Program for the rest of the course

- 16.4 Enzyme kinetics
- 17.4 Metabolic Control Analysis
- 19.4. Exercise session 5
- 23.4. Metabolic Control Analysis, cont.
- 24.4 Recap
- 27.4 Exercise session 6

How does an enzyme work?

An enzyme works by binding the substrate molecules into the so called active site. In the active site, the substrates end up in such a mutual geometric conformation that the reaction occurs effectively.

The occurence of the reaction causes the enzyme to change its conformation, which releases the products. After that, the enzyme is ready to bind another set of substrates. The enzyme itself stays unchanged in the reaction.

Enzyme activity

The rate of certain enzyme-catalyzed reaction depends on the concentration (amount) of the enzyme and the specific activity of the enzyme (how fast a single enzyme molecule works).

The specific activity of the enzyme depends on

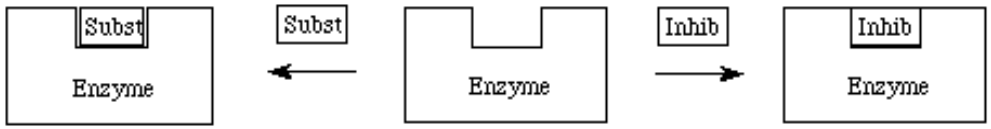
- pH and temperature
- positively on the concentration of the substrates
- negatively on the concentration of the end-product of the pathway (inhibition).

Note that transcription level gene regulation **directly** affects only the concentration of the enzyme.

Inhibition of Enzymes

The activity of enzymes is regulated in the metabolic level by inhibition: certain metabolites bind to the enzyme hampering its ability of catalysing reactions.

Inhibition can be competitive or non-competitive. In competitive inhibition, the inhibitor allocates the active site of the enzyme, thus stopping the substrate from entering the active site.



In non-competitive inhibition, the inhibitor molecule binds to the enzyme outside the active site, causing the active site to change conformation and making the catalysis less efficient.

Enzyme kinetics

Enzyme kinetics, the study of dynamic properties of enzymatic reaction systems, dates back over 100 years, 50 years prior to the discovery of DNA structure.

Via enzyme kinetics one aims for accurately predicting the behaviour of a enzymatics reaction system. In particular we might be interested in preicting the reaction rate of some enzymatic reaction.

The quantities of interest in a deterministic kinetic model of an individual biochemical reaction are

- Concentration S of substance S (slight abuse of notation): the number n of molecules of the substance per volume V, and
- The rate v of a reaction (the change of concentration per time t)

Modeling assumptions

The following simplifying assumptions are made

- Individual molecules are not considered, we assume that there are enough of the molecules of the substance so that the average behaviour of the molecules can be captured by the model
- We will assume spatial homogeneity, i.e. the concentration of S does not depend on the physical location in the cell or cell population
- The rate v is not directly dependent on time, only via the concentration: $v(t) = v(S(t))$, i.e. the system is assumed to have "no memory".

Law of mass action (1/3)

Law of mass action is one of the most fundamental and very well known kinetic model for a reaction

It is based on the following ideas:

- In order a reaction to happen, the reactants need to meet, or collide
- Assuming the molecules are well-mixed, the likelihood (or frequency) of a single molecule to occupy a certain physical location is proportional to its concentation
- Assuming the molecules occupy the locations independently from each other, the probability of two molecules (e.g. a reactant and an enzyme) to meet is proportional to the *product* of their concentrations.

Law of mass action (2/3)

Consider a reaction of the form

$$S_1 + S_2 \rightleftharpoons P_1 + P_2$$

Under the Law of mass action, the reaction rate satisfies

$$v = v_+ - v_- = k_+ S_1 \cdot S_2 - k_- P_1 \cdot P_2$$

where v_+ is the rate of the forward reaction, v_- is the rate of the backward reaction, and k_+, k_- are so called rate constants.

The general law of mass action for q substrates and r products follows the same pattern: $v = k_+ S_1 \cdots S_q - k_- P_1 \cdots P_r$

Law of mass action (3/3)

From the law of mass action,

$$v = k_+ S_1 \cdot S_2 - k_- P_1 \cdot P_2$$

we can deduce that the net rate of the reaction satisfies

- $v > 0$ if and only if $\frac{P_1 \cdot P_2}{S_1 \cdot S_2} < \frac{k_+}{k_-}$,
- $v = 0$ if and only if $\frac{P_1 \cdot P_2}{S_1 \cdot S_2} = \frac{k_+}{k_-}$, and
- $v < 0$ if and only if $\frac{P_1 \cdot P_2}{S_1 \cdot S_2} > \frac{k_+}{k_-}$.

