The control of enzyme activity

Enzyme Engineering

Two ways to control enzyme activity

1. Controlling concentration of enzyme : Regulation of transcription and translation → Long-term
2. Controlling activities of enzyme → Rapid responses

6.2 Control of the activities of single enzymes

1. By covalent bonds
2. By reversible binding of 'regulator'
3. Inhibitor, [S], product inhibition, etc…

6.2 Control of the activities

1. Control by covalent bond
   - Irreversible, mostly intracellular regulation
   - Example: phosphorylation, ubiquitination, ...

1.1 Irreversible changes in covalent bond (Proteolysis)

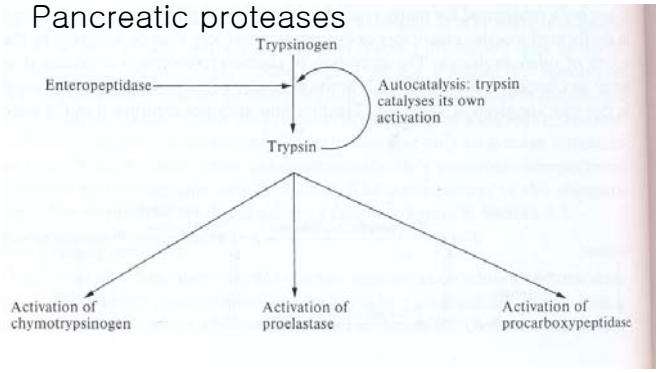
Table 6.1 Some enzymes activated by proteolytic action (mostly for extracellular regulation)

Enzyme	Precursor	Function
Trypsin Chymotrypsin Elastase Carboxypeptidase	Trypsinogen Chymotrypsinogen Proelastase Procarboxypeptidase	Pancreatic secretion (see Section 6.2.1.1)
Phospholipase A_2[7]	Prophospholipase A_2	Pancreatic secretion
Pepsin	Pepsinogen	Secreted into gastric juice: most active in pH range 1–5
Thrombin	Prothrombin	Part of the blood coagulation system (see Section 6.2.1.1)
Clr	Clr	Part of the first component of the complement system (see Section 6.2.1.1)
Chitin synthase[8]	Zymogen	Involved in the formation of the septum during budding and cell division in yeast

6.2.1.1 Control by irreversible changes in covalent bond

Signal amplification mechanism of proteolysis

1) Pancreatic proteases



2) Blood coagulation

prothrombin → thrombin → Fibrinogen → Fibrin

6.2.1.2 Control by reversible changes in covalent bond

- Phosphorylation-dephosphorylation
- Adenylation or ADP-ribosylation also regulate enzyme activity
- Methylation, acetylation, tyrosinolation, etc... do not regulate activity

1. Phosphorylation

- Kinase : phosphorylation of other protein
- Protein phosphatase : dephosphorylation
- Human has 2000 kinase and 1000 protein phosphatase
- Phosphorylation occurs on serine/threonine, tyrosine using ATP(GTP) as a substrate

Enzyme or protein	Modification	Biological function
Glycogen phosphorylase	Phosphorylation	Glycogen metabolism (see Section 6.4.2)
Glycogen synthase	Phosphorylation	
Phosphorylase kinase	Phosphorylation	
Phosphatase inhibitor protein	Phosphorylation	
Fructose 2,6-bisphosphatase/6-phosphofructo-2-kinase	Phosphorylation	Regulation of glycolysis (see Section 6.4.1.1)
Pyruvate dehydrogenase complex (mammalian)	Phosphorylation	Entry of pyruvate into tricarboxylic-acid cycle (see chapter 7, Section 7.7.5)
Branched chain 2-oxoacid dehydrogenase complex	Phosphorylation	Breakdown of leucine, isoleucine, and valine
Acetyl-CoA carboxylase	Phosphorylation	Synthesis of fatty acids
Troponin-1	Phosphorylation	Muscular contraction
Myosin light chain	Phosphorylation	
cdc kinase	Phosphorylation	Regulation of mitosis[15] (see Chapter 9, Section 9.7.4)
Glutamine synthetase (*E. coli*)	Adenylylation	Glutamine acts as N donor in a wide range of biosynthetic reactions
Glutamine synthetase (mammalian)	ADP-ribosylation	
RNA polymerase (*E. coli*)	ADP-ribosylation	On infection by T4 phage, an Arg side chain in the α subunit becomes modified. This shuts off transcription of the host genes
G-protein	ADP-ribosylation	G-proteins can act as transducing agents relaying the effect of hormone binding to the activation of adenylate cyclase (see Chapter 8, Section 8.4.5)
Nitrogenase	ADP-ribosylation	Regulation in response to ammonia in nitrogen-fixing bacteria[16]
Fructose 2,6-bisphosphatase/6-phosphofructo-2-kinase	ADP-ribosylation	Possible regulation of glycolysis[17] (see Section 6.4.1.2)

