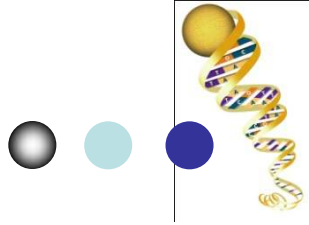


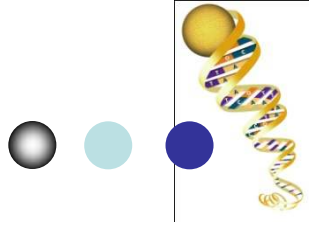
Introduction to

metabolic flux analysis (MFA) and metabolic control analysis (MCA):

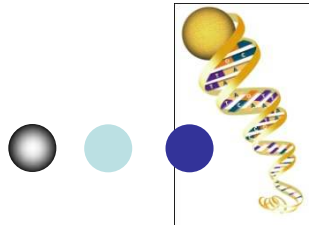


CONTENTS

1. Introduction to metabolic engineering
2. Metabolic flux analysis (MFA)
3. Metabolic control analysis (MCA)
4. Metabolic network modeling

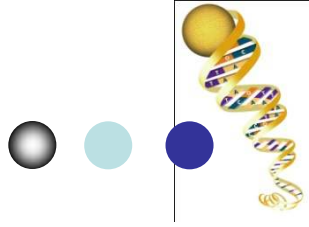


Introduction to Metabolic Engineering



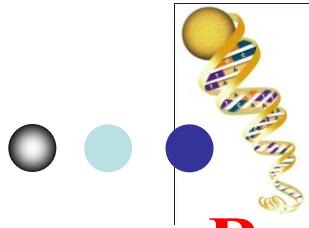
What is Metabolism?

- Anabolism
- Catabolism
- Transportation
- Regulation (genes, enzymes, and metabolites)



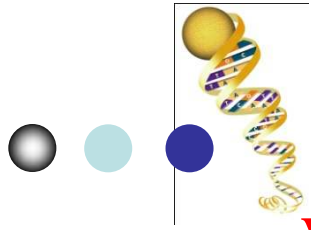
Target Molecules or Pathways

- **Primary metabolite** (during the growth phase)
- **Secondary metabolite: antibiotics, pigment**
(near the end of the growth phase or during stationary phase)
- **Signal transduction: sporulation, cell differentiation, cancer metastasis**



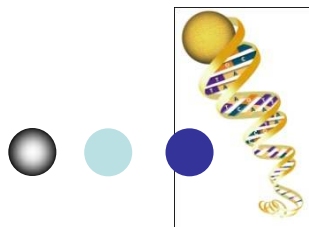
Requirements for understanding metabolism

- ✓ All pathways for substrates, products and intermediary metabolites
- ✓ All chemical reactions in cell
- ✓ Stoichiometry of the pathway reactions
- ✓ Enzyme reaction kinetics
- ✓ Regulatory interactions



Why Is Metabolic Analysis Needed?

- ✓ Estimation of *in vivo* flux
- ✓ Understanding the control of the flux
- ✓ Revealing the degree of pathway engagement in the overall metabolic process

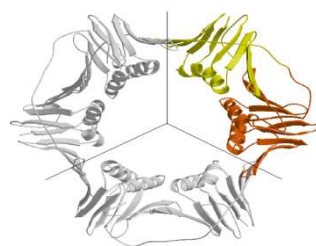


Assembly and analysis of genome sequences

ATCTCCGC
CGCTAGCTAA
AATATG
GCTAGCTAATA
TCTCCGCTA
ATCTCCGCTAGCTAATATG

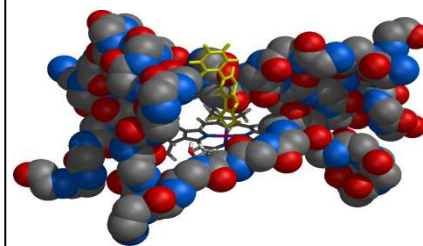
Consensus DNA sequence
from shotgun sequencing

Prediction of protein structure



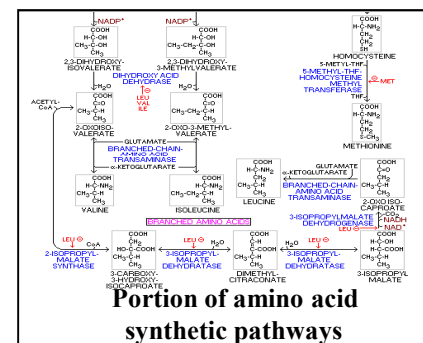
Predicted structure for
DNA clamping protein

Molecular modeling of biochemical reactions

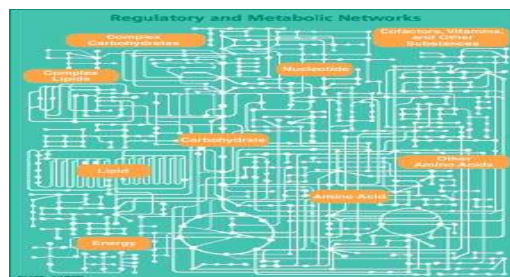


Enzymatic activation of
environmental mutagen

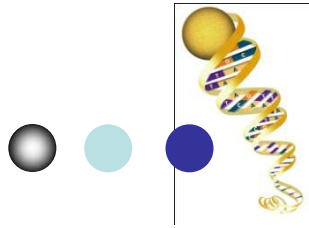
Process simulation of biological systems



Metabolic engineering



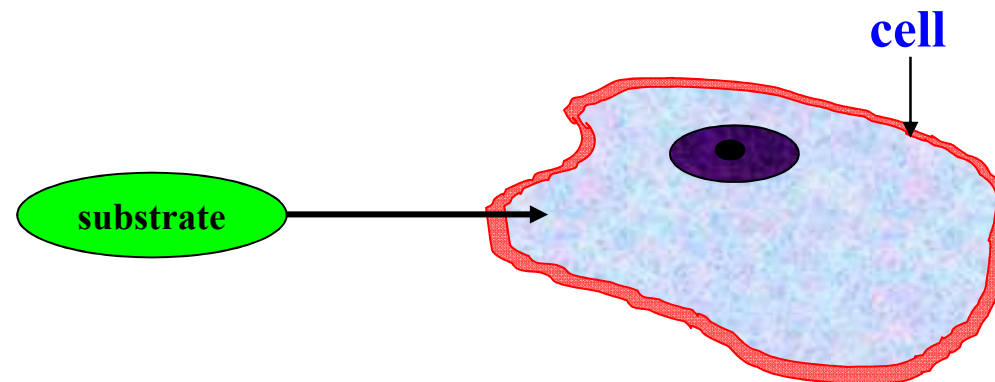
Metabolic network

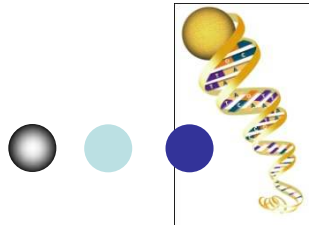


Targets of Metabolic Engineering

Transport machinery

- ✓ Extending the range of substrate spectrum
- ✓ Changing the regulation mechanism of transport
- ✓ Modifying the transport network for overproduction

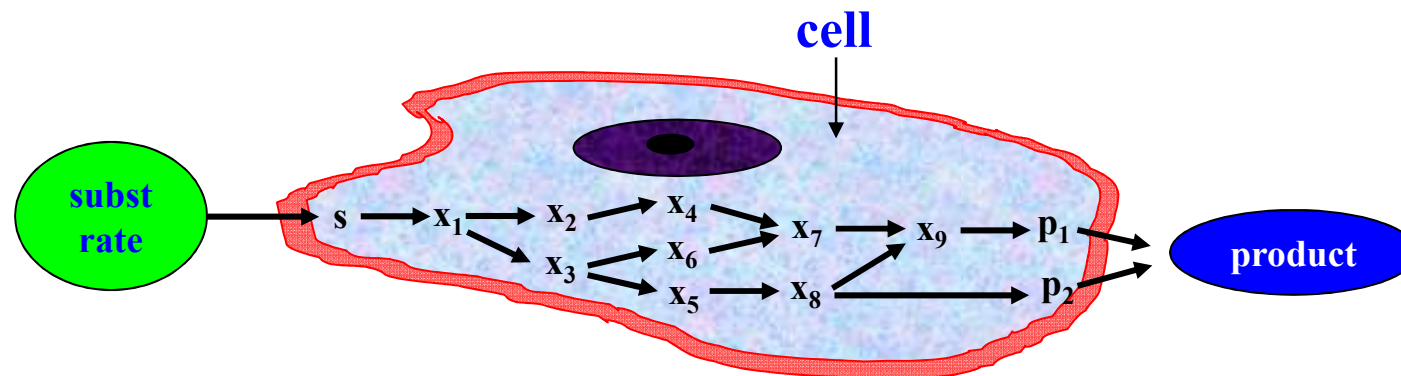


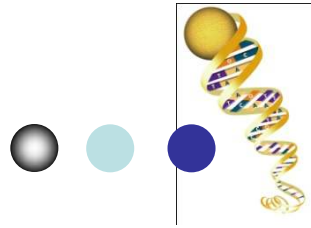


Targets of Metabolic Engineering

Intermediary metabolism

- ✓ Self-cloning
- ✓ Metabolic flux control
- ✓ Changing energy efficiencies
- ✓ Signal transduction pathway

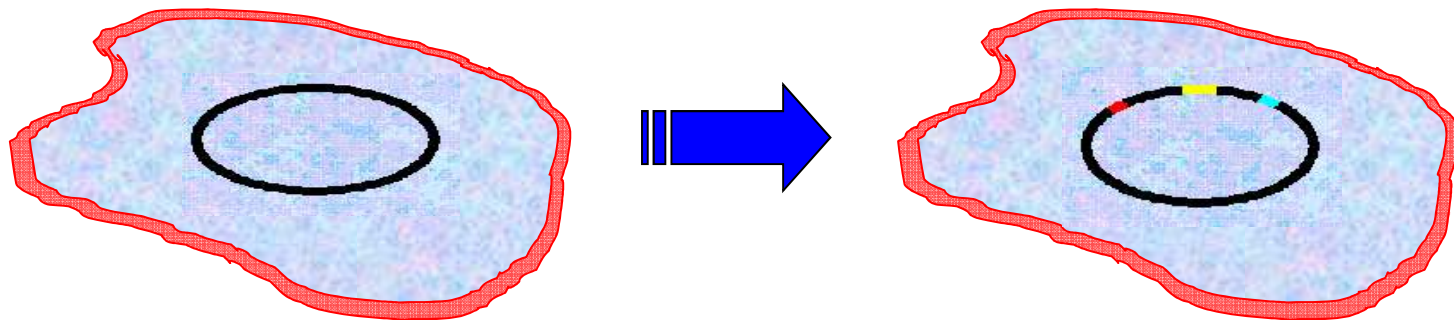


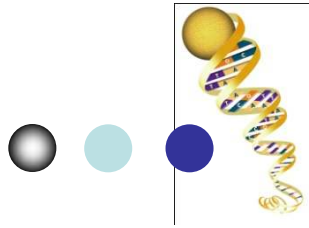


Targets of Metabolic Engineering

Metabolic redesign

- ✓ recruiting the non-indigenous metabolic activities
- ✓ Improve efficiency of host cell metabolism

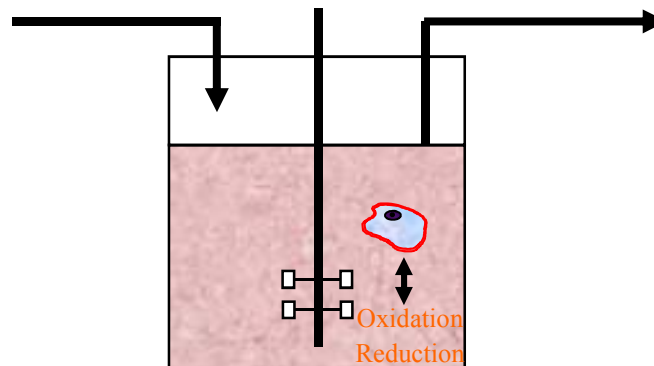


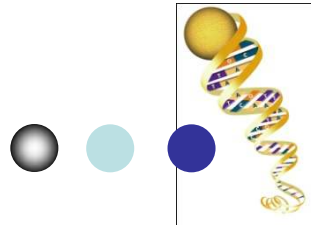


Targets of Metabolic Engineering

Global metabolism and physiological properties

- ✓ Changing the redox state in cell
- ✓ Endowing and enhancing whole-cell catalytic capacities
- ✓ Growth phase engineering
- ✓ Adaptability to down-stream processing conditions





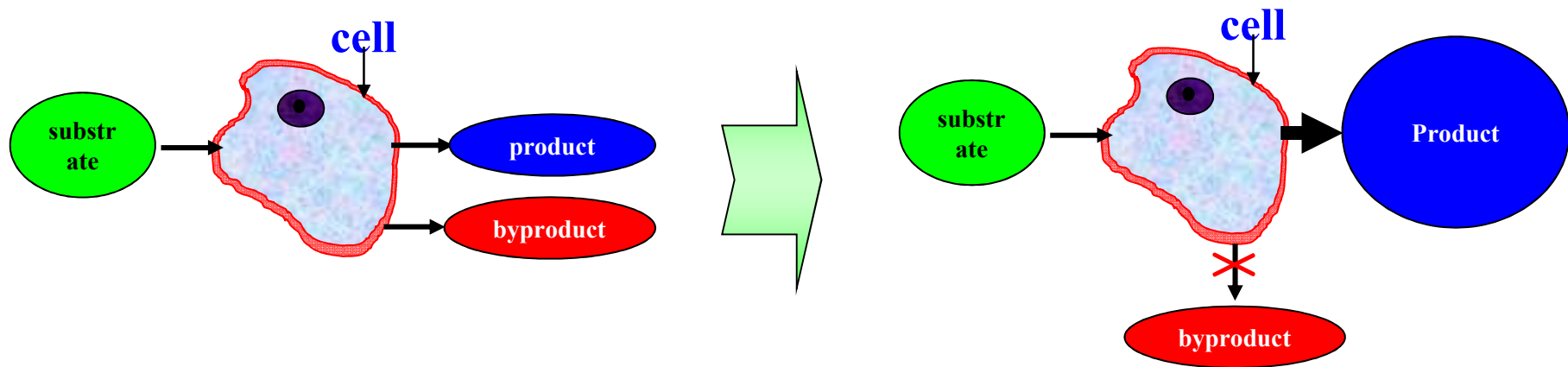
Targets of metabolic engineering

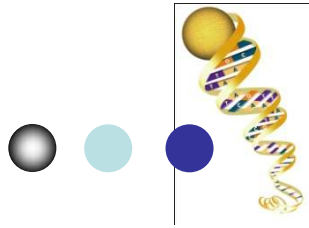
Byproducts metabolism

- ✓ Blocking and redirecting to less harmful or valuable (by)products

Secretion

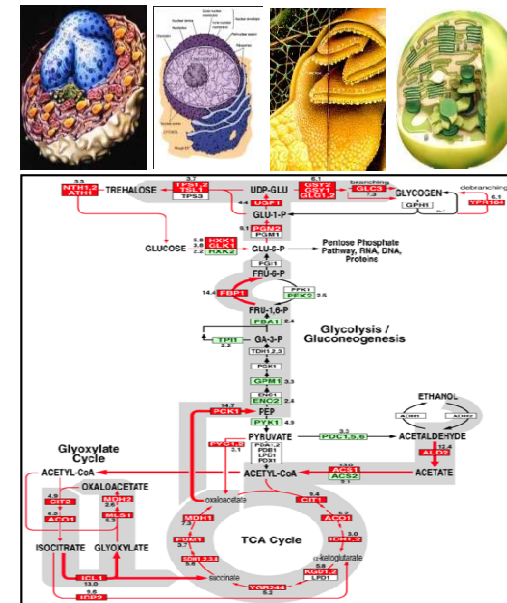
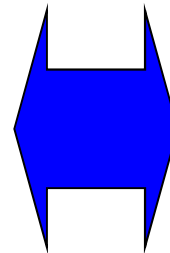
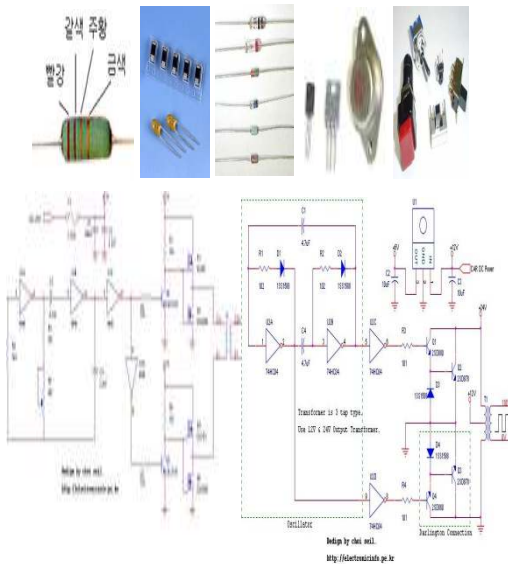
- ✓ Modifying secretion mechanism for overproduction

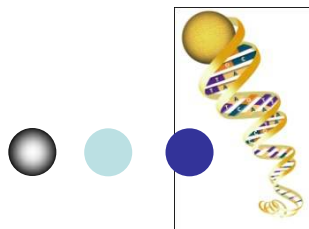




Electrical vs. Metabolic systems

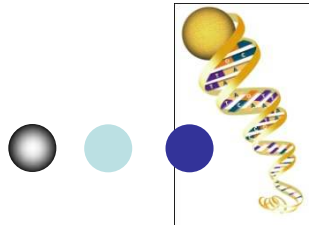
- ✓ Components can be isolated
- ✓ Limited number of components (resistance, condenser, coil, etc)
 - A few laws are applied
- ✓ Interactions are exactly known





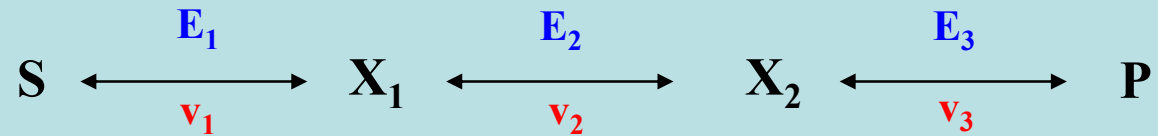
Metabolic Flux Analysis

MFA



Metabolic Flux

MFA



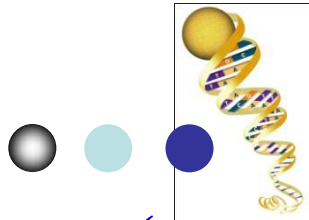
Rates of reaction $v_1 = f(S, X_1, E_1, \dots)$

$$v_2 = \dots$$

$$v_3 = \dots$$

Assumption of steady state

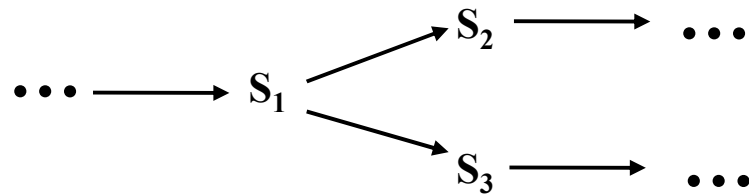
$$v_1 = v_2 = v_3 = \text{Flux} = \frac{dp}{dt}$$



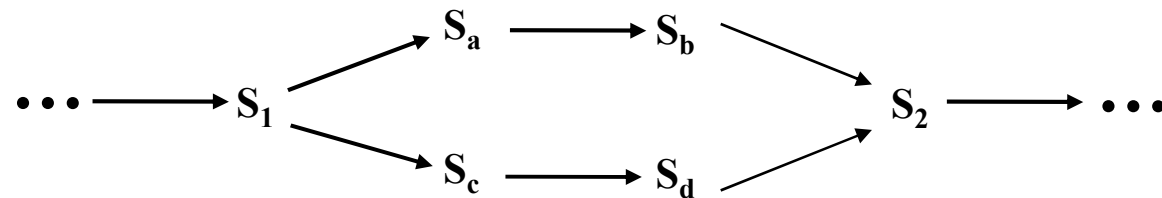
Modes in Metabolic Pathway

MFA

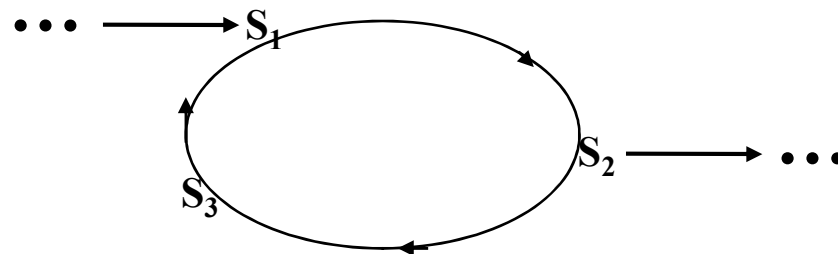
✓ branch point control (nodal rigidity)

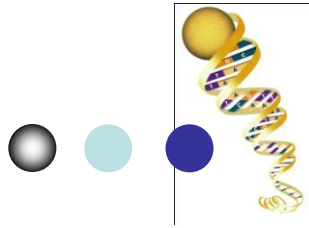


✓ alternative pathway



✓ cyclic pathway

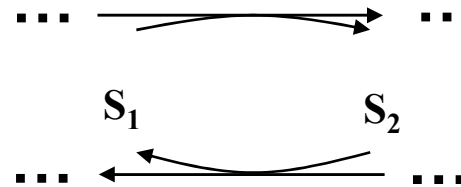




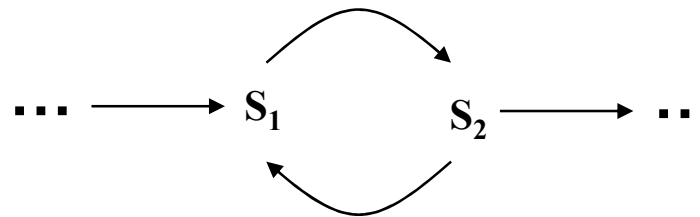
Modes in Metabolic Pathway

MFA

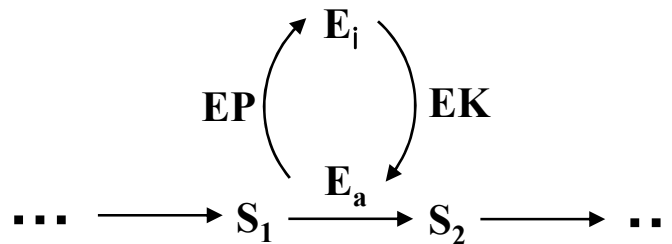
✓ moiety-conserved cycle

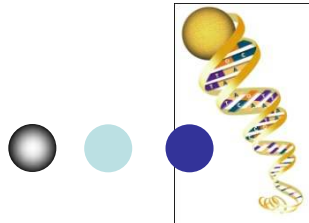


✓ substrate cycle



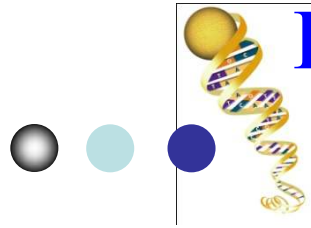
✓ interconvertible enzyme cascades





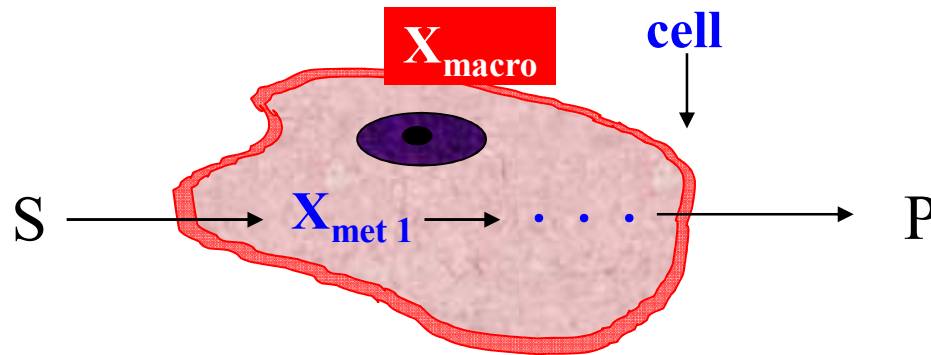
Determination of intracellular metabolic fluxes

