Vorlesung "Modellierung von Zellprozessen"

Wolfram Liebermeister, WS 2009/2010

3 Metabolic Models

3.1 Kinetic models and their dynamic behaviour in a nutshell

Examples: Metabolic network; transport reactions; signaling system; gene expression

Stoichiometric coefficients n_{ir}
Stoichiometric coefficients denote the proportions, with which the molecules of substrates and products enter the biochemical reactions. One can summarise the stoichiometric coefficients in a matrix $\boldsymbol{N}$. The rows refer to the substances, the columns refer to the reactions. External metabolites are usually not included in $\boldsymbol{N}$.

ODE system for internal substance concentrations

$$\frac{\mathrm{d}c_i(t)}{\mathrm{d}t} = \sum_r n_{ir} v_r(c(t)) \tag{1}$$

- Stoichiometric matrix $\boldsymbol{N} = n_{ir}, i = 1...n; r = 1...m.$
- Vector of metabolite concentrations $c = (c_1, ..., c_n)^{\mathrm{T}}$
- Vector of reaction rates $v = (v_1, ..., v_m)^{\mathrm{T}}$
- Parameter vector $p = (p_1, ..., p_q)$
- External substance concentrations are fixed ($c_{\mathrm{ext}} = const$) or predefined as time series ($c_{\mathrm{ext}} = c_{\mathrm{ext}}(t)$)
- Coupling with other variables (e.g., cell volume) → may lead to additional terms

Solution determined by:

- Parameters given (possibly time-dependent → non-autonomous ODE system)
- Initial conditions
- Variables (concentrations, fluxes) are then determined by system equations

Limitations of kinetic models:

- No spatial resolution (other possibilities: (i) Compartment models; (ii) Reaction-diffusion models (partial differential equations))
- Deterministic (other possibilities: (i) Nondeterministic model containing only constraints, describing not facts, but possibilities. (ii) Stochastic model, describing random events. Here, every simulation is different!)

Dynamic analysis: An ODE model (or biochemical system structure).

- **Simulation:**
  What behaviour will the system show for a certain choice of parameters and initial conditions?
- **Qualitative behaviour/bifurcations**
  What behaviour will the system show for which parameter choices?
- **Sensitivity/MCA**: How does the behaviour change due to small parameter perturbations?
- **Structure and behaviour**: How do the network structure and the form of the system equations predetermine the behaviour (irrespective of the parameter choice)?

3.2 Substance with simple production and degradation

One way to define time constants is by observing a system's relaxation to steady state like in the following example. We consider a substance that is produced at a rate v and linearly degraded with rate constant λ; its concentration s satisfies the rate equation

$$\frac{\mathrm{d}s(t)}{\mathrm{d}t} = v(t) - \lambda\, s(t). \tag{2}$$

If the production rate v is constant, the the concentration s will relax from an initial value $s(0) = s_0$ to its steady state value $s^{\mathrm{st}} = v/\lambda$ according to

$$s(t) = s^{\mathrm{st}} + (s_0 - s^{\mathrm{st}})\mathrm{e}^{-\lambda t} \tag{3}$$

We define the response time $\tau = 1/\lambda$ as the time at which the initial deviation $\Delta s(t) = s(t) - s^{\mathrm{st}}$ from the steady state has decreased by a factor 1/e. The response time is closely related to the response half time $\tau_{(1/2)} = \ln 2/\lambda$, at which half of the relaxation has been reached.

3.3 Kinetic models with varying volumes

If volumes are changing, we need to pay some attention to the correct conversion between substance amounts and concentrations.