Thus the reaction seeks to balance the concentrations of substrates and products to a specific constant ratio.

COPASI Simulation: Law of mass action

Equilibrium constant

When

$$v = v_+ - v_- = 0,$$

that is, the forward and backward rates are equal, we say that the reaction is in equilibrium.

From the law of mass action, we find that this happens when the reactant and product concentrations satisfy

$$\frac{P_1 \cdots P_r}{S_1 \cdots S_q} = \frac{k_+}{k_-} = K_{eq},$$

where K_{eq} is the so called equilibrium constant.

In practise, K_{eq} is an unknown parameter that only can be estimated.

Change of free energy

Whether a reaction occurs spontaneously, is coverned by the change of free energy

$$\Delta G = \Delta H - T\Delta S$$

- $H = U + PT$ is the enthalpy, where U is the internal energy of the compound (sum of kinetic energy of the molecule and energy contained in the chemical bonds and vibration of the atoms), P is pressure and T is the temperature (typically constant)
- ΔS is the change in entropy (disorder of the system)

Free energy and reactions

If

- $\Delta G < 0$, the reaction proceeds spontaneusly and releases energy.
- $\Delta G = 0$, the reaction is in equilibrium
- $\Delta G > 0$, the reaction will not occurr spontaneusly. The reaction can only happen if it obtains energy

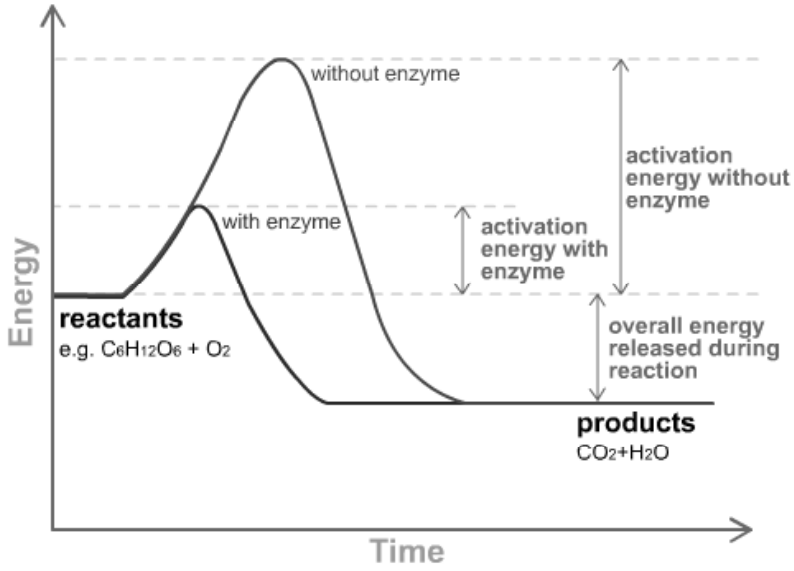
Roughly stated, the likelihood of a reaction occurring spontaneuosly is the larger

- the more it decreases the internal energy of the system
- the more it increases entropy of the system

Role of enzymes

Typically reactions involve transition states that are energetically unfavourable, that is the ΔG to the transition state requires energy input.

- An enzyme cannot change the free energy of the reactants of products, nor their difference
- Instead, the enzyme changes the reaction path so that the high energy transition state is avoided, and the reaction proceeds more easily



Kinetic model of an enzymatic reaction

The kinetic equation for an enzymatic reaction typically involves an intermediary state where the substrate S is bound to an enzyme E, forming a complex ES.

A simple model of a irreversible enzymatic reaction is

$$E + S \rightleftharpoons ES \rightarrow E + P$$

Each of the individual reaction steps have their own kinetic parameters k_1, k_{-1} for the forward and backward reaction of the first (reversible) step and k_2 for the second (irreversible) step

Kinetic model of an enzymatic reaction

The rate of change of the compounds are given by ordinary differential equations (ODE):

$$\frac{dS}{dt} = -k_1 E \cdot S + k_{-1} ES$$

$$\frac{dES}{dt} = k_1 E \cdot S - (k_{-1} + k_2) ES$$

$$\frac{dE}{dt} = -k_1 E \cdot S + (k_{-1} + k_2) ES$$

$$\frac{dP}{dt} = k_2 ES$$

The reaction rate satisfies:

$$v = -\frac{dS}{dt} = \frac{dP}{dt}$$

Unfortunately, the above system cannot be solved analytically, hence the reaction rate cannot be simply computed