1. From the reaction stoichiometry
2. Setting mass balance around intracellular metabolites
3. Measurement of metabolite reaction rates



I. Stoichiometric Mass Balance Modeling

MFA



S : Substrate

P : Product

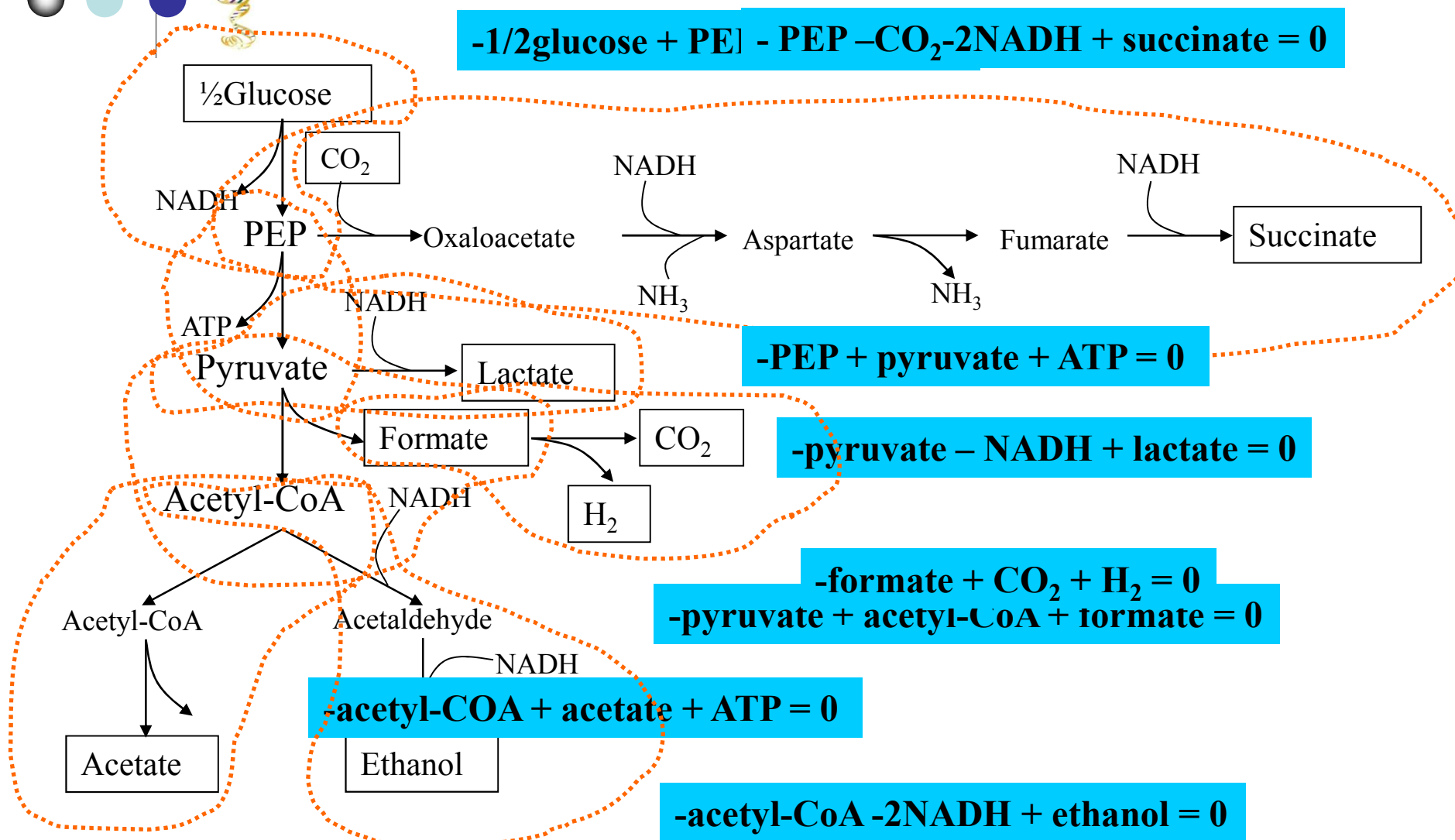
X_{macro} : Biomass constituent

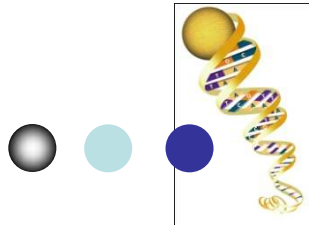
X_{met} : Intracellular metabolite

$$AS + BP + C.X_{macro} + G.X_{met} = 0$$

A, B, C, G : stoichiometry matrix

Example: Set-Up of Elementary Reactions





Example (continued)

MFA

Stoichiometric mass balance equations

$$-1/2\text{glucose} + \text{PEP} + \text{NADH} = 0 \quad (1)$$

$$- \text{PEP} - \text{CO}_2 - 2\text{NADH} + \text{succinate} = 0 \quad (2)$$

$$-\text{PEP} + \text{pyruvate} + \text{ATP} = 0 \quad (3)$$

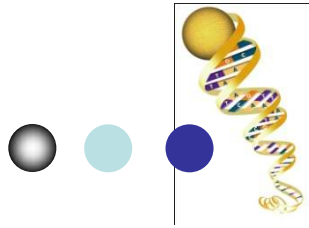
$$-\text{pyruvate} - \text{NADH} + \text{lactate} = 0 \quad (4)$$

$$-\text{pyruvate} + \text{acetyl-CoA} + \text{formate} = 0 \quad (5)$$

$$-\text{formate} + \text{CO}_2 + \text{H}_2 = 0 \quad (6)$$

$$-\text{acetyl-CoA} + \text{acetate} + \text{ATP} = 0 \quad (7)$$

$$-\text{acetyl-CoA} - 2\text{NADH} + \text{ethanol} = 0 \quad (8)$$



Example (end)

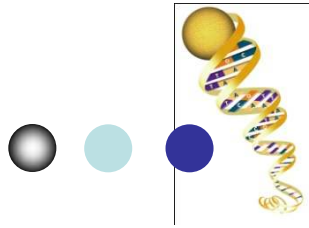
MFA

Matrix form of stoichiometric equations

$$\begin{pmatrix} -\frac{1}{2} \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} S_{\text{glc}} + \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} P_{\text{suc}} \\ P_{\text{CO}_2} \\ P_{\text{lac}} \\ P_{\text{for}} \\ P_{\text{H}_2} \\ P_{\text{ac}} \\ P_{\text{et}} \end{pmatrix} + \begin{pmatrix} 1 & 0 & 0 & 0 & 1 \\ -1 & 0 & 0 & 0 & -2 \\ -1 & 1 & 0 & 1 & 0 \\ 0 & -1 & 0 & 0 & -1 \\ 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 \\ 0 & 0 & -1 & 0 & -2 \end{pmatrix} \begin{pmatrix} X_{\text{PEP}} \\ X_{\text{pyr}} \\ X_{\text{AcCoA}} \\ X_{\text{ATP}} \\ X_{\text{NADH}} \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

$$AS + BP + C \cdot X_{\text{macro}} + G \cdot X_{\text{met}} = 0$$

A, B, C, G : stoichiometry matrix



II. Reaction Rate Modeling

MFA

Substrates

$$r_{s,i} = - \sum_{j=1}^J a_{ji} v_j$$

Matrix notation



$$\mathbf{r}_s = -\mathbf{A}^T \mathbf{v}$$

products

$$r_{p,i} = \sum \beta_{ji} v_j$$



$$\mathbf{r}_p = \mathbf{B}^T \mathbf{v}$$

**biomass
constituents**

$$r_{macro,i} = \sum_{j=1}^J \gamma_{ji} v_j$$



$$\mathbf{r}_{macro} = \mathbf{C}^T \mathbf{v}$$

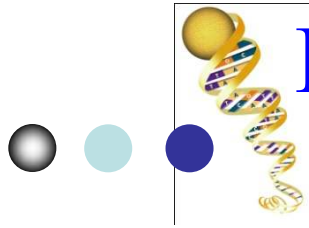
**intermediate
metabolite**

$$r_{met,i} = \sum_{j=1}^J g_{ji} v_j$$



$$\mathbf{r}_{met} = \mathbf{G}^T \mathbf{v}$$

A, B, C, G : stoichiometry matrix



III. Dynamic Rate Modeling

MFA

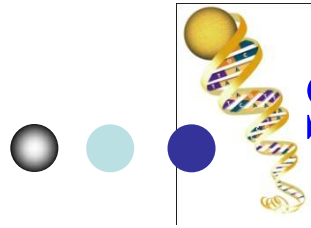
**Steady-State Approximation:
including bioreactor mass balance**

$$\text{Accumulation term} \quad \frac{dX_{met}}{dt} = \text{Generation term} \quad r_{met} - \text{Dilution term} \quad \mu X_{met}$$

X_{met} : concentration vector for the intracellular metabolites (or pathway intermediates)

r_{met} : vector containing the net rates of formation of the intracellular metabolites

μ : specific growth rate



Steady State Approximation

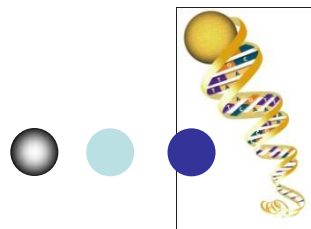
MFA

- ✓ No metabolite accumulation (pseudo-steady state)

$$0 = r_{met} - \mu X_{met}$$

- ✓ Second term(dilution effect) can be neglected: intermediate concentrations in the cell are negligible

$$0 = r_{met} = G^T v$$



Degree of freedom (D.O.F)

MFA

$$\mathbf{F} = \mathbf{J} - \mathbf{K}$$

F: degree of freedom

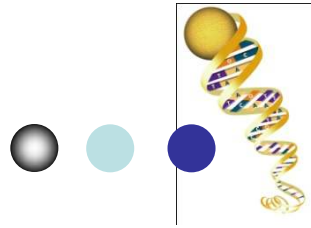
J: no. of reactions (or pathway fluxes)

K: no. of balance equations

1) Determined system: $\mathbf{J} = \mathbf{K}$

2) Overdetermined system: $\mathbf{J} < \mathbf{K}$

3) Underdetermined system: $\mathbf{J} > \mathbf{K}$



Determining unknown fluxes

MFA

If F numbers of fluxes in $\mathbf{v}$ are measured, the system becomes **determined** and the solution is unique.

$$0 = G^T v = G_m^T v_m + G_c^T v_c$$

$$v_c = - (G_c^T)^{-1} G_m^T v_m$$

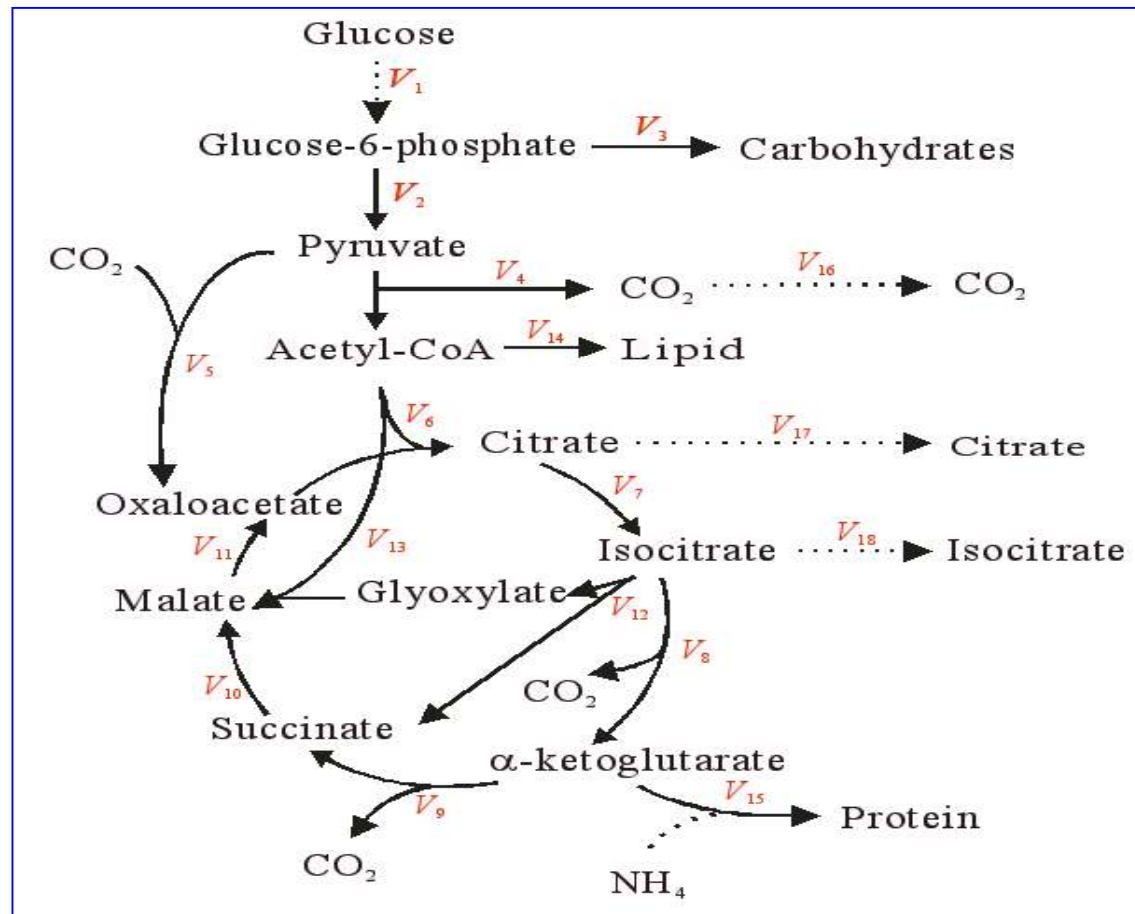
Where (**m**: measured **c**: calculated)

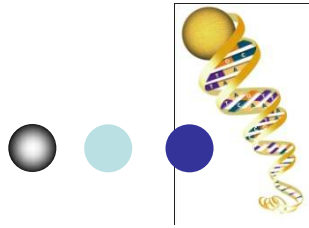


Case 1

MFA

Metabolic Flux Analysis of citric acid fermentation by *Candida lipolytica*
(by Aiba and Matsuoka(1979)): In the form of rates or fluxes





Case 1

MFA

< Solution >

- ✓ 18 reaction rates
- ✓ 11 balance equations

$$\text{G6P: } v_1 - v_2/2 - v_3 = 0$$

$$\text{Pyr: } v_2 - v_4 - v_5 = 0$$

$$\text{AcCoA: } v_4 - v_6 - v_{13} - v_{14} = 0$$

$$\text{CIT: } v_6 - v_7 - v_{17} = 0$$

$$\text{ICT: } v_7 - v_8 - v_{12} - v_{18} = 0$$

$$\text{OGT: } v_8 - v_9 - v_{15} = 0$$

$$\text{SUC: } v_9 - v_{10} + v_{12} = 0$$

$$\text{MAL: } v_{10} - v_{11} + v_{13} = 0$$

$$\text{GOX: } v_{12} - v_{13} = 0$$

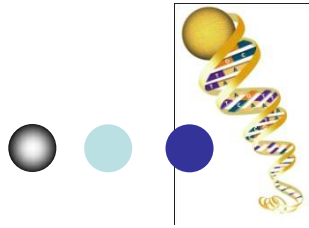
$$\text{OAA: } v_5 + v_{11} - v_6 = 0$$

$$\text{CO}_2: v_4 + v_8 + v_9 - v_{16} = 0$$

- ✓ Degree of freedom

$$\text{DOF} = J - K$$

$$7 = 18 - 11$$



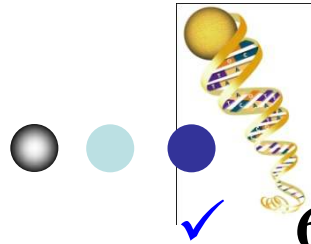
Case 1

MFA

Formulation in matrix form

$$G^T v = 0$$

$$\begin{bmatrix}
 1 & -0.5 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 1 & 0 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & -1 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0
 \end{bmatrix}
 \begin{bmatrix}
 v_1 \\
 v_2 \\
 v_3 \\
 \cdot \\
 \cdot \\
 \cdot \\
 v_{14} \\
 v_{15} \\
 v_{16} \\
 v_{17} \\
 v_{18}
 \end{bmatrix}
 =
 \begin{bmatrix}
 0 \\
 0 \\
 0 \\
 0 \\
 0 \\
 0 \\
 0 \\
 0 \\
 0 \\
 0 \\
 0
 \end{bmatrix}$$



Case 1

MFA

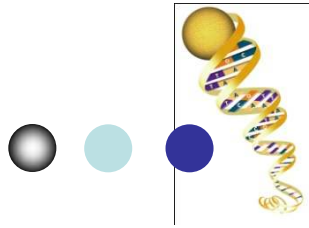
6 measurable reaction rates

- ① glucose uptake rate ($r_{\text{glc}}=v_1$)
- ② carbon dioxide production rate ($r_c=v_{16}$)
- ③ citric acid production rate ($r_{\text{cit}}=v_{17}$)
- ④ isocitric acid production rate ($r_{\text{ict}}=v_{18}$)
- ⑤ protein synthesis rate ($r_{\text{prot}}=v_{15}$)
- ⑥ carbohydrate synthesis rate ($r_{\text{car}}=v_3$)

✓ assumption

- ① The glyoxylate shunt is inactive. ($v_{12}=0$)

➡ Determined System



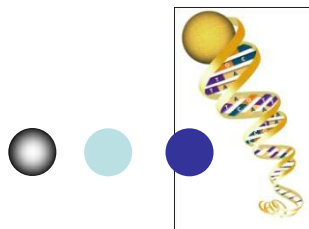
Case 1

MFA

$$0 = G^T v = G_m^T v_m + G_c^T v_c$$

$$v_c = - (G_c^T)^{-1} G_m^T v_m$$

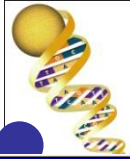
$$\begin{bmatrix} v_2 \\ v_4 \\ v_5 \\ v_6 \\ v_7 \\ v_8 \\ v_9 \\ v_{10} \\ v_{11} \\ v_{13} \\ v_{14} \end{bmatrix} = \begin{bmatrix} 2 & -2 & 0 & 0 & 0 & 0 & 0 \\ 2 & -2 & 1 & -1 & 0 & -1 & -1 \\ 0 & 0 & -1 & 1 & 0 & 1 & 1 \\ -1 & 1 & 0 & 1.5 & 0.5 & 2 & 2 \\ -1 & 1 & 0 & 1.5 & 0.5 & 1 & 2 \\ -1 & 1 & -1 & 1.5 & 0.5 & 1 & 1 \\ -1 & 1 & -1 & 0.5 & 0.5 & 1 & 1 \\ -1 & 1 & 0 & 0.5 & 0.5 & 1 & 1 \\ -1 & 1 & 1 & 0.5 & 0.5 & 1 & 1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 3 & -3 & 0 & -2.5 & -0.5 & -3 & -3 \end{bmatrix} \begin{bmatrix} r_{glc} \\ r_{car} \\ 0 \\ r_{prot} \\ r_c \\ r_{cit} \\ r_{ict} \end{bmatrix}$$



Overdetermined System

$$E\mathbf{r} = E_m\mathbf{r}_m + E_c\mathbf{r}_c = 0$$

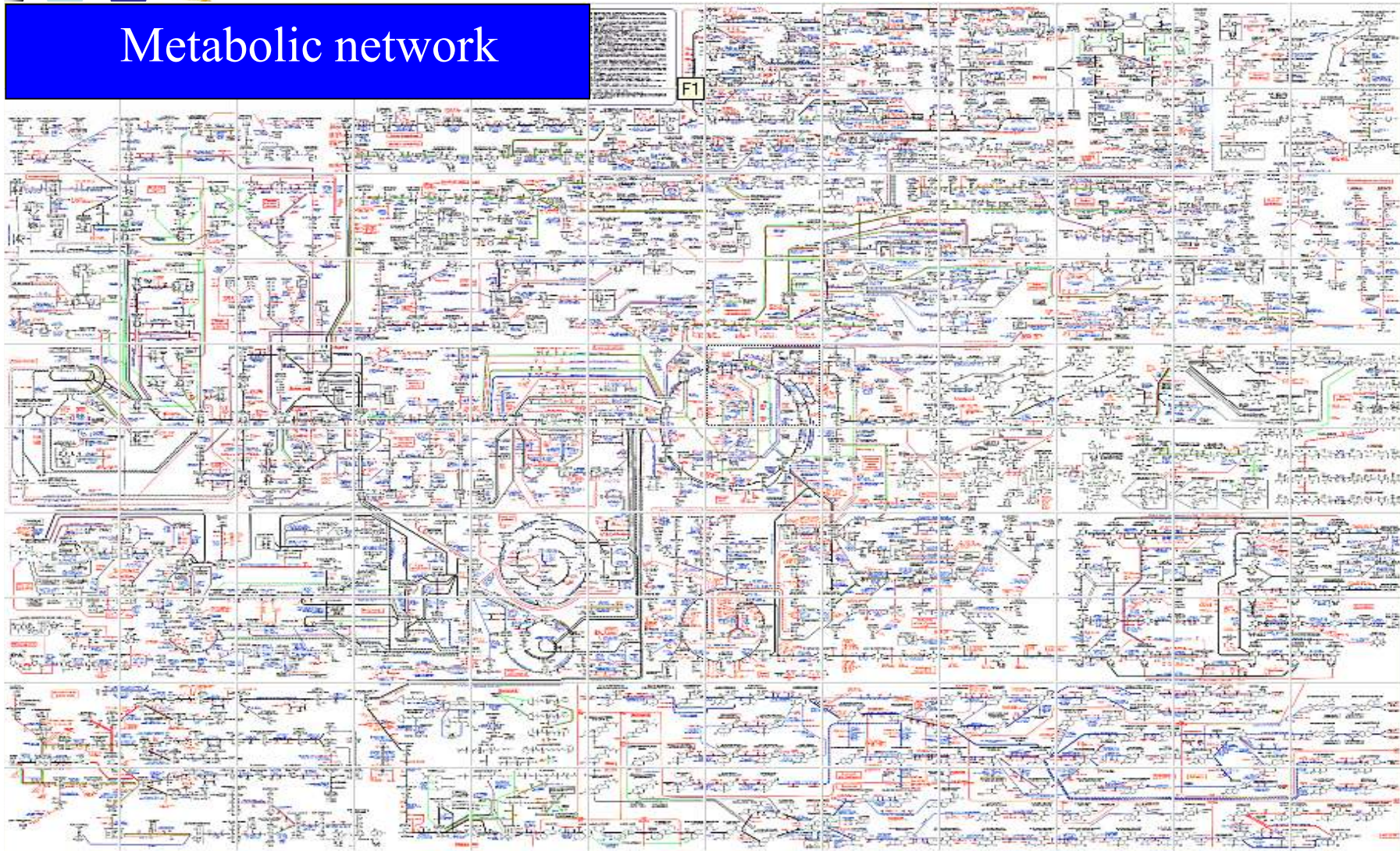
$$\mathbf{r}_c = -E_c^{-1} E_m\mathbf{r}_m$$

