically (Reder 1988).

First we have to reorder the rows in the stoichiometric matrix $N$ as well as in the concentration vector $S$ such that a set of independent rows is on top and the dependent rows are at the bottom. Then the matrix $N$ is split into the independent part $N^0$ and the dependent part $N^\prime$, and a link matrix $L$ is introduced in the following way:

\[
N = \begin{pmatrix} N^0 \\ N^\prime \end{pmatrix} = LN^0 = \begin{pmatrix} I_{\text{Rank } N} \\ L \end{pmatrix} N^0.
\]

(5-91)
\( I_{\text{RankN}} \) is the identity matrix of size \( \text{RankN} \). The differential equation system may be rewritten accordingly

\[
\dot{S} = \begin{pmatrix} \dot{S}_{\text{indep}} \\ \dot{S}_{\text{dep}} \end{pmatrix} = \begin{pmatrix} I_{\text{RankN}} \\ L' \end{pmatrix} N^0 v, \tag{5-92}
\]

and the dependent concentrations fulfill

\[
\dot{S}_{\text{dep}} = L' \cdot \dot{S}_{\text{indep}}. \tag{5-93}
\]

Integration leads to

\[
S_{\text{dep}} = L' \cdot S_{\text{indep}} + \text{const}. \tag{5-94}
\]

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

\[
\dot{S}_{\text{indep}} = N^0 v \tag{5-95}
\]

supplemented with the set of algebraic equations (Eq. (5-94)).

### Example 5-16

For the reaction system

\[
\begin{array}{c}
\overset{v_1}{\longrightarrow} & S_1 & \overset{v_2}{\longrightarrow} & S_2 & \overset{v_3}{\longrightarrow} \\
S_3 & \overset{v_4}{\longrightarrow} & S_4
\end{array}
\tag{5-96}
\]

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

\[
N = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & -1 & 0 & -1 \\ 0 & 1 & 0 & -1 \end{pmatrix}, \quad N^0 = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & -1 & 0 & 1 \end{pmatrix}, \quad L = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -1 \end{pmatrix}, \quad L' = \begin{pmatrix} 0 & 0 & -1 \end{pmatrix}.
\]

The conservation relation \( S_3 + S_4 = \text{const.} \) is expressed by \( G = (0 \quad 0 \quad 1) \). The ODE system

\[
\begin{align*}
\dot{S}_1 &= v_1 - v_2 \\
\dot{S}_2 &= v_2 - v_3 \\
\dot{S}_3 &= v_4 - v_2 \\
\dot{S}_4 &= v_2 - v_4
\end{align*}
\]

can be replaced by the differential-algebraic system

\[
\dot{S}_1 = v_1 - v_2
\]
\[
\begin{align*}
\dot{S}_2 &= v_2 - v_3 \\
\dot{S}_3 &= v_4 - v_2 \\
S_3 + S_4 &= \text{const.}
\end{align*}
\]

which has one differential equation less.

### 5.2.6

**Compartments and Transport across Membranes**

Eukaryotic cells contain a variety of organelles, e.g., the nucleus, mitochondria, or vacuoles, that are separated by membranes. Reaction pathways may cross the compartment boundaries. If a substance, say malate, occurs in two different compartments, e.g., in the cytosol and in mitochondria, the respective concentrations can be assigned to two different variables, \(c^\text{mito}_{\text{malate}}\) and \(c^\text{cytosol}_{\text{malate}}\) (see Figure 5.9). Transport across the membrane has to be considered formally as a reaction with rate \(v\). It is important to note that both compartments have different volumes, \(V^\text{mito}\) and \(V^\text{cytosol}\). Thus transport of a certain amount of malate from one compartment into the other changes the concentrations by a different amount:

\[
V^\text{mito} \cdot \frac{d}{dt} c^\text{mito}_{\text{malate}} = v \quad \text{and} \quad V^\text{cytosol} \cdot \frac{d}{dt} c^\text{cytosol}_{\text{malate}} = -v, \tag{5.97}
\]

where \(V \cdot c\) denotes the substance amount in moles.

### 5.2.7

**Characteristic Times**

An important feature of metabolism is the wide range of timescales in which cellular processes may occur. Some modifications may happen within seconds, while other processes take hours or even longer. Even on the level of enzymatic reactions, we may find large differences in the time they need to respond to changes. For the metabolic reactions the time regime is characterized by the kinetic constants. We will present different quantitative measures for their temporal description.

A time constant for the isolated first-order reaction

\[
A \xrightarrow{k_+}{k_-} B \tag{5.98}
\]

[Fig. 5.9] Metabolites may be present in different organelles of the cell, e.g., malate is present in cytosol and mitochondrion. In this case it is appropriate to consider two compartments and assign malate in the different compartments two different species (malate\(^c\), malate\(^m\))
can be derived simply in the following way. Assume that the reaction is in equilib-rium with \( A = A_0 \), \( B = B_0 \) determined by the temperature \( T \). A small increase of \( T \) leads to a perturbation of the equilibrium, a concentration shift \( x(t) = A_0 - A(t) \), and eventually a new equilibrium: \( A = A_n \), \( B = B_n \). Due to the mass action law, during relaxation it holds that

\[
\frac{d (B_n - x)}{dt} = k_+ (A_n + x) - k_- (B_n - x). \tag{5-99}
\]

In the new equilibrium, the concentration changes vanish

\[
\frac{dB_n}{dt} = k_+ A_n - k_- B_n = 0 \tag{5-100}
\]

and it results in

\[
- \frac{dx}{dt} = (k_+ + k_-) x. \tag{5-101}
\]

Integration leads to

\[
x = x_0 e^{-(k_+ + k_-)t}. \tag{5-102}
\]

The initial shift of \( x \) at \( t = 0 \) is given by

\[
x_0 = A_0 - A_n. \tag{5-103}
\]

Setting

\[
\frac{1}{\tau} = k_+ + k_- \tag{5-104}
\]

determines \( \tau \) as the relaxation time for the decrease of \( x \) from its initial value to the \( 1/e \)-fold value. It results in

\[
x = (A_0 - A_n) e^{-t/\tau}. \tag{5-105}
\]

The relaxation time as introduced in Eqs. (5-104) and (5-105) concerns concentration changes. In general, one can distinguish between time constants for reaction rates and time constants for the change of concentrations.

A more general definition of a time constant for reactions is given by Higgins (1965). The response time is defined as

\[
\tau_j = \left( \sum_i \left( \frac{n_i}{\partial v_j / \partial S} \right)^{-1} \right).
\]

5.2 Metabolic Networks
This definition is applicable to reactions with more than one substrate or product and even with nonlinear rate expressions. For example, the response time for the reaction

\[ \text{A} + \text{B} \rightleftharpoons \text{C} + \text{D} \]  

(5-107)
is given by

\[ \tau = (k_+ (A + B) + k_- (C + D))^{-1}. \]  

(5-108)

Time constants for metabolite concentrations have been defined in different ways. Reich and Sel’kov (1975), considered the turnover time, i.e., the time that is necessary to convert a metabolite pool once:

\[ \tau_{\text{turn}}^i = \frac{S_i}{\sum_{j=1}^{n} n_{ij}^+ v_j^+ + n_{ij}^- v_j^-}. \]  

(5-109)

To this end, every reaction is split into a forward \((v_j^+)\) and a backward \((v_j^-)\) reaction with \(v_j = v_j^+ - v_j^-.\) Accordingly, the stoichiometric coefficients of substance \(S_i\) are assigned to the individual reaction directions \((n_{ij}^+, n_{ij}^-).\)

Assume that we have an “empty” pathway, i.e., a pathway that consists of a set of enzymes but has no metabolites available. At once, the first substrate is added. Following Easterby (1973, 1981), the transition time describes the time necessary to build up the intermediate pools. It is a measure of the time it takes to reach steady-state concentrations. For each intermediate it holds that

\[ \tau_i = \frac{S_{iSS}}{J}, \]  

(5-110)

where \(S_{iSS}\) and \(J\) denote concentration and flux in the final steady state. The transition time of the complete pathway is the sum of the transition time of all intermediates, \(\tau = \sum_{i=1}^{n} \tau_i.\)

A measure for the time necessary to return to a steady state after a small perturbation is the transition time according to Heinrich and Rapoport (1975). Let \(\delta (t) = S(t) - \bar{S}\) be the deviation from steady-state concentrations. Then the transition time is defined as

\[ \tau = \frac{\int_0^\infty t \cdot \delta (t) dt}{\int_0^\infty \delta (t) dt}. \]  

(5-111)

This definition is applicable if \(\delta (t)\) vanishes asymptotically for large \(t.\)
Llorens and et al. (1999) have introduced a more general definition. Let $f$ denote a function such as a flux or a concentration that shall be analyzed after perturbation. The characteristic time can be calculated in analogy to center of mass as

$$T = \frac{\int_0^\infty t \cdot \frac{df}{dx} \, dt}{\int_0^\infty \left| \frac{df}{dx} \right| \, dt}$$  \hspace{1cm} (5-112)$$

This definition may be applied even for oscillating response to the perturbation.