Michaelis-Menten kinetics

The reaction rate becomes solvable if a simplifying assumption is made that the concentration of enzyme-substrate complex is approximately constant remains constant, or equivalently

$$\frac{dES}{dt} = 0$$

Denoting $E_{total} = E + ES$, from

$$\frac{dES}{dt} = k_1 E \cdot S - (k_{-1} + k_2)ES$$

we obtain

$$0 = \frac{dES}{dt} = k_1(E_{total} - ES) \cdot S - (k_{-1} + k_2)ES$$

which can be solved for ES:

$$ES = \frac{k_1 E_{total} S}{S + (k_{-1} + k_2)/k_1}$$

Michaelis-Menten kinetics

For the reaction rate $v = \frac{dP}{dt} = k_2 ES$ we obtain:

$$v = \frac{k_2 E_{total} S}{S + (k_{-1} + k_2)/k_1} = \frac{V_{max} S}{S + K_m}$$

This equation is the expression for Michaelis-Menten kinetics.

- $V_{max} = k_2 E_{total}$ is the *maximum velocity* obtained when the substrate completely saturates the enzyme and
- $K_m = (k_{-1} + k_2)/k_1$ is called the Michaelis constant

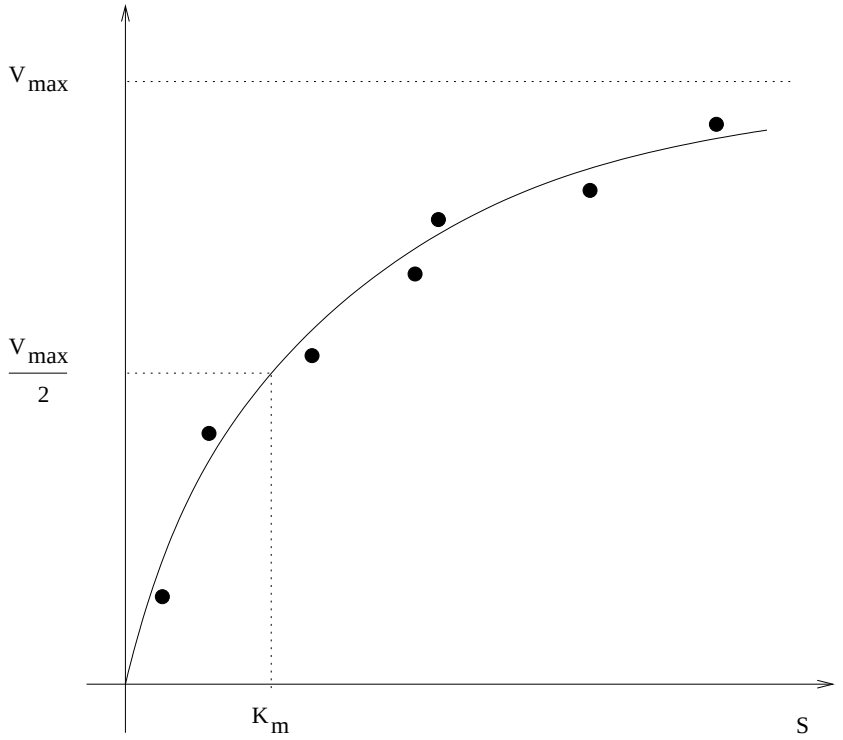
Parameters of Michaelis-Menten model

Values for K_m and V_{max} can be estimated for an isolated enzyme (in test tube) by measuring the initial rates given different initial concentrations S.

This yields a concave curve that tends asymptotically to V_{max} as the function of initial concentration S.