6.2.1.2 Control by reversible changes in covalent bond

- Ser/Thr phosphorylation involves in metabolic control, while Tyr phosphrylation in cell growth/differentiation

Enzyme family	Amino-acid acceptor	Regulator	Processes regulated
cAMP-dependent PK (cAPK)	Ser/Thr	cAMP	glycolysis, gluconeogenesis, triglyceride and cholestrol metabolism, catecholamine metabolism
cGMP-dependent PK (cGPK)	Ser/Thr	cGMP	similar to cAPK but restricted distribution, e.g. smooth muscle
protein kinase C	Ser/Thr	diacylglycerol Ca^{2+}	exact role unknown, control of intracellular $[Ca^{2+}]$, phosphorylation of EGF receptor
Ca^{2+}/calmodulin PK	Ser/Thr	Ca^{2+}	phosphodiesterase, Ca^{2+}/Mg^{2+} ATPase, myosin LC kinase, phosphorylase kinase
Cyclin-dependent kinase	Ser/Thr	cyclins	cell cycle regulation by phosphorylation of lamins, vimentin, histone H1, and others
Mitogen activated protein kinases (MAP kinases)	Ser/Thr	growth factors cytokines, pheromones	translocation to nucleus to activate transcriptional factors
Receptor tyrosine kinases	Tyr	growth factors	activation of enzymes including phosphatidylinositol 3-kinase, GTPase activation protein, MAP kinases
Cytosolic tyrosine kinases	Tyr	cytokines	transcriptional activation

6.2.1.2 Control by reversible changes in covalent bond

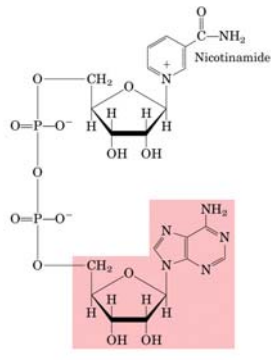
- Adenylylation on Tyr of glutamine synthase → Reducing enzyme activity
- Nitrogenase from nitrogen-fixing bacteria is regulated by ADP-ribosylation on Arg → Reducing enzyme activity

Features

1. Rapid response and signal amplification
2. Continuous response

   Ser/thr phos → in balance

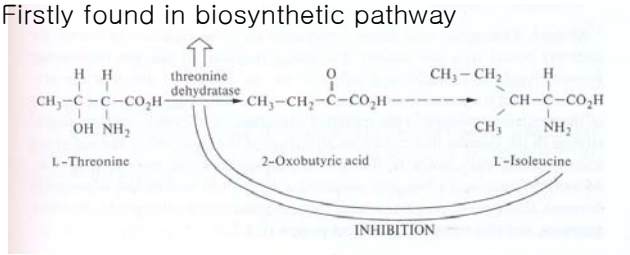
   Tyr phos → shift to dephosphorylation, meaning the transient response

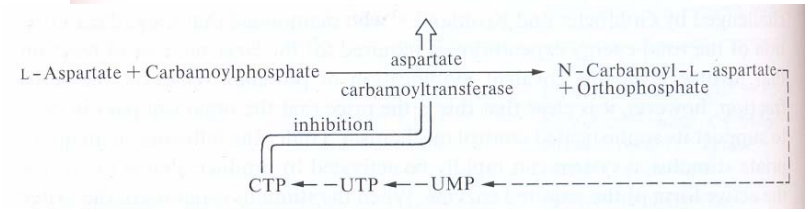


Nicotinamide adenine dinucleotide (NAD^+)

6.2.2 Control by Ligand binding

- Conformation changes by ligand binding regulates enzyme activity
- Firstly found in biosynthetic pathway

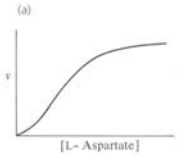


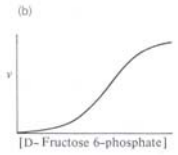


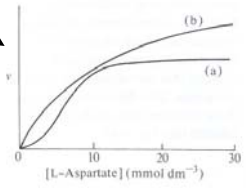
6.2.2 Control by Ligand binding

From Monod and Jacob

1. Effectors are structurally distinct from substrate or product, so they are unlikely bound to active site (allosteric)
2. Enzyme kinetics are sigmoidal rather than hyperbolic
3. Some treatments desensitize the enzymes to effector without losing enzyme activity
4. In general, the regulated enzymes are multimeric (structurally complex)