As an example, the characteristic times for Gluc6P, Fruc6P, and Fruc1,6P$_2$ in the running example are given in Tab. 5.4. Whatever definition we apply, characteristic times may differ by orders of magnitude for reactions or concentrations in realistic reaction networks.

5.2.8 Approximations Based on Timescale Separation

Differing timescales in reaction networks allow for reducing the systems in terms of reducing the number of differential equations (and replacing them by algebraic equations). This is implicitly based on the assumption that we choose to consider a window of the timescale while neglecting faster or slower processes. Assume that we consider a process that takes place within minutes. Variables that are changed by faster processes (say within seconds) reach a quasi-steady state after a short transitional period. Variables that are changed by slower processes (processes proceeding within years, e.g., seasonal changes) may be considered as constant. A common example for neglecting slow processes in metabolic modeling is to assume that enzyme concentrations are constant. Their production and degradation is considered to be much slower than the reactions they catalyze. Two types of approximations are used: quasi-steady-state approximations and quasi-equilibrium approximations. A more general mathematical consideration of the effect of timescale separation is given in Heinrich and Schuster (1996).

5.2.8.1 The Quasi-steady-state Approximation

Consider a complex system of processes, e.g., a reaction network. If a metabolite participates in a very fast process (compared to the other processes), it may approach a steady state very quickly. After this transitional period, its concentration $S$ may be

Tab. 5.4 Characteristic times (in min) for the metabolites of the running example. For the meaning of the quantities, see text.

<table>
<thead>
<tr>
<th></th>
<th>$\tau_i$ (Eq. (5-110))</th>
<th>$\tau$ (Eq. (5-111))</th>
<th>$T$ (Eq. (5-112))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluc6P</td>
<td>0.0855</td>
<td>0.0724</td>
<td>0.0890</td>
</tr>
<tr>
<td>Fruc6P</td>
<td>0.0096</td>
<td>0.0171</td>
<td>0.0583</td>
</tr>
<tr>
<td>Fruc1,6P$_2$</td>
<td>0.1095</td>
<td>0.1653</td>
<td>0.1657</td>
</tr>
</tbody>
</table>
considered as constant, i.e. $\dot{S} = 0$. Since this variable is still involved in the complex system, this state is considered as quasi-steady state. The condition $\dot{S} = 0$ allows one to replace the differential equation for the change of $S$ by an algebraic equation describing how $S$ depends on the other variables of the system.

**Example 5-17**

Assume a pathway consisting of faster and slower reactions

\begin{equation}
\text{P} \rightarrow_{k_1} S_1 \rightarrow_{k_2}^{\text{fast}} S_2 \rightarrow_{k_3} S_3
\end{equation}

(5-113)

described by the differential equation system

\begin{align*}
\frac{dS_1}{dt} &= Pk_1 - S_1 k_2 \\
\frac{dS_2}{dt} &= S_1 k_2 - S_2 k_3
\end{align*}

(5-114)

If the second reaction is fast, i.e., $k_2 \gg k_1, k_3$, we may assume a quasi-steady state for $S_1$:

\begin{equation}
\frac{dS_1}{dt} = 0.
\end{equation}

(5-115)

This enables us to calculate the concentration of $S_1$ and to simplify the differential equation system. Now it comprises only one differential equation and one algebraic expression:

\begin{align*}
S_1 &= \frac{P k_1}{k_2} \\
\frac{dS_2}{dt} &= Pk_1 - S_2 k_3
\end{align*}

(5-116) (5-117)

In Fig. 5.10, one can see that the introduced simplification describes the real behavior sufficiently well after an initial period.

5.2.8.2 Quasi-equilibrium Approximation

We again assume a coupling of slow and fast processes. The pathway is supposed to contain two substrates that can be converted rapidly and reversibly one into the other, such as the hexoses glucose-6-phosphate and fructose-6-phosphate in glycolysis. After a short transition period, their concentrations reach equilibrium. In mathematical terms, the differential equation for the concentration change of one of the substrates may be replaced by an algebraic expression for the concentration ratios in equilibrium. Since both substrates are still involved in the larger network, this is again only a quasi-equilibrium.
Example 5-18

The pathway

\[ P \xrightarrow{k_1} S_1 \xrightarrow{k_{+2}} S_2 \xrightleftharpoons[k_{-2}]{k_3} \]

is described by the differential equation system

\[
\frac{dS_1}{dt} = Pk_1 - S_1 k_{+2} + S_2 k_{-2} \\
\frac{dS_2}{dt} = S_1 k_{+2} - S_2 k_{-2} - S_2 k_3 .
\] (5-119)

The conversion is much faster than the other reactions, i.e., \( k_{+2}, k_{-2} \gg k_1, k_3 \). After a short period of time, it holds that

\[
\frac{S_2}{S_1} \approx \frac{k_{+2}}{k_{-2}} = q_2 ,
\] (5-120)

where \( q_2 \) is the equilibrium constant of the second reaction. The second reaction operates close to equilibrium, although a non-vanishing flux (\( v_2 \neq 0 \)) may pass this reaction. We may sum up the concentrations of \( S_1 \) and \( S_2 \), and it holds that

\[
\frac{d(S_1 + S_2)}{dt} = \frac{d(1/q_2 + 1)}{dt} S_2 = Pk_1 - S_2 k_3 .
\] (5-121)

The simplified equation system consists of a differential equation and an algebraic equation.
\[
\frac{dS_2}{dt} = \frac{q_2 (P k_1 - S_2 k_3)}{1 + q_2} \quad (5-122)
\]

\[
S_1 = \frac{S_2}{q_2} \quad (5-123)
\]

Symbolically, the reaction scheme reduces to

\[
P \xrightarrow{\dot{k}_1} S_1 + S_2 \xrightarrow{k_1}. \quad (5-124)
\]

As demonstrated, both types of approximations reduce the number of ODEs in the mathematical model by replacing some of them by algebraic equations. Applying these approximations systematically leads to skeleton models of the considered metabolic pathway, which preserve important features of the general models but are easier to analyze and perceive.

5.3 Metabolic Control Analysis

Metabolic control analysis (MCA) is a powerful quantitative and qualitative framework for studying the relationship between steady-state properties of a network of biochemical reactions and the properties of the individual reactions. It investigates the sensitivity of steady-state properties of the network to small parameter changes. MCA is a useful tool for theoretical and experimental analysis of control and regulation in cellular systems.


Metabolic networks are very complex systems that are highly regulated and exhibit interactions such as feedback inhibition or common substrates for distant reactions. Many mechanisms and regulatory properties of isolated enzymatic reactions are known. The development of MCA was motivated by a series of questions like the fol-
Can their properties or their behavior in the metabolic network be predicted from the knowledge about isolated reactions? Which individual steps control a flux or a steady-state concentration? Is there a rate-limiting step? Which effectors or modifications have the most prominent effect on the reaction rate? In biotechnological production processes, it is of interest which enzyme(s) are to be activated in order to increase the rate of synthesis of a desired metabolite. There are also related problems in health care. For example, concerning metabolic disorders such as overproduction of a metabolite, which reactions should be modified in order to downregulate this metabolite while perturbing the rest of the metabolism as weakly as possible?