Balance equations for amounts and concentrations
It is practical to start with the amounts a_i (where the subscript i indicates a substance localised in a compartment). With the reaction velocities v^* (in mol/s), we obtain the rate equation

$$\frac{\mathrm{d}a_i}{\mathrm{d}t} \quad = \quad \sum_r n_{ir}\; v_r^*(a). \tag{4}$$

Each amount a_i is defined in a compartment with volume V_i (of course: same volumes for all substances in the same compartment). After introducing the concentrations $c_i = a_i/V_i$, we can rewrite the time derivative in Eq. (4) as

$$\frac{\mathrm{d}a_i}{\mathrm{d}t} \quad = \quad \frac{\mathrm{d}}{\mathrm{d}t}(V_i\, c_i) = V_i\, \frac{\mathrm{d}c_i}{\mathrm{d}t} + \frac{\mathrm{d}V_i}{\mathrm{d}t}\, c_i \tag{5}$$

By solving for $\frac{\mathrm{d}c_i}{\mathrm{d}t}$ and inserting equation (4), we obtain the rate equation for concentrations

$$\frac{\mathrm{d}c_i}{\mathrm{d}t} = \sum_r \frac{n_{ir}}{V_i}\; v_r^*(\boldsymbol{s}) - \frac{\mathrm{d}V_i/\mathrm{d}t}{V_i} c_i. \tag{6}$$

Concentration changes can be caused by chemical reactions (first term) and volume changes (second term).

If all compartments in Eq. (6) have the same, time-independent volume, we can replace v_l^*/V_i by the usual reaction velocity v_r in mM/s; the second term vanishes, so we obtain the usual form of kinetic models.

Transport of a substance between compartments of different volume
If the volume sizes are constant in time, the second term vanishes and we obtain

$$V_1 \frac{\mathrm{d}c_1}{\mathrm{d}t} = -V_2 \frac{\mathrm{d}c_2}{\mathrm{d}t}. \tag{7}$$

The minus sign stems from the stoichiometric coefficients. The volumes play an important role in transport between cells and the external medium: intra- and extracellular concentrations are converted to each other by the volume ratio $V_{\mathrm{cell}}/V_{\mathrm{ext}}$, where V_{cell} is the volume of a single cell and V_{ext} denotes the extracellular volume divided by the number of cells.

Effect of volume changes The second term in Eq. (6) describes the effect of temporal volume changes: substances in growing cells are diluted, so their concentration will decrease even if they are not consumed by chemical reactions. If a cell population grows at a rate $\mu(t)$, the total cell volume V increases according to

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \mu(t)V, \tag{8}$$

so the prefactor in the second term in Eq. (6) is just the growth rate $\mu(t)$. The dilution of molecules in a growing cell population formally resembles linear degradation, with the cell growth rate μ appearing as an effective degradation rate.

3.4 Stationary states

3.4.1 Stoichiometric Analysis

We asssume that the steady state condition

$$0 = \boldsymbol{N} v(s, p) \tag{9}$$

has a solution $s = s(p)$. In this case, stationary fluxes are given by $J(p) = v(s(p), p)$; steady-state metabolite concentrations s and fluxes J depend on kinetic parameters.

The big problem
The equation system $\boldsymbol{N} v(c, p) = 0$ is usually nonlinear in c and cannot be solved analytically. In any case, the solution requires knowledge of the kinetic equations $v(\cdot, \cdot)$. What can we learn from the network structure alone?

Stoichiometric Analysis

- Is only based on the chemical reaction sum formulas, i.e. the stoichiometric matrix
- Characterises the reaction network
- Yields information about possible pathways, stationary flux distributions, and substance conversions
- Enzyme kinetics are not considered
- If flux directions are known, they can be enforced
  Remark: physically, every reaction is reversible; the direction depends on the metabolites' chemical potentials (see below). In living cells - physiologically - some reaction directions are restricted, and the chemical potentials are adjusted to ensure the "right" direction.

3.4.2 Stationary flux distributions and the kernel matrix K

In a stationary state, substance concentrations must be constant in time (by definition!). For the internal metabolites, this means that incoming and outgoing fluxes must be balanced. Mathematically, we require $\boldsymbol{N} v = 0$ (where the rows of $\boldsymbol{N}$ refer to the internal metabolites only).