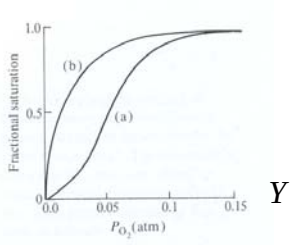






6.2.2.2 Kinetics of Ligand binding

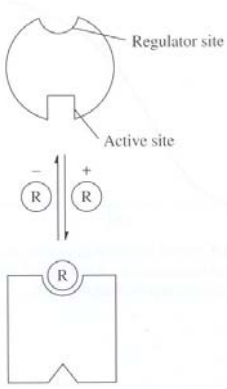
- Starting from Hb model



$$P + A \rightleftharpoons PA$$

$$K = \frac{[P][A]}{[PA]}$$

$$Y = \frac{[PA]}{[P]+[PA]} = \frac{[A]}{[A]+K}$$



Allosteric effect

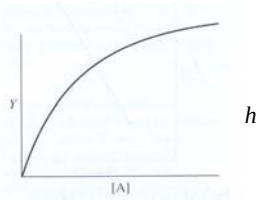
6.2.2.2 Kinetics of Ligand binding

- Hill eq. (1910)

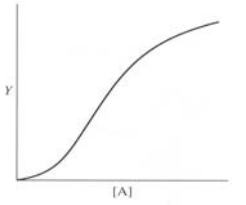
$$P + nA \rightleftharpoons PA_n$$

$$K = \frac{[P][A]^n}{[PA_n]}$$

$$Y = \frac{[PA_n]}{K+[PA_n]} = \frac{[A]^h}{[A]^h + K}$$



$h = 1$



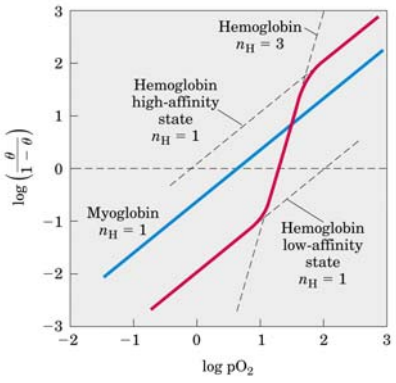
$h > 1$

6.2.2.2 Kinetics of Ligand binding

- *h* : cooperativity
- *h* is generally smaller than *n*
- *h* can be obtained by experiment

$$\frac{Y}{1-Y} = \frac{[A]^h}{K}$$

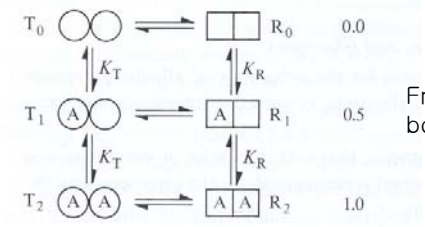
$$\log(\frac{Y}{1-Y}) = h\log[A] - \log K$$



6.2.2.2 Kinetics of Ligand binding

- Monod, Wyman, and Changeux (MWC) model

1. At lease one axis of symmetry
2. The conformation of each subunit is affected by others
3. Two conformation states, R and T
4. Either in R or T state, the symmetry is conserved – no 'hybrid' state



Fraction of subunit bound to substrate

6.2.2.2 Kinetics of Ligand binding

- Two parameters are defined

a; conc. Free ligand, $c=K_R/K_T$, $L=[T_0]/[R_0]$

$$[R_1]=\frac{4[F]}{K_R}[R_0]=4\alpha[R_0] \qquad [T_1]=\frac{4c[F]}{K_R}L[R_0]=4\alpha cL[R_0]$$

$$[R_2]=\frac{6[F]^2}{K_R^2}[R_0]=6\alpha^2[R_0] \qquad [T_2]=\frac{6c^2[F]^2}{K_R^2}L[R_0]=6\alpha^2c^2L[R_0]$$

(a) the fraction of protein in the R state ($\bar{R}$ = *function of state* R):

$$\bar{R}=\frac{[R_0]+[R_1]+[R_2]+[R_3]\cdots+[R_n]}{([R_0]+[R_1]+[R_2]+[R_3]\cdots+[R_n])+([T_0]+[T_1]+[T_2]+[T_3]\cdots+[T_n])} \quad (13.31)$$

(b) the fraction of sites actually bound by the ligand (Y_F = *saturation function*):

$$Y_F=\frac{([R_1]+2[R_2]+3[R_3]\cdots+n[R_n])+([T_1]+2[T_2]+3[T_3]\cdots+n[T_n])}{n([R_0]+[R_1]+[R_2]+[R_3]\cdots+[R_n])+n([T_0]+[T_1]+[T_2]+[T_3]\cdots+[T_n])} \quad (13.32)$$

