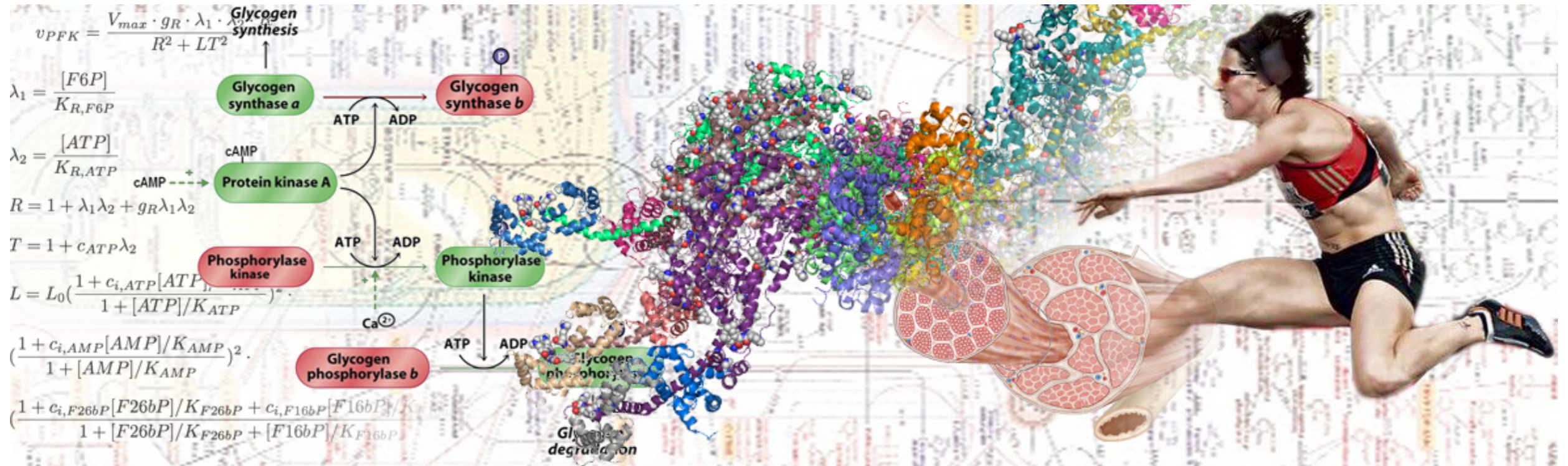


Biochemistry 714 Mini-course: Molecular Systems Biology



Prof Johann Rohwer (lectures, tutorials & data analysis)
 Dr Dawie van Niekerk (practical and data analysis)

February – March 2026

Thus far

- First Lecture: Chemical kinetics
- Direction of reaction: ΔG , Γ/K_{eq}
- How far: K_{eq} , ΔG^0
- How fast: mass action kinetics
- Second Lecture: Enzyme kinetics
- Derivation of rate equations: equilibrium binding, steady-state approximation
- V_{max} , K_{m} , saturation, cooperativity, allostery, reversibility, product inhibition
- Third Lecture: Coupled reactions
- Parameter estimation; initial rates, progress curves
- Closed, open systems; equilibrium, steady state, rate characteristics
- Kinetic model of simple pathways in steady state