In metabolic networks, the steady-state variables, i.e., the fluxes and the metabolite concentrations, are controlled by parameters such as enzyme concentrations, kinetic constants (e.g., Michaelis constants and maximal activities), and other model-specific parameters. The relations between steady-state variables and kinetic parameters are usually nonlinear. Up to now, no general theory exists that predicts the effect of large parameter changes in a network. The approach presented here is basically restricted to small parameter changes. Mathematically, the system is linearized at steady state, which yields exact results if the parameter changes are infinitesimally small.

We will first define a set of mathematical expressions that are useful to quantify control. Later we will show the relations between these functions and their application for prediction of reaction network behavior.

### 5.3.1 The Coefficients of Control Analysis

Biochemical reaction systems are networks of metabolites connected by chemical reactions. Their behavior is determined by the properties of their components – the individual reactions and their kinetics – as well as by the network structure – the involvement of compounds in different reactions or, in short, the stoichiometry. Hence, the effect of a perturbation exerted at a reaction in this network will depend on both the local properties of this reaction and the embedding of this reaction in the global network.

Let $y(x)$ denote a quantity that depends on another quantity $y$. The effect of the change $\Delta x$ on $y$ is expressed in terms of coefficients:

$$c^y_x = \left( \frac{x \Delta y}{y \Delta x} \right)_{\Delta x \to 0}.$$  \hspace{1cm} (5-125)

In practical applications, $\Delta x$ might be identified, e.g., with one percent change of $x$ and $\Delta y$ with the percentage change of $y$. The pre-factor $x/y$ is a normalization factor that makes the coefficient independent of units and the magnitude of $x$ and $y$. In the limiting case $\Delta x \to 0$, the coefficient defined in Eq. (5-125) can be written as
which is mathematically equivalent to

\[ c_x^y \frac{\partial y}{\partial x} \]

(5-126)

Two distinct types of coefficients, local and global coefficients, reflect the relations among local and global changes. Elasticity coefficients (sensitivities) are local coefficients pertaining to individual reactions. They can be calculated in any given state. Control coefficients and response coefficients are global quantities. They refer to a given steady state of the entire system. After a perturbation of \( y \), the relaxation of \( x \) to a new steady state is considered.

The general form of the coefficients in control analysis as defined in Eq. (5-126) contains the normalization \( x/y \). The normalization has the advantage that we get rid of units and can compare, e.g., fluxes belonging to different branches of a network. The drawback of the normalization is that \( x/y \) is not defined as soon as \( y = 0 \), which may happen for certain parameter combinations. In those cases it is favorable to work with non-normalized coefficients. Throughout this chapter we will usually consider normalized quantities. If we use non-normalized coefficients, they are flagged as \( \text{nonc} \). In general, the use of one or the other type of coefficient is also a matter of the personal choice of the modeler.

A graphical representation of changes reflected in the different coefficients is shown in Fig. 5.11.
5.3.1.1 The Elasticity Coefficients

An elasticity coefficient quantifies the sensitivity of a reaction rate to the change of a concentration or a parameter. It measures the direct effect on the reaction velocity, while the rest of the network is kept fixed. The sensitivity of the rate \( v_k \) of a reaction to the change of the concentration \( S_i \) of a metabolite is calculated by the \( \epsilon \)-elasticity:

\[
\epsilon_i^k = \frac{S_i \frac{\partial v_k}{\partial S_i}}{v_k}.
\]  

(5-128)

While the \( \epsilon \)-elasticity involves the derivative with respect to a variable, the metabolite concentration, the \( \pi \)-elasticity

\[
\pi_m^k = \frac{p_m}{v_k} \frac{\partial v_k}{\partial p_m}
\]

is defined with respect to parameters \( p_m \) such as kinetic constants, concentrations of enzymes, or external metabolites.

Example 5-19

In the glycolysis model, the rate of reaction 1 depends on the ATP concentration. The sensitivity is given by the elasticity

\[
\epsilon_{ATP}^1 = \frac{ATP}{v_1} \frac{\partial v_1}{\partial ATP}.
\]  

(5-130)

Since Eq. (5-2) defines the dependency of \( v_1 \) on the ATP concentration, it is easy to calculate that

\[
\epsilon_{ATP}^1 = \frac{ATP}{v_1} \frac{\partial}{\partial ATP} \left( \frac{V_{max,1}ATP}{K_{ATP,1}+ATP} \right) = \frac{ATP}{v_1} \frac{V_{max,1}(K_{ATP,1}+ATP) - V_{max,1}ATP}{(K_{ATP,1}+ATP)^2} = \frac{ATP}{K_{ATP,1}+ATP}.
\]  

(5-131)

The elasticities of all (other) rates with respect to any metabolite concentration can be calculated similarly. Whenever the rate does not depend directly on a concentration (like \( v_1 \) and AMP), the elasticity is zero.

Example 5-20

Typical values of elasticity coefficients will be explained for an isolated reaction transforming substrate \( S \) into product \( P \). The reaction is catalyzed by enzyme \( E \) with the inhibitor \( I \) and the activator \( A \) as depicted below:
Usually, the elasticity coefficients for metabolite concentrations are in the following range:

\[
\varepsilon^v_S = \frac{\partial \ln v}{\partial \ln S} > 0 \quad \text{and} \quad \varepsilon^v_P = \frac{\partial \ln v}{\partial \ln P} \leq 0. \tag{5-133}
\]

In most cases, the rate increases with the concentration of the substrate (compare, e.g., Eq. (5-131)) and decreases with the concentration of the product. An exception from \(\varepsilon^v_S > 0\) occurs with substrate inhibition (Eq. (5-44)), where the elasticity will become negative for \(S > S_{\text{opt}}\). The relation \(\varepsilon^v_P = 0\) holds if the reaction is either irreversible or the product concentration is kept fixed at zero by external mechanisms. The elasticity coefficients with respect to effectors I or A should obey

\[
\varepsilon^v_A = \frac{\partial \ln v}{\partial \ln A} > 0 \quad \text{and} \quad \varepsilon^v_I = \frac{\partial \ln v}{\partial \ln I} < 0, \tag{5-134}
\]

since this is essentially what the notions activator and inhibitor mean. For the most kinetic types, the reaction rate \(v\) is proportional to the enzyme concentration \(E\). For example, \(E\) is a multiplicative factor in the mass action rate law (Eq. (5-12)) as well as in the maximal rate of the Michelis-Menten rate law (Eq. (5-33)). Therefore, it holds that

\[
\varepsilon^v_E = \frac{\partial \ln v}{\partial \ln E} = 1. \tag{5-135}
\]

More complicated interactions between enzymes and substrates, such as metabolic channeling (direct transfer of the metabolite from one enzyme to the next without release to the medium), may lead to exceptions to this rule.

### 5.3.1.2 Control Coefficients

When defining control coefficients, we refer to a stable steady state of the metabolic system characterized by steady-state concentrations \(S = S(p)\) and steady-state fluxes \(J = v(S(p), p)\). Any sufficiently small perturbation of an individual reaction rate by a parameter change, \(v_k \rightarrow v_k + \Delta v_k\), drives the system to a new steady state in close proximity with \(J \rightarrow J + \Delta J\) and \(S \rightarrow S + \Delta S\). A measure for the change of fluxes and concentrations are the control coefficients.
Example 5-21
Consider the glycolysis model in the running example. We may ask, what is the impact of a change in the rate of ATP consumption, reaction 7, on the steady flux through the upper glycolysis, i.e., through reaction 1 or through reactions 3, 4, and 5? Or, what is the effect of an acceleration of the rate of reaction 3 on the concentrations of ATP or of Fruc6P? We cannot calculate these effects directly as we did for the elasticities in Example 5-19.

The flux-control coefficient for the control of rate $v_k$ over flux $J_j$ is defined as

$$C_j^k = \frac{v_k}{J_j} \frac{\partial J_j}{\partial v_k},$$

(5-136)

while the concentration-control coefficient of concentration $S_i$ with respect to $v_k$ reads

$$C_i^k = \frac{v_k}{S_i} \frac{\partial S_i}{\partial v_k}.$$  

(5-137)

The control coefficients quantify the control that a certain reaction $v_k$ exerts on the steady-state flux $J$ or on the steady state concentration $S_i$, respectively.