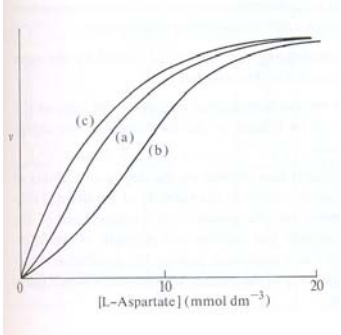
6.2.2.2 Kinetics of Ligand binding

$$\bar{Y}=\frac{Lc\alpha(1+c\alpha)^{n-1}+\alpha(1+\alpha)^{n-1}}{L(1+c\alpha)^n+(1+\alpha)^n}, \quad (6.7)$$

$$\bar{R}=\frac{(1+\alpha)^n}{L(1+c\alpha)^n+(1+\alpha)^n}. \quad (6.8)$$

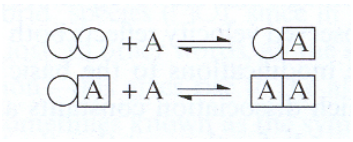
- *L* and *c* can be obtained experimentally (in hemoglobin, L=9050, c=0.014)
- K system and V system

a) No Substrate, no Effector
b) Adding substrate
c) Adding effector

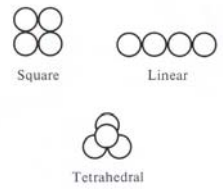


6.2.2.2 Kinetics of Ligand binding

- Koshland, Nemethy, and Filmer (KNF) model
  - "Induced-fit" hypothesis

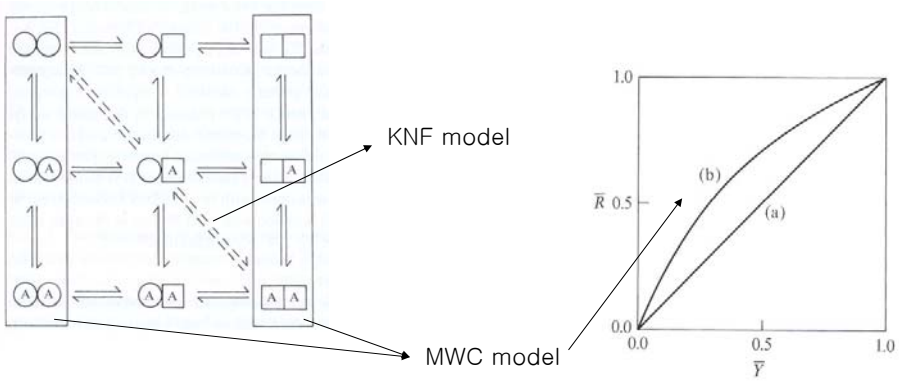


1. Without substrate, the protein exist in one state
2. The conformational change is sequential
3. The intxns b/n subunits can be positive/negative



6.2.2.2 Kinetics of Ligand binding

- Differences between MWC and KNF models



6.2.2.2 Kinetics of Ligand binding

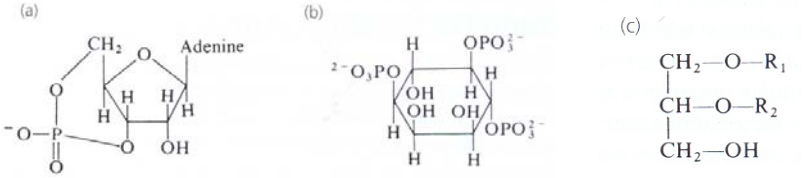
- The significance of the cooperativity in enzyme kinetics : small changes in $[S]$ induce large changes in v

$$v = \frac{V_{max}[S]^h}{K_m + [S]^h}$$

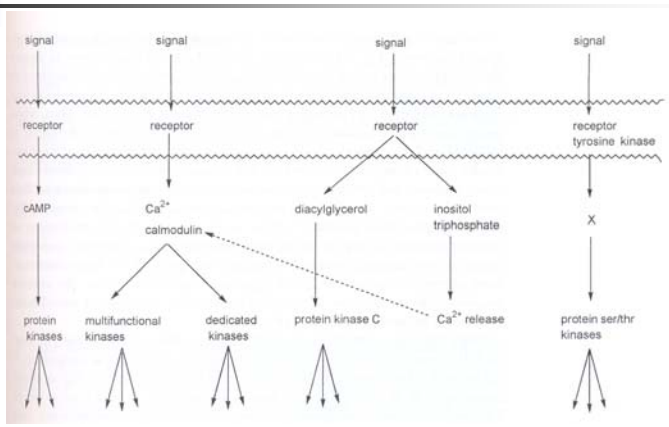
Value of h in eqn (6.9)	Required change in [S] to increase velocity from 10 per cent of V_{max} to 90 per cent of V_{max}
1	81-fold
2	9-fold
3	4.33-fold
4	3-fold
0.5	6561-fold

6.3 Control of metabolic pathways

- Intrinsic control vs extrinsic control
- Intrinsic control : Control of metabolic activity by metabolite concentrations (Unicellular organism)
- Extrinsic control : Control of metabolic activity by extracellular signals, such as hormones or nervous stimulation (Multicellular organism)
  - Secondary signaling molecules : cyclic AMP, Ca^{2+}, inositol 1,4,5-triphosphate, and diacylglycerol