The kernel matrix $\boldsymbol{K}$
Non-trivial solutions (called "stationary flux distributions") v can only exist if the columns of $\boldsymbol{N}$ are linearly dependent ($Rank(\boldsymbol{N}) < m$, number of reactions). Mathematically, the linear dependencies can be expressed in the form $\boldsymbol{N}\boldsymbol{K} = 0$ with a *kernel matrix* $\boldsymbol{K}$. Each of its columns k ("kernel vectors", "nullspace vectors", "stationary flux distributions") satisfies the equation $\boldsymbol{N}k = 0$. The dimension of the null space ("kernel") of $\boldsymbol{N}$, i.e., the number of basis vectors is $m - Rank(\boldsymbol{N})$.

Calculation of $\boldsymbol{K}$
The kernel matrix $\boldsymbol{K}$ can be calculated with the Gaussian elimination algorithm for the solution of homogeneous linear equation systems. (In practice, simply calculate $\boldsymbol{K}$ with computer programmes!)

Representation of $\boldsymbol{K}$: The kernel matrix $\boldsymbol{K}$ is not uniquely determined. A matrix $\boldsymbol{K'} = \boldsymbol{K}\boldsymbol{Q}$ containing linear combinations of the original columns is also a possible solution, i.e., matrix multiplication with a regular matrix $\boldsymbol{Q}$ from right yields another kernel matrix. For some applications one needs a simple ("canonical") representation of the kernel matrix (with integer and many zero entries).

Informations contained in kernel matrix $\boldsymbol{K}$

- **Admissible fluxes in steady state** are given by columns k and their linear combinations
- **Equilibrium reactions** If all elements of a row in $\boldsymbol{K}$ vanish, then the respective reaction is in equilibrium in every steady state.
- **Unbranched reaction sequences** A column k (and any stationary flux vector v) has the same entry for all reactions forming an unbranched reaction sequence. Such unbranched reaction sequences can be lumped (= replaced by a single reaction) for further analysis.
- **Elementary modes** are stationary flux modes with a maximal number of zero entries. Irreversible reactions can be considered (by choosing the right reaction direction in the elementary modes and requiring positive coefficients in the linear combinations).

3.4.3 Constraint-based flux optimization

Constraint-based flux prediction methods are the most popular modeling approaches for metabolic systems. Flux optimization methods do not describe *how* a certain flux distribution is realised (by kinetics or enzyme regulation), but *which* flux distribution yields an optimal result for the cell - e.g. the highest rate of biomass production at a limited inflow of external nutrients.

Even if we do not know anything about the enzyme kinetics, we can predict which metabolites the network can produce and which precursors are needed to produce biomass. Given a number of nutrients and a optimality requirement (e.g. for fast biomass production), we can try to predict an optimal flux distribution in the network.

Flux balance analysis Flux balance analysis is based on a number of simplifying model assumptions (which are not always justified!):

1. A steady state in which all internal metabolites are balanced:

$$\boldsymbol{N}v = 0. \tag{10}$$

   (Assumption possibly valid at certain timescales, for the average over a cell population) Also note that for the stationarity condition, external metabolites have to be defined. One possibility is to consider the extracellular metabolites external and to assume a number of transport reactions in the model.

2. Irreversibility of certain reactions under physiological conditions; this yields predefined signs for some fluxes v_r (Assumption based on thermodynamics and physiology).

3. Upper bounds on certain reaction fluxes (justified by limited capacity of enzyme molecules and need for keeping enzyme levels low). For example, in the case of a Michaelis-Menten-type enzyme, the reaction rate is limited by the maximal rate set by the enzymes' concentration and turnover number. In general, the constraints on individual metabolic fluxes read

$$v_r^{\min} \leq v_r \leq v_r^{\max}. \tag{11}$$

Partial perturbation of enzymes can be modelled by tighter maximality constraints leading to reduced maximal rates.

4. Together, these constraints confine the steady-state fluxes to a feasible set (a convex polyhedron in flux space), but usually do not yield a unique solution. Thus, as a fourth requirement, an optimality assumption is added: the flux distribution has to maximise an objective function $f(v)$

$$\max \stackrel{!}{=} f(v) = \sum_{i=1}^{r} z_r \, v_r \tag{12}$$

where the coefficients z_r represent weights for the individual rates v_r. Examples of such objective functions are maximization of ATP production, minimization of nutrient uptake, maximal yield of a desired product, maximal biomass yield, or a combination thereof.