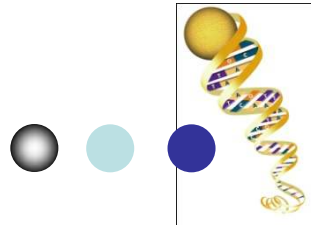


Case 2: Extension to whole cell

MFA

Metabolic network



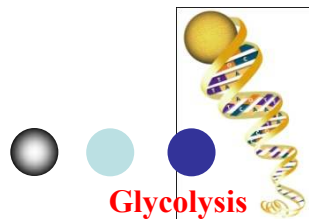


Case 2

MFA

J. Pramanik, J. D. Keasling(1997)

- ✓ Metabolic network was described by metabolic reactions for *Escherichia coli* on glucose
- ✓ The model incorporates 153 reversible and 147 irreversible reactions
- ✓ 289 metabolites from several metabolic data bases for the biosynthesis of the macromolecules



Case 2 (Pramanik & Keasling, 1997)

MFA

Glycolysis

Phosphotransferase system <i>ptsI, ptsH</i>	$GLC + PEP \rightarrow PYR + G6P$
Phosphoglucose isomerase <i>pgi</i>	$G6P \leftrightarrow F6P$
Phosphofructokinase <i>pfk</i>	$F6P + ATP \leftrightarrow ADP + F16P$
Fructose-1,6-bisphosphate aldolase <i>fba</i>	$F16P + H_2O \rightarrow F6P + DHAP$
Fructose-1,6-bisphosphatase <i>fba</i>	$F16P \leftrightarrow T3P1 + T3P2$
Triosephosphate isomerase <i>tpi</i>	$T3P1 \leftrightarrow T3P2$
Glyceraldehyde-3-phosphate dehydrogenase <i>gap</i>	$T3P1 + P_i + NAD^+ \leftrightarrow NADH + 1,3P2DG$
Phosphoglycerate kinase <i>pgk</i>	$1,3P2DG + ADP \leftrightarrow ATP + 3PGDGL$
Phosphoglycerate mutase <i>gpm</i>	$3PGDGL \leftrightarrow 2PDGL$
Enolase <i>eno</i>	$2PDGL \leftrightarrow PEP$
Pyruvate kinase <i>pyk</i>	$PEP + ADP \rightarrow ATP + PYR$
PEP carboxykinase <i>pck</i>	$OA + ATP \leftrightarrow PEP + CO_2 + ADP$
PEP carboxylase <i>ppc</i>	$PEP + CO_2 \rightarrow OA + P_i$
Pyruvate dehydrogenase <i>pdh</i>	$PYR + COA + NAD^+ \rightarrow NADH + CO_2 + ACCOA$
PEP synthase <i>pps</i>	$PYR + ATP \rightarrow PEP + AMP + P_i$

Pentose phosphate shunt

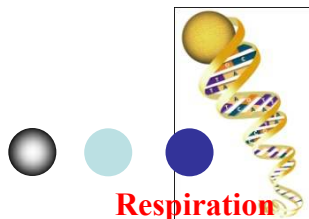
Glucose-6-phosphate dehydrogenase <i>zwf</i>	$G6P + NADP^+ \rightarrow D6PGL + NADPH$
6-Phosphogluconolactonase <i>pgl</i>	$D6PGL \rightarrow D6PGC$
6-Phosphogluconate dehydrogenase <i>gnd</i>	$D6PGC + NADP^+ \rightarrow NADPH + CO_2 + RL5P$
Ribose-5-phosphate isomerase <i>rpi</i>	$RL5P \leftrightarrow R5P$
Ribose-5-phosphate epimerase <i>rpe</i>	$RL5P \leftrightarrow X5P$
Transketolase 1 <i>tktA</i>	$R5P + X5P \leftrightarrow T3P1 + S7P$
Transaldolase <i>tal</i>	$T3P1 + S7P \leftrightarrow E4P + F6P$
Transketolase 2 <i>tktB</i>	$X5P + E4P \leftrightarrow F6P + T3P1$
6-Phosphogluconate dehydratase <i>edd</i>	$D6PGC \rightarrow 2K3D6PG$
2-Keto-3-deoxy-6-phosphogluconate aldolase <i>eda</i>	$2K3D6PG \rightarrow T3P1 + PYR$

Glycogen metabolism

Phosphoglucomutase <i>pgm</i>	$G6P \leftrightarrow G1P$
Glycogen synthase <i>glgA</i>	$G1P + ATP \rightarrow ADP + PPI + GLYCOGEN$
Glycogen phosphorylase <i>glg</i>	$P \text{ GLYCOGEN} + P_i \rightarrow G1P$
Dissimilation of pyruvate	
Lactate dehydrogenase <i>ldh</i>	$PYR + NADH \leftrightarrow NAD + LAC$
Alcohol dehydrogenase <i>adh</i>	$ACAL + NADH \leftrightarrow ETHANOL + NAD$
Acetaldehyde dehydrogenase <i>adh</i>	$AC + NADH \leftrightarrow NAD + ACAL$
Pyruvate formate lyase <i>pfl</i>	$PYR + COA \rightarrow ACCOA + FORMATE$
Phosphotransacetylase <i>pta</i>	$ACCOA + P_i \leftrightarrow ACTP + COA$
Acetate kinase <i>ackA</i>	$ACTP + ADP \leftrightarrow ATP + AC$
Formate hydrogen lyase <i>fhl</i>	$FORMATE \rightarrow CO_2$

TCA cycle and glyoxylate bypass

Citrate synthase <i>glcA</i>	$ACCOA + OA \leftrightarrow COA + CIT$
Aconitase <i>can</i>	$CIT \leftrightarrow ICIT$
Isocitrate dehydrogenase <i>idh</i>	$ICIT + NAD^+ \rightarrow CO_2 + NADH + AKG$
2-Ketoglutarate dehydrogenase <i>sucAB</i>	$AKG + NAD^+ + COA \leftrightarrow CO_2 + NADH + SUCCOA$
Succinate thiokinase <i>sucCD</i>	$SUCCOA + GDP + P_i \leftrightarrow GTP + COA + SUCC$
Succinate dehydrogenase <i>sdhABCD</i>	$SUCC + FAD \rightarrow FADH_2 + FUM$
Fumarate reductase <i>frdABCD</i>	$FUM + FADH_2 \rightarrow SUCC + FAD$
Fumarase <i>fumAB</i>	$FUM \leftrightarrow MAL$
Malate dehydrogenase <i>mdh</i>	$MAL + NAD^+ \leftrightarrow NADH + OA$
Malic enzyme <i>mez</i>	$MAL + NADP^+ \rightarrow CO_2 + NADPH + PYR$
Malic enzyme <i>mez</i>	$MAL + NAD^+ \rightarrow CO_2 + NADH + PYR$
Isocitrate lyase <i>aceA</i>	$ICIT \rightarrow GLX + SUCC$
Malate synthase <i>aceB</i>	$ACCOA + GLX \rightarrow COA + MAL$



Case 2 (Pramanik & Keasling, 1997)

MFA

Respiration

NADH dehydrogenase II *ndh* $\text{NADH} + \text{Q} \rightarrow \text{NAD} + \text{QH}_2$

NADH dehydrogenase I *ndh* $\text{NADH} + \text{Q} \rightarrow \text{NAD} + \text{QH}_2 + 4 \text{ HEXT}$

Formate dehydrogenase $\text{FORMATE} + \text{Q} \rightarrow 2 \text{ HEXT} + \text{QH}_2 + \text{CO}_2$

Cytochrome oxidase bo3 $\text{QH}_2 + 1/2 \text{ O}_2 \rightarrow \text{Q} + 4 \text{ HEXT}$

Cytochrome oxidase bd $\text{QH}_2 + 1/2 \text{ O}_2 \rightarrow \text{Q} + 2 \text{ HEXT}$

Succinate dehydrogenase complex $\text{FADH}_2 + \text{Q} \leftrightarrow \text{FAD} + \text{QH}_2$

ATP synthesis

F0F1-ATPase *unc* $\text{ATP} \leftrightarrow \text{ADP} + \text{PI} + 3 \text{ HEXT}$

Biosynthesis of aspartate

Aspartate transaminase *aspC* $\text{OA} + \text{GLU} \leftrightarrow \text{ASP} + \text{AKG}$

Biosynthesis of asparagine

Glutamine-dependent asparagine *asnB* $\text{ASP} + \text{ATP} + \text{GLN} \rightarrow \text{GLU} + \text{ASN} + \text{AMP} + \text{PPI}$
synthetase

Ammonia-dependent asparagine *asnA* $\text{ASP} + \text{ATP} + \text{NH}_3 \rightarrow \text{ASN} + \text{AMP} + \text{PPI}$
synthetase

Biosynthesis of proline

Glutamyl kinase *proB* $\text{GLU} + \text{ATP} \rightarrow \text{ADP} + \text{GLUP}$

Glutamate-5-semialdehyde

Dehydrogenase *proA* $\text{GLUP} + \text{NADPH} \leftrightarrow \text{NADP} + \text{PI} + \text{GLUGSAL}$

Pyrroline-5-carboxylate reductase *proC* $\text{GLUGSAL} + \text{NADPH} \leftrightarrow \text{PRO} + \text{NADP}$

Ornithine oxoacid transaminase $\text{ORN} + \text{AKG} \leftrightarrow \text{GLU} + \text{GLUGSAL}$

Biosynthesis of threonine and lysine

Aspartate kinase *thrA* $\text{ASP} + \text{ATP} \leftrightarrow \text{ADP} + \text{BASP}$

Aspartate semialdehyde *asd* $\text{BASP} + \text{NADPH} \leftrightarrow \text{NADP} + \text{PI} + \text{ASPSA}$
dehydrogenase

Homoserine dehydrogenase *thrA* $\text{ASPSA} + \text{NADPH} \leftrightarrow \text{NADP} + \text{HSER}$

Homoserine kinase *thrB* $\text{HSER} + \text{ATP} \rightarrow \text{ADP} + \text{PHSER}$

Threonine synthase *thrC* $\text{PHSER} \rightarrow \text{PI} + \text{THR}$

Dihydrodipicolinate synthase *dapA* $\text{ASPSA} + \text{PYR} \rightarrow \text{D23PIC}$

Dihydrodipicolinate reductase *dapB* $\text{D23PIC} + \text{NADPH} \rightarrow \text{NADP} + \text{PIP26DX}$

Tetrahydrodipicolinate succinylase *dapD* $\text{PIP26DX} + \text{SUCCOA} \rightarrow \text{COA} + \text{NS2A6O}$

Succinyl diaminopimelate *dapC* $\text{NS2A6O} + \text{GLU} \leftrightarrow \text{AKG} + \text{NS26DP}$
aminotransferase

Succinyl diaminopimelate *dapE* $\text{NS26DP} \rightarrow \text{SUCC} + \text{D26PIM}$
desuccinylase

Diaminopimelate epimerase *dapF* $\text{D26PIM} \leftrightarrow \text{MDAP}$

Diaminopimelate decarboxylase *lysA* $\text{MDAP} \rightarrow \text{CO}_2 + \text{LYS}$

Biosynthesis of methionine

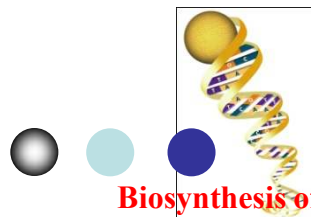
Homoserine transsuccinylase *metA* $\text{HSER} + \text{SUCCOA} \rightarrow \text{COA} + \text{OSLHSER}$

Cystathionine synthase *metB* $\text{OSLHSER} + \text{CYS} \rightarrow \text{SUCC} + \text{HCYS} + \text{PYR} + \text{NH}_3$

Cystathionase *metC* $\text{HCYS} + \text{ADN} \leftrightarrow \text{SAH}$

Methionine synthase *metEH* $\text{HCYS} + \text{MTHF} \rightarrow \text{MET} + \text{THF}$

Methionyl adenosyl transferase *metK* $\text{MET} + \text{ATP} \rightarrow \text{PPI} + \text{PI} + \text{SAM}$



Case 2 (Pramanik & Keasling, 1997)

MFA

Biosynthesis of arginine, putrescine, and spermidine

N-Acetylglutamate synthase *argA* $\text{GLU} + \text{ACCOA} \rightarrow \text{COA} + \text{NAGLU}$
N-Acetylglutamate kinase *argB* $\text{NAGLU} + \text{ATP} \rightarrow \text{ADP} + \text{NAGLUYP}$
N-Acetylglutamate phosphatereductase *argC* $\text{NAGLUYP} + \text{NADPH} \leftrightarrow \text{NADP} + \text{PI} + \text{NAGLUSAL}$
 Acetylornithine aminotransferase *argD* $\text{NAGLUSAL} + \text{GLU} \leftrightarrow \text{AKG} + \text{NAARON}$
N-Acetylornithinase *argE* $\text{NAARON} \rightarrow \text{AC} + \text{ORN}$
 Carbamoyl phosphate synthetase *carAB* $\text{GLN} + 2 \text{ATP} + \text{CO}_2 \rightarrow \text{GLU} + \text{CAP} + 2 \text{ADP} + \text{PI}$
 Ornithine carbamoyl transferase *argFI* $\text{ORN} + \text{CAP} \leftrightarrow \text{CITR} + \text{PI}$
 Argininosuccinate synthase *argG* $\text{CITR} + \text{ASP} + \text{ATP} \leftrightarrow \text{AMP} + \text{PPI} + \text{ARGSUCC}$
 Argininosuccinase *argH* $\text{ARGSUCC} \leftrightarrow \text{FUM} + \text{ARG}$
 Ornithine decarboxylase *speC* $\text{ORN} \rightarrow \text{PTRSC} + \text{CO}_2$
 Spermidine synthase *speE* $\text{PTRSC} + \text{DSAM} \rightarrow \text{SPRMD} + 5\text{MTA}$
 Adenosylmethionine decarboxylase *speD* $\text{SAM} \leftrightarrow \text{DSAM} + \text{CO}_2$
 Unknown pathway $5\text{MTA} \rightarrow \text{ADN} + \text{MET}$
 Agmatine decarboxylase *speA* $\text{ARG} \rightarrow \text{CO}_2 + \text{AGM}$
 Agmatine ureohydrolase *speB* $\text{AGM} \rightarrow \text{UREA} + \text{PTRSC}$

Biosynthesis of histidine synthesis

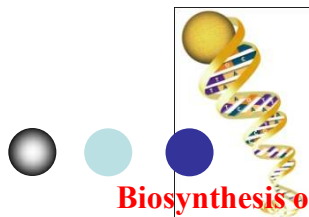
Phosphoribosyl pyrophosphatesynthetase *prs* $\text{R5P} + \text{ATP} \leftrightarrow \text{PRPP} + \text{AMP}$
 Phosphoribosyl pyrophosphate *hisG* $\text{PRPP} + \text{ATP} \rightarrow \text{PPI} + \text{PRBATP}$
 PR-ATP pyrophosphohydrolase *hisI* $\text{PRBATP} \rightarrow \text{PPI} + \text{PRBAMP}$
 PR-AMP cyclohydrolase *hisI* $\text{PRBAMP} \rightarrow \text{PRFP}$
 5-ProFAR isomerase *hisA* $\text{PRFP} \rightarrow \text{PRLP}$
 Imidazoleglycerol phosphate synthase *hisFH* $\text{PRLP} + \text{GLN} \rightarrow \text{GLU} + \text{AICAR} + \text{DIMGP}$
 IGP dehydratase *hisB* $\text{DIMGP} \rightarrow \text{IMACP}$
 L-Histidinol phosphateaminotransferase *hisC* $\text{IMACP} + \text{GLU} \rightarrow \text{AKG} + \text{HISOLP}$
 Hol-*P*-phosphatase *hisB* $\text{HISOLP} \rightarrow \text{PI} + \text{HISOL}$
 Hol dehydrogenase *hisD* $\text{HISOL} + 2 \text{NAD} \rightarrow 2 \text{NADH} + \text{HIS}$

Biosynthesis of branched-chain amino acids

Isopropylmalate synthase *leuA* $\text{ACCOA} + \text{OIVAL} \rightarrow \text{COA} + \text{CBHCAP}$
 Isopropylmalate isomerase *leuCD* $\text{CBHCAP} \leftrightarrow \text{IPPMAL}$
 3-Isopropylmalate dehydrogenase *leuB* $\text{IPPMAL} + \text{NAD} \rightarrow \text{NADH} + \text{OICAP} + \text{CO}_2$
 Transaminase C *ilvE* $\text{OICAP} + \text{GLU} \rightarrow \text{AKG} + \text{LEU}$
 Acetyohydroxy acid synthase *ilvB* $2 \text{PYR} \rightarrow \text{CO}_2 + \text{ACLAC}$
 Acetohydroxy acid isomeroeductase *ilvC* $\text{ACLAC} + \text{NADPH} \rightarrow \text{NADP} + \text{DHVAL}$
 Dihydroxy acid dehydratase *ilvD* $\text{DHVAL} \rightarrow \text{OIVAL}$
 Transaminase C *ilvE* $\text{OIVAL} + \text{GLU} \leftrightarrow \text{AKG} + \text{VAL}$
 Threonine deaminase *ilvA* $\text{THR} \rightarrow \text{NH}_3 + \text{OBUT}$
 Acetohydroxy acid synthase *ilvB* $\text{OBUT} + \text{PYR} \rightarrow \text{ABUT} + \text{CO}_2$
 Acetohydroxy acid isomeroeductase *ilvC* $\text{ABUT} + \text{NADPH} \rightarrow \text{NADP} + \text{DHMVA}$
 Dihydroxy acid dehydratase *ilvD* $\text{DHMVA} \rightarrow \text{OMVAL}$
 Transaminase B *ilvE* $\text{OMVAL} + \text{GLU} \leftrightarrow \text{AKG} + \text{ILE}$
 Amino acid oxidase $\text{O}_2 + \text{ILE} \rightarrow \text{OMVAL} + \text{NH}_3$

Biosynthesis of cysteine

ATP sulfhydrylase *cysDN* $\text{H}_2\text{SO}_4 + \text{ATP} \rightarrow \text{PPI} + \text{APS}$
 ATS kinase *cysC* $\text{APS} + \text{ATP} \rightarrow \text{ADP} + \text{PAPS}$
 PAPS sulfotransferase *cysHP* $\text{PAPS} + \text{NADPH} \rightarrow \text{NADP} + \text{H}_2\text{SO}_3 + \text{PAP}$
 NAHPH-sulfite reductase *cysGIJ* $\text{H}_2\text{SO}_3 + 3 \text{NADPH} \leftrightarrow 3 \text{NADP} + \text{H}_2\text{S}$
 Serine transacetylase *cysE* $\text{SER} + \text{ACCOA} \leftrightarrow \text{COA} + \text{ASER}$
O-Acetylserine (thiol)-lyase *cysKM* $\text{ASER} + \text{H}_2\text{S} \rightarrow \text{AC} + \text{CYS}$
 Sulfotransferase $\text{PAP} + \text{H}_2\text{SO}_3 \leftrightarrow \text{PAPS}$
 Adenylyl sulfate kinase $\text{PAPS} + \text{ADP} \rightarrow \text{ATP} + \text{APS}$



Case 2 (Pramanik & Keasling, 1997)

MFA

Biosynthesis of aromatic amino acids

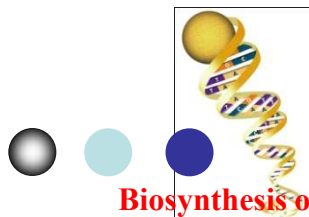
3-Deoxy-D-arabinoheptulosonate-7-phosphate synthase *aroFGH* E4P + PEP → PI + 3DDAH7P
 3-Dehydroquinate synthase *aroB* 3DDAH7P → DQT + PI
 3-Dehydroquinate dehydratase *aroD* DQT ↔ DHSK
 Shikimate dehydrogenase *aroE* DHSK + NADPH ↔ SME + NADP
 Shikimate kinase *aroKL* SME + ATP → ADP + SME5P
 5-Enolpyruvylshikimate-3-phosphatesynthase *aroA* SME5P + PEP ↔ 3PSME + PI
 Chorismate synthase *aroC* 3PSME → PI + CHOR
 Chorismate mutase *pheA* CHOR → PHEN
 Prephenate dehydratase *pheA* PHEN → CO₂ + PHPYR
 Phenylalanine aminotransferase *tyrB* PHPYR + GLU ↔ AKG + PHE
 Prephanate dehydrogenase *tyrA* PHEN + NADP → HPHPYR + CO₂ + NADPH
 Tyrosine aminotransferase *tyrB* HPHPYR + GLU ↔ AKG + TYR
 Anthranilate synthase *trpDE* CHOR + GLN → GLU + PYR + AN
 Anthranilate phosphoribosyltransferase *trpD* AN + PRPP → PPI + NPRAN
 Phosphoribosyl anthranilateisomerase *trpC* NPRAN → CPAD5P
 Indoleglycerol phosphate synthetase *trpC* CPAD5P → CO₂ + IGP
 Tryptophan synthetase *trpAB* IGP + SER → T3P1 + TRP

Biosynthesis of THF

Methylene THF reductase *metF* METTHF + NADH → NAD + MTHF
 Methylene THF dehydrogenase *folD* METTHF + NADP ↔ METHF + NADPH
 Methenyl tetrahydrofolate cyclehydrilase *folD* METHF ↔ FTHF