6.3 Control of metabolic pathways



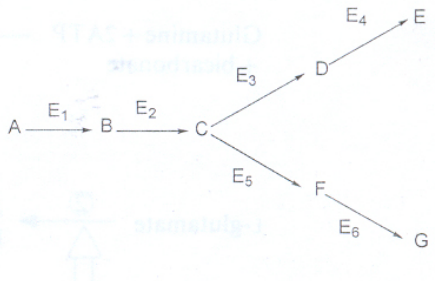
1. Amplification
2. Signaling depending on the # of receptors/cell
3. One protein kinase activating many different enzymes

6.3 Control of metabolic pathways

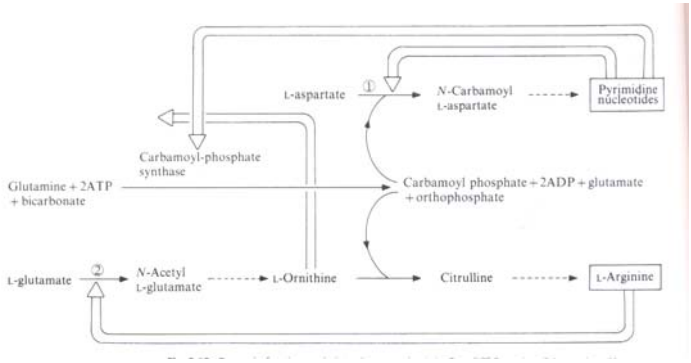
6.3.1 General aspects of intrinsic control

- Finding most economic way to regulate the flux

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} E \xrightarrow{E_5} F.$$



6.3 Control of metabolic pathways

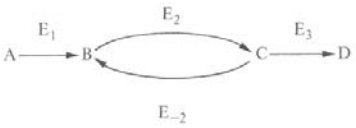


- Carbamoyl-phosphate synthase is inhibited by pyrimidines, but not by arginine
- Carbamoyl-phosphate synthase is activated by orinithine
- Other methods of regulation include isoenzymes (ex. Aromatic amino acid pathway)

6.3 Control of metabolic pathways

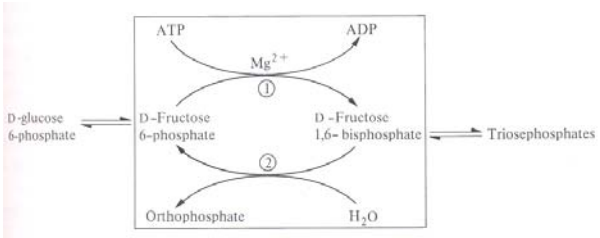
6.3.2 Amplification of signals

1. Substrate cycles



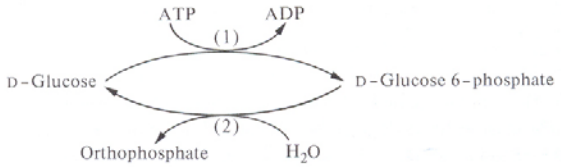
If E_2 and E_{-2} are catalyzed by different enzymes,

AMP activates 1 and inhibits 2



6.3 Control of metabolic pathways

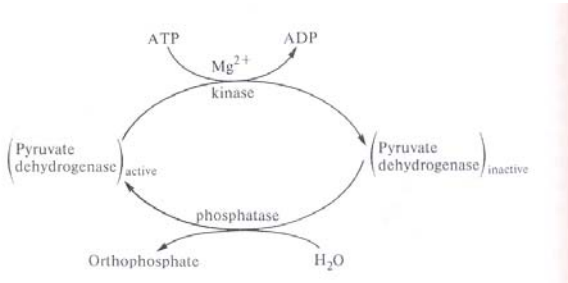
Control of glucose/glycogen metabolism in liver



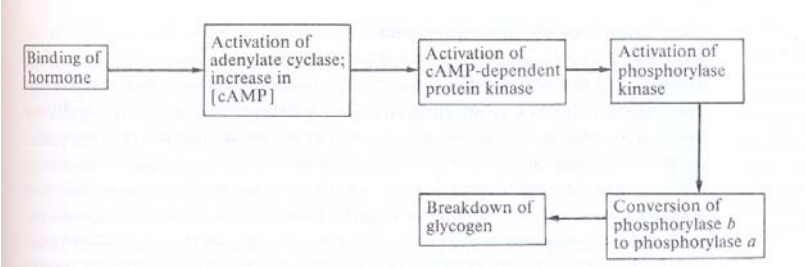
- Other examples include PEP/pyruvate interconversion, fatty acid/triglycerol in adipose tissue, etc...
- Sometimes working as futile cycle

6.3 Control of metabolic pathways

2. Interconvertible enzyme cycle
   - Control of enzyme activities by covalent modification
   - 0.5% changes of modifier can regulate enzyme activity from 10 to 90%
   - High ratios of [NADH]/[NAD+] and [acetylCoA]/[CoA] activate kinase and inactivates protein phosphatase