K_m is the concentration of S where the curve intersects $V_{max}/2$ which can be seen by substituting $S = K_m$ in the rate equation

$$v = \frac{V_{max} S}{S + K_m}$$



Reversible reaction

In practice most biochemical reactions are reversible:

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

with four rate constants k_1, k_{-1}, k_2, k_{-2} corresponding to the forward and backward direction of the first and second reaction step, respectively

The following expression for the reaction rate can be obtained

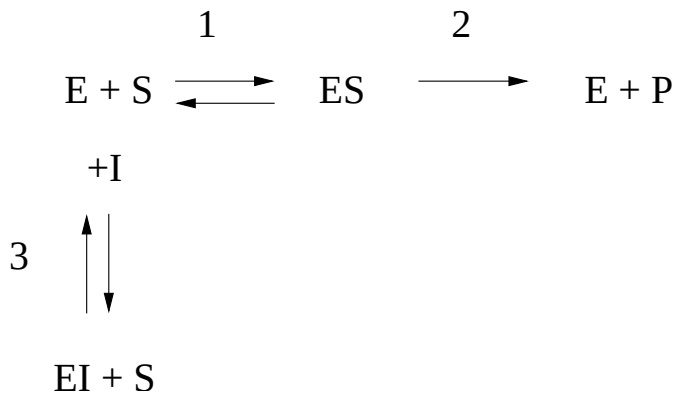
$$v = \frac{V_{max}^{for}/K_{mS}S - V_{max}^{back}/K_{mP}}{1 + S/K_{mS} + P/K_{mP}},$$

where $V_{max}^{for}, V_{max}^{back}$ are maximum rates in forward and backward directions and K_{mS} and K_{mP} are concentrations causing half the maximal rate.

Kinetics of competitive inhibition

In competitive inhibition

- the inhibitor only can bind to the free enzyme E, not to the complex ES
- product is released from the ES complex



Rate equation:

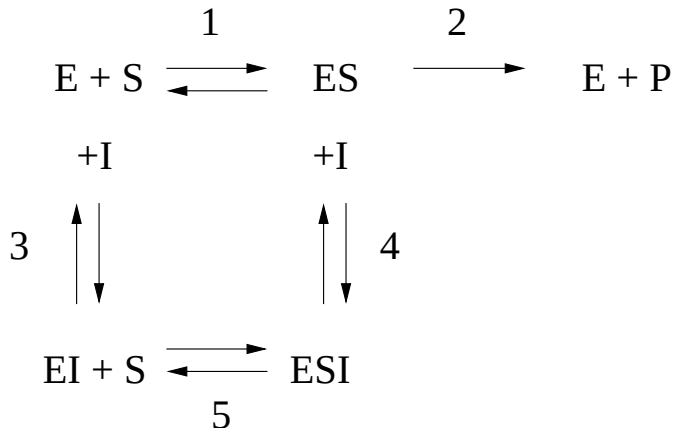
$$v = \frac{V_{max} S}{K_m(1 + I k_3/k_{-3}) + S}$$

COPASI Simulation: competitive inhibition

Kinetics of non-competitive inhibition

In non-competitive inhibition

- the inhibitor can bind both to the free enzyme E and the complex ES
- product is released from the ES complex
- The rate constants for step (3) and (4) are assumed to be equal



Rate equation:

$$v = \frac{V_{max} S}{(K_m + S)(1 + I k_3 / k_{-3})}$$

Problems of mechanistic kinetic models

While mechanistic kinetic models are the most faithful models to the biochemistry, they have several drawbacks:

- A mechanistic model even for a small system becomes complicated, and analytical solution of the reaction rates is not possible, instead we have to resort to numerical simulation.
- Kinetic parameters are too many to be reliably estimated from restricted number of experiments
- Values estimated for isolated enzymes (in test tube) may not reflect the reality in the living cell, thus the predictions of the model may have significant biases

Metabolic Control Analysis (MCA)

So far, we have looked at metabolism from to extreme views:

- Kinetic modeling, which aims at accurate mechanistic models of enzymatic reactions. Limited to small systems in prectise
- Steady-state flux analysis, where large systems can be studied but in a limited setting where the effect of regulation is side-stepped in the modeling

Metabolic control analysis can be seen as middle ground of the two extremes: in MCA, we can model the network behaviour of the reactions and consider regulation at the same time.

Metabolic Control Analysis (MCA)

- The restriction imposed by MCA is that we only study effects of small perturbations: what will happen if we 'nudge' the metabolic system slightly of its current steady state
- Mathematically, we employ a linearized system around the steady state, thus ignoring the non-linearity of the kinetics.
- The predictions are local in nature; in general different for each steady state

Questions of interest

- How does the change of enzyme activity affect the fluxes?
- Which individual reaction steps control the flux or concentrations?
- Is there a bottle-neck or rate-limiting step in the metabolism?
- Which effector molecules (e.g. inhibitors) have the greatest effect?
- Which enzyme activities should be down-regulated to control some metabolic disorder? How to distrub the overall metabolism the least?