The above assumptions lead to a linear programming problem with constraints

$$\begin{array}{rcl} z^{\mathrm{T}} \, v & \stackrel{!}{=} & \max \\ \boldsymbol{N} v & = & 0 \\ \begin{pmatrix} \boldsymbol{I} \\ -\boldsymbol{I} \end{pmatrix} v & \geq & \begin{pmatrix} v^{\min} \\ -v^{\max} \end{pmatrix}. \end{array} \tag{13}$$

The latter inequality represents the constraints (11). This is a standard problem of linear optimization which can be solved by the simplex algorithm.

Geometric interpretation of flux balance analysis We can imagine each possible flux distribution v as a point in a multidimensional flux space. Each dimension in this space corresponds to a reaction in the network and represents its velocity (see Figure 1).

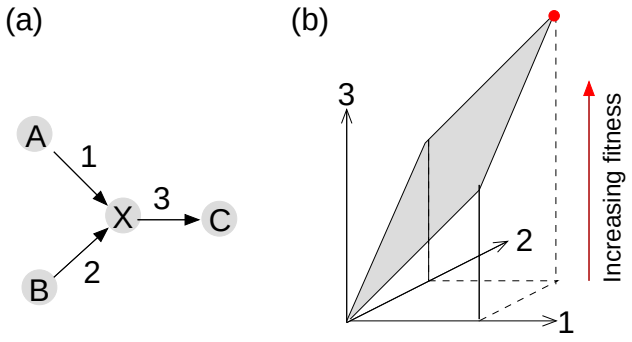
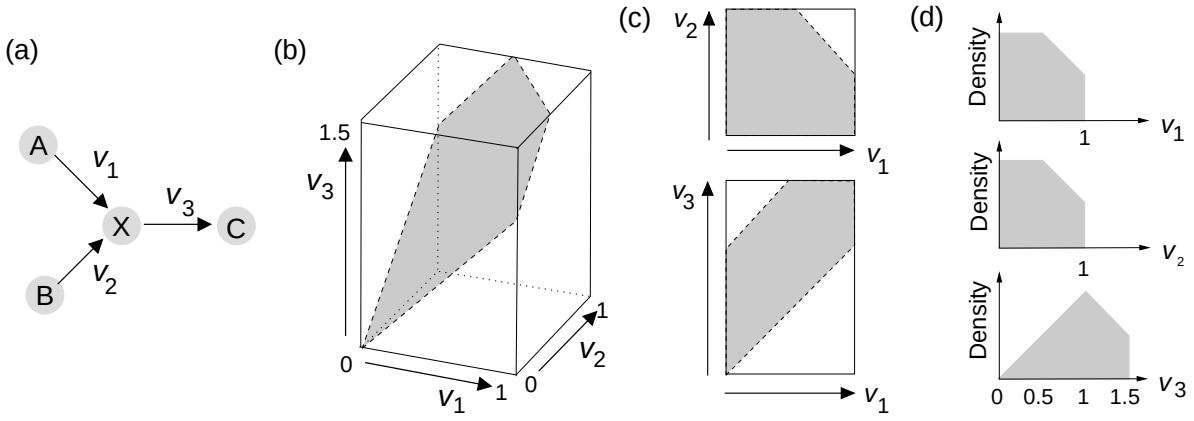


Figure 1: Geometric interpretation of flux balance analysis for a simple example system. (a) Simple metabolic branch point (letters denote metabolites, numbers reactions). In stationary state, the internal metabolite X must be balanced, so $v_1 + v_2 = v_3$. (b) Geometric interpretation: the feasible fluxes form a two-dimensional rhombus in $\mathbb{R}^3$. The fitness function $f = v_3$ in this example is maximised in the upper corner (red dot).

The stationary fluxes, which are constrained by the linear equations $\boldsymbol{N}v = 0$, form a hyperplane. If all individual fluxes are constrained by lower and upper bounds, the resulting region of allowed flux distributions is a convex polyhedron (Figure 1 (b)): any combination $\lambda v_\alpha + (1-\lambda) v_\beta$ of two allowed flux distributions v_α and v_β with $0 \leq \lambda \leq 1$ is again an allowed flux distribution.