Biosynthesis of purine nucleotides

Glutamine PRPP amidotransferase *purF* PRPP + GLN → PPI + GLU + PRAM
 GAR synthetase *purD* PRAM + ATP + GLY ↔ ADP + PI + GAR
 GAR transformylase *purNT* GAR + FTHF → THF + FGAR
 FGAM synthetase *purL* FGAR + ATP + GLN → GLU + ADP + PI + FGAM
 AIR synthetase *purM* FGAM + ATP → ADP + PI + AIR
 RCAIM synthetase *purK* AIR + CO₂ ↔ RCAIM
 PRSCAIM synthetase *purE* RCAIM + ATP + ASP ↔ ADP + PI + PRSCAIM
 Adenylosuccinate lyase *purB* PRSCAIM ↔ FUM + AICAR
 AICAR transformylase *purH* AICAR + FTHF ↔ THF + PRFICA
 IMP cyclohydrilase *purH* PRFICA ↔ IMP
 Adenylosuccinate synthetase *purA* IMP + GTP + ASP → GDP + PI + ASUC
 Adenylosuccinate lyase *purB* ASUC ↔ FUM + AMP
 AMP phosphatase AMP → PI + ADN
 Adenylate kinase *adk* ATP + ADN → ADP + AMP
 Adenylate kinase *adk* ATP + AMP → 2 ADP
 IMP dehydrogenase *guaB* IMP + NAD → NADH + XMP
 GMP synthetase *guaA* XMP + ATP + GLN → GLU + AMP + PPI + GMP
 GMP kinase *gmK* GMP + ATP ↔ GDP + ADP
 GDP kinase *gmK* GDP + ATP ↔ GTP + ADP
 Ribonucleotide reductase (ADP) *deoD* ADP + NADPH → DADP + NADP
 Ribonucleotide reductase (GDP) *deoD* GDP + NADPH → DGDP + NADP
 Ribonucleotide reductase (ATP) *deoD* ATP + NADPH → NADP + DATP
 Ribonucleotide reductase (GTP) *deoD* GTP + NADPH → NADP + DGTP
 dADP kinase *nck* DADP + ATP ↔ DATP + ADP
 dGDP kinase *ndk* DGDP + ATP ↔ DGTP + ADP
 dAMP kinase *ndk* DAMP + ATP ↔ ADP + DADP



Case 2 (Pramanik & Keasling, 1997)

MFA

Biosynthesis of pyrimidines

Aspartate carbamoyl transferase *pyrBI* CAP + ASP → CAASP + PI
 Dihydroorotase *pyrC* CAASP ↔ DOROA
 Dihydroorotate dehydrogenase *pyrD* DOROA + NAD ↔ NADH + OROA
 Orotate phosphoribosyl transferase *pyrE* OROA + PRPP ↔ PPI + OMP
 OMP decarboxylase *pyrF* OMP → CO₂ + UMP
 UMP kinase *pyrH* UMP + ATP ↔ UDP + ADP
 UDP kinase *ndk* UDP + ATP ↔ UTP + ADP
 CTP synthetase *pyrG* UTP + GLN + ATP → GLU + CTP + ADP + PI
 CMP kinase *ndk* CMP + ATP ↔ CDP + ADP
 CDP kinase *ndk* CDP + ATP ↔ CTP + ADP
 Deoxycytidilate deaminase *cdd* DCMP → NH₃ + DUMP
 Ribonucleotide reductase (CDP) *nrdAB* CDP + NADPH → DCDP + NADP
 Ribonucleotide reductase (UDP) *nrdAB* UDP + NADPH → DUDP + NADP
 Ribonucleotide reductase (CTP) *nrdAB* CTP + NADPH → DCTP + NADP
 Ribonucleotide reductase (UTP) *nrdAB* UTP + NADPH → NADP + DUTP
 dCMP kinase *ndk* DCMP + ATP ↔ ADP + DCDP
 dCDP kinase *ndk* DCDP + ATP ↔ DCTP + ADP
 dUDP kinase *ndk* DUDP + ATP ↔ DUTP + ADP
 dUTP pyrophosphatase *dut* DUTP → PPI + DUMP
 dUMP kinase *ndk* DUMP + ATP ↔ ADP + DUDP
 Thymidilate synthetase *thyA* DUMP + METTHF → DHF + TMP Matthews,
 TMP kinase *tmk* TMP + ATP ↔ ADP + TDP
 TDP kinase *ndk* TDP + ATP ↔ ADP + TTP

Biosynthesis of isoprenoids

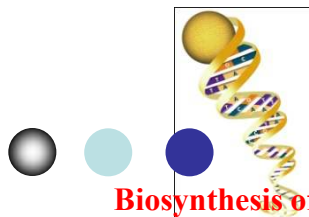
Aldose reductase GL + NADP ↔ NADPH + GLAL
 Glyceraldehyde kinase GLAL + ATP → ADP + T3P1
 Hydroxymethyl-glutaryl-CoAsynthase 3 ACCOA → 2 COA + HMGCOA
 3-Methyl-glutaconyl-CoA hydratase HMGCOA ↔ TMGCOA
 IPP synthase HMGCOA + 2 NADPH + 3 ATP → COA + 2 NADP + 3 ADP + 1 PI + CO₂ + IPPP
 GGPP synthase 4 IPPP → 3 PPI + GGPP
 Methylcrotonyl-CoA carboxylase MCCOA + ATP + CO₂ ↔ ADP + TMGCOA + PI
 Acyl-CoA dehydrogenase ISOVCOA + Q ↔ QH₂ + MCCOA
 2-Keto-isocaproate decarboxylase OICAP + COA + NADP → NADPH + CO₂ + ISOVCOA

Biosynthesis of quinone

Chorismate pyruvate-lyase *ubiC* CHOR → 4HBZ + PYR
 Hydroxybenzoate octaprenyltransferase
 2O6H synthetase *ubiB* 2PPP + O₂ → 2O6H
 QH₂ synthetase *ubiEFGH* 2O6H + 2 O₂ + 3 SAM → 3 SAH + QH₂

Biosynthesis of riboflavin

GTP cyclohydrolase *ribA* GTP → D6RP5P + CO₂ + PPI
 Pyrimidine deaminase *ribD* D6RP5P → A6RP5P + NH₃
 Pyrimidine reductase *ribD* A6RP5P + NADPH → A6RP5P₂ + NADP
 Phosphatase A6RP5P₂ → A6RP + PI
 3,4-Dihydroxy-2-butanone-4-phosphatesynthase *ribB* A6RP → DB4P + FORMATE
 6,7-Dimethyl-8-ribityllumazinesynthase *ribE* DB4P + A6RP → D8RL + PI
 Riboflavin synthase *ribC* 2 D8RL → RIBOFLAVIN + A6RP
 Riboflavin kinase *ribF* RIBOFLAVIN + ATP → FMN + ADP
 FAD synthetase *ribF* FMN + ATP → FAD + PPI



Case 2 (Pramanik & Keasling, 1997)

MFA

Biosynthesis of folate

GTP cyclohydrolase *folE* $\text{GTP} \rightarrow \text{FORMATE} + \text{AHTD}$

H2Neopterin triphosphate pyrophosphatase $\text{AHTD} \rightarrow 3 \text{ PI} + \text{DHP}$

H2Neopterin aldolase $\text{DHP} \rightarrow \text{AHHMP} + \text{GLAL}$

6-Hydroxymethyl H2pterin pyrophosphokinase *folK* $\text{AHHMP} + \text{ATP} \rightarrow \text{AMP} + \text{AHHMD}$

H2pteroate synthase *folP* $\text{AN} + \text{AHHMD} \rightarrow \text{PPI} + \text{DHD}$

Dihydrofolate reductase *folA* $\text{DHD} + \text{ATP} + \text{GLU} \rightarrow \text{ADP} + \text{PI} + \text{DHF}$

Biosynthesis of coenzyme A

CoA Synthase *panBCDE* $\text{OIVAL} + \text{METTHF} + \text{NADPH} + \text{ALA} + \text{CTP} + 4 \text{ ATP} + \text{CYS} \rightarrow \text{THF} + \text{NADP} + \text{AMP} + 2 \text{ PPI} + 2 \text{ ADP} + \text{CO}_2 + \text{COA}$

(Lumped pathway)

ACP Synthase *acpS* $\text{COA} \rightarrow 35 \text{ ADP} + \text{ACP}$

3,5-ADP phosphatase $35 \text{ ADP} \rightarrow \text{AMP} + \text{PI}$

Biosynthesis of NAD

Quinolinate synthase *nadAB* $\text{ASP} + \text{FAD} + \text{T3P}_2 \rightarrow \text{FADH}_2 + \text{PI} + \text{QNL}$

Quinolinate phosphoribosyl transferase *nadC* $\text{QNL} + \text{PRPP} \rightarrow \text{PPI} + \text{NICNT} + \text{CO}_2$

NAMN adenyl transferase *nadD* $\text{NICNT} + \text{ATP} \rightarrow \text{PPI} + \text{DANAD}$

Deamido-NAD ammonia ligase *nadE* $\text{DANAD} + \text{ATP} + \text{NH}_3 \rightarrow \text{AMP} + \text{PPI} + \text{NAD}$

NAD kinase $\text{NAD} + \text{ATP} \rightarrow \text{NADP} + \text{ADP}$

NADP phosphatase $\text{NADP} \rightarrow \text{NAD} + \text{PI}$

Biosynthesis of porphyrins and hemes

GSA synthetase *gltX, hemA* $\text{GLU} + \text{ATP} + \text{NADPH} \rightarrow \text{GSA} + \text{AMP} + \text{PPI} + \text{NADP}$

GSA aminotransferase *hemL* $\text{GSA} \rightarrow \text{ALAV}$

Porphyrinogen synthetase *hemBCD* $8 \text{ ALAV} \rightarrow \text{PORPH} + \text{NH}_3$

Polyphosphate and pyrophosphate metabolism

Pyrophosphatase *ppa* $\text{PPI} \rightarrow 2 \text{ PI}$

Polyphosphate kinase *ppk* $1000 \text{ ATP} \leftrightarrow 1000 \text{ ADP} + \text{POLYP}$

Polyphosphatase *ppx* $\text{POLYP} \rightarrow 1000 \text{ PI}$

Transport reactions

Ammonia transport $\text{NH}_3 \text{ext} + \text{Hext} \leftrightarrow \text{NH}_3$

Sulfate transport $\text{H}_2\text{SO}_4 \text{ext} \leftrightarrow \text{H}_2\text{SO}_4$

Phosphate transport *pit* $\text{Plext} + \text{Hext} \leftrightarrow \text{PI}$

Acetate transport $\text{ACext} + \text{Hext} \leftrightarrow \text{AC}$

Lactate transport $\text{LACext} + \text{Hext} \leftrightarrow \text{LAC}$

Formate transport $\text{FORMATEext} + \text{Hext} \leftrightarrow \text{FORMATE}$

Ethanol transport $\text{ETHANOLext} \leftrightarrow \text{ETHANOL}$

Succinate transport $\text{SUCCext} + \text{Hext} \leftrightarrow \text{SUCC}$

D-Glyceraldehyde transport $\text{GLALext} + \text{Hext} \leftrightarrow \text{GLAL}$

Glucose transport $\text{GLCext} \leftrightarrow \text{GLC}$

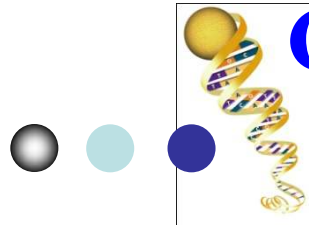
Carbon dioxide transport $\text{CO}_2 \text{ext} \leftrightarrow \text{CO}_2$

Oxygen transport $\text{O}_2 \text{ext} \leftrightarrow \text{O}_2$

Glycerol metabolism

Glycerol kinase $\text{GL} + \text{ATP} \leftrightarrow \text{GL}_3\text{P} + \text{ADP}$

Glycerol-3-phosphate dehydrogenase $\text{GL}_3\text{P} + \text{FAD} \rightarrow \text{T3P}_2 + \text{FADH}_2$

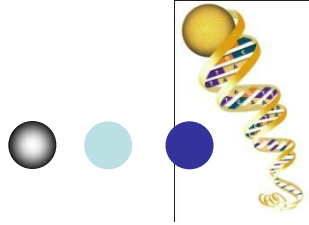


Case 3: (S.Y. Lee, KAIST, 2002)

MFA

MetaFluxNet 1.6 개발, version 1.8 available

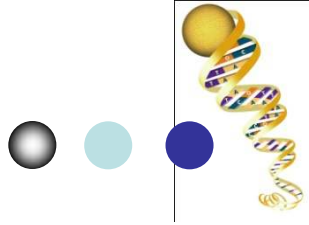
- ✓ Metabolic network was described by metabolic reaction for *Escherichia coli* on glucose
- ✓ The model incorporates 127 reversible and 174 irreversible
- ✓ 294metabolites from several metabolic data base for the biosynthesis of the macromolecular



Applications of MFA

MFA

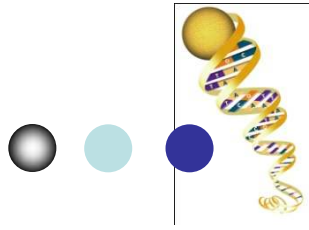
- ✓ Quantification of various **fluxes inside cell**
- ✓ Identification of possible rigid **branch points** (nodes) in the pathways
- ✓ Calculation of non-measured extracellular fluxes
- ✓ Influence of **alternative pathways** on the distribution of fluxes
- ✓ Calculation of maximum theoretical yields
- ✓ Optimum growth media design
- ✓ **Metabolic modification**



Problems of MFA

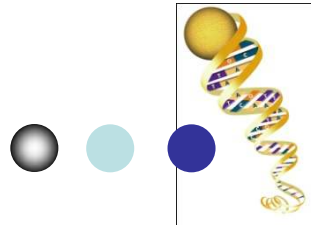
MFA

- ✓ **Linear model**: since most of the biological systems are highly *non-linear and complex*.
- ✓ **No kinetic or regulatory** terms are explicitly used in the model.
- ✓ It is a steady state model, **not *dynamic model***.



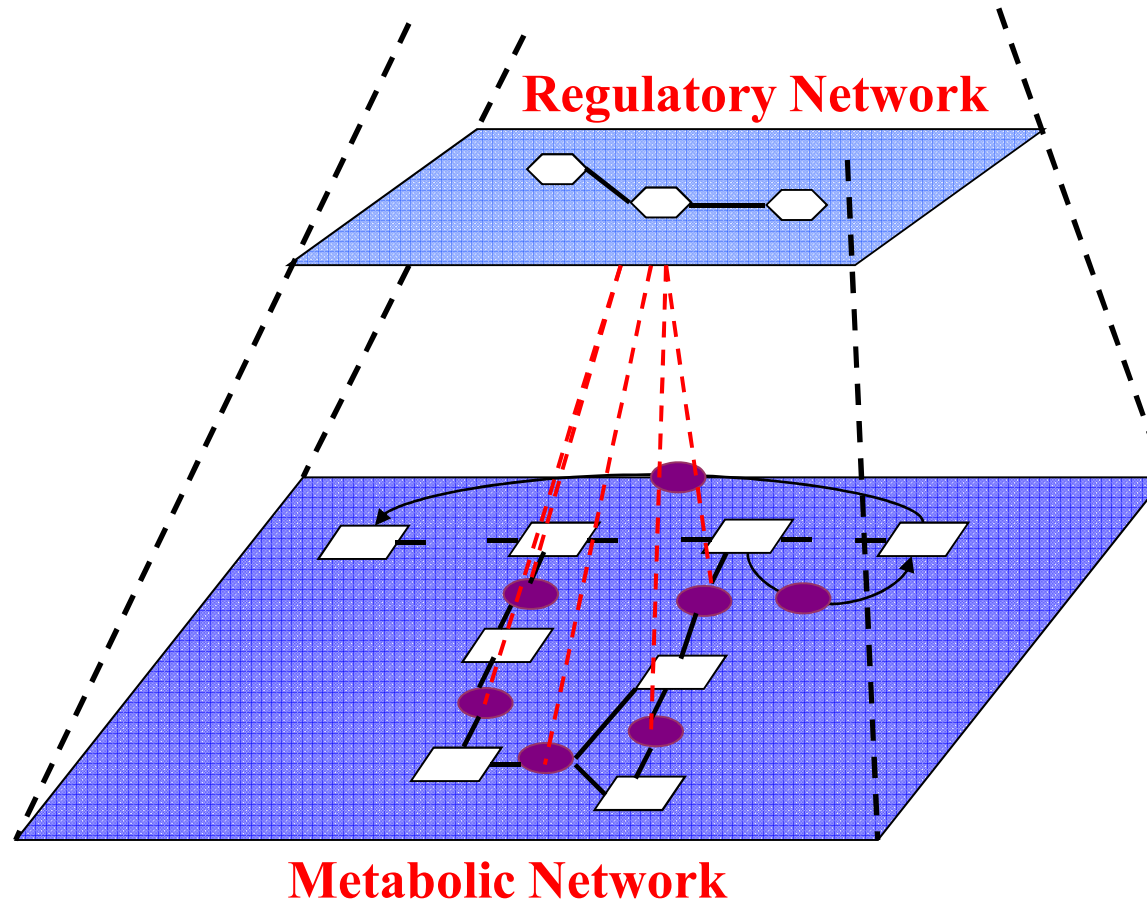
Metabolic control analysis

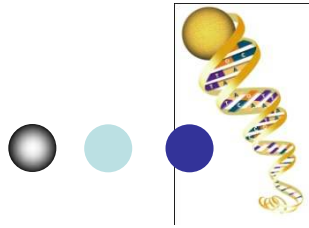
MCA



Metabolic Control Analysis

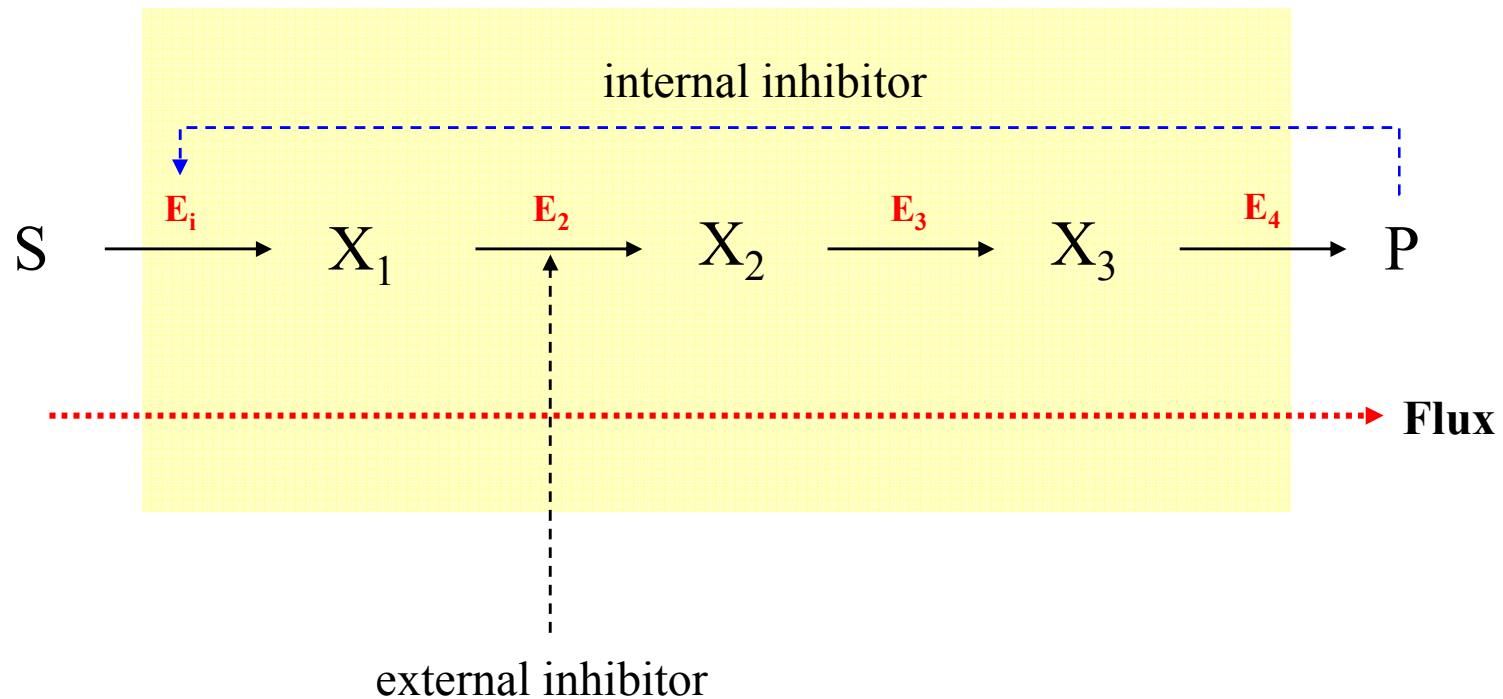
MCA

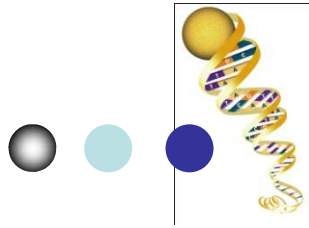




What can MCA tell us ?

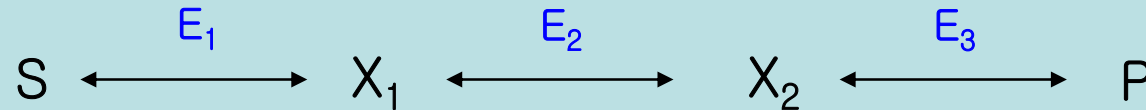
MCA





Metabolic system

MCA



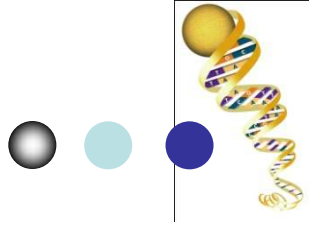
■ Variables

1. Concentrations of molecular species
2. Fluxes

■ Parameters

1. Enzyme levels
2. Kinetic constants
3. Boundary conditions

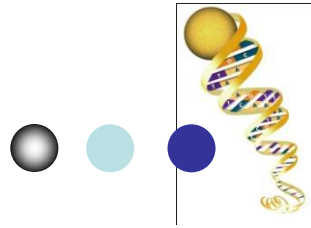
✓ MCA provides the information of the relationship between the variables and parameters in a biochemical network.



Introduction

MCA

- ✓ MCA strictly applies only to steady-state condition (enzyme catalyzing).
- ✓ MCA is aiming to study the influence of enzyme activity in steady- state.

