

## Computational Biology

part IV Modelling metabolic pathways  
Jaap Kaandorp

### Metabolism, 2 types of reactions

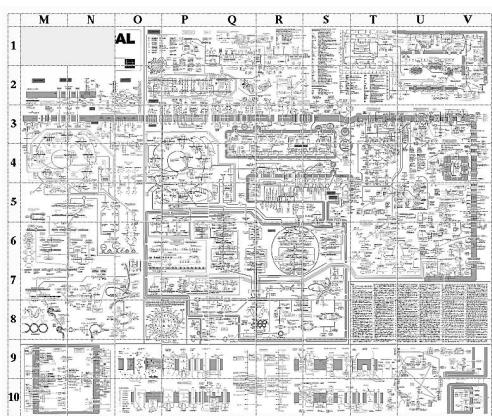
- Catabolic reactions: breakdown of complex compounds to get energy and building blocks
- Anabolic reactions: construction of complex compounds used in cellular functioning

### Metabolism, 3 levels of abstraction

- Enzyme kinetics: dynamic properties of the individual reactions in isolation
- The network character of metabolism: stoichiometric analysis considering the balance of compound production and degradation
- Metabolic control analysis quantifies the effect of perturbations in the network employing the individual dynamics of concentration changes and their integration in the network

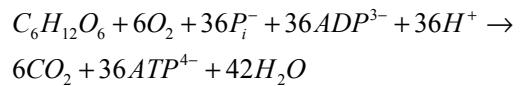
Note that modelling approaches for metabolic networks also apply for other types of networks and biochemical reactions, for example

- Signalling cascades
- Binding of transcription factors to DNA

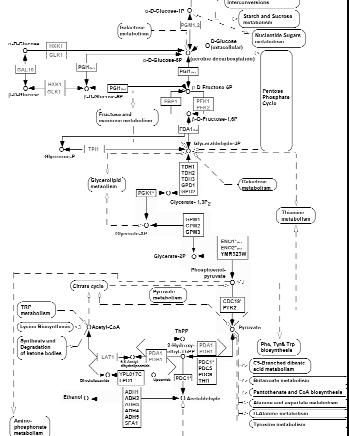


### Example glycolysis I

Aerobic oxidation of glucose in the mitochondria



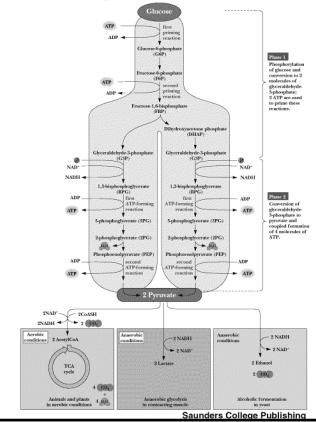
## Example glycolysis II



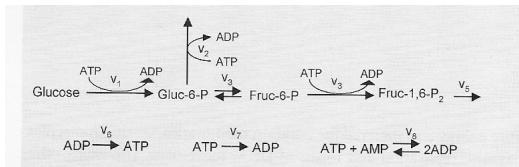
## Example glycolysis II

Overview of the glycolysis

Garrett & Grisham: Biochemistry, 2/e  
Figure 19.1



## Example glycolysis III, Upper part of the glycolysis



## Example glycolysis III, Upper part of the glycolysis

Gluc-6-P	Glucose-6-phosphate
Fruc-6-P	Fructose-6-phosphate
Fruc-1,6-P	Fructose-1,6-bisphosphate
ATP	Adenosine-triphosphate
ADP	Adenosine-biphosphate
AMP	Adenosine-monophosphate
v1	hexokinase
v2	consumption of Gluc-6-P by other pathways
v3	phosphoglucomutase
v4	phosphofructokinase
v5	aldolase
v6	ATP production in lower glycolysis
v7	ATP consumption in other pathways
v8	adenylate kinase

## Role of enzymes

- Enzymes catalyze biochemical reactions. Enzymes are proteins, have a catalytic center, usually highly specific, remain unchanged by the reaction. One enzyme molecule catalyzes 1000 reaction per second, this leads to a rate acceleration of about  $10^6$  to  $10^{12}$  fold compared to uncatalyzed, spontaneous reaction.