Flux balance analysis maximises a linear function within this polyhedron. The optimum has to lie somewhere on the surface: depending on the direction of the fitness gradient, there is a unique optimum in a corner of the polyhedron, or the fitness function is maximised on an entire surface.



Flux sampling The pictures above show how stationarity and lower and upper bounds can define a convex set of admissible flux distributions in flux space. Uniform sampling of this flux set yields non-uniform distributions of individual reaction fluxes (right).

3.5 Linear conservation relations and the left-kernel matrix G

If compounds or chemical groups do not enter or leave a reaction system, their total amount must remain constant.

Examples:

- Michaelis-Menten kinetics $\frac{\mathrm{d}}{\mathrm{d}t}([ES]+[E])=0$
  Relation $\rightarrow [ES]+[E]=\text{const.}$
- Isolated reaction 2 A $\leftrightarrow$ 3 B
  Relation $2[A]+3[B]=\text{const.}$.
- Several reactions: Pyruvate kinase (ATP production), Na/K-ATPase (ATP consumption)
  Relation [ATP] + [ADP] = const..

Linear conservation relations - calculation If there exist linear dependencies between the rows of the stoichiometric matrix $\boldsymbol{N}$, then one can find a *left kernel matrix* $\boldsymbol{G}$ satisfying $\boldsymbol{G}\,\boldsymbol{N}=0$. We obtain

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}c &= \boldsymbol{N}v\\ \boldsymbol{G}\frac{\mathrm{d}}{\mathrm{d}t}c &= \boldsymbol{G}\,\boldsymbol{N}v=0\end{aligned}$$

Integration of this equation yields the conservation relations

$$\boldsymbol{G}\,c=\text{const.}$$

All linear conservation relations can be determined from the matrix $\boldsymbol{G}$.

The number of independent row vectors g (= number of independent conservation relations) is given by $n-$ Rank($\boldsymbol{N}$) (n = number of rows of the stoichiometric matrix = number of metabolites) $\boldsymbol{G}^{\mathrm{T}}$ is the right kernel matrix of $\boldsymbol{N}^{\mathrm{T}}$, and can be found in the same way as $\boldsymbol{K}$ (Gaussian elimination algorithm).

Again, the matrix $\boldsymbol{G}$ is not unique: $\boldsymbol{G}' = \boldsymbol{P}\,\boldsymbol{G}$ with a regular quadratic matrix $\boldsymbol{P}$ is also conservation matrix.

If the conservation relations are supposed to contain external metabolites, then the total stoichoimetric matrix $\boldsymbol{N}^{\mathrm{tot}}$ has to be used.

3.6 Thermodynamic conditions for kinetic models

3.6.1 Chemical potentials and reaction rates

According to the second law of thermodynamics, chemical reactions at constant pressure p and temperature T need to be driven by a consumption of Gibbs free energy $G(p,T)$. The Gibbs free energy of a biochemical system at given pressure p and temperature T is associated with the amount and types of molecules via

$$G = \sum_i n_i \mu_i \tag{14}$$

where μ_i and n_i, respectively, denote the the chemical potential and the amount (in mol) of substance i. The chemical potential of substance i is defined as

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right) \tag{15}$$

where all other substance amounts (as well as pressure p and absolute temperature T) are kept constant.

Chemical potentials and concentrations The chemical potentials are related to the substance concentrations: for an ideal mixture (with vanishing mixing enthalpy), the chemical potential of substance i at pressure p and temperature T reads

$$\mu_i(p,T) = \mu_i^0(p,T) + RT \ln c_i, \tag{16}$$

where c_i denotes the concentration of metabolite i in mM and $R \approx 8.3 J/(K mol)$ is Boltzmann's gas constant.