This lecture: Network analysis

- For a given system, derive N , K , L matrices, interpret them
- Flux modes

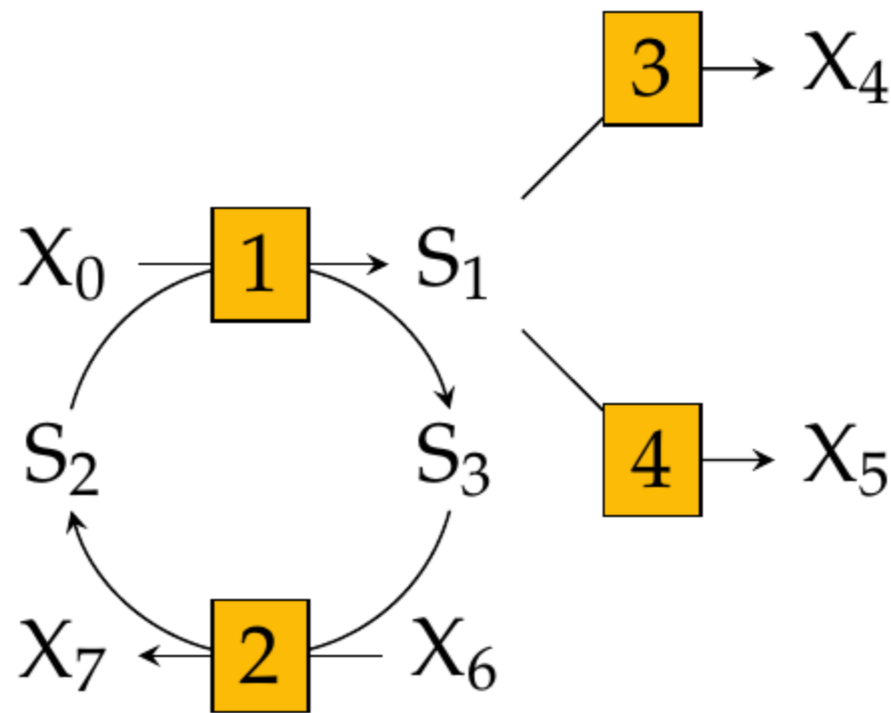
Kinetic model of a coupled reaction network

$$\frac{d\mathbf{s}}{dt} = \mathbf{N}\mathbf{v}[\mathbf{s}, \mathbf{p}]$$

where, for a system of n coupled reactions that interconvert m metabolites:

- \mathbf{s} = m -dim vector of metabolite concentrations
- \mathbf{N} = $m \times n$ matrix of stoichiometric coefficients
- \mathbf{v} = n -dim vector of reaction rates
- \mathbf{p} = p -dim vector of parameters

Analysis of the stoichiometric matrix, \mathbf{N}



$$ds_1/dt = v_1 - v_3 - v_4$$

$$ds_2/dt = v_2 - v_1$$

$$ds_3/dt = v_1 - v_2$$

Stoichiometry matrix, \mathbf{N}

	R ₁	R ₂	R ₃	R ₄
S ₁	1	0	-1	-1
S ₂	-1	1	0	0
S ₃	1	-1	0	0

$$\frac{d\mathbf{s}}{dt} = \begin{bmatrix} 1 & 0 & -1 & -1 \\ -1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix}$$

$$\frac{d\mathbf{s}}{dt} = \mathbf{N}\mathbf{v}[\mathbf{s}, \mathbf{p}]$$

The nullspace of \mathbf{N} and flux-relationships

In steady state $\frac{ds}{dt} = \mathbf{N}\mathbf{v} = 0$ and rates, \mathbf{v} , are now called fluxes, \mathbf{J} . If J_3 and J_4 (the R_3 and R_4 -columns without pivots) are chosen as the independent fluxes, \mathbf{J}_i :

$$\begin{bmatrix} J_3 \\ J_4 \\ J_1 \\ J_2 \end{bmatrix} = \begin{bmatrix} J_3 \\ J_4 \\ J_3 + J_4 \\ J_3 + J_4 \end{bmatrix} = \begin{bmatrix} 1 \\ 0 \\ 1 \\ 1 \end{bmatrix} J_3 + \begin{bmatrix} 0 \\ 1 \\ 1 \\ 1 \end{bmatrix} J_4 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} J_3 \\ J_4 \end{bmatrix}$$

or

$$\mathbf{J} = \mathbf{K}\mathbf{J}_i \quad \text{or} \quad \begin{bmatrix} \mathbf{J}_i \\ \mathbf{J}_d \end{bmatrix} = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{K}_0 \end{bmatrix} \mathbf{J}_i$$

where \mathbf{K} is the nullspace or kernel of \mathbf{N} .

The link-matrix, \mathbf{L} , and ODE relationships

Either S_2 or S_3 can be chosen as the dependent metabolite, S_i .
Choose S_3 .

$$\begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \\ \dot{s}_3 \end{bmatrix} = \begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \\ -\dot{s}_2 \end{bmatrix} = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} \dot{s}_1 + \begin{bmatrix} 0 \\ 1 \\ -1 \end{bmatrix} \dot{s}_2 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{bmatrix} \begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \end{bmatrix}$$

or

$$\frac{d\mathbf{s}}{dt} = \mathbf{L} \frac{d\mathbf{s}_i}{dt} \quad \text{or} \quad \frac{d}{dt} \begin{bmatrix} \mathbf{s}_i \\ \mathbf{s}_d \end{bmatrix} = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{L}_0 \end{bmatrix} \frac{d\mathbf{s}_i}{dt}$$

where \mathbf{L} is the link-matrix

The reduced stoichiometry matrix

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

From the stoichiometry matrix \mathbf{N} , delete the rows that correspond to dependent metabolites (here S_3):

$$\mathbf{N}_R = \begin{bmatrix} 1 & 0 & -1 & -1 \\ -1 & 1 & 0 & 0 \end{bmatrix}$$