## Example glycolysis II

The ODE system

$$\begin{aligned} \frac{d}{dt} \text{Gluc6P} &= v_1 - v_2 - v_3 \\ \frac{d}{dt} \text{Fruc6P} &= v_3 - v_4 \\ \frac{d}{dt} \text{Fruc1,6P}_2 &= v_4 - v_5 \\ \frac{d}{dt} \text{ATP} &= -v_1 - v_2 - v_4 + v_6 - v_7 - v_8 \\ \frac{d}{dt} \text{ADP} &= v_1 + v_2 + v_4 - v_6 + v_7 + 2v_8 \\ \frac{d}{dt} \text{AMP} &= -v_8 \end{aligned}$$

### Example glycolysis III

The individual rates

$$v_1 = \frac{V_{max,1} ATP(t) \cdot Glucose}{1 + \frac{ATP(t)}{K_{ATP,1}} + \frac{Glucose}{K_{Glucose,1}}} \quad \text{or} \quad v_1 = \frac{V_{max,1} ATP(t)}{K_{ATP,1} + ATP(t)} \quad (5.2)$$

$$v_2 = k_2 ATP(t) \cdot Gluc6P(t) \quad (5.3)$$

$$v_3 = \frac{\frac{V'_{max,3}}{K_{Gluc6P,3}} Gluc6P(t) - \frac{V'_{max,3}}{K_{Fruc6P,3}} Fruc6P(t)}{1 + \frac{Gluc6P(t)}{K_{Gluc6P,3}} + \frac{Fruc6P(t)}{K_{Fruc6P,3}}} \quad (5.4)$$

$$v_4 = \frac{V_{max,4} (Fruc6P(t))^2}{K_{Fruc6P,4} \left( 1 + \kappa \left( \frac{ATP(t)}{AMP(t)} \right)^2 \right) + (Fruc6P(t))^2} \quad (5.5)$$

$$v_5 = k_5 Fruc1,6P_2(t) \quad (5.6)$$

$$v_6 = k_6 ADP(t) \quad (5.7)$$

$$v_7 = k_7 / ATP(t) \quad (5.8)$$

$$v_8 = k_8 f ATP(t) \cdot AMP(t) - k_9 r (ADP(t))^2, \quad (5.9)$$

### Example glycolysis IV

The parameters

$$Glucose = 12.8174 \text{ mM}, V_{max,1} = 1398.00 \text{ mM} \cdot \text{min}^{-1}, K_{ATP,1} = 0.10 \text{ mM}, \\ K_{Glucose,1} = 0.37 \text{ mM}, V_{max,1} = 50.2747 \text{ mM} \cdot \text{min}^{-1}$$

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

$$V'_{max,3} = 140.282 \text{ mM} \cdot \text{min}^{-1}, V'_{max,3} = 140.282 \text{ mM} \cdot \text{min}^{-1}, K_{Gluc6P,3} = 0.80 \text{ mM}, \\ K_{Fruc6P,3} = 0.15 \text{ mM}$$

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

$$k_3 = 6.04662 \text{ min}^{-1}$$

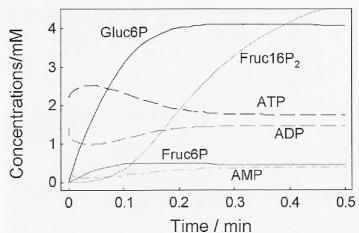
$$k_6 = 68.48 \text{ min}^{-1}$$

$$k_7 = 3.21 \text{ min}^{-1}$$

$$k_8 f = 432.9 \text{ mM}^{-1} \cdot \text{min}^{-1}, k_9 r = 133.33 \text{ mM}^{-1} \cdot \text{min}^{-1}$$

### Example glycolysis V

Time course of concentrations



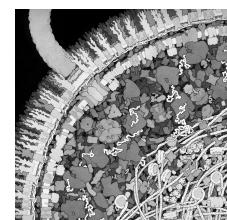
### Basic assumptions in enzyme kinetics