It should be noted that the rate change, $\Delta v_k$, is caused by the change of a parameter $p_k$ that has a direct effect solely on $v_k$. Thus it holds that

$$\frac{\partial v_k}{\partial p_k} \neq 0 \quad \text{and} \quad \frac{\partial v_l}{\partial p_k} = 0 \quad (l \neq k).$$

(5-138)

Example 5-22
In the glycolysis model, the Michaelis constant of ATP in reaction 1, $K_{ATP,1}$, is a parameter that directly influences only reaction 1.

Such a parameter might be the enzyme concentration, a kinetic constant, or the concentration of a specific inhibitor or effector. Hence, the definition of the flux-control coefficients in extenso is

$$C_j^k = \frac{v_k}{J_j} \frac{\partial J_j/\partial p_k}{\partial v_k/\partial p_k}.$$  

(5-139)

As long as Eq. (5-138) holds, the value of the control coefficient does not depend on the choice of the perturbed parameter.
5.3.1.3 **Response Coefficients**

The steady state is determined by the values of the parameters. A third type of coefficient expresses the direct dependence of steady-state variables on parameters. The response coefficients are defined as

\[
R^j_m = \frac{p_m}{J_j} \frac{\partial J_j}{\partial p_m} \quad \text{and} \quad R^i_m = \frac{p_m}{S_i} \frac{\partial S_i}{\partial p_m}.
\]

(5-140)

where the first coefficient expresses the response of the flux to a parameter perturbation while the latter describes the response of a steady-state concentration.

**Example 5-23**

What is the influence of a perturbation of the Michaelis constant of ATP in reaction 1, $K_{ATP,1}$, on the steady-state flux of reaction 6 or on the concentration of Fruc1,6P2?

5.3.1.4 **Matrix Representation of the Coefficients**

Control, response, and elasticity coefficients are defined with respect to all rates, steady-state concentrations, fluxes, or parameters in the metabolic system and in the respective model. They can be arranged in matrices:

\[
C^J = \{C^J_i\}, \quad C^S = \{C^S_i\}, \quad R^J = \{R^J_m\}, \quad R^S = \{R^S_m\}, \quad \varepsilon = \{\varepsilon^k_i\}, \quad \pi = \{\pi^k_m\}.
\]

(5-141)

Matrix representation can also be chosen for the response coefficients as well as for all types of non-normalized coefficients. The arrangement in matrices allows applying of matrix algebra in control analysis. In particular, the matrices of normalized control coefficients can be calculated from the matrices of non-normalized control coefficient as follows:

\[
C^J = (dg J)^{-1} \cdot \non C^J \cdot dg J
\]

\[
C^S = (dg S)^{-1} \cdot \non C^J \cdot dg J
\]

\[
R^J = (dg J)^{-1} \cdot \non R^J \cdot dg p
\]

\[
R^S = (dg S)^{-1} \cdot \non R^S \cdot dg p
\]

\[
\varepsilon = (dg J)^{-1} \cdot \non \varepsilon \cdot dg S
\]

\[
R^S = (dg S)^{-1} \cdot \non R^S \cdot dg p.
\]

(5-142)

The symbol $dg$ stands for the diagonal matrix, e.g., $dg J = \begin{pmatrix} J_1 & 0 & 0 \\ 0 & J_2 & 0 \\ 0 & 0 & J_r \end{pmatrix}$. 

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5 Metabolism
The Theorems of Metabolic Control Theory

We are interested in calculating the control coefficients for a system under investigation. Usually, the steady-state fluxes or concentrations cannot be expressed explicitly as a function of the reaction rates. Therefore, flux- and concentration-control coefficients cannot simply be determined by taking the respective derivatives, as we did for the elasticity coefficients in Example 5-19.

Fortunately, the work with control coefficients is eased by a set of theorems. The first type of theorem, the summation theorems, makes a statement about the total control over a flux or a steady-state concentration. The second type of theorem, the connectivity theorems, relates the control coefficients to the elasticity coefficients. Both types of theorems together with dependency information encoded in the stoichiometric matrix contain enough information to calculate all control coefficients as a function of the elasticities.

We will first introduce the theorems and then present a hypothetical perturbation experiment to illustrate the summation theorem. Finally, the theorems will be mathematically derived.

5.3.2.1 The Summation Theorems

The summation theorems make a statement about the total control over a certain steady-state flux or concentration. The flux-control coefficients fulfill

\[
\sum_{k=1}^{r} C^{f}_{vk} = 1, \tag{5-143}
\]

where \( r \) is the number of reactions. The flux-control coefficients of a metabolic network for one steady-state flux sum up to 1. This means that all enzymatic reactions can share the control over this flux. For the concentration-control coefficients, we have

\[
\sum_{k=1}^{r} C^{s}_{vk} = 0. \tag{5-144}
\]

The control coefficients of a metabolic network for one steady-state concentration are balanced. This means again that the enzymatic reactions can share the control over this concentration, but some exert a negative control while others exert a positive control. Both relations can also be expressed in matrix formulation. For the flux-control coefficients, we have

\[
C^{f} \cdot \mathbf{1} = \mathbf{1}, \tag{5-145}
\]

and for the concentration control coefficients, we have

\[
C^{s} \cdot \mathbf{1} = \mathbf{0}. \tag{5-146}
\]
The symbols $\mathbf{1}$ and $\mathbf{0}$ denote column vectors with $r$ rows containing as entries only ones or zeros, respectively.

The summation theorems for the non-normalized control coefficients read

$$\text{non} \mathbf{C}^J \cdot \mathbf{K} = \mathbf{K}$$

and

$$\text{non} \mathbf{C}^S \cdot \mathbf{K} = \mathbf{0}.$$  \hspace{3cm} (5-148)

A more intuitive derivation of the summation theorems is given in the following example according to Kacer and Burns (1973).

**Example 5-24**

The summation theorem for flux-control coefficients can be derived using a thought experiment. Consider the unbranched pathway

$$P_0 \xleftrightarrow{v_1} S_1 \xleftrightarrow{v_2} S_2 \xleftrightarrow{v_3} P_3.$$  \hspace{3cm} (5-149)

What happens to steady-state fluxes and metabolite concentrations if we perform a directed experimental manipulation of all three reactions leading to the same fractional change $\alpha$ of all three rates?

$$\frac{\delta v_1}{v_1} = \frac{\delta v_2}{v_2} = \frac{\delta v_3}{v_3} = \alpha.$$  \hspace{3cm} (5-150)

The flux must increase to the same extent:

$$\frac{\delta J}{J} = \alpha.$$  \hspace{3cm} (5-151)

But since producing and degrading reactions increase to the same amount, the concentrations of the metabolites remain constant

$$\frac{\delta S_1}{S_1} = \frac{\delta S_2}{S_2} = 0.$$  \hspace{3cm} (5-152)

The combined effect of all changes in local rates on the system variables $S_1$, $S_2$, and $J$ can be written as the sum of all individual effects caused by the local rate changes. For the flux it holds that

$$\frac{\delta J}{J} = C_1^j \frac{\delta v_1}{v_1} + C_2^j \frac{\delta v_2}{v_2} + C_3^j \frac{\delta v_3}{v_3}.$$  \hspace{3cm} (5-153)

Using Eqs. (5-155) and (5-156), it follows that

$$\alpha = \alpha \left( C_1^j + C_2^j + C_3^j \right),$$  \hspace{3cm} (5-154)
and, therefore, it holds that

\[ 1 = C_1^J + C_2^J + C_3^J. \tag{5-155} \]

This is just a special case of Eq. (143). In the same way, for the change of concentration \( S_1 \), we obtain

\[ \frac{\delta S_1}{S_1} = \frac{C_{S_1}^{\delta v_1}}{v_1} + \frac{C_{S_1}^{\delta v_2}}{v_2} + \frac{C_{S_1}^{\delta v_3}}{v_3}. \tag{5-156} \]

By means of Eqs. (5-155) and (5-152), we find

\[ 0 = C_{1}^{S_1} + C_{2}^{S_1} + C_{3}^{S_1}. \tag{5-157} \]

A similar result holds for the change of concentration \( S_2 \):

\[ 0 = C_{1}^{S_2} + C_{2}^{S_2} + C_{3}^{S_2}. \tag{5-158} \]

Although shown here only for a special case, these properties hold in general for systems without conservation relations. The general derivation is given in Section 5.3.2.3.