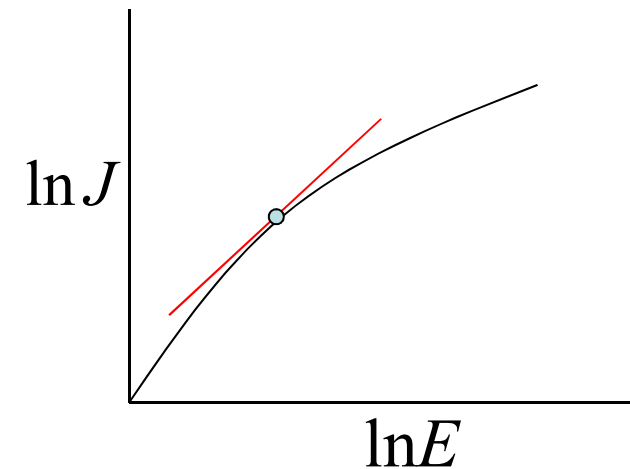


Flux control coefficient (FCC)

MCA

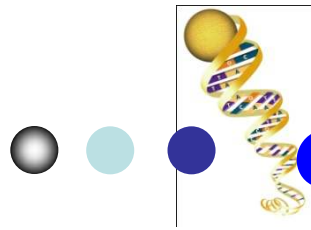


$$V = f(S, E, \dots) = \text{Flux, } J$$



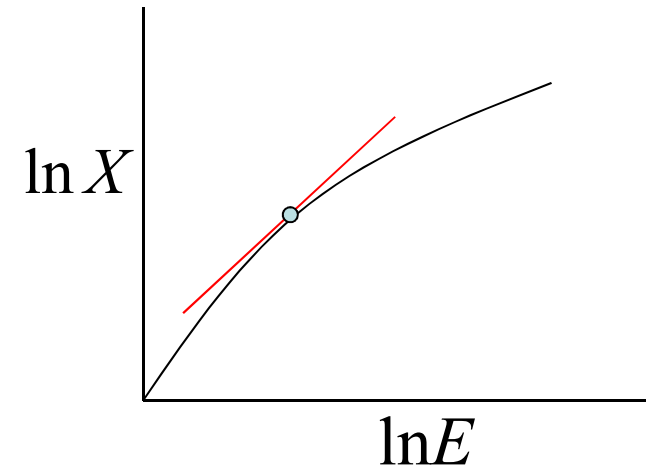
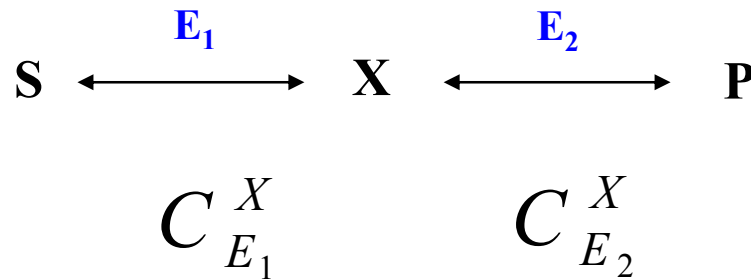
Flux control coefficient: global properties

$$C_i^J = \frac{E}{J} \frac{dJ}{dE} = \frac{d \ln J}{d \ln E}$$



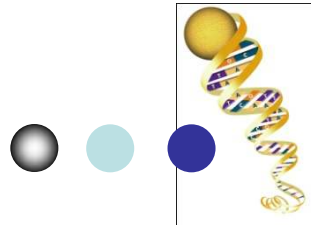
MCA

Concentration Control Coefficient (CCC)



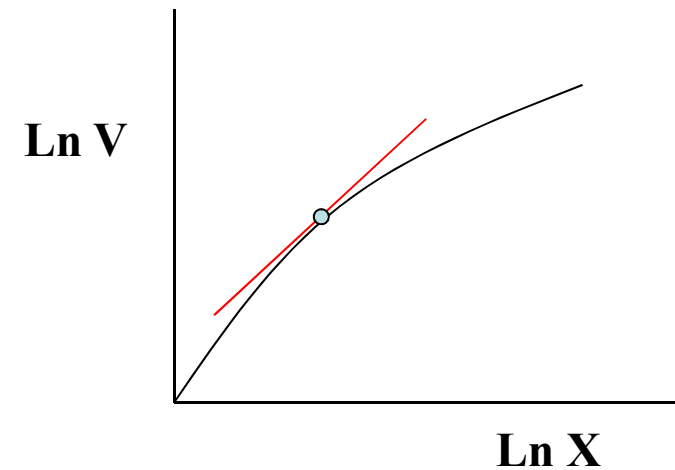
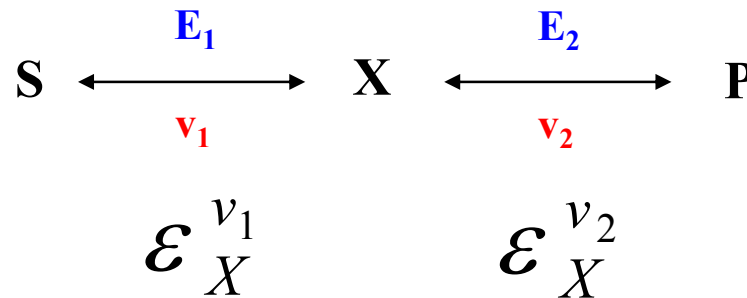
Concentration control coefficient

$$C_E^X = \frac{E}{X} \frac{dX}{dE} = \frac{d \ln X}{d \ln E}$$



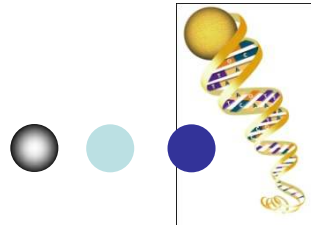
Elasticity Coefficient

MCA



Elasticity Coefficient: Kinetic property of individual enzymes,
local property

$$\mathcal{E}_X^v = \frac{v}{X} \cdot \frac{\delta X}{\delta v} = \frac{\delta \ln |v|}{\delta \ln X}$$



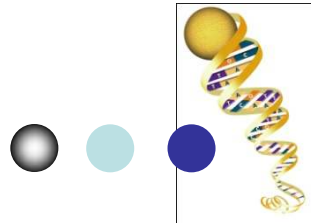
Parameter elasticity coefficient

MCA

- ✓ Parameter elasticity coefficient as measures of enzymatic sensitivity to changes in other parameters

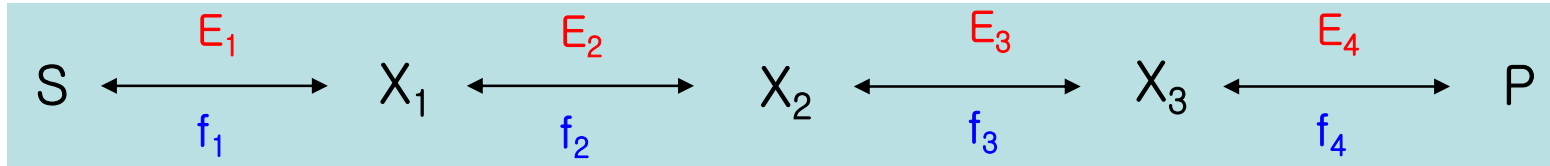
ex) enzyme activity, inhibition (that is not a metabolite here)

$$\pi_{p_l}^i = \frac{p_l}{v_i} \frac{dv_i}{dp_l} = \frac{d \ln v_i}{d \ln p_l}$$



The summation theorem

MCA

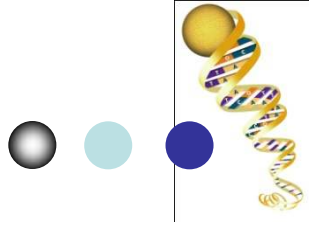


- ✓ General form (for FCC)

$$\sum_i^L C_i^J = 1$$

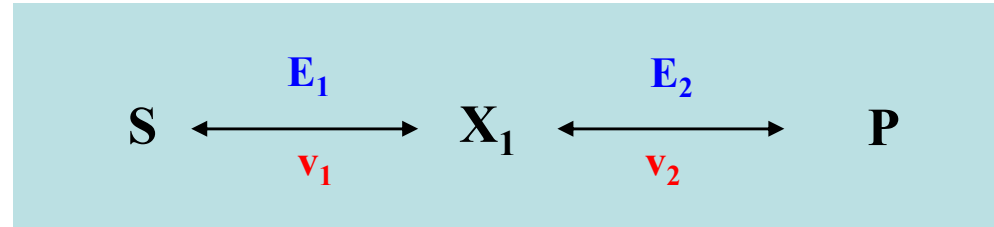
- ✓ General form (for CCC)

$$\sum_i^L C_i^{X_j} = 0 \quad j \in \{1, 2, \dots, K\}$$



Connectivity theorem for FCC

MCA

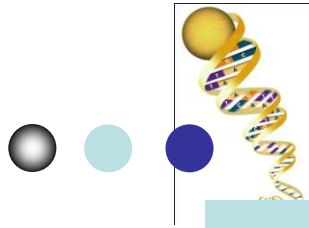


$$C_1^J \varepsilon_{X_1}^1 + C_2^J \varepsilon_{X_1}^2 = 0$$

General form

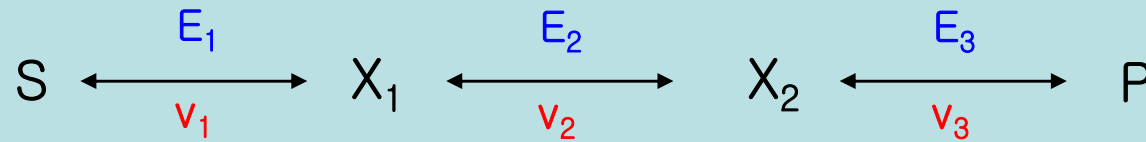
$$\sum_{i=1}^L C_i^{J_k} \varepsilon_{X_j}^i = 0$$

$J_k \rightarrow$ Steady state flux
 $v_1 = v_2$



Connectivity theorem for CCC

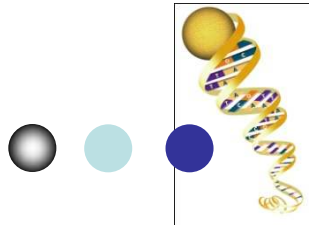
MCA



$$\sum_{i=1}^L C_i^{X_j} \cdot \varepsilon_{X_j}^i = -1, \quad j \in \{1, 2, \dots, K\}$$

$$C_1^{X_1} \varepsilon_{X_1}^1 + C_2^{X_1} \varepsilon_{X_1}^2 + C_3^{X_1} \varepsilon_{X_1}^3 = -1$$

$$C_1^{X_2} \varepsilon_{X_2}^1 + C_2^{X_2} \varepsilon_{X_2}^2 + C_3^{X_2} \varepsilon_{X_2}^3 = -1$$



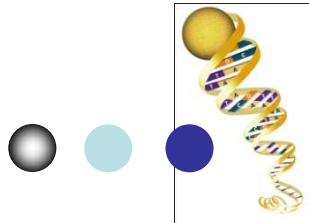
Connectivity theorem for CCC

MCA

General form

$$\sum_{i=1}^L C_i^{X_l} \cdot \varepsilon_{X_j}^i = 0, j \neq l$$

$$\sum_{i=1}^L C_i^{X_l} \cdot \varepsilon_{X_j}^i = -1, j = l$$



Generalization of MCA theorems **MCA**

For FCC

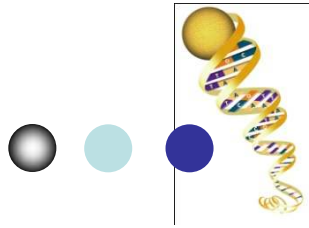
$$C_i^J = \frac{E}{J} \frac{dJ}{dE}$$

$$C_i^{J_k} = \frac{E_i}{J_k} \left(\sum_l \frac{dJ_k}{dP_l} \frac{dp_l}{dE_i} + \sum_{j=1}^k \frac{dJ_k}{dc_j} \frac{dc_j}{dE_i} \right)$$

$$C_i^{J_k} = \pi_i^j + \sum_{j=1}^k \varepsilon_X^k C_i^{X_j}$$



$$C^J = P + E \cdot C^X$$

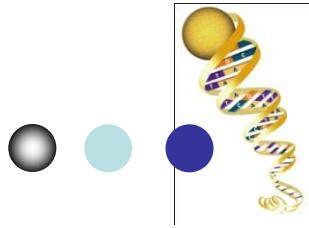


Generalization of MCA theorems MCA

$$\begin{pmatrix} C_1^{J_1} C_2^{J_1} \cdots C_L^{J_1} \\ C_1^{J_2} C_2^{J_2} \cdots C_L^{J_2} \\ \vdots \\ C_1^{J_L} C_2^{J_L} \cdots C_L^{J_L} \end{pmatrix} = \begin{pmatrix} \pi_{E_1}^1 \pi_{E_2}^1 \cdots \pi_{E_L}^1 \\ \pi_{E_1}^2 \pi_{E_2}^2 \cdots \pi_{E_L}^2 \\ \vdots \\ \pi_{E_1}^L \pi_{E_2}^L \cdots \pi_{E_L}^L \end{pmatrix} + \begin{pmatrix} \varepsilon_{X_1}^1 \varepsilon_{X_2}^1 \cdots \varepsilon_{X_K}^1 \\ \varepsilon_{X_1}^2 \varepsilon_{X_2}^2 \cdots \varepsilon_{X_K}^2 \\ \vdots \\ \varepsilon_{X_1}^L \varepsilon_{X_2}^L \cdots \varepsilon_{X_K}^L \end{pmatrix} \times$$

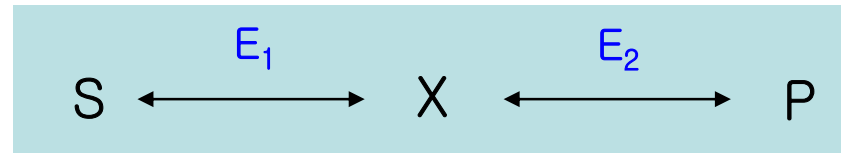
$$C^J = P + E \cdot C^X$$

$$\begin{pmatrix} C_1^{X_1} C_2^{X_1} \cdots C_L^{X_1} \\ C_1^{X_2} C_2^{X_2} \cdots C_L^{X_2} \\ \vdots \\ C_1^{X_K} C_2^{X_K} \cdots C_L^{X_K} \end{pmatrix}$$



Example

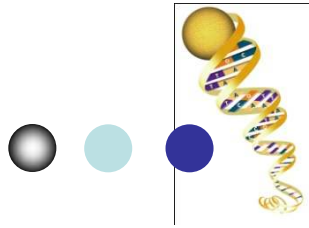
MCA



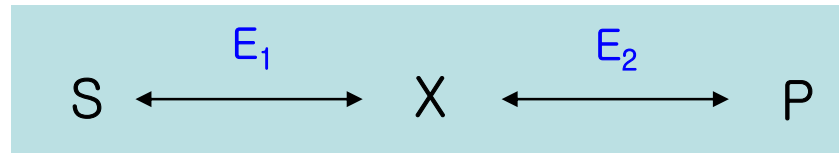
$$C^J = P + E \cdot C^X$$

$$v_1 = v_2$$

$$\begin{pmatrix} C_1^{J_1} C_2^{J_1} \\ C_1^{J_2} C_2^{J_2} \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} + \begin{pmatrix} \varepsilon_{X_1}^1 \\ \varepsilon_{X_1}^2 \end{pmatrix} \times \begin{pmatrix} C_1^{X_1} C_2^{X_1} \end{pmatrix}$$

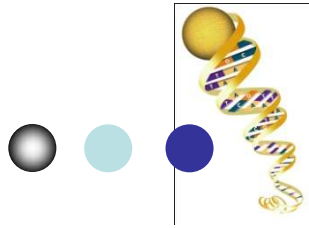


Example (end)

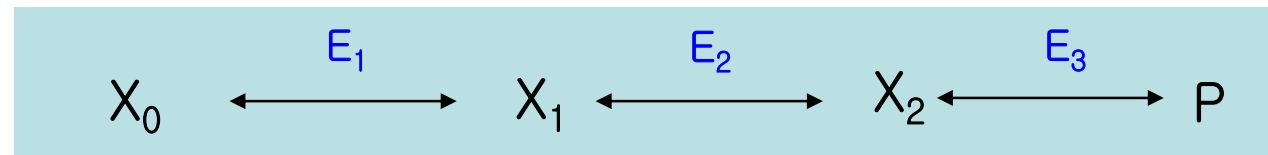


$$\begin{pmatrix} 1 & 1 \\ \varepsilon_X^1 & \varepsilon_X^2 \end{pmatrix} \begin{pmatrix} C_1^J - C_1^X \\ C_2^J - C_2^X \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$$

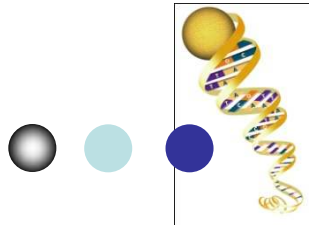
$$\begin{pmatrix} C_1^J & C_1^X \\ C_2^J & C_2^X \end{pmatrix} = \begin{pmatrix} \frac{\varepsilon_X^2}{\varepsilon_X^2 - \varepsilon_X^1} & \frac{1}{\varepsilon_X^2 - \varepsilon_X^1} \\ -\frac{\varepsilon_X^1}{\varepsilon_X^2 - \varepsilon_X^1} & -\frac{1}{\varepsilon_X^2 - \varepsilon_X^1} \end{pmatrix}$$



Example 2

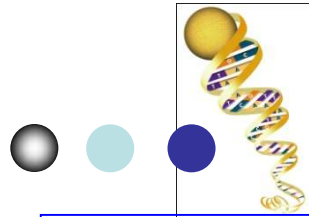


Write summation theorem and connectivity theorem



Example 2: Branched Theorem

- 1) Branched Theorem: $J_r = J_1/J_2$ at steady state $J_r = a/(1-a)$
- 2) Flux CC: $J_2 \sum C_{ji}(\text{pathway 1}) - J_1 \sum C_{ji}(\text{pathway 2}) = 0$ etc.

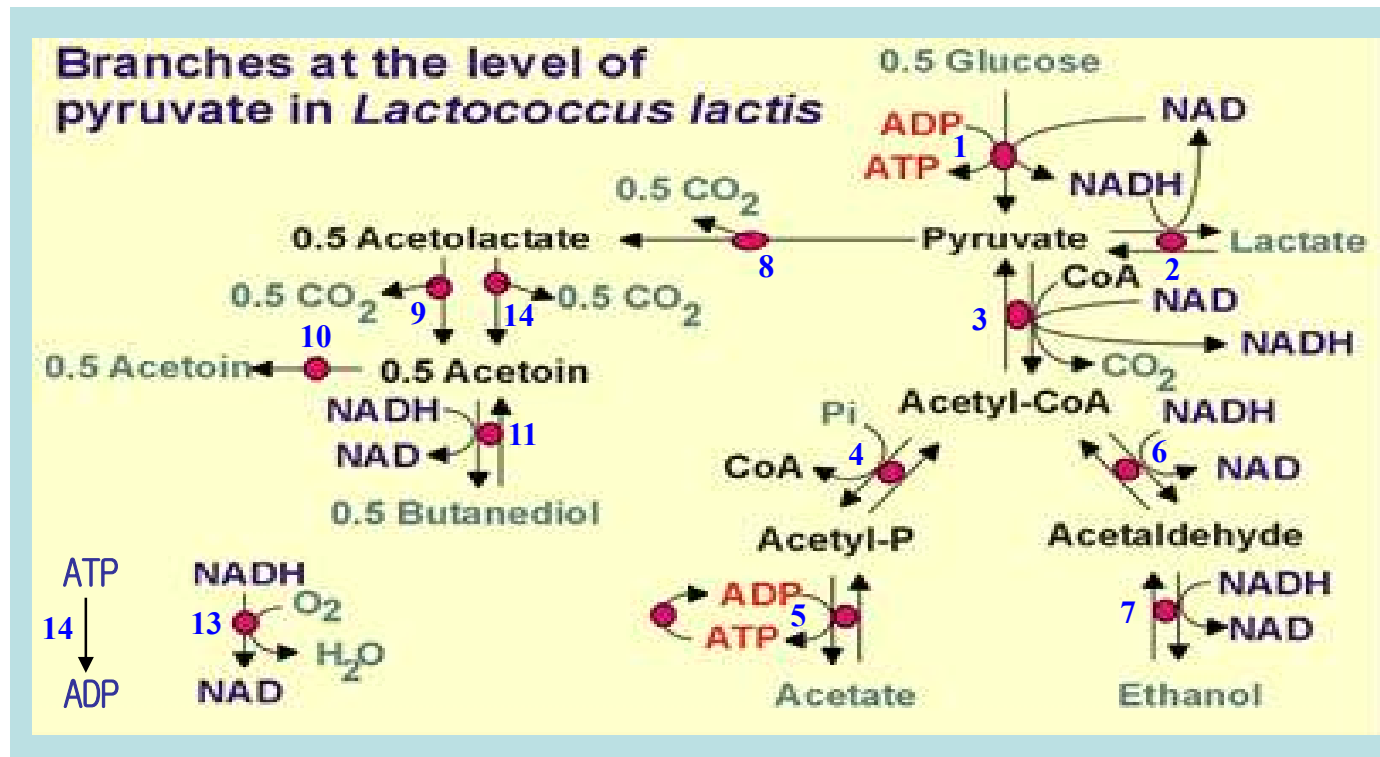


Case 1 (Hoefnagel *et al.*)

MCA

Metabolic engineering of lactic acid bacteria, the combined approach:
kinetic modelling, metabolic control and experimental analysis

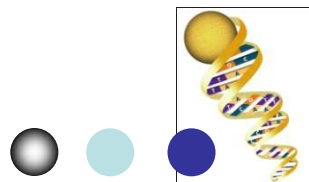
(by Marcel H. N. Hoefnagel *et al.* (2002))



Case 1 (Hoefnagel *et al.*)

MCA

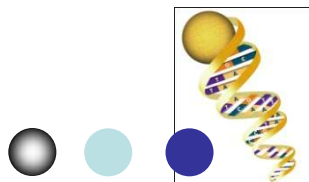
	Kinetic model
1	$\frac{d[PYR]}{dt} = v_{GLYC} - v_{LDH} - v_{PDH} - 2v_{ALS}$
2	$\frac{d[ACP]}{dt} = v_{PTA} - v_{ACK}$
3	$\frac{d[ACAL]}{dt} = v_{ACALDH} - v_{ADH}$
4	$\frac{d[ACLAC]}{dt} = v_{ALS} - v_{ALDC} - v_{NEALC}$
5	$\frac{d[ACET]}{dt} = v_{ALDC} + v_{NEALC} - v_{ACETDH} - v_{ACETEFF}$
6	$\frac{d[ATP]}{dt} = v_{GLYC} - v_{ACK} - v_{ATP_{ase}}$
7	$[ADP] + [ATP] = CONSTANT$
8	$\frac{d[NADH]}{dt} = v_{GLYC} + v_{PDH} - v_{LDH} - v_{ACALDH} - v_{ADH} - v_{ACETDH} - v_{NOX}$
9	$[NAD] + [NADH] = CONSTANT$
10	$\frac{d[ACCOA]}{dt} = v_{PDH} - v_{ACALDH} - v_{PTA}$
11	$[COA] + [ACCOA] = CONSTANT$