- The basic assumption is that the reaction rate  $v$  at a certain point in space and time can be expressed as a unique function of the concentrations of all substances at this point in space and time. We assume spatial homogeneity ('`well-stirred'') and no direct dependency of the rate on time:  $v(t) = v(S(t))$
- The concentration  $S$  of a substance,  $v$  is the rate of a reaction (change of concentration  $S$  per time  $t$ )
- Macroscopic modelling or phenomenological approach (no single molecules are considered)

### Basic assumptions in enzyme kinetics I (are these assumptions valid?)

- Stochastic fluctuations due to low molecule numbers (for example enzymes!)
- Localization of chemical agents: cell is not a well-stirred medium, many reactions are for example membrane-bound, many spatial inhomogeneities, formation of gradients due to diffusion, cellular structures hinder free movement of molecules

### Major challenge how to model the cell (for example E.coli) and biochemical pathways?



Detail of the E. coli cell

## Law of mass action

- Reaction rate is proportional to the probability of a collision of the reactants. The probability is in turn proportional to the concentration of reactions to the power of the molecularity (number in which they are involved in the reaction)

## Law of mass action II

- General mass action rate law for a reaction with substrate concentrations  $S_i$  and product  $P_j$  concentrations

$$v = v_+ - v_- = k_+ \prod_i S_i^{m_i} - k_- \prod_j P_j^{m_j}$$

- Where  $m_i$  and  $m_j$  are the respective molecularities of  $S_i$  and  $P_j$ ,  $v$  is the net rate,  $v_+$  rate of the forward reaction,  $v_-$  backward reaction,  $k_+$  and  $k_-$  are the rate constants

## Law of mass action III

- The equilibrium constant

$$K_{eq} = \frac{k_+}{k_-} = \frac{\prod P_{eq}}{\prod S_{eq}}$$

- Where  $S_{eq}$  and  $P_{eq}$  are respectively substrate and product concentrations in equilibrium

## Reaction kinetics and thermodynamics

- A distinction can be made between energy-supplying reactions, energy demanding reactions and energetically neutral reactions
- Change of G Gibbs free energy:

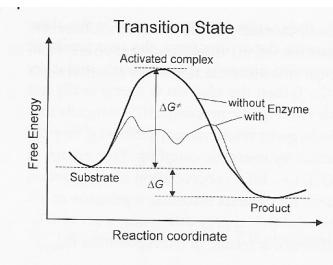
$$\Delta G = \Delta H - T\Delta S$$

- Where  $\Delta H$  is the change in enthalpy,  $\Delta S$  is the change in entropy,  $T$  absolute temperature

## Reaction kinetics and thermodynamics $\Delta G$ in a number of important reactions

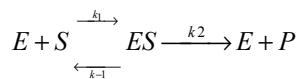
Reaction	$\Delta G^\circ / (\text{kJ mol}^{-1})$
$2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$	-474
$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$	-99
$\text{PP}_i + \text{H}_2\text{O} \rightarrow 2\text{P}_i$	-33.49
$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i$	-30.56
$\text{Glucose-6-phosphate} + \text{H}_2\text{O} \rightarrow \text{Glucose} + \text{P}_i$	-13.82
$\text{Glucose} + \text{P}_i \rightarrow \text{Glucose-6-phosphate} + \text{H}_2\text{O}$	+13.82
$\text{Glucose-1-phosphate} \rightarrow \text{Glucose-6-phosphate}$	-7.12
$\text{Glucose-6-phosphate} \rightarrow \text{Fructose-6-phosphate}$	+1.67
$\text{Glucose} + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$	-2890

## Change of free energy along the course of a reaction



### Michaelis-Menten kinetics I

- Reaction with enzyme E, substrate S, enzyme-substrate complex ES, product P



- Construct system of ODEs (on blackboard)

### Michaelis-Menten kinetics II

- Rate of the reaction is

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

- Assumption Michaelis and Menten: conversion of E and S to ES much faster than decomposition of ES, quasi-equilibrium  $k_1, k_{-1} \gg k_2$

### Michaelis-Menten kinetics III

- Assumption initial concentration of S is much larger than concentration of E, ES complex remains constant, quasi-steady state for ES:

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

- From ODEs for ES and E:

$$\frac{dES}{dt} + \frac{dE}{dt} = 0 \quad \text{or} \quad E_{total} = E + ES$$

### Michaelis-Menten kinetics IV

- From previous equations (on blackboard) under steady state assumption for ES:

$$v = \frac{k_2 E_{total} S}{S + \frac{k_{-1} + k_2}{k_1}}$$

### Michaelis-Menten kinetics V

Simpler form

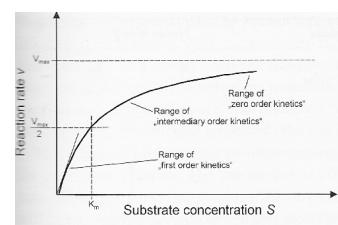
$$v = \frac{v_{max} S}{S + K_m}$$

Maximal velocity

Michaelis constant

$$v_{max} = k_2 E_{total} \quad K_m = \frac{k_{-1} + k_2}{k_1}$$

### Plot of rate versus substrate concentrations



### Example glycolysis III

Note v1 is described with Michealis-Menten kinetics

$$v_1 = \frac{V_{max,1} ATP(t) \cdot \text{Glucose}}{1 + \frac{ATP(t)}{K_{ATP,1}} + \frac{\text{Glucose}}{K_{Glucose,1}}} \quad \text{or} \quad v_1 = \frac{V_{max,1} ATP(t)}{K_{ATP,1} + ATP(t)} \quad (5.2)$$

$$v_2 = k_2 ATP(t) \cdot \text{Fruc6P}(t) \quad (5.3)$$

$$v_3 = \frac{\frac{V'_{max,3}}{K_{GlucP,3}} \text{Gluc6P}(t) - \frac{V'_{max,3}}{K_{FrucP,3}} \text{Fruc6P}(t)}{1 + \frac{\text{Gluc6P}(t)}{K_{Gluc6P,3}} + \frac{\text{Fruc6P}(t)}{K_{Fruc6P,3}}} \quad (5.4)$$

$$v_4 = \frac{V_{max,4} (\text{Fruc6P}(t))^2}{K_{Fruc6P,4} \left(1 + \kappa \left(\frac{ATP(t)}{AMP(t)}\right)^2\right) + (\text{Fruc6P}(t))^2} \quad (5.5)$$

$$v_5 = k_5 \text{Fruc1,6P}_2(t) \quad (5.6)$$

$$v_6 = k_6 ADP(t) \quad (5.7)$$

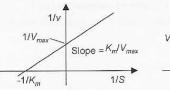
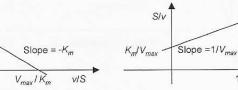
$$v_7 = k_7 ATP(t) \quad (5.8)$$

$$v_8 = k_8 ATP(t) \cdot AMP(t) - k_9 (ADP(t))^2, \quad (5.9)$$

### How to derive a rate equation

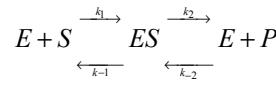
- 1. Construct reaction equation, containing all substrates and product (S and P) and free or bound enzyme species (E and ES)
- 2. Construct the set of ODEs for the concentration changes. The rates follow mass action kinetics
- 3. Sum of all enzyme-containing species is equal to the total enzyme concentrations  $E_{total}$ , this constitutes one equation
- 4. The assumption of a quasi-steady state for the enzyme species, together with the equation in 3, results in equations for the enzyme species
- 5. The reaction rate is equal to the rate of product formation

### Parameter estimation and linearization of the Michaelis-Menten equation

	Lineweaver-Burk	Eadie-Hofstee	Hanes-Woolf
Transformed equation	$\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{S} + \frac{1}{V_{max}}$	$v = V_{max} - K_m \frac{v}{S}$	$\frac{S}{v} = \frac{S}{V_{max}} + \frac{K_m}{V_{max}}$
New variables	$\frac{1}{v} = \frac{1}{V_{max}} \frac{1}{S}$	$v = \frac{v}{S}$	$\frac{S}{v} = \frac{S}{V_{max}} \cdot S$
Graphical representation			