6.3 Control of metabolic pathways



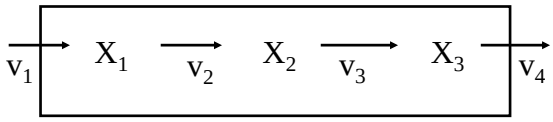
- 1% increase of [cAMP] can convert 50% of phosphorylase *b* to *a* within 2 seconds

6.3 Control of metabolic pathways

3. Theoretical approach to analyze the metabolic pathways
1. Metabolic flux analysis
2. Metabolic control analysis

Metabolic Flux Analysis

Example 1



$$2X_2 \rightarrow X_3$$

$$\frac{dX_1}{dt} = v_1 - v_2$$

$$\frac{dX_2}{dt} = v_2 - 2v_3$$

$$\frac{dX_3}{dt} = v_3 - v_4$$

$$\frac{dX}{dt} = S \cdot v$$

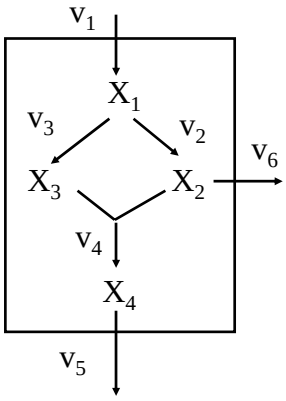
$$S = \begin{array}{c} \begin{matrix} V_1 & V_2 & V_3 & V_4 \end{matrix} \\ \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -2 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \end{array} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

Quasi Steady State Assumption

$$\frac{dX}{dt} = S \cdot v = 0$$

$$\begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -2 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix} = 0$$

Example 2



$$\frac{dX}{dt} = S \cdot v = 0$$

$$\begin{bmatrix} 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 1 & 0 & -2 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{bmatrix} = 0$$

$$2X_2 + X_3 \rightarrow X_4$$

$$\begin{bmatrix} 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 1 & 0 & -2 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{bmatrix} = 0$$

Type I Operation

$$\begin{bmatrix} -1 & -1 & 0 & 0 & 1 & 0 \\ 1 & 0 & -2 & 0 & 0 & -1 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \end{bmatrix} \begin{bmatrix} v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_1 \\ v_6 \end{bmatrix} = 0$$

$$\left[\begin{array}{cccc:cc} -1 & -1 & 0 & 0 & 1 & 0 \\ 1 & 0 & -2 & 0 & 0 & -1 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \end{array}\right]\left[\begin{array}{c} v_2 \\ v_3 \\ v_4 \\ v_5 \\ \hdashline v_1 \\ v_6 \end{array}\right] = 0$$

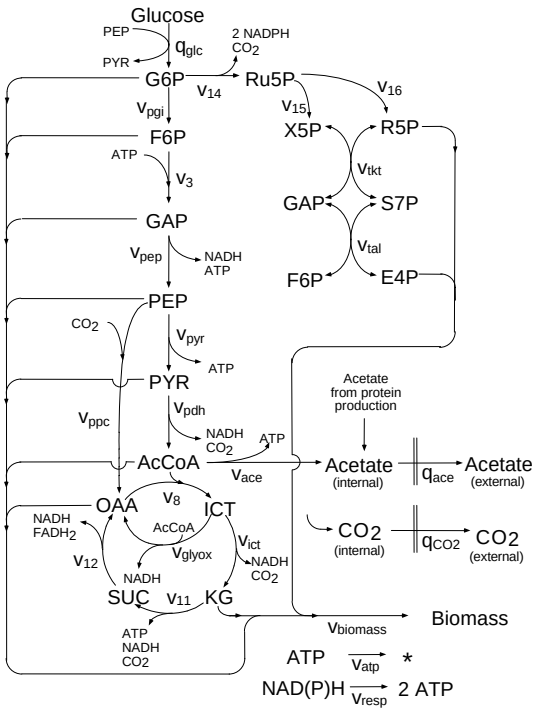
$$\begin{bmatrix} S_u & S_m \end{bmatrix}\begin{bmatrix} V_u \\ V_m \end{bmatrix} = 0$$