5.3.2.2 The Connectivity Theorems

Flux-control coefficients and elasticity coefficients are related by the expression

\[ \sum_{k=1}^{r} C_{v_k}^{J} e_{S_i}^{v_k} = 0. \tag{5-159} \]

Note that the sum runs over all rates \( v_k \). Considering the concentration \( S_i \) of a fixed metabolite and a fixed flux \( J_j \), each term contains the elasticity \( e_{S_i}^{v_k} \) describing the direct influence of a change of \( S_i \) on the rates \( v_k \) and the control coefficient expressing the control of \( v_k \) over \( J_j \).

The connectivity theorem between concentration-control coefficients and elasticity coefficients reads

\[ \sum_{k=1}^{r} C_{v_k}^{S_i} e_{S_i}^{v_k} = -\delta_{hi}. \tag{5-160} \]

Again, the sum runs over all rates \( v_k \), while \( S_h \) and \( S_i \) are the concentrations of two fixed metabolites. The symbol \( \delta_{hi} = \begin{cases} 0, & \text{if } h \neq i \\ 1, & \text{if } h = i \end{cases} \) is the so-called Kronecker symbol.

In matrix formulation, the connectivity theorems read

\[ C^J \cdot e = 0 \tag{5-161} \]
and
\[ \mathbf{C}^S \cdot \mathbf{\varepsilon} = -\mathbf{I} \]  
(5-162)

where \( \mathbf{I} \) denotes the identity matrix of size \( n \times n \). For non-normalized coefficients, it holds that
\[ \text{non} \mathbf{C}^J \cdot \text{non} \mathbf{\varepsilon} \cdot \mathbf{L} = \mathbf{0} \]  
(5-163)

and
\[ \text{non} \mathbf{C}^S \cdot \mathbf{\varepsilon} \cdot \mathbf{L} = -\mathbf{L} \]  
(5-164)

where \( \mathbf{L} \) is the link matrix that expresses the relation between independent and dependent rows in the stoichiometric matrix (Eq. (5-91)). A very comprehensive representation of both summation and connectivity theorems for non-normalized coefficients is given by the following equation:
\[
\begin{pmatrix} \text{non} \mathbf{C}^J \\ \text{non} \mathbf{C}^S \end{pmatrix} \cdot \begin{pmatrix} K & 0 \\ 0 & -L \end{pmatrix} = \begin{pmatrix} \mathbf{K} \\ \mathbf{0} \end{pmatrix} . 
\]  
(5-165)

As mentioned above, the summation and connectivity theorems together with the structural information of the stoichiometric matrix are sufficient to calculate the control coefficients for a metabolic network as function of the elasticities. This shall be illustrated for a small network in the next example.

**Example 5-25**

To calculate the control coefficients, we study the following reaction system:
\[
\begin{align*}
\text{P}_0 & \underset{\nu_1}{\overset{\nu_2}{\longleftrightarrow}} \text{S}_1 & \text{S}_1 & \overset{\nu_2}{\underset{\nu_1}{\longleftrightarrow}} \text{P}_2 .
\end{align*}
\]  
(5-166)

The flux-control coefficients obey the theorems
\[
\mathbf{C}_1^J + \mathbf{C}_2^J = 1 \quad \text{and} \quad \mathbf{C}_1^J \mathbf{\varepsilon}_S + \mathbf{C}_2^J \mathbf{\varepsilon}_S = 0 ,
\]  
(5-167)

which can be solved for the control coefficients
\[
\mathbf{C}_1^J = \frac{\mathbf{\varepsilon}_S}{\mathbf{\varepsilon}_S^1 - \mathbf{\varepsilon}_S^3} \quad \text{and} \quad \mathbf{C}_2^J = \frac{-\mathbf{\varepsilon}_S^3}{\mathbf{\varepsilon}_S^2 - \mathbf{\varepsilon}_S^3} .
\]  
(5-168)

Since usually \( \mathbf{\varepsilon}_S^1 < 0 \) and \( \mathbf{\varepsilon}_S^3 > 0 \) (see Example 5-20), both control coefficients assume positive values \( \mathbf{C}_1^J > 0 \) and \( \mathbf{C}_2^J > 0 \). This means that both reactions exert a positive control over the steady-state flux and that acceleration of any of them leads to increase of \( J \), which is in accordance with common intuition.
The concentration control coefficients fulfill

\[ C^S_1 + C^S_2 = 0 \quad \text{and} \quad C^{S^1}_1 + C^{S^2}_2 = -1, \quad (5-169) \]

which yields

\[ C^S_1 = \frac{1}{\varepsilon^S_2 - \varepsilon^S_1} \quad \text{and} \quad C^J_2 = \frac{-1}{\varepsilon^S_2 - \varepsilon^S_1}. \quad (5-170) \]

With \( \varepsilon^S_1 < 0 \) and \( \varepsilon^S_2 > 0 \), we get \( C^S_1 > 0 \) and \( C^S_2 < 0 \), i.e., increase of the first reaction causes a rise in the steady-state concentration of \( S \), while acceleration of the second reaction leads to the opposite.

### 5.3.2.3 Derivation of Matrix Expressions for Control Coefficients

After having introduced the theorems of metabolic control analysis, we will derive expressions for the control coefficients in matrix form. These expressions are suited for calculating the coefficients even for large-scale models. We start from the steady-state condition

\[ N v(S(p), p) = 0. \quad (5-171) \]

Implicit differentiation with respect to the parameter vector \( p \) yields

\[ N \frac{\partial v}{\partial S} \frac{\partial S}{\partial p} + N \frac{\partial v}{\partial p} = 0. \quad (5-172) \]

Since we have chosen reaction-specific parameters for perturbation, the matrix of non-normalized parameter elasticities contains nonzero entries in the main diagonal and zeros elsewhere (compare Eq. (5-138)).

\[
\frac{\partial v}{\partial p} = \begin{pmatrix}
\frac{\partial v_1}{\partial p_1} & 0 & 0 \\
0 & \frac{\partial v_2}{\partial p_2} & 0 \\
0 & 0 & \ldots & \frac{\partial v_r}{\partial p_r}
\end{pmatrix}.
\quad (5-173)
\]

Therefore, this matrix is regular and has an inverse. Furthermore, we consider the following Jacobian matrix:

\[ M = N \frac{\partial v}{\partial S}. \quad (5-174) \]
The Jacobian matrix \( \mathbf{M} \) is a regular matrix if the system is asymptotically stable and contains no conservation relations. The case with conservation relations is considered below. Here, we may pre-multiply Eq. (5-172) by the inverse of \( \mathbf{M} \) and rearrange to get

\[
\frac{\partial \mathbf{S}}{\partial \mathbf{p}} = -\left( \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{p}} = -\mathbf{M}^{-1} \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \equiv \text{non} \mathbf{R} \mathbf{S}^i. \tag{5-175}
\]

As indicated, \( \mathbf{\partial S}/\partial \mathbf{p} \) is the matrix of non-normalized response coefficients for concentrations. Post-multiplication by the inverse of the non-normalized parameter elasticity matrix gives us

\[
\frac{\partial \mathbf{S}}{\partial \mathbf{p}} \left( \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \right)^{-1} = -\left( \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N} = \text{non} \mathbf{C} \mathbf{S}. \tag{5-176}
\]

This is the matrix of non-normalized concentration-control coefficients. The right (middle) side contains no parameters. This means that the control coefficients do not depend on the particular choice of parameters to exert the perturbation as long as Eq. (5-138) is fulfilled. The control coefficients are dependent on the structure of the network, represented by the stoichiometric matrix \( \mathbf{N} \), and on the kinetics of the individual reactions, represented by the non-normalized elasticity matrix \( \partial \mathbf{v}/\partial \mathbf{S} \).