### Michaelis-Menten kinetics for reversible reactions I

- Reaction with enzyme E, substrate S, enzyme-substrate complex ES, product P



- Construct system of ODEs

### Michaelis-Menten kinetics for reversible reactions II

Product (P) formation

$$\frac{dP}{dt} = k_2 ES - k_{-2} P = v$$

Rate equation

$$v = E_{total} \frac{S_q - P}{\frac{Sk_1}{k_{-1}k_{-2}} + \frac{1}{k_{-2}} + \frac{k_2}{k_{-1}k_{-2}} + \frac{P}{k_{-1}}} = \frac{\frac{v_{max}^{for}}{K_{mS}} S - \frac{v_{max}^{back}}{K_{mP}} P}{1 + \frac{S}{K_{mS}} + \frac{P}{K_{mP}}}$$

### Michaelis-Menten kinetics for reversible reactions III

Rate equation

$$v = E_{total} \frac{S_q - P}{\frac{Sk_1}{k_{-1}k_{-2}} + \frac{1}{k_{-2}} + \frac{k_2}{k_{-1}k_{-2}} + \frac{P}{k_{-1}}} = \frac{\frac{v_{max}^{for}}{K_{mS}} S - \frac{v_{max}^{back}}{K_{mP}} P}{1 + \frac{S}{K_{mS}} + \frac{P}{K_{mP}}}$$

Equilibrium constant

$$K_{eq} = \frac{v_{max}^{for} K_{mP}}{v_{max}^{back} K_{mS}}$$

### Example glycolysis III

Note  $v_3$  is described with the (reversible) Michealis Menten kinetics

$$v_1 = \frac{V_{max,1} ATP(t) \cdot Glucose}{1 + \frac{ATP(t)}{K_{ATP,1}} + \frac{Glucose}{K_{Glucose,1}}} \quad \text{or} \quad v_1 = \frac{V_{max,1} ATP(t)}{K_{ATP,1} + ATP(t)} \quad (5.2)$$

$$v_2 = k_2 ATP(t) \cdot Fruc6P(t) \quad (5.3)$$

$$v_3 = \frac{\frac{V'_{max,3}}{K_{Gluc6P,3}} Gluc6P(t) - \frac{V'_{max,3}}{K_{Fruc6P,3}} Fruc6P(t)}{1 + \frac{Gluc6P(t)}{K_{Gluc6P,3}} + \frac{Fruc6P(t)}{K_{Fruc6P,3}}} \quad (5.4)$$

$$v_4 = \frac{V_{max,4} (Fruc6P(t))^2}{K_{Fruc6P,4} \left(1 + \kappa \left(\frac{ATP(t)}{AMP(t)}\right)^2\right) + (Fruc6P(t))^2} \quad (5.5)$$

$$v_5 = k_5 Fruc1,6P_2(t) \quad (5.6)$$

$$v_6 = k_6 ADP(t) \quad (5.7)$$

$$v_7 = k_7 ATP(t) \quad (5.8)$$

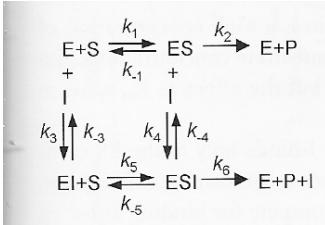
$$v_8 = k_8 ATP(t) \cdot AMP(t) - k_9 (ADP(t))^2, \quad (5.9)$$

### Modelling enzyme kinetics, some more extensions of the model I (see for details Klipp et al, 2005)

- Regulation of enzyme activity by protein interaction
- Inhibition by irreversible binding of inhibitor to enzyme
- Substrate inhibition
- Inhibition by binding of inhibitor to substrate
- Binding of ligands to proteins
- Positive Homotropic Cooperativity and the Hill equation

### Modelling enzyme kinetics, some more extensions of the model II

- Regulation of enzyme activity by protein interaction



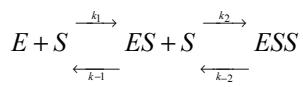
### Modelling enzyme kinetics, some more extensions of the model III

- Inhibition by irreversible binding of inhibitor to enzyme



### Modelling enzyme kinetics, some more extensions of the model IV

- Substrate inhibition



### Modelling enzyme kinetics, some more extensions of the model V

- Substrate inhibition

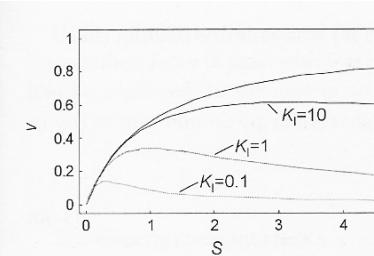
$$v = k_2 ES = \frac{v_{max} S}{K_m + S(1 + \frac{S}{K_I})}$$

