

Regulation of metabolism

- ▶ So far in this course we have assumed that the metabolic system is in steady state
- ▶ For the rest of the course, we will abandon this assumption, and look at techniques for analyzing the regulation of metabolism
- ▶ The general approaches examined are:
 - ▶ Enzyme kinetics, where the target is accurate analysis of an individual enzyme or a small system of enzymes
 - ▶ Metabolic control analysis that the response of a larger metabolic system to a small perturbation

Enzyme kinetics

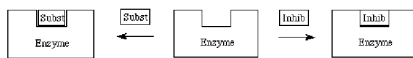
- ▶ Enzyme kinetics, the study of dynamic properties of enzymatic reaction systems, dates back over 100 years, 50 years prior to the discovery of DNA structure.
- ▶ Via enzyme kinetics one aims for accurately predicting the behaviour of a enzymatics reaction system. In particular we might be interested 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)

Enzyme activity

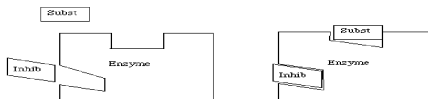
- ▶ The rate of certain enzyme-catalyzed reaction depends on the concentration (amount) of the enzyme and the specific activity of the enzyme (how fast a single enzyme molecule works).
- ▶ The specific activity of the enzyme depends on
 - ▶ pH and temperature
 - ▶ positively on the concentration of the 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 & Metabolic-level regulation

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



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)

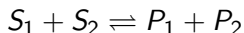
Law of mass action is one of the most fundamental and very well known kinetic model for a reaction

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 concentration
- ▶ 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



- ▶ 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.

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.

COPASI simulation

- ▶ Numerical simulation of a time course of a single reversible reaction obeying the law of mass action
- ▶ Using the COPASI software (www.copasi.org)

Change of free energy

Whether a reaction occurs spontaneously, is covered 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 spontaneously and releases energy.
- ▶ $\Delta G = 0$, the reaction is in equilibrium
- ▶ $\Delta G > 0$, the reaction will not occur spontaneously. The reaction can only happen if it obtains energy

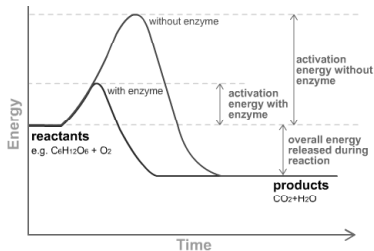
Roughly stated, the likelihood of a reaction occurring spontaneously is the larger

- ▶ 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 or 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



- ▶ 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):

$$\frac{dS}{dt} = -k_1 E \cdot S + k_{-1} ES, \quad \frac{dP}{dt} = k_2 ES$$

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

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

- ▶ The reaction rate satisfies:

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

- ▶ Unfortunately, the above system cannot be solved analytically, instead numerical simulation is required

Michaelis-Menten kinetics

- ▶ The reaction rate becomes solvable if a simplifying assumption is made that the concentration of enzyme-substrate complex is approximately 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:

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

Michaelis-Menten kinetics

For the reaction rate $v = \frac{dP}{dt} = k_2ES$ we obtain:

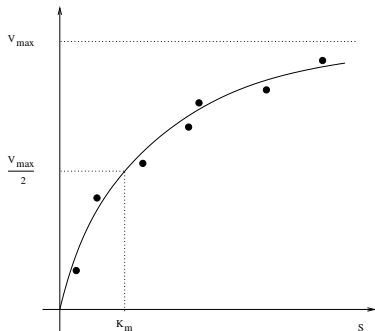
$$v = \frac{k_2 E_{total} S}{S + (k_{-1} + k_2)/k_1} = \frac{V_{max} S}{S + K_m}$$

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

- ▶ 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 $V_{max}/2$



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 two extreme views:

- ▶ Kinetic modeling, which aims at accurate mechanistic models of enzymatic reactions. Limited to small systems in precise
- ▶ 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 disturb the overall metabolism the least?

Coefficients of control analysis

The central concept in MCA is the *control coefficient* between two quantities (fluxes, concentrations, activities, ...) y and x :

$$c_x^y = \left(\frac{x}{y} \frac{\Delta y}{\Delta x} \right)_{\Delta x \rightarrow 0}$$

- ▶ Intuitively, c_x^y is the relative change of y in response of infinitely small change to x

Coefficients of control analysis

The limit can be written as

$$c_x^y = \frac{x}{y} \frac{\partial y}{\partial x} = \frac{\partial \ln y}{\partial \ln x},$$

by using the derivation rule $d/dz \ln z = 1/z$, for $z = x, y$

- ▶ The normalization factor x/y makes the coefficient independent of units, the same value will be obtained regardless of in which units y and x are expressed.
- ▶ Unnormalized coefficients $\frac{\partial y}{\partial x}$ are sometimes used as well as some mathematical derivations become easier

Types of coefficients

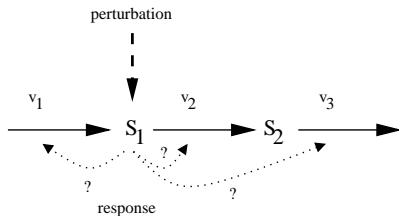
- ▶ Elasticity coefficients quantify the sensitivity of a reaction rate to the change of concentration or a parameter.
- ▶ Flux control coefficients quantify the change of a flux along a pathway in response to a change in the rate of a reaction
- ▶ Concentration control coefficients quantify the change of concentration of some metabolite S_i in response of a change in the rate of a reaction
- ▶ Response coefficients quantify the change of a flux in response to a change in a parameter (e.g. kinetic parameters of an enzyme)

ϵ -elasticity coefficient

- ▶ ϵ -elasticity coefficient

$$\epsilon_i^k = \frac{S_i}{v_k} \frac{\partial v_k}{\partial S_i}$$

quantifies the change of a reaction rate v_k in response to a change in the concentration S_i , while everything else is kept fixed.