Chemical potential balances A chemical reaction will change the substance amounts according to the stoichiometric coefficients n_{ir}, and the resulting Gibbs free energy change (in kJ per mole reaction events) can be expressed by the chemical potential difference

$$\Delta_{\mathrm{r}}\mu_r = \sum_i \mu_i \; n_{ir}. \tag{17}$$

The symbol Δ_{r} denotes changes associated with chemical reactions. Important: here, we consider *all* metabolites (internal and external ones), i.e., we use the full stoichiometric matrix $\boldsymbol{N}^{\mathrm{tot}}$

The negative value $A_r = \Delta_{\mathrm{r}}\mu_l$, called the *reaction affinity*, can be seen as the thermodynamic driving force. The vector of chemical potential differences satisfies the condition

$$(\Delta_{\mathrm{r}}\mu)^{\mathrm{T}}\boldsymbol{K} = 0. \tag{18}$$

where $\boldsymbol{K}^{\mathrm{tot}}$ is a right kernel matrix of the total stoichiometric matrix $\boldsymbol{N}^{\mathrm{tot}}$, satisfying $\boldsymbol{N}^{\mathrm{tot}}\,\boldsymbol{K}^{\mathrm{tot}} = 0$.

Proof? (This equation is an example of a *Wegscheider condition* imposed by the network structure).

Chemical potentials and reaction directions As stated above, the Gibbs free energy must decrease in any occuring reaction, so for a forward reaction, the difference of chemical potentials (17) must be negative. In general, for a reaction r, we obtain the condition

$$\Delta\mu_r v_r \quad = \quad \sum_i \mu_i \; n_{ir} \; v_r \leq 0 \tag{19}$$

Consequences for flux prediction:

- A given flux pattern $v = (v_1, ..., v_n)^{\mathrm{T}}$ is only feasible if condition (19) can be satisfied by some vector $(\mu_1, ..., \mu_m)$ of chemical potentials.
- If the standard chemical potentials μ_i^0 are known (e.g. calculated by the group contribution method), Eq. (19) translates into constraints between flux directions and substance concentrations.

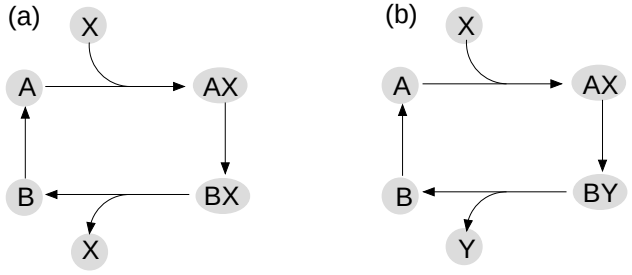


Figure 2: Unfeasible and feasible flux distribution. (a) The circular flux is infeasible because the total balance of all metabolite numbers in this flux is zero, and therefore no Gibbs free energy is consumed. In terms of chemical potentials, a forward flux would require $\mu_A + \mu_X > \mu_{AX} > \mu_{BX} > \mu_B + \mu_X$, as well as $\mu_B > \mu_A$, which leads to a contradiction. (b) Feasible flux distribution: with chemical potentials $\mu_X > \mu_Y$, Gibbs free energy is consumed and can drive the reactions.

3.6.2 Example: chemical potentials, concentrations, and flux direction

Example reaction: A + B $\leftrightarrow$ C

The mass-action kinetics would read

$$v = k_+ \, a \, b - k_- \, c. \tag{20}$$

In chemical equilibrium, it holds that

$$0 = v = k_+ \, a^{\mathrm{eq}} \, b^{\mathrm{eq}} - k_- \, c^{\mathrm{eq}}, \tag{21}$$

which fixes the equilibrium constant (defined as $k^{\mathrm{eq}} = \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}} \, b^{\mathrm{eq}}}$) to a value of

$$k^{\mathrm{eq}} = \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}} \, b^{\mathrm{eq}}} = \frac{k_+}{k_-} \tag{22}$$

and we obtain the relationship between rate and concentrations:

$$\frac{c}{a \, b} > \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}} \, b^{\mathrm{eq}}} \qquad \Rightarrow \qquad v < 0 \tag{23}$$

$$\frac{c}{a \, b} = \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}} \, b^{\mathrm{eq}}} \qquad \Rightarrow \qquad v = 0 \tag{24}$$

$$\frac{c}{a \, b} < \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}} \, b^{\mathrm{eq}}} \qquad \Rightarrow \qquad v > 0 \tag{25}$$

$$\tag{26}$$

The ratio $\frac{c}{a \, b}$ is called *mass action ratio*.