This expression has a maximum for

$$S_{opt} = \sqrt{K_m K_I}$$

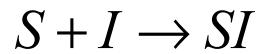
$$v_{opt} = \frac{v_{max}}{1 + 2\sqrt{K_m / K_I}}$$

### Modelling enzyme kinetics, some more extensions of the model Vb, substrate inhibition



### Modelling enzyme kinetics, some more extensions of the model VI

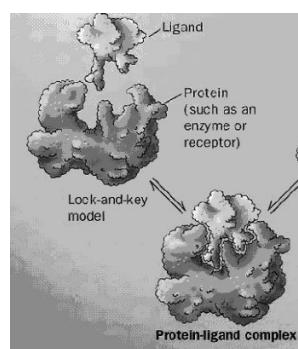
- Inhibition by binding of inhibitor to substrate



### Modelling enzyme kinetics, some more extensions of the model VII

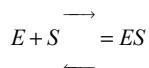
- Binding of ligands to proteins

### Enzyme substrate



### Modelling enzyme kinetics, some more extensions of the model VIII

- Binding of ligands to proteins, case binding of 1 ligand (S) to a protein (E)

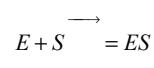


- Binding constant

$$K_B = \frac{ES}{E \cdot S}$$

### Modelling enzyme kinetics, some more extensions of the model IX

- Binding of ligands to proteins, case binding of 1 ligand (S) to a protein (E)



- Fractional saturation Y for one subunit

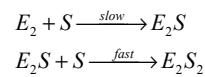
$$Y = \frac{ES}{E_{total}} = \frac{ES}{ES + E} = \frac{K_B \cdot S}{K_B \cdot S + 1}$$

### Modelling enzyme kinetics, some more extensions of the model X

- Binding of ligands to proteins, case binding of more ligand (S) to a protein (E)
- Positive cooperativity: increase of the protein to bind ligands
- Negative cooperativity: decrease of the protein to bind ligands

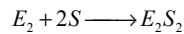
### Modelling enzyme kinetics, some more extensions of the model XI

- Binding of ligands to proteins, case binding of 2 monomeric ligands (S) to a dimeric protein ( $E_2$ ), positive cooperativity



### Modelling enzyme kinetics, some more extensions of the model XII

- Binding of ligands to proteins, case binding of 2 monomeric ligands (S) to a dimeric protein ( $E_2$ ), complete cooperativity

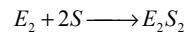


- Binding constant

$$K_B = \frac{E_2S_2}{E_2 \cdot S^2}$$

### Modelling enzyme kinetics, some more extensions of the model XIII

- Binding of ligands to proteins, case binding of 2 monomeric ligands (S) to a dimeric protein ( $E_2$ ), complete cooperativity



- Fractional saturation:

$$Y = \frac{2E_2S_2}{2E_{2,\text{total}}} = \frac{E_2S_2}{E_2 + E_2S_2} = \frac{K_B S^2}{1 + K_B S^2}$$

### Modelling enzyme kinetics, some more extensions of the model XIV

- Binding of ligands to proteins, case binding of n ligands (S) to a protein (E), complete cooperativity (Hill equation)

$$v = V_{\max} Y = \frac{V_{\max} K_B S^n}{1 + K_B S^n}$$

### Modelling enzyme kinetics, some more extensions of the model XIV

- Binding of ligands to proteins, case binding of n ligands (S) to a protein (E), complete cooperativity , example: binding of oxygen to Hemoglobin (Hb). A plot of the fractional saturation of Hb against O<sub>2</sub> shows a sigmoid shape. Hb has 4 binding sites, cooperativity is not complete.