$$S_u V_u + S_m V_m = 0$$

$$S_u V_u = -S_m V_m$$

$$V_u = -S_u^{-1} S_m V_m$$

Example 3



	J_{glc}	J_{pgi}	J_3	J_{pep}	J_{pyk}	J_{pdh}	J_{ace}	J_8	J_{ict}	J_{11}	J_{12}	J_{ppc}	J_{14}	J_{15}	J_{16}	J_{tkt}	J_{tal}	J_{resp}	J_{atp}	Q_{co2}	Q_{ace}	$J_{biomass}$
G6P	1	-1	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	-205
F6P	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	-70.9
GAP	0	0	2	-1	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	-1625
PEP	-1	0	0	1	-1	0	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	-519.1
PyR	1	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-2832.8
AcCoA	0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	-4028.8
OAA	0	0	0	0	0	0	0	-1	0	0	1	1	0	0	0	0	0	0	0	0	0	-1786.7
ICT	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
KG	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	-1078.9
SUC	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0
Ru5P	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0
R5P	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	-1	0	0	0	0	0	-897.7
X5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0
S7P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0
E4P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	-361
NAD(H)P	0	0	0	1	0	1	0	0	1	1	1	0	2	0	0	0	0	-1	0	0	0	-14678
ATP	0	0	-1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	2	-1	0	0	-18485
CO2	0	0	0	0	0	1	0	0	1	1	0	-1	1	0	0	0	0	0	0	-1	0	1793
acetate	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	387

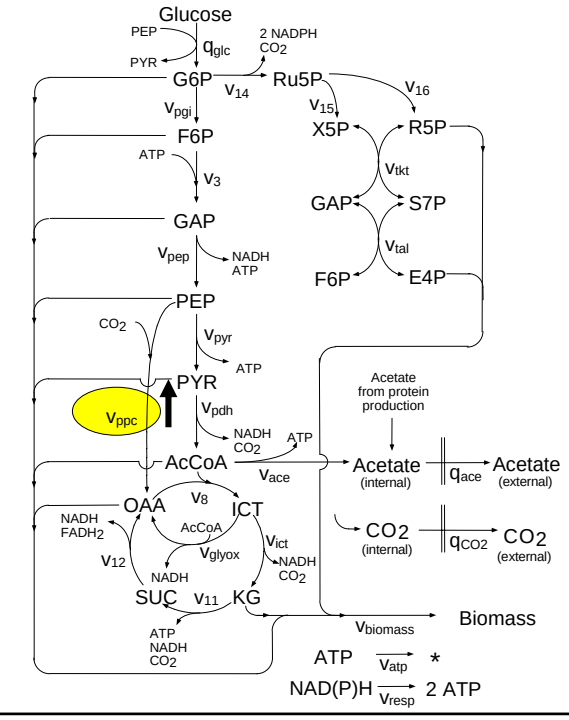
Assumption : $V_{glyox} = 0$, Otherwise, S_u is singular

$$V_u = S_u^{-1} S_m V_m$$

$$V_m = \begin{bmatrix} J_{glc} \\ q_{ace} \\ q_{CO_2} \end{bmatrix} = \begin{bmatrix} 1 \\ 0.3 \\ 2.2 \end{bmatrix}$$

$$V_u = \begin{bmatrix} J_{pgi} \\ J_3 \\ J_{pep} \\ J_{pyr} \\ J_{pdh} \\ J_{ace} \\ J_8 \\ J_{ict} \\ J_{11} \\ J_{12} \\ J_{ppc} \\ J_{14} \\ J_{15} \\ J_{16} \\ J_{tkt} \\ J_{tal} \\ J_{resp} \\ J_{atp} \\ J_{Biomass} \end{bmatrix} = \begin{bmatrix} 0.856 \\ 0.879 \\ 1.63 \\ 0.363 \\ 1.14 \\ 0.271 \\ 0.551 \\ 0.551 \\ 0.466 \\ 0.466 \\ 0.226 \\ 0.128 \\ 0.0992 \\ 0.0285 \\ 0.0285 \\ 0.0285 \\ 3.35 \\ 7.56 \\ 0.0000788 \end{bmatrix}$$

$$V_u = \begin{bmatrix} J_{pgi} \\ J_3 \\ J_{pep} \\ J_{pyr} \\ J_{pdh} \\ J_{ace} \\ J_8 \\ J_{ict} \\ J_{11} \\ J_{12} \\ J_{ppc} \\ J_{14} \\ J_{15} \\ J_{16} \\ J_{tkt} \\ J_{tal} \\ J_{resp} \\ J_{atp} \\ J_{Biomass} \end{bmatrix} = \begin{bmatrix} 0.856 \\ 0.879 \\ 1.63 \\ 0.363 \\ 1.14 \\ 0.271 \\ 0.551 \\ 0.551 \\ 0.466 \\ 0.466 \\ 0.226 \\ 0.128 \\ 0.0992 \\ 0.0285 \\ 0.0285 \\ 0.0285 \\ 3.35 \\ 7.56 \\ 0.0000788 \end{bmatrix}$$



$$V_u = S_u^{-1} S_m V_m$$

$$V_m = \begin{bmatrix} J_{glc} \\ J_{ppc} \\ J_{resp} \end{bmatrix} = \begin{bmatrix} 1 \\ 0.237 \\ 3.35 \end{bmatrix}$$