The implicit differentiation of

\[
\mathbf{J} = \mathbf{v}(\mathbf{S}(\mathbf{p}), \mathbf{p}) \tag{5-177}
\]

with respect to the parameter vector \( \mathbf{p} \) leads to

\[
\frac{\partial \mathbf{J}}{\partial \mathbf{p}} = \frac{\partial \mathbf{v}}{\partial \mathbf{p}} + \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \frac{\partial \mathbf{S}}{\partial \mathbf{p}} = \left( \mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \left( \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N} \right) \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \equiv \text{non} \mathbf{R} \mathbf{J}. \tag{5-178}
\]

This yields, after some rearrangement, an expression for the non-normalized flux-control coefficients:

\[
\frac{\partial \mathbf{J}}{\partial \mathbf{p}} \left( \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \right)^{-1} = \mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \left( \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N} = \text{non} \mathbf{C} \mathbf{J}. \tag{5-179}
\]

The normalized control coefficients are (by use of Eq. (5-142))

\[
\mathbf{C}^J = \mathbf{I} - (dgJ)^{-1} \left( \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \left( \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N} \right) (dgJ) \tag{5-180}
\]

and

\[
\mathbf{C}^S = -(dgS)^{-1} \left( \left( \mathbf{N} \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N} \right) (dgJ). \tag{5-181}
\]
These equations can easily be implemented for numerical calculation of control coefficients or used for analytical computation (see Example 5-26). They are also suited for derivation of the theorems of MCA. The summation theorems for the control coefficients follow from Eq. (5-180) or Eq. (5-181) by, respectively, post-multiplying with the vector $\mathbf{1}$ (the row vector containing only 1’s) and consideration of the relations $(\mathbf{dgJ}) \cdot \mathbf{1} = \mathbf{J}$ and $\mathbf{N} \mathbf{J} = 0$. The connectivity theorems result from post-multiplying Eq. (5-180) or Eq. (5-181) with the elasticity matrix $\mathbf{e} = (\mathbf{dgJ})^{-1} \cdot \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \cdot \mathbf{dgS}$, and using that multiplication of a matrix with its inverse yields the identity matrix $\mathbf{I}$ of respective type.

If the reaction system involves conservation relations, we eliminate dependent variables as explained in Section 5.2.5. In this case the non-normalized coefficients read

$$
\text{non} \mathbf{C}^J = \mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \mathbf{L} \left( \mathbf{N}^0 \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N}^0 \quad (5-182)
$$

$$
\text{non} \mathbf{C}^S = -\mathbf{L} \left( \mathbf{N}^0 \frac{\partial \mathbf{v}}{\partial \mathbf{S}} \right)^{-1} \mathbf{N}^0 \quad (5-183)
$$

and the normalized control coefficients are obtained by applying Eq. (5-142).

**Example 5-26**

Consider the simple reaction system of Example 5-25 and let the rate equations be $v_1 = k_1 P_0 - k_{-1} S_1$ and $v_2 = k_2 S_1 - k_{-2} P_2$. Then, the steady-state condition $\mathbf{J} = v_1 = v_2$ results in

$$
\mathbf{J} = \frac{k_1 k_2 P_0 - k_{-1} k_{-2} P_2}{k_{-1} + k_2} \quad (5-184)
$$

and

$$
S_1 = \frac{k_1 P_0 + k_{-2} P_2}{k_{-1} + k_2} \quad (5-185)
$$

The stoichiometric matrix is $\mathbf{N} = (1 -1)$ and the non-normalized elasticity coefficients read

$$
\frac{\partial \mathbf{v}}{\partial \mathbf{S}} = \begin{pmatrix} \frac{\partial v_1}{\partial S_1} \\ \frac{\partial v_2}{\partial S_1} \end{pmatrix} = \begin{pmatrix} -k_{-1} \\ k_2 \end{pmatrix} \quad (5-186)
$$

Introducing these expressions into Eq. (5-180) yields
\[
C^f = \begin{pmatrix} \frac{\kappa_1 \kappa_2 P_0 - \kappa_{-1} \kappa_{-2} P_2}{k_{-1} + k_2} & 0 \\ \frac{\kappa_1 \kappa_2 P_0 - \kappa_{-1} \kappa_{-2} P_2}{k_{-1} + k_2} & \frac{\kappa_1 \kappa_2 P_0 - \kappa_{-1} \kappa_{-2} P_2}{k_{-1} + k_2} \end{pmatrix}^{-1} \quad (5-187)
\]

\[
\begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} - \left( \frac{-k_{-1}}{k_2} \right) \left( \begin{pmatrix} 1 & -1 \\ 0 & 0 \end{pmatrix} \right)^{-1} \left( \begin{pmatrix} 1 & -1 \\ 0 & 0 \end{pmatrix} \right) (dg) = \left( \begin{pmatrix} 0 & k_2 \\ \frac{1}{k_{-1} + k_2} (k_2 & k_{-1}) \end{pmatrix} \right) (dg)
\]

meaning that the flux-control coefficients are \( C^f_1 = \frac{k_2}{k_{-1} + k_2}, \ C^f_2 = \frac{k_{-1}}{k_{-1} + k_2} \). For the concentration-control coefficients we have

\[
C^s = \begin{pmatrix} \frac{\kappa_1 \kappa_2 P_0 - \kappa_{-1} \kappa_{-2} P_2}{k_{-1} + k_2} & 0 \\ \frac{\kappa_1 \kappa_2 P_0 - \kappa_{-1} \kappa_{-2} P_2}{k_{-1} + k_2} & \frac{\kappa_1 \kappa_2 P_0 - \kappa_{-1} \kappa_{-2} P_2}{k_{-1} + k_2} \end{pmatrix}^{-1} \left( \begin{pmatrix} 1 & -1 \\ 0 & 0 \end{pmatrix} \right)^{-1} \left( \begin{pmatrix} 1 & -1 \\ 0 & 0 \end{pmatrix} \right) (dg) = \left( \begin{pmatrix} \frac{-1}{k_{-1} + k_2} (1 & -1) \\ \frac{1}{k_{-1} + k_2} (1 & -1) \end{pmatrix} \right)
\]

\[
\begin{pmatrix} \frac{k_1 k_2 P_0 - k_{-1} k_{-2} P_2}{k_{-1} + k_2} & 0 \\ 0 & \frac{k_1 k_2 P_0 - k_{-1} k_{-2} P_2}{k_{-1} + k_2} \end{pmatrix}^{-1} \left( \begin{pmatrix} 1 & -1 \\ \frac{1}{k_{-1} + k_2} (1 & -1) \end{pmatrix} \right)
\]

\[
= \frac{(k_1 k_2 P_0 - k_{-1} k_{-2} P_2)}{(k_{-1} + k_2) \cdot (k_1 P_0 + k_{-2} P_2)} \begin{pmatrix} 1 & -1 \\ \frac{1}{k_{-1} + k_2} (1 & -1) \end{pmatrix}
\]

or

\[
C^s_1 = \frac{k_1 k_2 P_0 - k_{-1} k_{-2} P_2}{(k_{-1} + k_2) \cdot (k_1 P_0 + k_{-2} P_2)}, C^s_2 = \frac{-(k_1 k_2 P_0 - k_{-1} k_{-2} P_2)}{(k_{-1} + k_2) \cdot (k_1 P_0 + k_{-2} P_2)} \quad (5-189)
\]

To investigate the implications of control distribution, we will now analyze the control pattern in an unbranched pathway

\[
P_0 \xleftrightarrow{v_1} S_1 \xleftrightarrow{v_2} S_2 \cdots S_{r-1} \xleftrightarrow{v_r} P_r
\]

with linear kinetics \( v_i = k_i S_{i-1} - k_- S_i \) (with \( S_0 = P_0, S_r = P_r \)) and equilibrium constants \( q_i = k_i / k_{-i} \). In this case, one can calculate an analytical expression for the steady-state flux.
as well as an analytical expression for the flux control coefficients

\[
C_i^f = \frac{1}{\sum_{i=1}^{r} \frac{1}{k_i} \prod_{m=1}^{r} q_m} \frac{1}{k_i} \prod_{j=i}^{r} q_j .
\]  

\(5-192\)

Let us consider two very general cases. First, assume that all reactions have the same individual kinetics, \(k_+ = k_i, k_- = k_{-i}\) for \(i = 1, \ldots, r\), and that for the equilibrium constants, which are also equal, it holds that \(q = k_+/k_- > 1\). In this case the ratio of two subsequent flux-control coefficients is

\[
\frac{C_i^f}{C_{i+1}^f} = \frac{k_{i+1}}{k_i} q_i = q > 1 .
\]  

\(5-193\)

Hence, the control coefficients of the preceding reactions are bigger than the control coefficients of the succeeding reactions, or flux-control coefficients are higher in the beginning of a chain than in the end. This conforms to the frequent observation that flux control resides in the upper part of an unbranched reaction pathway.