## Stoichiometric analysis of a metabolic network I

- With such a description we can study the balance of fluxes and moieties in the network
- Basic components are:
  - Substances and their concentrations
  - Reactions and transport processes

## Stoichiometric analysis of a metabolic network II

- For a metabolic network consisting of m substances and r reactions, the system dynamics is described by:
- $$\frac{dS_i}{dt} = \sum_j n_{ij} v_j$$
- Where  $n_{ij}$  are the stoichiometric coefficients of metabolite i in reaction j
  - Assumption: reactions are only reasons for concentration changes, no mass flow due to convection and diffusion

## Stoichiometric analysis of a metabolic network III, compartments

## Stoichiometric analysis of a metabolic network IV

- For a metabolic network consisting of m substances and r reactions, the system dynamics is described by, the stoichiometric matrix N can be constructed:
- $$N = \{n_{ij}\}$$
- For  $i = 1, \dots, m$  and  $j = 1, \dots, r$

## Stoichiometric analysis of a metabolic network V

- For a metabolic network consisting of m substances and r reactions, the balance equation is:

$$\frac{dS}{dt} = Nv$$

## Stoichiometric analysis of a metabolic network VI

- The glycolysis example

$$S = \begin{Bmatrix} Gluc6P \\ Fru6P \\ Frud6P_2 \\ ATP \\ ADP \\ AMP \end{Bmatrix} \quad v = (v_1, v_2, \dots, v_8)^T$$

$$P = (GlucoseV_{max1}, K_{ATP1}, K_{glucose1}, k_2, V'_{max3}, V'_{max3}, K_{Gluc6P3}, K_{Fru6P3}, V_{max4}, K_{F6P4}, K_4, K_5, K_6, K_7, K_8, K_{8r})^T$$

- Where S is the concentration vector, v the vector with reaction rates and P the parameter vector

## Stoichiometric analysis of a metabolic network VII

- The glycolysis example (cont), the stoichiometric matrix

$$\mathbf{N} = \begin{pmatrix} 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ -1 & -1 & 0 & -1 & 0 & 1 & -1 & -1 \\ 1 & 1 & 0 & 1 & 0 & -1 & 1 & 2 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{pmatrix}$$

## Stoichiometric analysis of a metabolic network VIII, Information in the stoichiometric matrix

- We can calculate which combinations of individual fluxes are possible in steady state
- Discover dead ends and unbranched reaction pathways
- Conservation relations for the included reactants

## Stoichiometric analysis of a metabolic network VIII, Information in the stoichiometric matrix

- In steady state

$$\frac{d\mathbf{S}}{dt} = \mathbf{N}\mathbf{v} = 0$$

- Nontrivial solutions only for  $\text{rank } \mathbf{N} < r$ , the kernel matrix  $\mathbf{K}$ :

$$\mathbf{NK} = 0$$

## Stoichiometric analysis of a metabolic network VIII, Information in the stoichiometric matrix

- The kernel  $\mathbf{K}$  can be determined with the Gaussian algorithm, it contains  $r-\text{rank } \mathbf{N}$  basis vectors. Every possible set of steady state fluxes can be determined as a linear combination of the columns  $\mathbf{k}_i$  of  $\mathbf{K}$

$$\mathbf{J} = \sum_{i=1}^{r-\text{rank } \mathbf{N}} \alpha_i \cdot \mathbf{k}_i$$

## Stoichiometric analysis of a metabolic network VIII, Information in the stoichiometric matrix, glycolysis example

$\mathbf{K} = (k_1 \ k_2 \ k_3)$  with

$$k_1 = \begin{pmatrix} -1 \\ -1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 2 \\ 0 \end{pmatrix}, k_2 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, k_3 = \begin{pmatrix} 0 \\ -1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 0 \\ 0 \end{pmatrix}$$

## Stoichiometric analysis of a metabolic network VIII, Information in the stoichiometric matrix, glycolysis example, some conclusions

- If entries in a certain row are zero in all basis vectors, then we have found an equilibrium reaction. In any steady state the net rate of this reaction ( $v_8$ ) must be zero
- The entries for the reaction 3,4 and 5 are equal for each column of  $\mathbf{K}$ , therefore reactions 3,4 and 5 constitute an unbranched pathway. In steady state they must have equal rates

## Metabolic pathways, modelling data bases

- Snoep & Olivier, Java web simulation (JWS): a web based database of kinetic models (2002) Mol. Biol. Rep. 29:259-263