Case 1

MCA

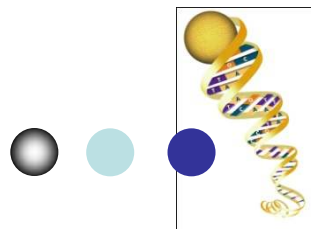
	Rate equation
1	$v_{GLYC} = \frac{V^+ \left(\frac{GLC}{K_{m, GLC}} \right) \times \left(\frac{NAD}{K_{m, NAD}} \right) \times \left(\frac{ADP}{K_{m, ADP}} \right)}{\left(1 + \frac{GLC}{K_{m, GLC}} + \frac{PYR}{K_{m, PYR}} \right) \times \left(1 + \frac{NAD}{K_{m, NAD}} + \frac{NADH}{K_{m, NADH}} \right) \times \left(1 + \frac{ADP}{K_{m, ADP}} + \frac{ATP}{K_{m, ATP}} \right)}$
2	$v_{LDH} = \frac{V^+ \left(\frac{1}{K_{m, PYR} \times K_{m, NADH}} \right) \times \left(PYR \times NADH - \frac{LAC \times NAD}{K_{eq}} \right)}{\left(1 + \frac{PYR}{K_{m, PYR}} + \frac{LAC}{K_{m, LAC}} \right) \times \left(1 + \frac{NADH}{K_{m, NADH}} + \frac{NAD}{K_{m, NAD}} \right)}$
3	$v_{PDH} = \frac{V^+ \left(\frac{1}{1 + K_i \times \frac{NADH}{NAD}} \right) \times \left(\frac{PYR}{K_{m, PYR}} \right) \times \left(\frac{NAD}{K_{m, NAD}} \right) \times \left(\frac{COA}{K_{m, COA}} \right)}{\left(1 + \frac{PYR}{K_{m, PYR}} \right) \times \left(1 + \frac{NAD}{K_{m, NAD}} + \frac{NADH}{K_{m, NADH}} \right) \times \left(1 + \frac{COA}{K_{m, COA}} + \frac{ACCOA}{K_{m, ACCOA}} \right)}$
4	$v_{PTA} = \frac{V^+ \left(\frac{1}{K_{L, ACCOA} \times K_{m, P}} \right) \times \left(ACCOA \times P - \frac{ACP \times COA}{K_{eq}} \right)}{\left(1 + \frac{ACCOA}{K_{L, ACCOA}} + \frac{P}{K_{L, P}} + \frac{ACP}{K_{L, ACP}} + \frac{COA}{K_{L, COA}} + ((ACCOA \times P) / (K_{L, ACCOA} \times K_{m, P}) + ((ACP \times COA) / (K_{m, ACP} \times K_{L, COA})) \right)}$
5	$v_{ACK} = \frac{V^+ \left(\frac{1}{K_{m, ADP} \times K_{m, ACP}} \right) \times \left(ACP \times ADP - \frac{AC \times ATP}{K_{eq}} \right)}{\left(1 + \frac{ACP}{K_{m, ACP}} + \frac{AC}{K_{m, AC}} \right) \times \left(1 + \frac{ADP}{K_{m, ADP}} + \frac{ATP}{K_{m, ATP}} \right)}$
6	$v_{ACALDH} = \frac{V^+ \left(\frac{1}{K_{m, ACCOA} \times K_{m, NADH}} \right) \times \left(ACCOA \times NADH - \frac{COA \times NAD \times ACAL}{K_{eq}} \right)}{\left(1 + \frac{NAD}{K_{m, NAD}} + \frac{NADH}{K_{m, NADH}} \right) \times \left(1 + \frac{ACCOA}{K_{m, ACCOA}} + \frac{COA}{K_{m, COA}} + \frac{ACAL}{K_{m, ACAL}} + \frac{ACAL \times COA}{K_{m, ACAL} \times K_{m, COA}} \right)}$
7	$v_{ADH} = \frac{V^+ \left(\frac{1}{K_{m, ACAL} \times K_{m, NADH}} \right) \times \left(ACAL \times NADH - \frac{ETOH \times NAD}{K_{eq}} \right)}{\left(1 + \frac{NAD}{K_{m, NAD}} + \frac{NADH}{K_{m, NADH}} \right) \times \left(1 + \frac{ACAL}{K_{m, ACAL}} + \frac{ETOH}{K_{m, ETOH}} \right)}$



Case 1

MCA

	Rate equation
8	$v_{ALS} = \frac{V^+ \left(\frac{PYR}{K_{m, PYR}} \right) \times \left(1 - \frac{ACLAC}{PYR \times K_{eq}} \right) \times \left(\left(\frac{PYR}{K_{m, PYR}} + \frac{ACLAC}{K_{m, ACLAC}} \right)^{N-1} \right)}{\left(1 + \left(\frac{PYR}{K_{m, PYR}} + \frac{ACLAC}{K_{m, ACLAC}} \right)^N \right)}$
9	$v_{ALDC} = \frac{V^+ \left(\frac{ACLAC}{K_{m, ACLAC}} \right)}{1 + \frac{ACLAC}{K_{m, ACLAC}} + \frac{ACET}{K_{m, ACET}}}$
10	$v_{ACETEF} = \frac{V^+ \left(\frac{ACET}{K_{m, ACET}} \right)}{1 + \left(\frac{ACET}{K_{m, ACET}} \right)}$
11	$v_{ACETDH} = \frac{V^+ \left(\frac{1}{K_{m, ACET} \times K_{m, NADH}} \right) \times \left(ACET \times NADH - \frac{BUT \times NAD}{K_{eq}} \right)}{\left(1 + \frac{ACET}{K_{m, ACET}} + \frac{BUT}{K_{m, BUT}} \right) \times \left(1 + \frac{NADH}{K_{m, NADH}} + \frac{NAD}{K_{m, NAD}} \right)}$
12	$v_{ATPase} = \frac{V^+ \left(\frac{ATP}{ADP \times K_{m, ATP}} \right)^N}{1 + \left(\frac{ATP}{ADP \times K_{m, ATP}} \right)^N}$
13	$v_{NOX} = \frac{V^+ \left(\frac{NADH \times O}{K_{m, NADH} \times K_{m, O}} \right)}{\left(1 + \frac{NADH}{K_{m, NADH}} + \frac{NAD}{K_{m, NAD}} \right) \times \left(1 + \frac{O}{K_{m, O}} \right)}$
14	$v_{NEALC} = k \times ACLAC$

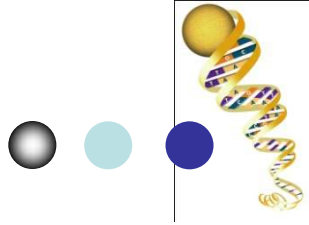


Case 1 (Hoefnagel *et al.*)

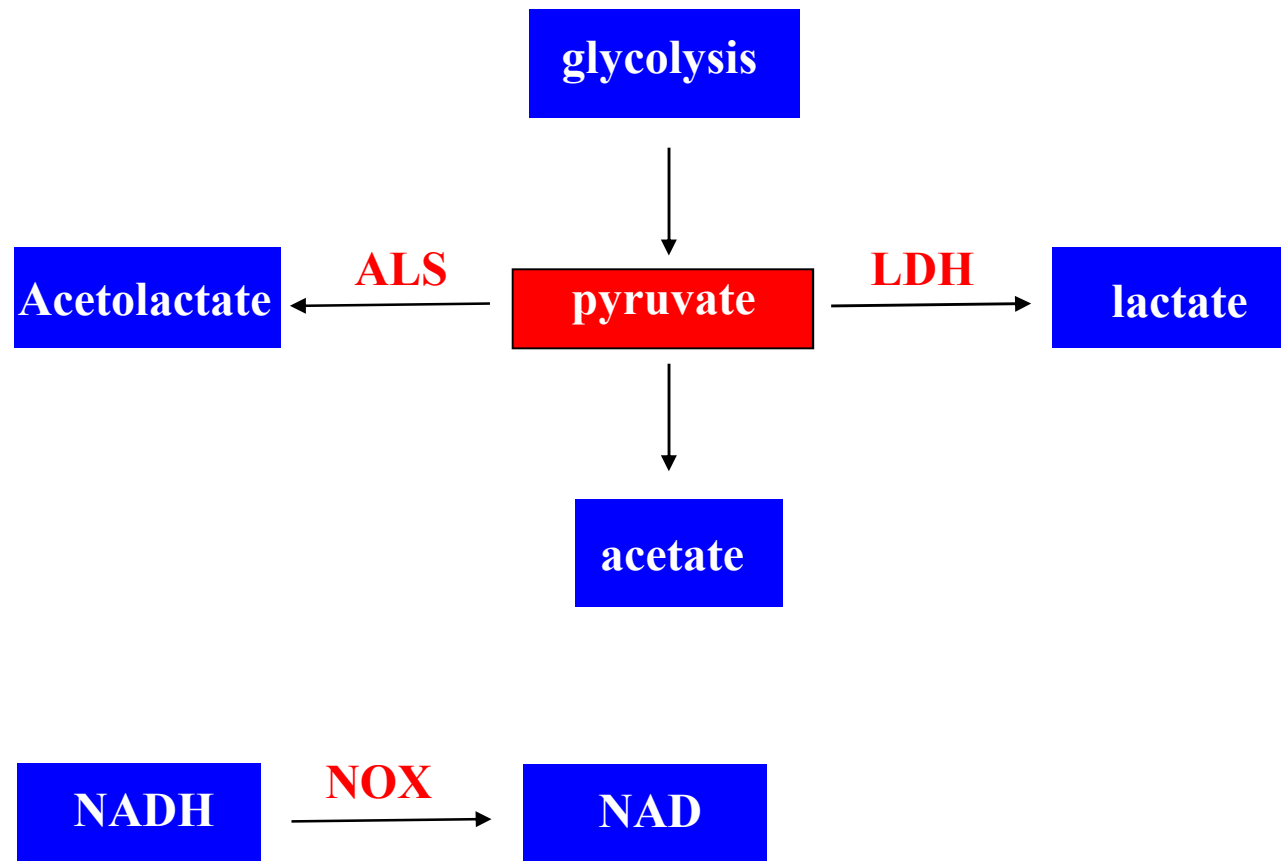
MCA

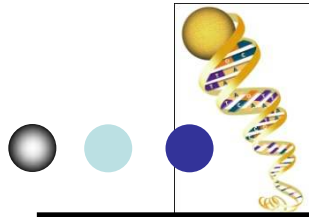
Reaction No.	Kinetic parameter	Value
1. v_{GLYC}	V^+	2397
	$K_{m, PYR}$	25
	$K_{m, GLC}$	0.1
	$K_{m, NAD}$	0.14
	$K_{m, NADH}$	0.09
	$K_{m, ADP}$	0.047
	$K_{m, ATP}$	0.019
2. v_{LDH}	V^+	21120
	K_{eq}	0.08
	$K_{m, NADH}$	1.5
	$K_{m, PYR}$	2.4
	$K_{m, NAD}$	100
	$K_{m, LAC}$	259

Reaction No.	Kinetic parameter	Value
3. v_{PDH}	V^+	259
	$K_{m, PYR}$	1
	$K_{m, NAD}$	0.4
	$K_{m, COA}$	0.014
	$K_{m, ACCOA}$	0.008
	$K_{m, NADH}$	0.1
	K_{K_i}	46.4
4. v_{PTA}	V^+	42
	$K_{i, ACCOA}$	0.2
	$K_{m, P}$	2.6
	$K_{i, P}$	2.6
	$K_{m, ACP}$	0.2
	$K_{i, ACP}$	0.029
	$K_{m, ACP}$	0.12
	K_{eq}	0.0281



Case 1 (Hoefnagel *et al.*)



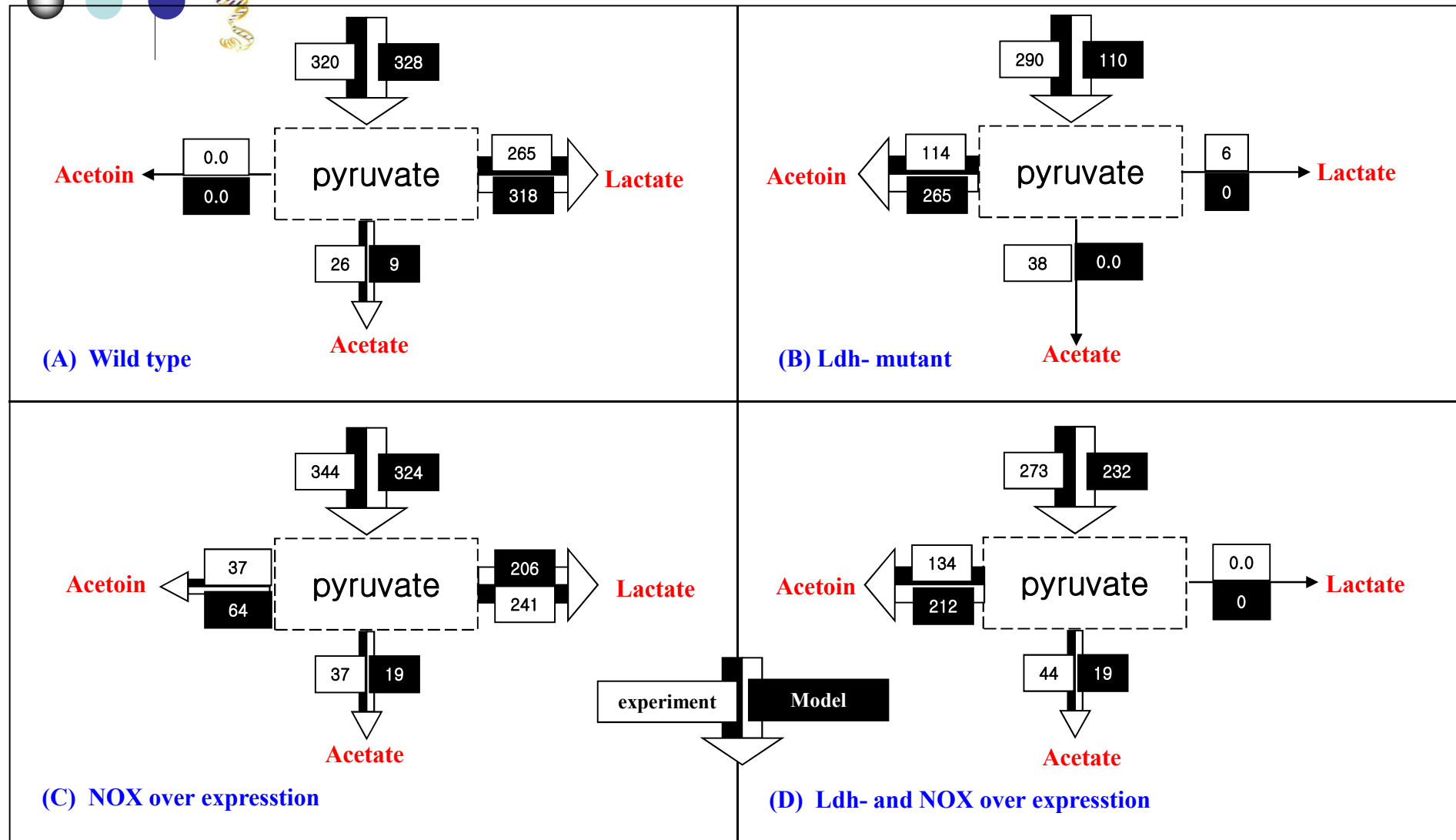


Case 1 (Hoefnagel *et al.*)

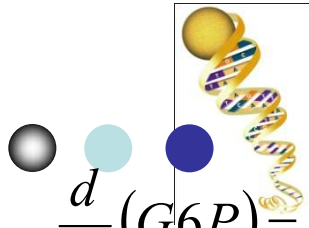
MCA

	Control coefficient			
	Wild-type	Ldh- mutant	NOX ⁺⁺ mutant	Ldh- NOX ⁺⁺ mutant
<i>Glycolysis</i>	1.6	0.0	1.0	0.0
<i>ATP_{ase}</i>	0.8	0.0	0.5	0.0
<i>LDH</i>	-2.3	0.0	-1.1	0.0
<i>NOX</i>	1.5	0.9	1.0	0.0
<i>ALS</i>	1.0	0.0	0.1	0.0
<i>Acetoindolac arboxylase</i>	0.0	0.0	0.0	1.0
<i>Phosphotransacetylase</i>	-0.6	0.0	-0.3	0.0
<i>Pyruvate dehydrogenase</i>	-0.8	0.0	-0.1	0.0

Case 1 (Hoefnagel *et al.*)







Glycolysis dynamic mass balances

$$\frac{d}{dt}(G6P) = v_{HK} - v_{PGI} - v_{G6PDH}$$

$$\frac{d}{dt}(F6P) = v_{PGI} - v_{PFK} + v_{TA} + v_{TKII}$$

$$\frac{d}{dt}(FDP) = v_{PFK} - v_{ALD}$$

$$\frac{d}{dt}(DHAP) = v_{ALD} - v_{TPI}$$

$$\frac{d}{dt}(GA3P) = v_{ALD} + v_{TPI} - v_{GAPDH} + v_{TKI} + v_{TKII} - v_{TA}$$

$$\frac{d}{dt}(1,3DPG) = v_{GAPDH} - v_{PGK} - v_{DPGM}$$

$$\frac{d}{dt}(3PG) = v_{PGK} - v_{PGM} + v_{DPGase}$$

$$\frac{d}{dt}(2PG) = v_{PGM} - v_{EN}$$

$$\frac{d}{dt}(PEP) = v_{EN} - v_{PK}$$

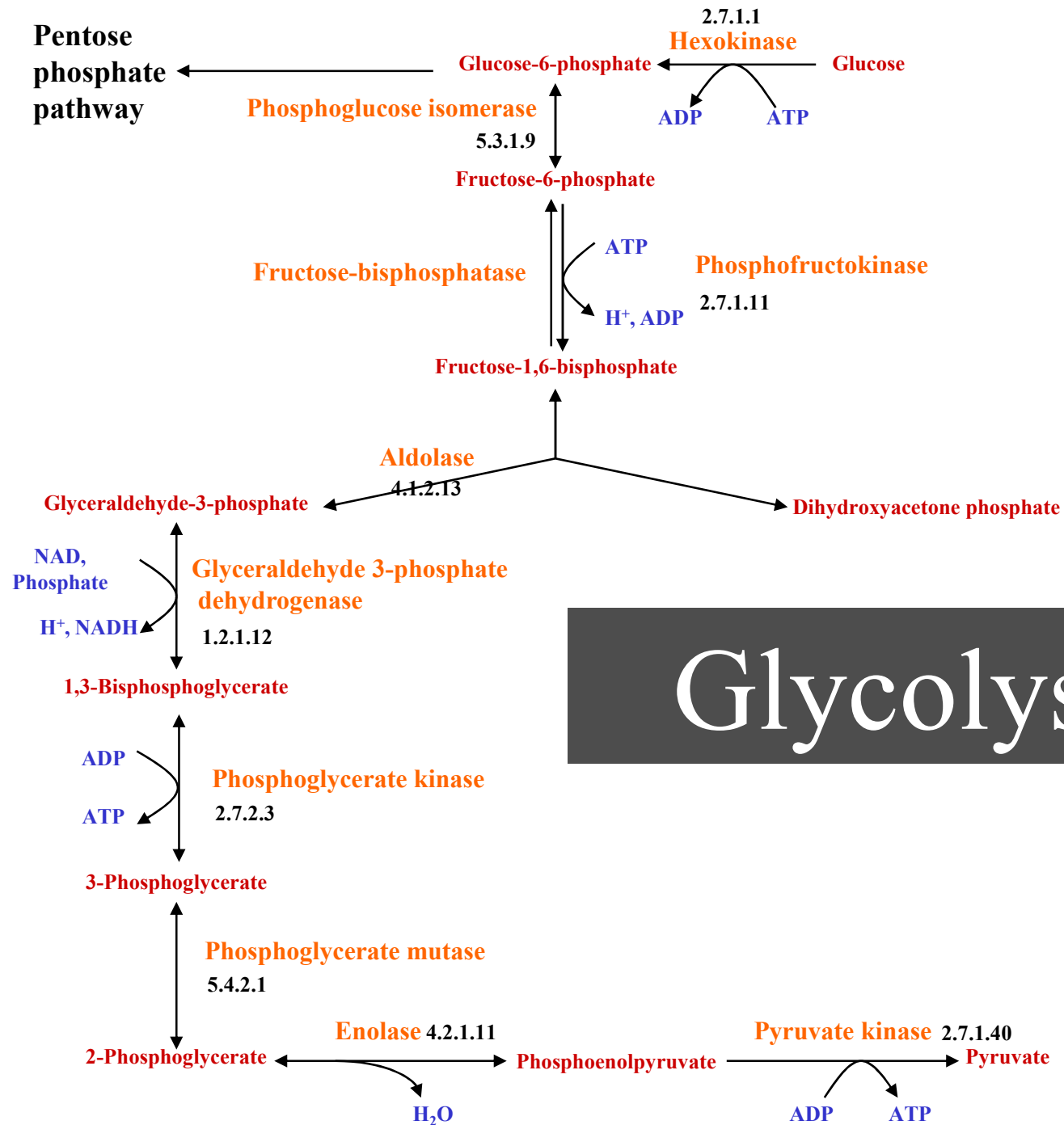
$$\frac{d}{dt}(PYR) = v_{PK} - v_{PYR_{ex}} - v_{LDH}$$

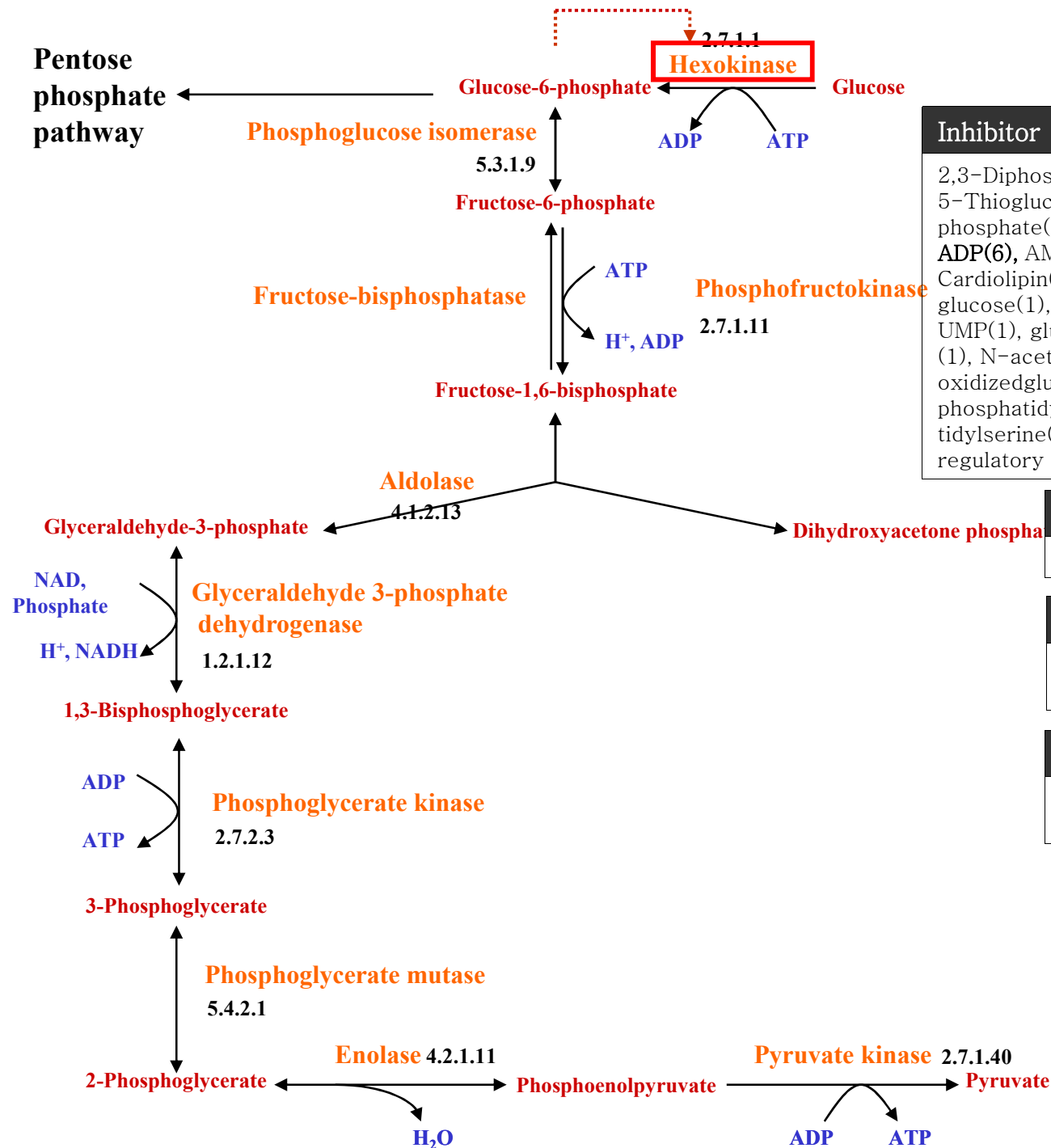
$$\frac{d}{dt}(LAC) = v_{LDH} - v_{LAC_{ex}}$$