Chemical potentials for an ideal mixture

$$\begin{aligned} \mu_A &= \mu_A^0 + RT \ln a \\ \mu_B &= \mu_B^0 + RT \ln b \\ \mu_C &= \mu_C^0 + RT \ln c \end{aligned}$$

Chemical potential difference

$$\begin{aligned}\Delta_{\mathrm{r}}\mu &= -\mu_A - \mu_B + \mu_C \\ &= -(\mu_A^0 + RT\ln a) - (\mu_B^0 + RT\ln b) + (\mu_C^0 + RT\ln c) \\ &= (-\mu_A^0 - \mu_B^0 + \mu_C^0) + RT(-\ln a - \ln b + \ln c) \\ &= \Delta_{\mathrm{r}}\mu^0 + RT\ln\frac{c}{a\,b}\end{aligned}$$

In equilibrium, $\Delta_{\mathrm{r}}\mu$ must vanish, so

$$0 = \Delta_{\mathrm{r}}\mu \quad = \quad \Delta_{\mathrm{r}}\mu^0 + RT\ln\frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}}\,b^{\mathrm{eq}}}$$

so the standard chemical potential difference can be written as

$$\Delta_{\mathrm{r}}\mu^0 = -RT\ln\frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}}\,b^{\mathrm{eq}}} = -RT\,\ln k^{\mathrm{eq}}$$

Inserting this above yields

$$\begin{aligned}\Delta_{\mathrm{r}}\mu &= -RT\,\ln k^{\mathrm{eq}} + RT\ln\frac{c}{a\,b} \\ &= RT[\ln\frac{c}{a\,b} - \ln k^{\mathrm{eq}}] \\ &= RT[\ln\frac{\frac{c}{a\,b}}{k^{\mathrm{eq}}}]\end{aligned}$$

A comparison to the equilibrium constant (mass action ratio in chemical equilibrium) determines the flux directions:

1. **Chemical equilibrium:**

$$\frac{c}{a\,b} = \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}}\,b^{\mathrm{eq}}} = k^{\mathrm{eq}} \qquad \Rightarrow \qquad \Delta_{\mathrm{r}}\mu = 0 \qquad \Rightarrow \qquad \Delta_{\mathrm{r}}v = 0$$

   Concentrations at equilibrium, no driving force, no net reaction

2. **Forward reaction (more substrate)**

$$\frac{c}{a\,b} < \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}}\,b^{\mathrm{eq}}} = k^{\mathrm{eq}} \qquad \Rightarrow \qquad \Delta_{\mathrm{r}}\mu < 0 \qquad \Rightarrow \qquad \Delta_{\mathrm{r}}v > 0$$

   Mass action ratio below equilibrium constant, positive driving force, forward net reaction

3. **Reverse reaction (more product)**

$$\frac{c}{a\,b} > \frac{c^{\mathrm{eq}}}{a^{\mathrm{eq}}\,b^{\mathrm{eq}}} = k^{\mathrm{eq}} \qquad \Rightarrow \qquad \Delta_{\mathrm{r}}\mu > 0 \qquad \Rightarrow \qquad \Delta_{\mathrm{r}}v < 0$$

   Mass action ratio above equilibrium constant, negative driving force, backward net reaction

This behaviour is realised by the mass-action kinetics (see above) and by any thermodynamically correct rate law.

Application: Energy Balance Analysis Flux balance analysis does not require that condition (19) is fulfilled and can therefore lead to incorrect flux signs. This problem can be avoided by fixing some of the flux directions, which will then restrict the solution space in advance.

Energy balance analysis, in contrast, ensures thermodynamically feasible fluxes by a joint optimization of the fluxes v_r and the chemical potential differences $\Delta_{\mathrm{r}}\mu$. Besides the conditions (13), it imposes the additional requirements (19) and (18), which leads to an optimization problem for v and $\Delta_{\mathrm{r}}\mu$ with nonlinear constraints (much harder to solve!).