ϵ -elasticity coefficient

- ▶ Consider a reaction catalyzed by an enzyme E , inhibited by effector I and activated by effector A
- ▶ Typical values (there are exceptions) for elasticity coefficients satisfy the following:

$$\epsilon_S^v = \frac{\partial \ln v}{\partial \ln S} > 0, \epsilon_P^v = \frac{\partial \ln v}{\partial \ln P} < 0$$

i.e. the more substrate the faster the rate, the more product the slower the rate

$$\epsilon_A^v = \frac{\partial \ln v}{\partial \ln A} > 0, \epsilon_I^v = \frac{\partial \ln v}{\partial \ln I} < 0$$

i.e. the higher activator concentration the faster the rate, the higher inhibitor concentration the slower the rate

Example: ϵ -elasticity of a simple reaction

- ▶ Consider an enzymatic reaction modelled with Michaelis-Menten kinetics

$$v_k = \frac{V_{max} S_i}{K_m + S_i}$$

- ▶ The elasticity with respect to the change in the substrate concentration is found to be

$$\epsilon_i^k = \frac{S_i}{v_k} \frac{\partial v_k}{\partial S_i} = \frac{K_m}{K_m + S_i}$$

by applying the derivation rule $\frac{d}{dx} \frac{f(x)}{g(x)} = \frac{f(x)'g(x) - g(x)'f(x)}{g(x)^2}$

- ▶ The change of reaction rate in response to change of concentration of the substrate is the lower the higher the concentration
- ▶ The reaction rate is a concave function of the substrate concentration

Control coefficients

We consider a vector

$$\mathbf{S} = \mathbf{S}(\mathbf{p})$$

of steady state concentrations and a vector

$$\mathbf{J} = \mathbf{v}(\mathbf{S}(\mathbf{p}), \mathbf{p})$$

of steady state fluxes, parametrized by \mathbf{p} , which includes kinetic parameters of enzymes and concentrations of external metabolites. Consider a small perturbation of a reaction rate v_k via perturbation of the parameters \mathbf{p} .

This will cause the system to seek a new steady state in the neighborhood of of the original: $\mathbf{J} \rightarrow \mathbf{J} + \Delta\mathbf{J}$, $\mathbf{S} \rightarrow \mathbf{S} + \Delta\mathbf{S}$

Questions of interest

We wish to capture the answers to the following questions

- ▶ What is the effect of rate change of a reaction to a particular flux?
- ▶ What is the effect of rate change of a reaction to a particular concentration?

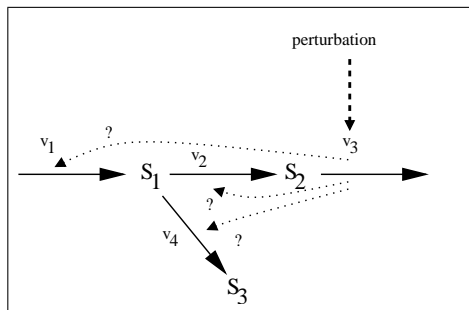
Answers to the above questions are characterized by so called control coefficients.

Flux control coefficients

The flux-control coefficient (FCC)

$$FCC_k^j = \frac{v_k}{J_j} \frac{\partial J_j}{\partial v_k}$$

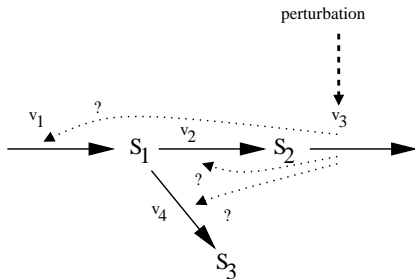
is defined as the change of flux J_j of a given pathway, in response to a change in the reaction rate v_k .



Flux control coefficients

- ▶ Unlike elasticity coefficients, FCC's are global: all reaction rate have control over all fluxes, the strength of control is quantified by the FCC.
- ▶ Note that the notion of 'control' does not in general mean direct regulatory relationship e.g. FCC_3^4 denoting the control

of v_3 to the flux from S_1 to S_3 will typically be non-zero

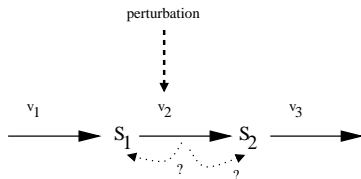


Concentration control coefficients

The concentration-control coefficient (CCC)

$$CCC_k^i = \frac{v_k}{S_i} \frac{\partial S_i}{\partial v_k}$$

is defined as the change of concentration S_i , in response to a change in the reaction rate v_k .



Theorems of MCA

- ▶ Unlike the elasticity coefficients, the control coefficients cannot be directly computed from the kinetic parameters of the reactions, even in principle.
- ▶ In order to determine the coefficients we need both some MCA theory and experimental data
- ▶ MCA theory consists of two sets of theorems:
 - ▶ Summation theorems make statements about the total control of a flux or a steady-state concentration
 - ▶ Connectivity theorems relate the control coefficients to the elasticity coefficients

Summation theorems

The first summation theorem says that for each flux J_j the flux-control coefficients must sum to unity

$$\sum_{k=1}^r FCC_k^j = 1$$

Thus, control of a flux is shared across all enzymatic reactions
For concentration control coefficients we have

$$\sum_{k=1}^r CCC_k^i = 0$$

Control of a concentration is shared across all enzymatic reactions, some exerting positive control, other exerting negative control.