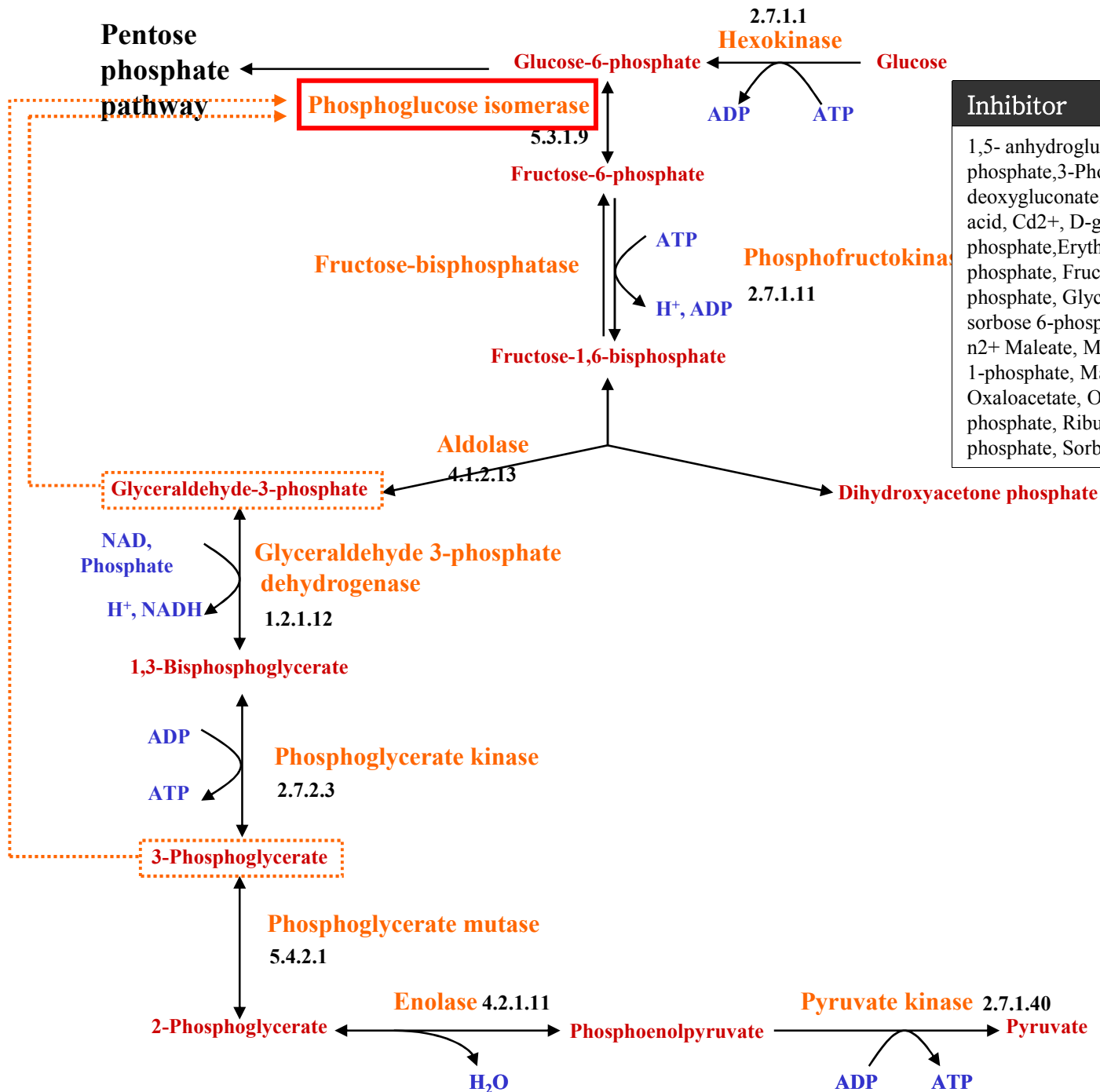
$$\frac{d}{dt}(NADH) = v_{GAPDH} - v_{LDH}$$

Rapoport-Leubering shunt

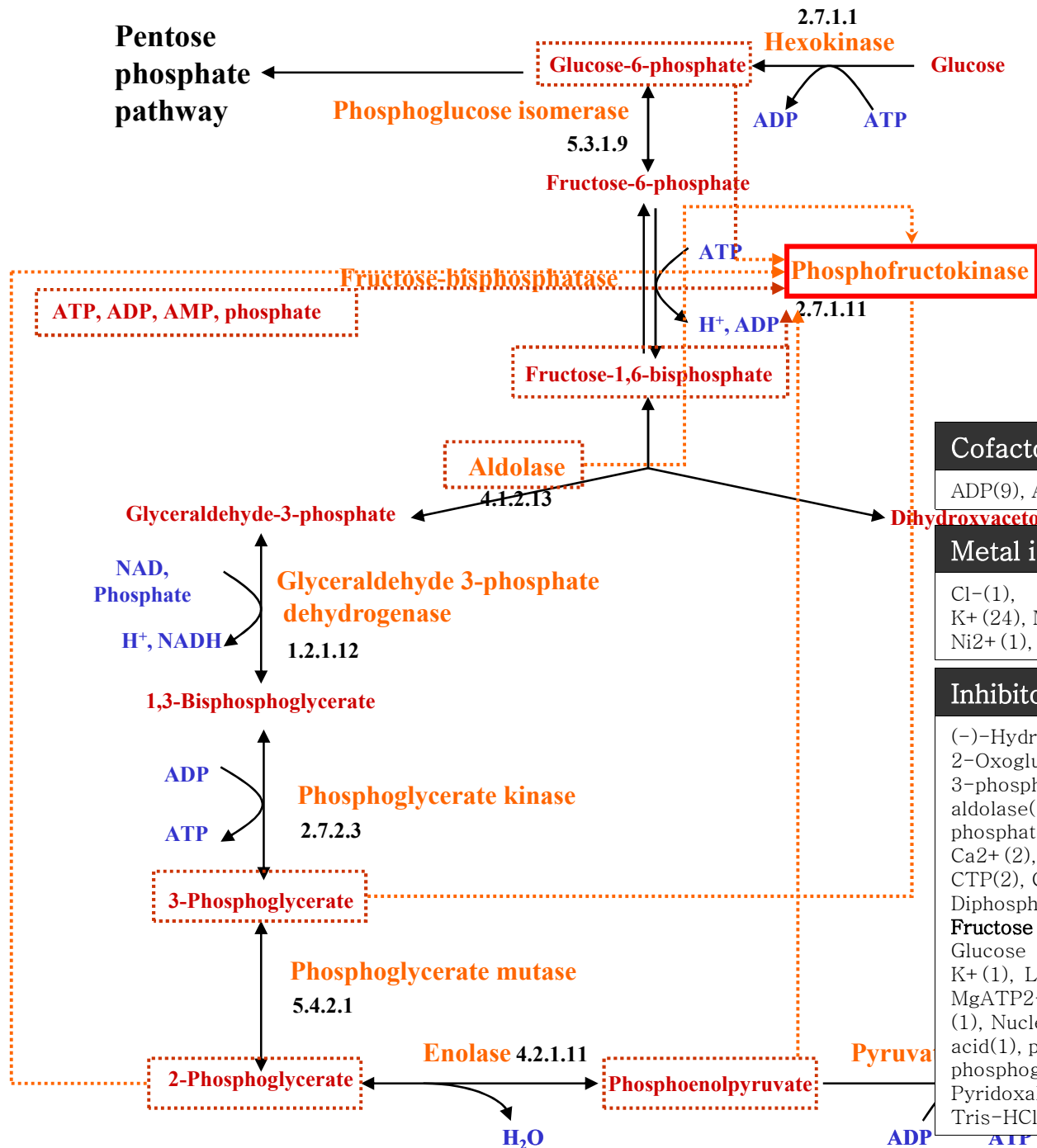
$$\frac{d}{dt}(2,3DPG) = v_{DPGM} - v_{DPG_{ase}}$$







Inhibitor
1,5- anhydroglucitol 6-phosphate, 2-deoxyglucose6-phosphate,3-Phosphoglycerate, 6-phospho-2-deoxygluconate, 6-phospho mannionate, agaricic acid, Cd2+, D-glucal 6-phosphate, Dihdroxy acetone phosphate,Erythritol 4-phosphate, Erythrose 4-phosphate, Fructose 1,6-diphosphate, Fructose 1-phosphate, Glyceraldehyde 3-phosphate, Hg2+, L-sorbose 6-phosphate, L-Xylulose 5-phosphate, Zn2+ Maleate, Maleic anhydride, Malonate, Mannitol 1-phosphate, Mannitol 6-phosphate, Mn2+, Ni2+, Oxaloacetate, Oxoglutarate, phosphate, Ribose 5-phosphate, Ribulose 5-phosphate, Sedoheptylose 7-phosphate, Sorbitol 6-phosphate, Sr2+, Suramin,



Activating compound

cAMP-dependent protein kinase(1),
Fructose 1,6-bisphosphate (5),
Fructose 2,6-bisphosphate (5),
 Glucose 1,6-bisphosphate (4),
 Glucose 6-phosphate (1), GSH
 Citrate(1)

Cofactor

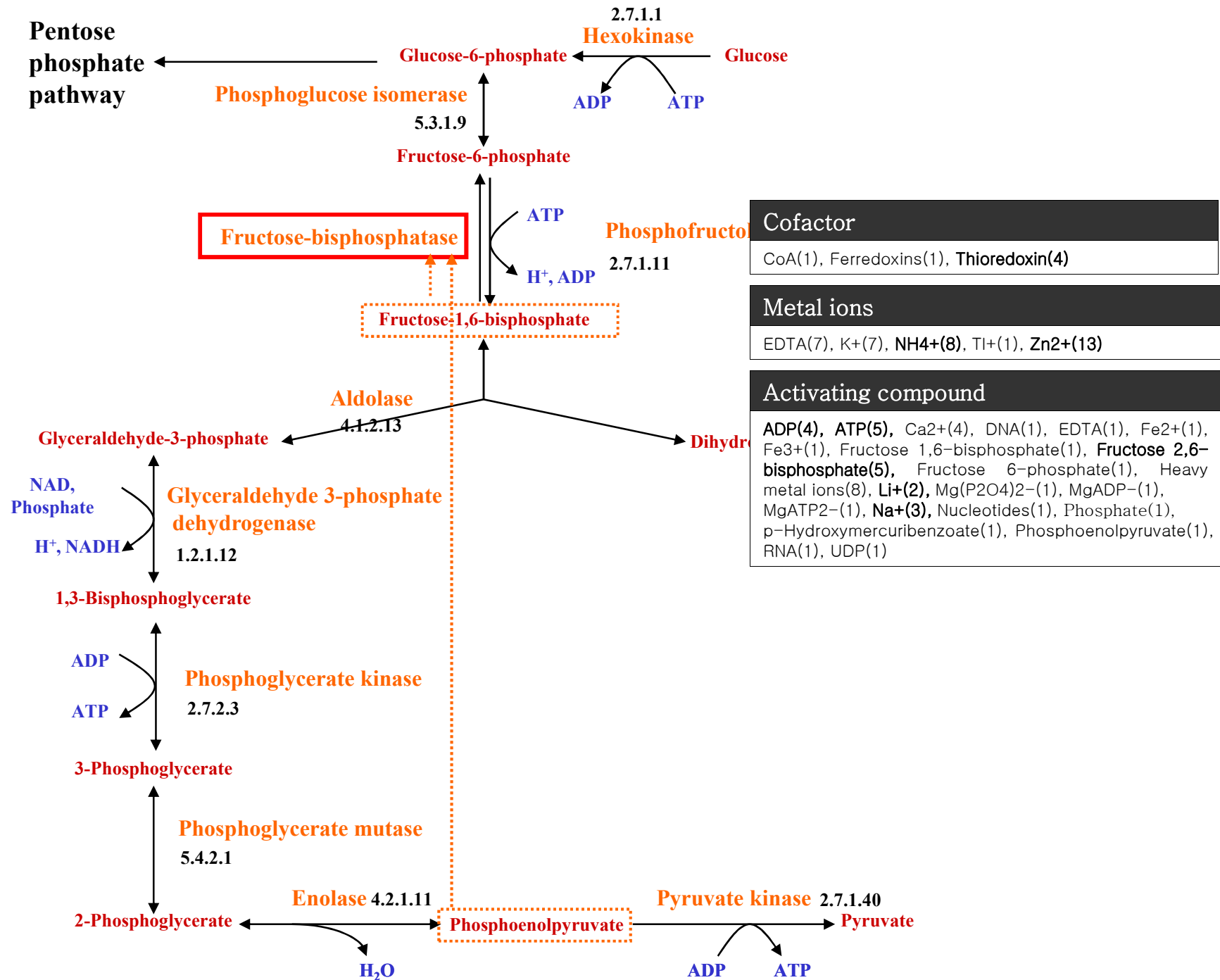
ADP(9), AMP(13), cAMP(3), GDP(1)

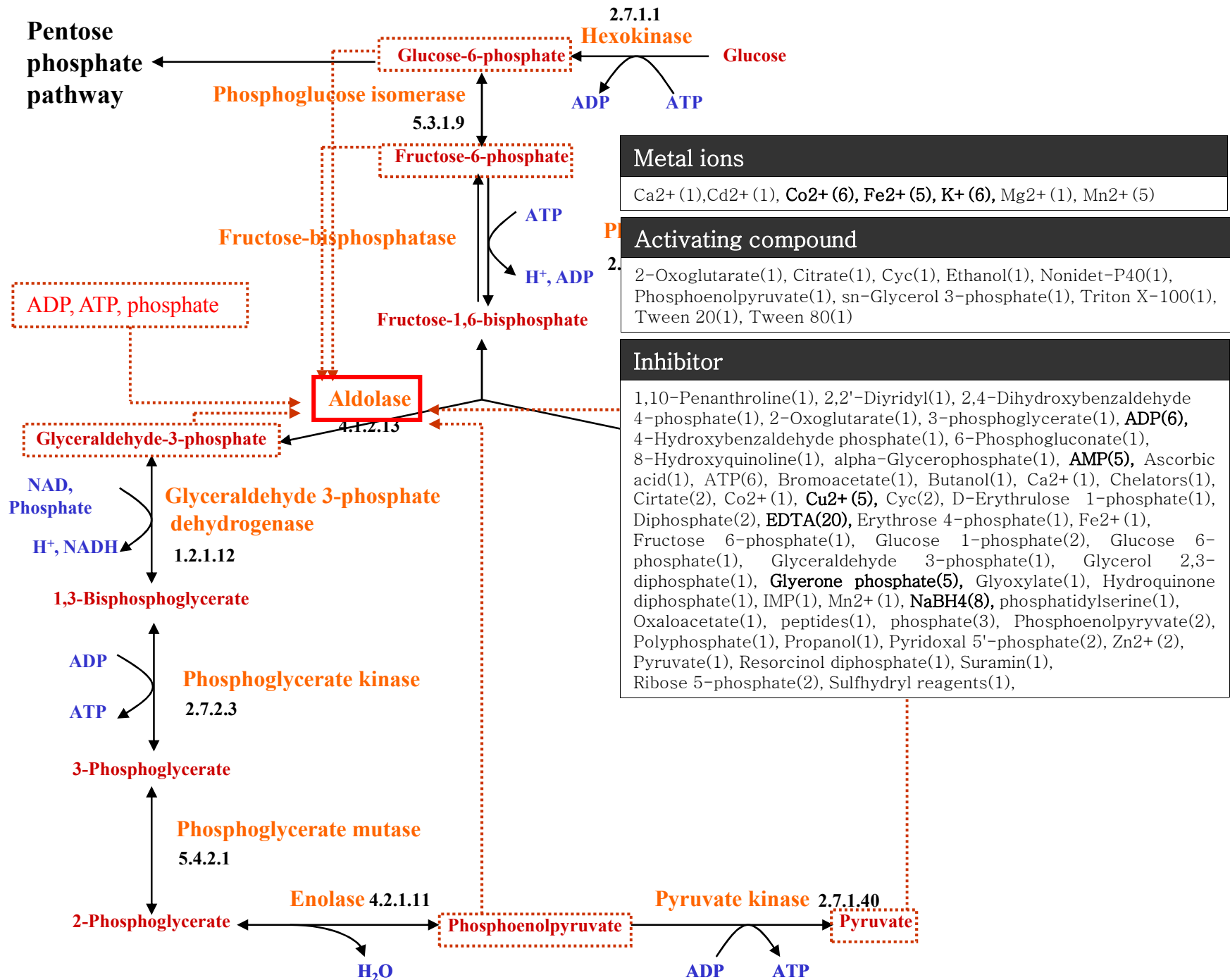
Metal ions

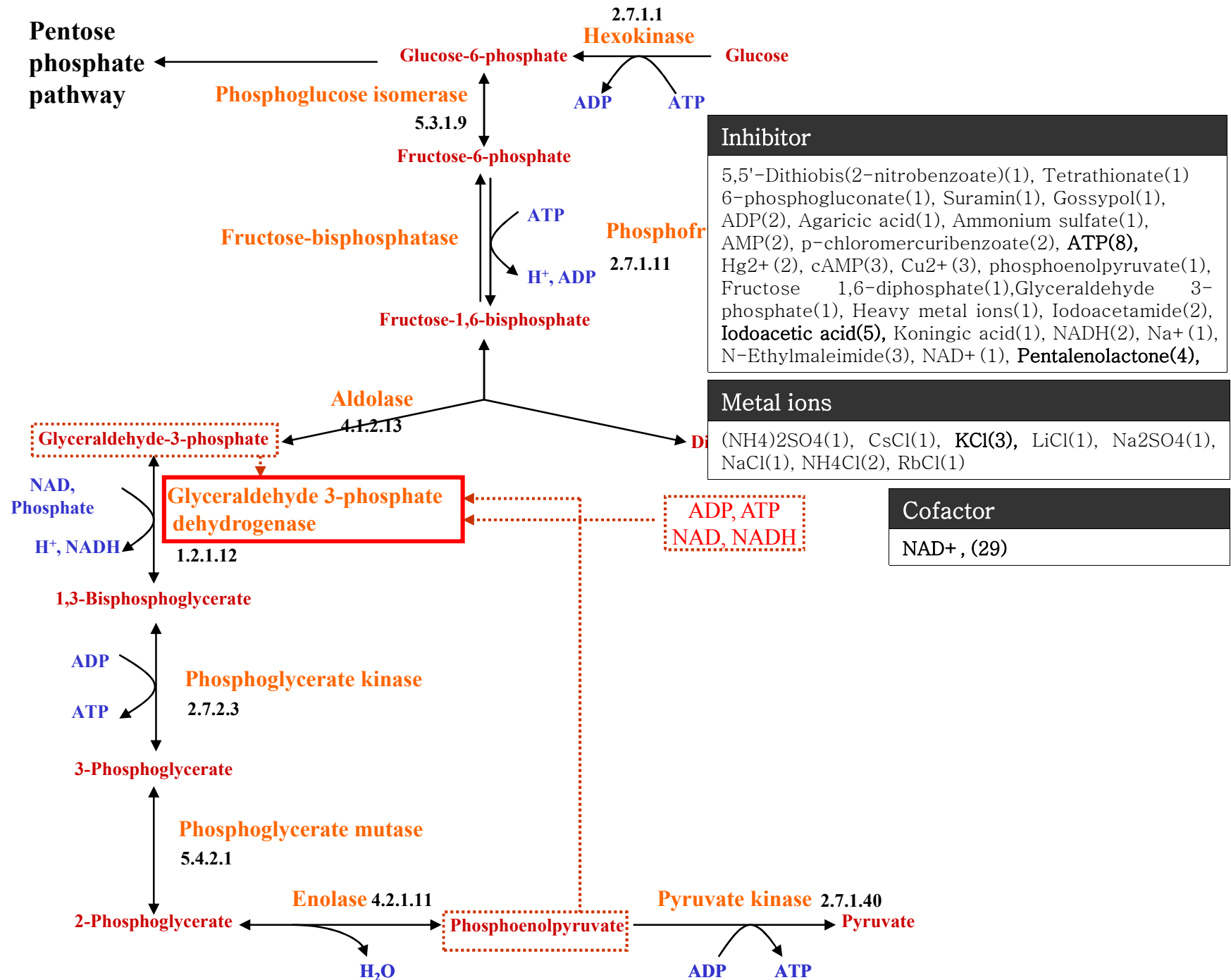
Cl-(1), Co2+ (24), Fe2+ (1), HAsO42-(1), HCO3-(1),
 K+ (24), Mg2+ (73), NH4+ (13)
 Ni2+ (1), NO3-(1), phosphate(9), SO42-(2), Zn2+ (2)