Now assume that the individual rate constants might be different but that all equilibrium constants are equal to 1, \(q_i = 1\) for \(i = 1, \ldots, r\). This implies that \(k_i = k_{-i}\). Furthermore, Eq. (5-192) simplifies to

\[
C_i^f = \frac{1}{\sum_{i=1}^{r} \frac{1}{k_i}}
\]

Now consider the relaxation time \(\tau_i = 1/(k_i + k_{-i})\) (compare Section 5.2.7) as a measure of the rate of an enzyme. The flux-control coefficient reads

\[
C_i^f = \frac{\tau_i}{\tau_1 + \tau_2 + \ldots + \tau_r} .
\]  

\(5-194\)

This expression helps to elucidate two aspects of metabolic control. First, all enzymes participate in the control since all enzymes have a relaxation time. No one enzyme has all the control or determines the flux through the pathway alone. Second, slow enzymes with a higher relaxation time exert in general more control than fast enzymes with a short relaxation time.

The predictive power of flux-control coefficients for directed changes of flux is illustrated in the following example.
Example 5-27

Assume that we can manipulate the pathway by changing the enzyme concentration in a predefined way. We would like to explore the effect of the perturbation of the individual enzymes. For a linear pathway of the type of Eq. (5-190) consisting of four consecutive reactions with \( v_i = E_i (k_i S_{i-1} - k_{-i} S_i) \), we will calculate the flux-control coefficients. For \( i = 1, \ldots, 4 \), it shall hold that (1) all enzyme concentrations \( E_i = 1 \), (2) the rate constants \( k_i = 2 \), \( k_{-i} = 1 \), and (3) the concentrations of the external reactants \( P_0 = P_4 = 1 \). The resulting flux is \( J = 1 \) and the flux-control coefficients are \( C^J = (0.533 \ 0.267 \ 0.133 \ 0.067)^T \) according to Eq. (5-180).

If we now slightly perturb the first enzyme, perhaps by performing a percentage change of its concentration, i.e., \( E_1 / C_63 E_1 + 1\% \), then Eq. (5-125) implies that the flux increases as \( J / C_63 J + C^J_1 \cdot 1\% \). In fact, the flux in the new steady state is \( JE_1 / C_63 1.01 E_1 = 1.00531 \). Increasing \( E_2 \), \( E_3 \), or \( E_4 \) by one percent leads to flux values of 1.00265, 1.00132, and 1.00066, respectively.

A strong perturbation would not yield similar effects. Assume that we can manipulate the system at will and can double the total amount of enzyme in the system. If we keep \( E_2 \) to \( E_4 \) constant but change \( E_1 / C_63 E_1 \), the resulting flux is \( JE_1 / C_63 5 E_1 = 1.7441 \). Changing instead \( E_4 / C_63 E_4 \) yields \( JE_4 / C_63 5 E_4 = 1.0563 \), a lower flux increase.

Equal distribution of changes to all enzymes \( E_i / C_63 E_i \), \( i = 1, \ldots, 4 \) results in \( JE_i / C_63 2 E_i = 2 \). The maximal value for the flux \( J_{\text{max}} = 2.2871 \) is obtained for the optimal distribution of enzyme concentration to the individual reactions, which is \( E_1 = 3.124 \), \( E_2 = 2.209 \), \( E_3 = 1.562 \), \( E_4 = 1.105 \) (see Chapter 12). Changes in enzyme concentrations and their effects on flux control are illustrated in Fig. 5.12.

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**Fig. 5.12** Effect of enzyme concentration change on steady-state flux and on flux-control coefficients in an unbranched pathway consisting of four reactions. In the reference state, all enzymes have the concentration 1 (in arbitrary units), the control distribution is the same as in case (c), and the steady-state flux is \( J = 1 \). (a) Change of \( E_1 \rightarrow 5 E_1 \) while keeping the other enzyme concentrations constant results in a remarkable drop of control of the first enzyme. The resulting flux is \( J^{E_1\rightarrow 5 E_1} = 1.7741 \). (b) The change \( E_4 \rightarrow 5 E_4 \) corresponds to \( J^{E_4\rightarrow 5 E_4} = 1.0563 \). There is only slight change of control distribution. (c) Equal enzyme concentrations with \( E_i \rightarrow 2 E_i \), \( i = 1, \ldots, 4 \) result in \( J^{E_i\rightarrow 2 E_i} = 2 \). (d) Optimal distribution of enzyme concentration \( E_1 = 3.124, E_2 = 2.209, E_3 = 1.562, E_4 = 1.105 \) resulting in the maximal steady-state flux \( J^{\text{max}}_{\text{max}} = 2.2871 \).
5.3.3 Extensions of Metabolic Control Analysis

5.3.3.1 Control Analysis for Variables other than Fluxes and Concentrations

Control coefficients are defined as the ratio of fractional changes in the system’s variables at steady state to fractional changes in the biochemical activity that causes the change. The concept of control analysis can also be applied to other relevant variables of the system besides fluxes and concentrations (Schuster 1996). Such variables can be the transition time (Section 5.2.7), free energy differences (Section 5.1.2, Westerhoff and van Dam 1987), growth rate (Kacser and Beeby 1984; Dykhuizen et al. 1987), and others. Let \( X \) be a vector of generalized state variables and \( Y \) a vector of generalized response variables. State variables \( X \) characterize the state of the system, which is not necessarily the steady state. Response variables \( Y = Y(X,p) \) can be calculated in terms of the state variables \( X \) and the parameters \( p \). The reaction rates \( v \) are also considered as functions of \( X \) and \( p \), \( v = v(X,p) \). The matrix of non-normalized control coefficients can be expressed as

\[
\text{non} C^Y = \frac{dY}{dp} \left( \frac{\partial v}{\partial p} \right)^{-1},
\]

and for the matrix of normalized control coefficients, it holds that

\[
C^Y = (dgY)^{-1} \frac{dY}{dp} \left( \frac{\partial v}{\partial p} \right)^{-1} (dgv).
\]

In Eqs. (5-195) and (5-196), the total derivative \( dY/dp \) has been used since not only the direct effect of \( p \) on \( Y \) but also the indirect effect via \( X \) must be taken into account. Schuster (1996) showed that the coefficients defined in Eq. (5-195) obey a unified summation theorem,

\[
\text{non} C^Y K = \frac{\partial Y}{\partial p} \left( \frac{\partial v}{\partial p} \right)^{-1} K,
\]

as well as a unified connectivity theorem,

\[
\text{non} C^Y \frac{\partial v}{\partial X} \frac{\partial X}{\partial X_a} = \left[ \frac{\partial Y}{\partial p} \left( \frac{\partial v}{\partial p} \right)^{-1} \frac{\partial v}{\partial X} - \frac{\partial Y}{\partial X} \right] \frac{\partial X}{\partial X_a},
\]

where \( X_a \) is a subvector of \( X \) containing only independent variables (compare Chapter 5, Section 5.2.5).
Example 5-28

In a metabolic system with conservation relations such as the conservation \( ATP + ADP = \text{const.} \) considered in Example 5-14, it could be of interest to interpret experimental results in terms of the concentration ratio \( X_1 = \frac{ATP}{ADP} \). The vector \( X = (ATP/ADP, \ ADP + ATP)^T \) can formally replace the concentration vector \( S = (ATP \ ADP)^T \). The coefficients expressing the control of reaction \( k \) over the concentration ratio can be expressed in terms of the concentration control coefficients

\[
\frac{\text{non} \ C^{ATP/ADP}_k}{k} = \frac{\partial (ATP/ADP)}{\partial v_k} = \frac{1}{ADP} \frac{\text{non} \ C^{ATP}_k}{k} - \frac{ATP}{ADP^2} \frac{\text{non} \ C^{ADP}_k}{k}. \tag{5-199}
\]

Since \( ATP + ADP = \text{const.} \), it holds that \( \frac{\text{non} \ C^{ATP}_k}{k} = -\frac{\text{non} \ C^{ADP}_k}{k} \), and therefore,

\[
\frac{\text{non} \ C^{ATP/ADP}_k}{k} = \frac{(ATP/ADP)}{ADP^2} \frac{\text{non} \ C^{ATP}_k}{k}. \tag{5-200}
\]

Coefficients for the control of molar free energy differences were defined by Westerhoff and Van Dam (Westerhoff et al. 1987). In non-normalized form, they read

\[
\frac{\text{non} \ C^{AG}_k}{k} = \frac{\partial AG_i}{\partial v_k}. \tag{5-201}
\]

and obey the following summation and connectivity theorems:

\[
\sum_{k=1}^{r} \frac{\text{non} \ C^{AG}_k}{k} \cdot J_k = 0 \tag{5-202}
\]

\[
\sum_{k=1}^{r} \frac{\text{non} \ C^{AG}_k}{k} \cdot v^k_{AG_j} = -\hat{\delta}_{ij}. \tag{5-203}
\]

The quantity \( v^k_{AG_j} \) denotes the elasticity of reaction rate \( \nu_k \) with respect to the free energy difference of reaction \( j \), and \( \delta_{ij} = \begin{cases} 1, & \text{if } i = j \\ 0, & \text{if } i \neq j \end{cases} \) is the Kronecker symbol.