$$V_u = \begin{bmatrix} J_{pgi} \\ J_3 \\ J_{pep} \\ J_{pyr} \\ J_{pdh} \\ J_{ace} \\ J_8 \\ J_{ict} \\ J_{11} \\ J_{12} \\ J_{14} \\ J_{15} \\ J_{16} \\ J_{tkt} \\ J_{tal} \\ J_{atp} \\ q_{CO_2} \\ q_{ace} \\ J_{Biomass} \end{bmatrix} = \begin{bmatrix} 0.849 \\ 0.873 \\ 1.61 \\ 0.331 \\ 1.10 \\ 0.174 \\ 0.590 \\ 0.590 \\ 0.500 \\ 0.500 \\ 0.134 \\ 0.104 \\ 0.0299 \\ 0.0299 \\ 0.0299 \\ 7.42 \\ 2.32 \\ 0.204 \\ 0.0000829 \end{bmatrix}$$

Metabolic Flux Analysis I

- Mass Balance Equations
- Quasi Steady State Approximation
- Removing the Linearly Dependent Reactions
- Measure the Fluxes as many as Degree of Freedom

Metabolic Flux Analysis II

- Mass Balance Equations
- Quasi Steady State Approximation
- Removing the Linearly Dependent Reactions
- Measure the Fluxes as many as Degree of Freedom

• More Constraints

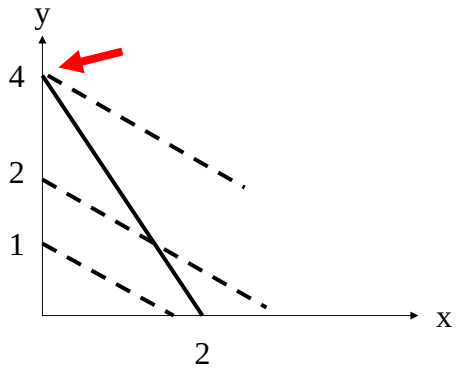
• Optimal Solution

$$\frac{dX}{dt} = S \cdot v = 0$$

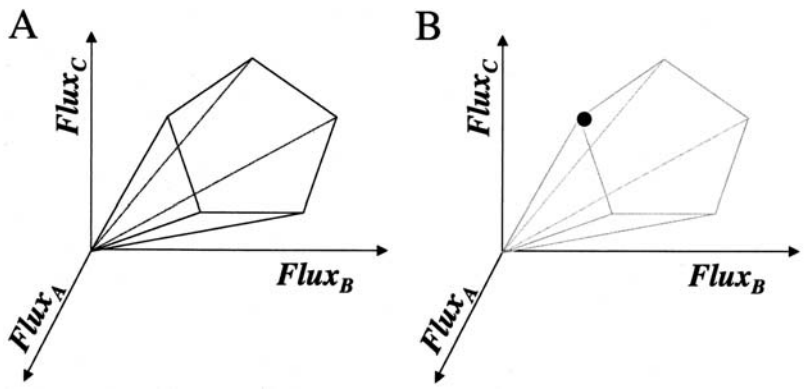
$$0 \le v_i \le v_{i,\max}$$
$$0 \le v_j \le v_{j,\max}$$
$$0 \le v_k \le v_{k,\max}$$
$$\vdots$$

$J_{biomass} \longrightarrow$ Maximum

$$2x + y = 4 \quad (x \ge 0, y \ge 0)$$



$$2x + 3y \rightarrow Max$$



From Edwards and Palsson, *PNAS*, **97**:5528

Summary : Metabolic Flux Analysis

- Metabolic Flux Distribution
- Maximum Yield
- Predicting Growth Rates (wild type and mutant)
- Relying on Biochemical Knowledge
- No Kinetics and Regulatory Information

Metabolic Control Analysis

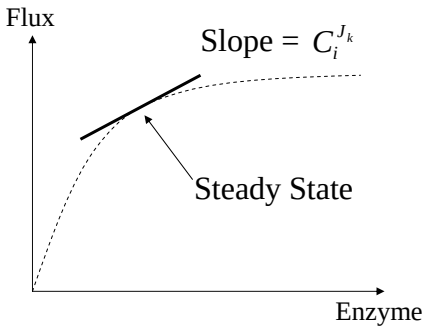
$$\xrightarrow{e_1} X_1 \xrightarrow{e_2} X_2 \xrightarrow{e_3} X_3 \xrightarrow{e_4}$$

Flux control coefficient

$$C_i^{J_k} = \frac{E_i}{J_k}\frac{dJ_k}{dE_i} = \frac{d\ln J_k}{d\ln E_i}$$

Elasticity coefficient

$$E_i^{v_k} = \frac{dv_k}{dS_i}$$



Theorems

$$\sum C_n^J = 1$$

$$\sum C_i^J E_S^i = 0$$

Metabolic Control Analysis

- Rate limiting enzyme
- How the activity change in one enzyme can affect on the overall pathway
- Hard to estimate parameters