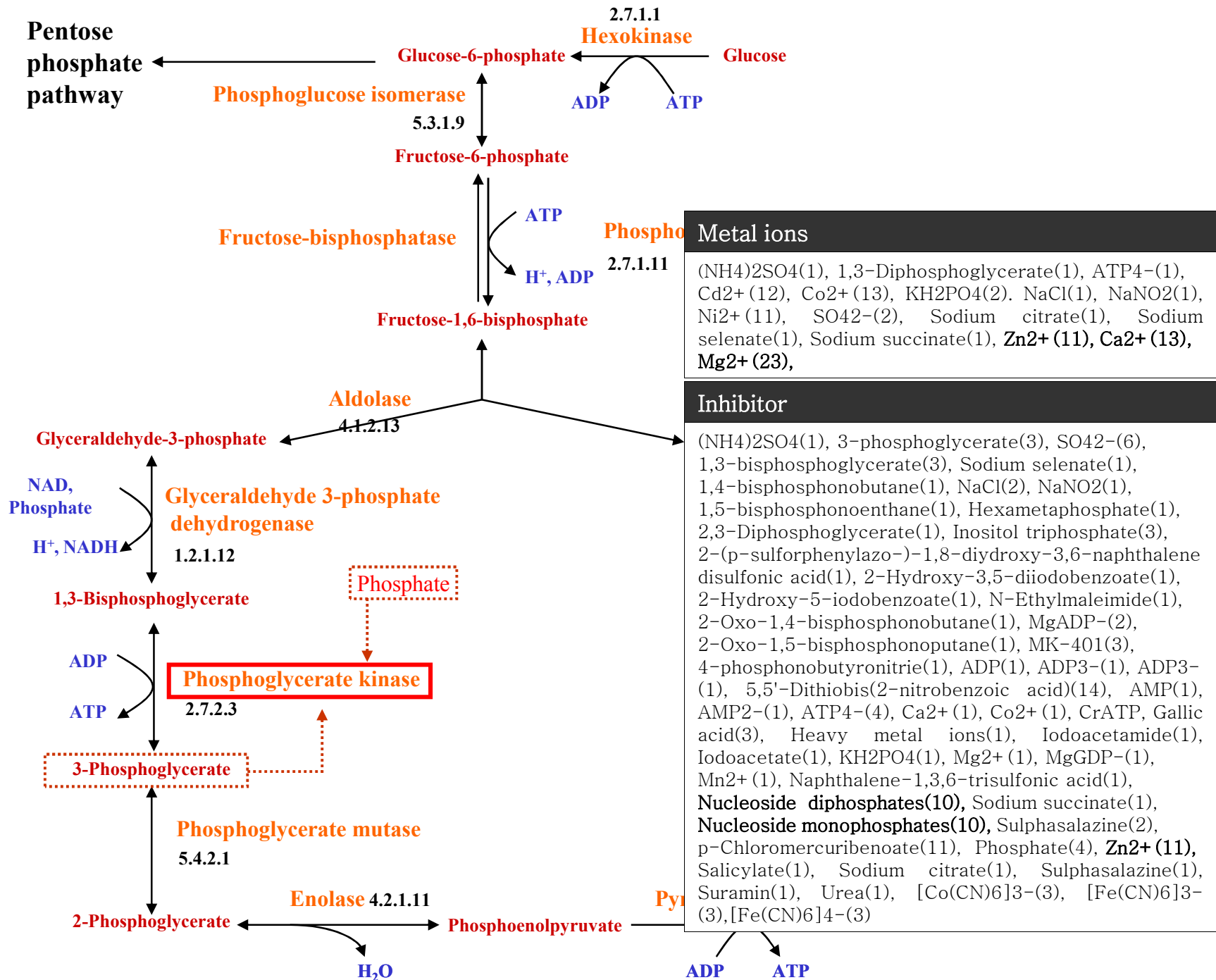
Inhibitor

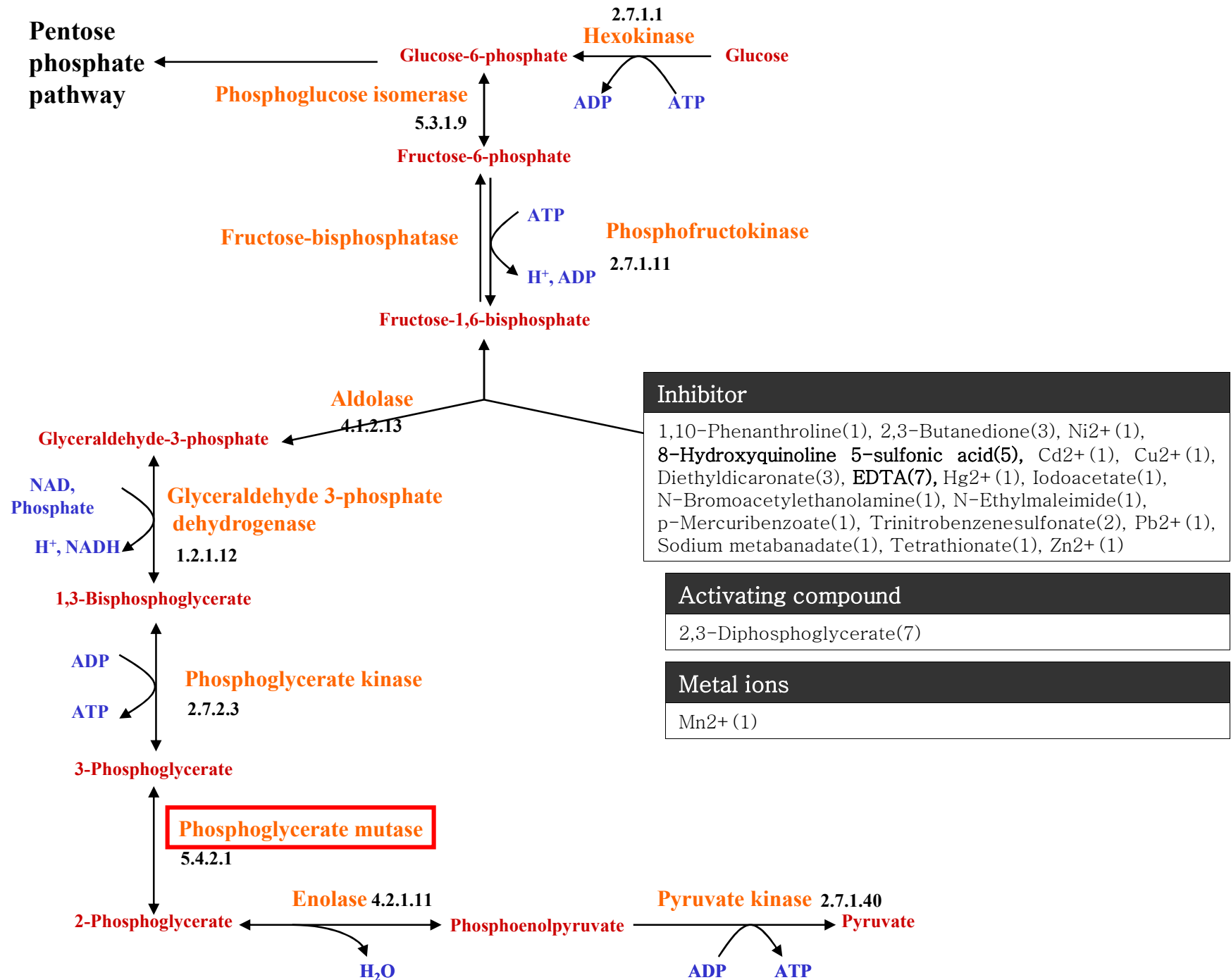
(-)-Hydroxycitrate(1), 2,3-Diphosphoglycerate(1),
 2-Oxoglutarate(1), 2-phosphoglycerate(3), Arginine,
 3-phosphoglycerate(3), **ADP(10)**, Agaric acid(1),
 aldolase(2), AMP(7), Antibodies againse rabbit muscle
 phosphate(1), **ATP(28)**, Aurintricarboxylic acid(1),
 Ca2+ (2), cAMP(1), cis-Aconitate(1), Citrate(16), Cl-(1),
 CTP(2), Cu2+ (1), Dephosphorylation with phosphatase(2),
 Diphosphate(2), DTNB(1), Fructose 1,6-bisphosphate(5),
Fructose 1-phosphate(23), Glucose 1,6-bisphosphate(1),
 Glucose 6-phosphate(1), GTP(4), Hg2+ (1), Isocitrate(1),
 K+ (1), Li+ (1), Malate(2), Maleic anhydride(3), Mg2+ (4),
 MgATP2-(4), Na+ (1), NH4+ (1), Ni2+ (1), NO2-(1), NO3-
 (1), Nucleoside triphosphates(3), p-Chloromercurisulfonic
 acid(1), phosphate(3), **phosphoenopyruvate(PEP)(12)**,
 phosphoglycolate(2), protein factor(1), Succinate(1),
 Pyridoxal phoshate(3), SO42-, Succinic anhydride(3),
 Tris-HCl buffer(1), UTP(1), Zn2+ (1)

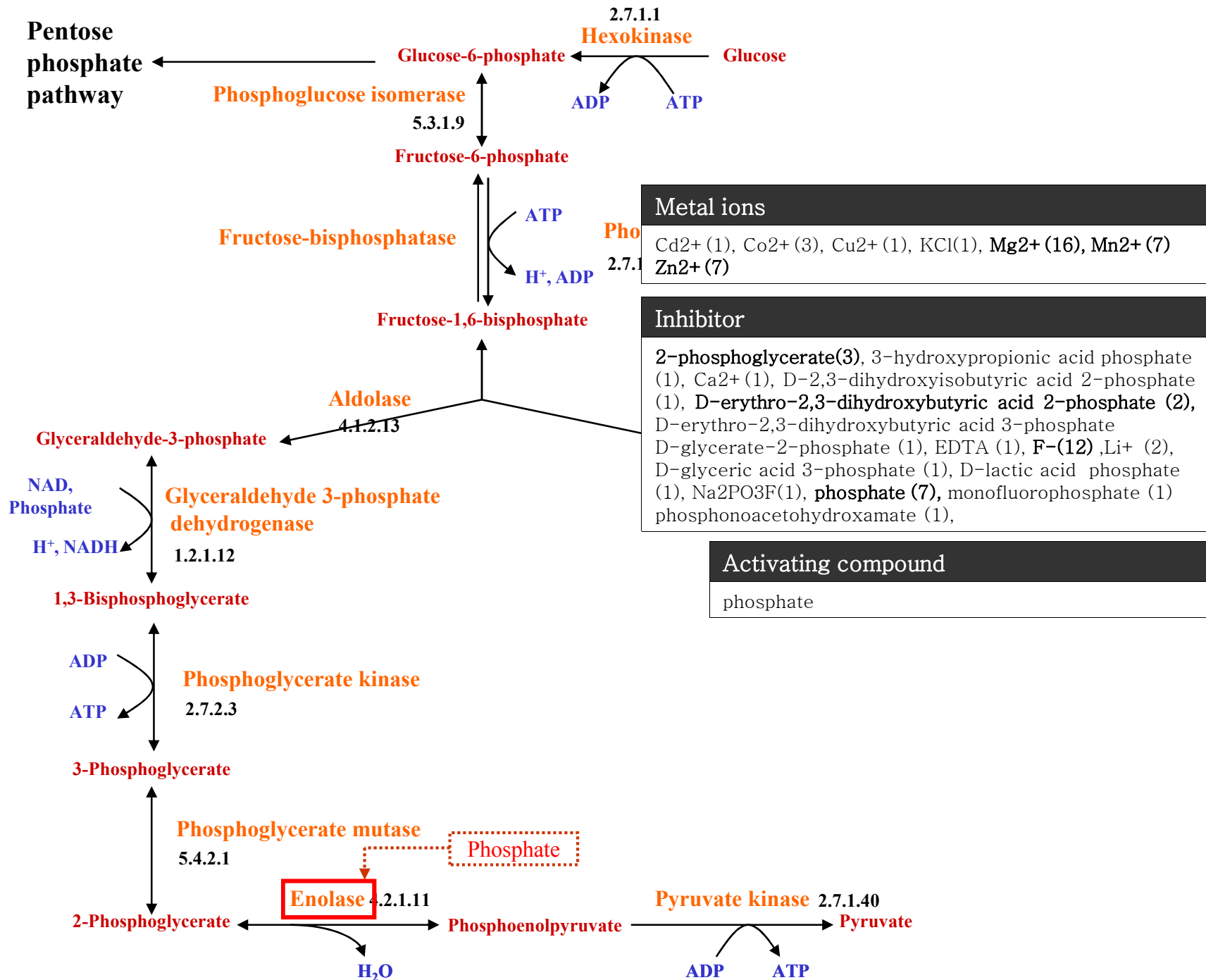






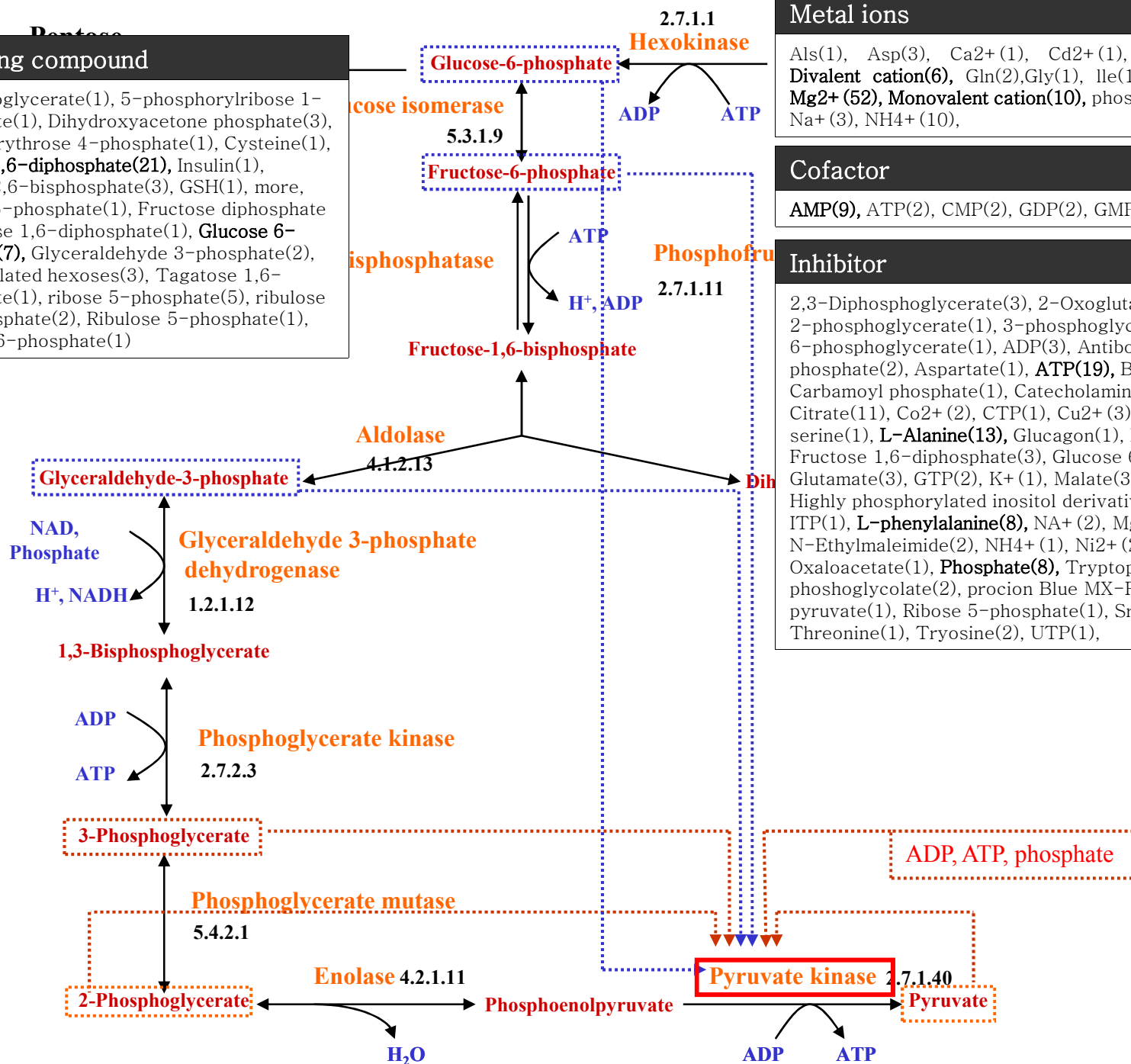






Activating compound

3-phosphoglycerate(1), 5-phosphorylribose 1-diphosphate(1), Dihydroxyacetone phosphate(3), DTT(2), Erythrose 4-phosphate(1), Cysteine(1), **Fructose 1,6-diphosphate(21)**, Insulin(1), Fructose 2,6-bisphosphate(3), GSH(1), more, Fructose 6-phosphate(1), Fructose diphosphate (8), Glucose 1,6-diphosphate(1), **Glucose 6-phosphate(7)**, Glyceraldehyde 3-phosphate(2), phosphorylated hexoses(3), Tagatose 1,6-diphosphate(1), ribose 5-phosphate(5), ribulose 1,5-diphosphate(2), Ribulose 5-phosphate(1), Tagatose 6-phosphate(1)



Metal ions

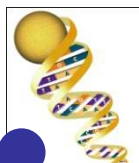
Al³⁺(1), Asp³⁻(3), Ca²⁺(1), Cd²⁺(1), CO₃²⁻(1), Co²⁺(3), **Divalent cation(6)**, Gln²⁻(2), Gly¹⁻(1), Ile¹⁻(1), **K⁺9(23)**, Met¹⁻(1), **Mg²⁺(52)**, **Monovalent cation(10)**, phosphate³⁻(3), Zn²⁺(1), Na⁺(3), NH₄⁺(10).

Cofactor

AMP(9), ATP(2), CMP(2), GDP(2), GMP(2), UDP(1)

Inhibitor

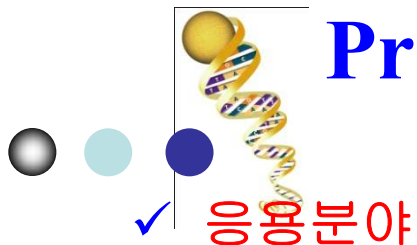
2,3-Diphosphoglycerate(3), 2-Oxoglutarate(1), Adenine(1), 2-phosphoglycerate(1), 3-phosphoglycerate(1), AMP(2), 6-phosphoglycerate(1), ADP(3), Antibody(1), Arginine phosphate(2), Aspartate(1), **ATP(19)**, Ba²⁺ (1), Ca²⁺ (5), Carbamoyl phosphate(1), Catecholamines(1), Cd²⁺ (2), Citrate(11), Co²⁺ (2), CTP(1), Cu²⁺ (3), Cysteine(1), D-serine(1), **L-Alanine(13)**, Glucagon(1), L-Lactate (2), Fructose 1,6-diphosphate(3), Glucose 6-phosphate (2), Glutamate(3), GTP(2), K⁺ (1), Malate(3), Mg²⁺ (1), Highly phosphorylated inositol derivatives(1), Isocitrate(3), ITP(1), **L-phenylalanine(8)**, NA⁺ (2), MgATP(3), Valine(1) N-Ethylmaleimide(2), NH₄⁺ (1), Ni²⁺ (2), Oxalate(4), Oxaloacetate(1), **Phosphate(8)**, Tryptophan(2), Zn²⁺ (3) phosphoglycolate(2), procion Blue MX-R(1), proline(1), pyruvate(1), Ribose 5-phosphate(1), Sr²⁺ (1), Sulfate(2), Threonine(1), Tyrosine(2), UTP(1),



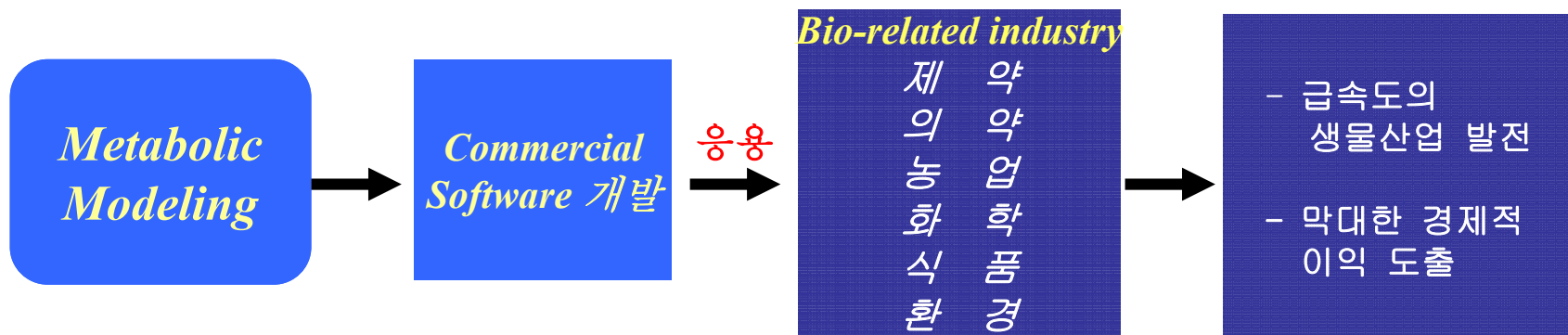
Simulation software

MCA

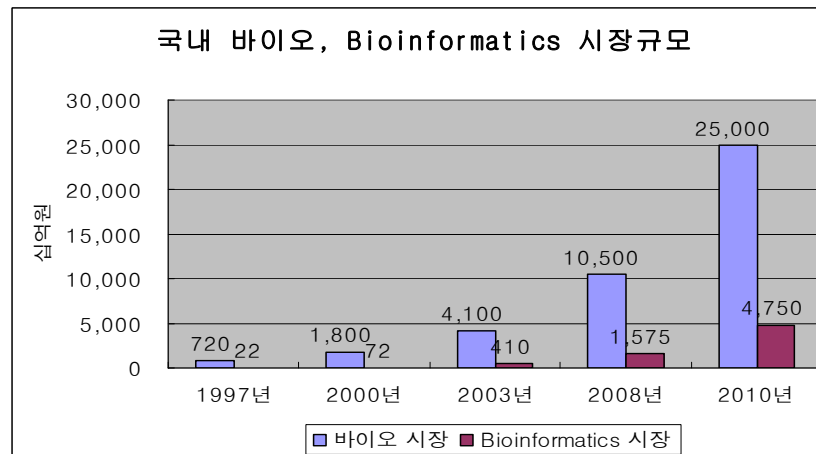
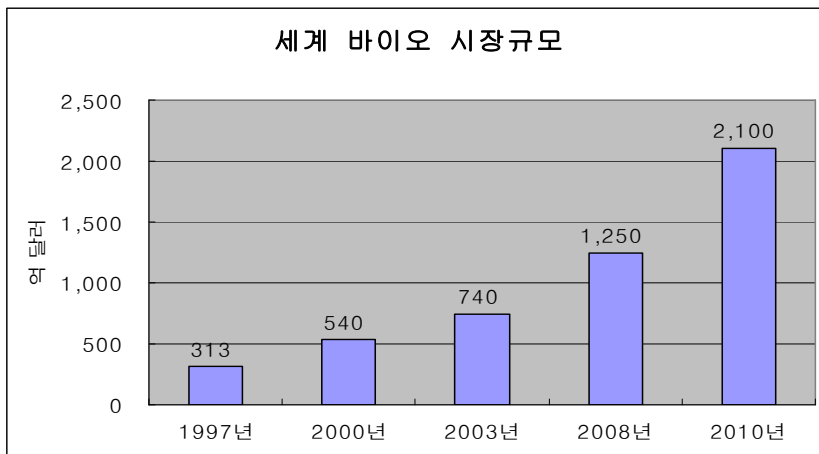
Name	Site	URL
Gepasi	Univ. of Wales Aberystwyth (USA)	http://www.gepasi.org/
MIST	Lund Univ. (Sweden)	http://www.cpb.dtu.dk/
SCAMP	Future Skill Software (UK)	http://www.fssc.demon.co.uk/Scamp/scamp.htm
MCELL	Carnegie Mellon Univ. (USA)	http://www.mcell.cnl.salk.edu/
A-CELL	Fuji Xerox Co., Ltd. (Japan)	http://www.fujixerox.co.jp/crc/cng/A-Cell/index.html
Virtual cell	University of Connecticut Health Center (USA).	http://www.nrcam.uchc.edu/
SCoP	Duke University Medical Center (USA)	http://www.simresinc.com/
E-CELL	E-CELL Project (Japan)	http://www.e-cell.org/
MetaFluxNet	KAIST (Korea)	http://mbel.kaist.ac.kr
KEGG	Institute for chemical research, Kyoto University	http://www.genome.ad.jp/kegg/
cellML	Auckland University, New Zeland	http://www.cellml.org/public/about/what_is_cellml.html



Promises of Metabolic Modeling



✓전망



<출처 : 산업연구원, OECD (2002. 11)>