It may also be of interest to study the control over characteristic times of the reaction network. For unbranched reaction sequences like the one presented in Eq. (5-190), the control coefficient for the transition time \( \tau_i \) of metabolite \( S_i \) (given in Eq. (5-109)) can be defined as

\[
C^{\tau_i}_k = \frac{\nu_k \ \hat{\tau}_i}{\tau_i \ \delta v_k}. \tag{5-204}
\]

Their properties have been investigated by Easterby, Melendez-Hevia, Schuster, and others (Easterby 1981, 1990; Melendez-Hevia et al. 1990; Schuster 1996). The following simple relation holds:
\[ C^*_k = C^S_k - C^I_k. \]  

(5-205)

The summation theorem reads

\[ \sum_{i=1}^{r} C^*_k = -1. \]  

(5-206)

This implies that the activation of all enzymes by the same fractional amount reduces the transition time by the same factor. The connectivity theorem for pathways without conservation relations reads

\[ \sum_{i=1}^{r} C^*_k \cdot e^*_j = -\delta_{ij}. \]  

(5-207)

5.3.3.2 Time-dependent Control Coefficients

Time-dependent control coefficients have been introduced for the case that the steady state is not reached within a reasonable time (Heinrich and Reder 1991). They are given as

\[ \text{non}C^I(t) = I + \frac{\partial v}{\partial S} \left( \exp \left( tN \frac{\partial v}{\partial S} \right) - I \right) \left( N \frac{\partial v}{\partial S} \right)^{-1} N \]  

(5-208)

and

\[ \text{non}C^S(t) = \left( \exp \left( tN \frac{\partial v}{\partial S} \right) - I \right) \left( N \frac{\partial v}{\partial S} \right)^{-1} N. \]  

(5-209)

The matrices \( \text{non}C^I(t) \) and \( \text{non}C^S(t) \) are time-dependent operators that transform the initial perturbation of the reaction rates into flux and concentration variations at time \( t \). Consideration of relaxation processes does not change the summation relationships

\[ \text{non}C^I(t) \cdot K = K \]

\[ \text{non}C^S(t) \cdot K = 0 \]  

(5-210)

but affects the connectivity relationships

\[ \text{non}C^I(t) \frac{\partial v}{\partial S} L = \frac{\partial v}{\partial S} L \cdot \exp \left( tN \frac{\partial v}{\partial S} \right) \]

\[ \text{non}C^S(t) \frac{\partial v}{\partial S} L = L \left( \exp \left( tN \frac{\partial v}{\partial S} \right) - I \right) \]  

(5-211)

(Heinrich and Reder 1991). An extension of metabolic control analysis to non-steady-state trajectories is presented by Ingalls and Sauro (2003).
5.3.3.3 Spatially Heterogeneous and Time-varying Cellular Reaction Networks

Peletier et al. (2003) took into account that during signaling processes the assumption of spatial homogeneity (Eq. (5-10)) does not necessarily hold. Instead, the concentrations of activated proteins show temporally changing spatial gradients. These gradients are due to protein diffusion from the receptor to the place of action. They play an important role in the transmission of the respective signal. To tackle this problem, protein concentrations $S_i$ are considered as functions of time and space. They may change due to occurrence of biochemical reactions with the rates $v_j$ or due to diffusion with the diffusion constant $D_i$. The evolution of concentrations is given by the balance equations (Katchalsky and Curran 1965)

$$\frac{\partial S_i}{\partial t} - D_i \Delta S_i = \sum_{j=1}^{r} n_{ij} v_j,$$  \hspace{1cm} (5-212)

where $r$ is the number of reactions, $\Delta = \frac{\partial^2}{\partial x_1^2} + \frac{\partial^2}{\partial x_2^2} + \frac{\partial^2}{\partial x_3^2}$ is the Laplacian operator, and $x_1, ..., x_3$ are the spatial Cartesian coordinates.

In addition to reaction and diffusion, molecules may be transported across system boundaries (the plasma membrane or membranes of organelles) with a flux $f_i$. The quantity $L$ denotes a characteristic length of the cell (e.g., the diameter) that may change due to cell swelling or growth. For modulation of these quantities around a reference state, dimensionless parameters $\alpha$ are introduced, such that

$$v_j \rightarrow \alpha_{vj} \cdot v_j, \quad D_i \rightarrow \alpha_{Di} \cdot D_i, \quad f_i \rightarrow \alpha_{fi} \cdot f_i, \quad \text{and} \quad L \rightarrow \alpha_{L} \cdot L.$$  \hspace{1cm} (5-213)

The parameters $\alpha$ assume the value 1 at the reference state. The control over a steady-state variable $q$ is expressed by the control coefficients

$$C^q_{vj} = \frac{\partial \ln q}{\partial \ln \alpha_{vj}}, \quad C^q_{Di} = \frac{\partial \ln q}{\partial \ln \alpha_{Di}}, \quad C^q_{fi} = \frac{\partial \ln q}{\partial \ln \alpha_{fi}}, \quad \text{and} \quad C^q_{L} = \frac{\partial \ln q}{\partial \ln \alpha_{L}},$$  \hspace{1cm} (5-214)

i.e., by the ratio of the fractional change in $q$ caused by the fractional change in $\alpha$. For systems with transport processes and a non-homogeneous distribution of concentrations, the generalized summation theorem for the steady-state flux takes the form

$$\sum_{j=1}^{r} C^f_{vj} + \sum_{i=1}^{n} C^f_{Di} + \sum_{i=1}^{n} C^f_{fi} = 1,$$  \hspace{1cm} (5-215)

i.e., the total control of 1 on any steady-state flux is shared by all diffusion, reaction, and transport processes.

A further extension of this concept takes into account a dependence on changing conditions in space or time. The space conditions may modify due to cell growth or shrinkage. The dependence of the system on time may be given if initial conditions alter with time. In an analogue way, as in Eq. (5-213), time $t$ is rescaled according to
The generalization of the summation theorem for flux-control coefficients for systems that depend on time reads

\[-C_i^{J(t)} + \sum_{j=1}^{r} C_{ij}^{J(t)} + \sum_{i=1}^{n} C_{Di}^{J(t)} + \sum_{i=1}^{n} C_{fi}^{J(t)} = 1. \] (5-216)

For control over concentrations, the following summation theorem holds:

\[-C_i^{S_k} + \sum_{j=1}^{r} C_{ij}^{S_k} + \sum_{i=1}^{n} C_{Di}^{S_k} + \sum_{i=1}^{n} C_{fi}^{S_k} = 0. \] (5-217)

where $S_k$ is the concentration depending on space and time. The control exerted by cell size is reflected in two further summation theorems, namely, for fluxes:

\[2 \sum_{i=1}^{n} C_{Di}^{J(t)} + \sum_{i=1}^{n} C_{fi}^{J(t)} + C_{L}^{J(t)} = 1. \] (5-218)

For control over concentrations, the following summation theorem holds:

\[2 \sum_{i=1}^{n} C_{Di}^{S_k} + \sum_{i=1}^{n} C_{fi}^{S_k} + C_{L}^{S_k} = 0. \] (5-219)

The theorems introduced by Peletier et al. (2003) are well suited for application in signaling networks.

**Suggested Further Reading**

References


References


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