The reduced stoichiometry matrix

$$\mathbf{N} = \mathbf{L}\mathbf{N}_R$$

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

Kinetic model of a coupled reaction network

$$\frac{d\mathbf{s}}{dt} = \mathbf{N}\mathbf{v}[\mathbf{s}, \mathbf{p}]$$

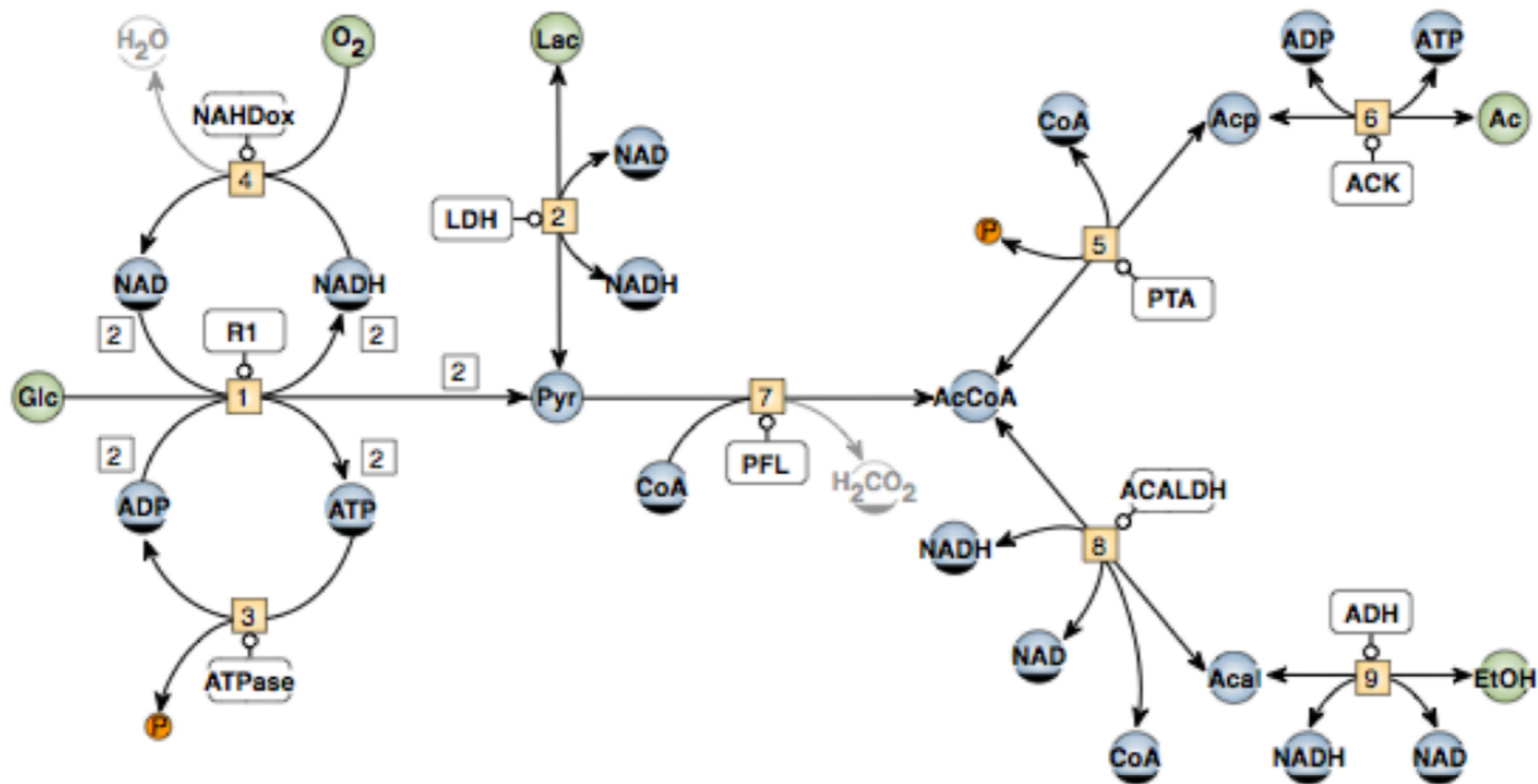
For independent metabolites:

$$\frac{d\mathbf{s}_i}{dt} = \mathbf{N}_R\mathbf{v}[\mathbf{s}, \mathbf{p}]$$

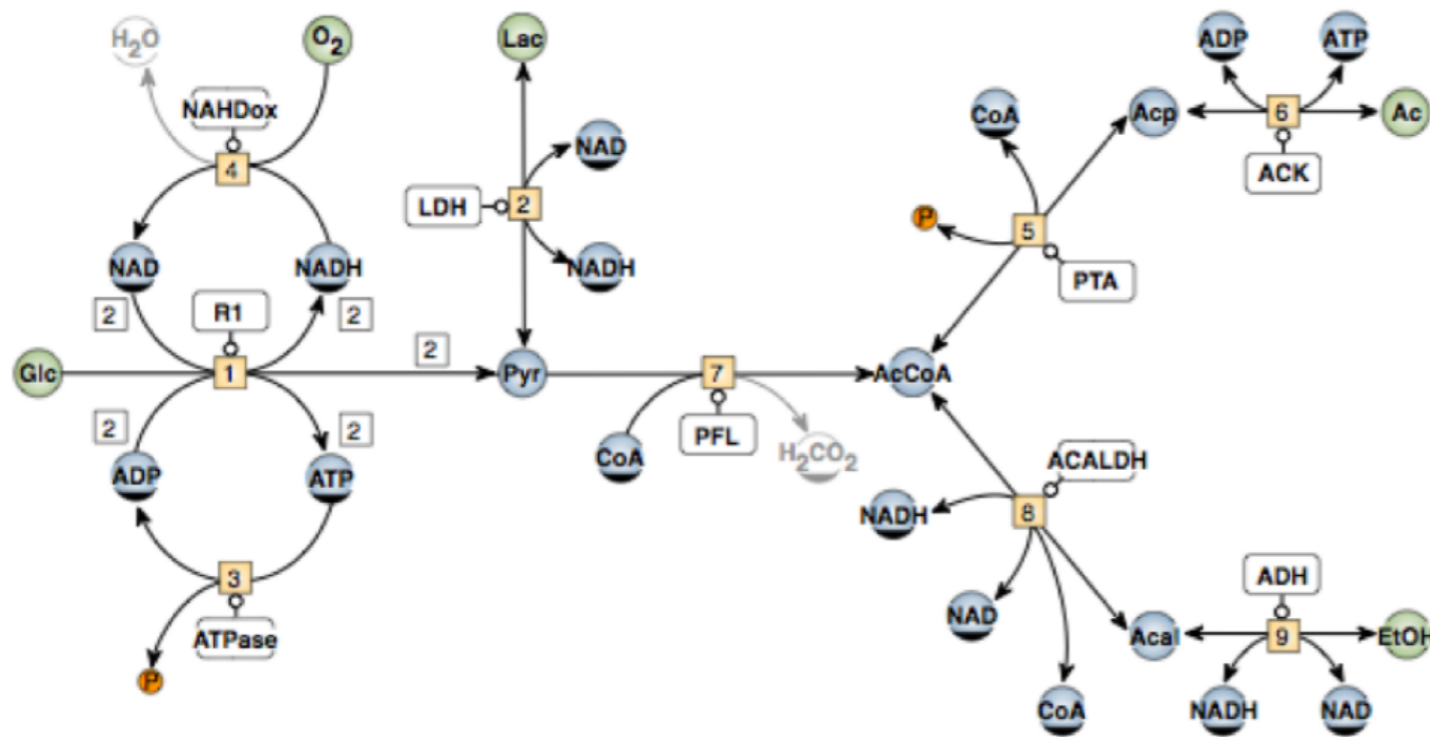
Combine

$$\frac{d\mathbf{s}}{dt} = \mathbf{L}\frac{d\mathbf{s}_i}{dt} = \mathbf{L}\mathbf{N}_R\mathbf{v}[\mathbf{s}, \mathbf{p}]$$

Reaction network



Reaction network: N matrix (stoichiometry)

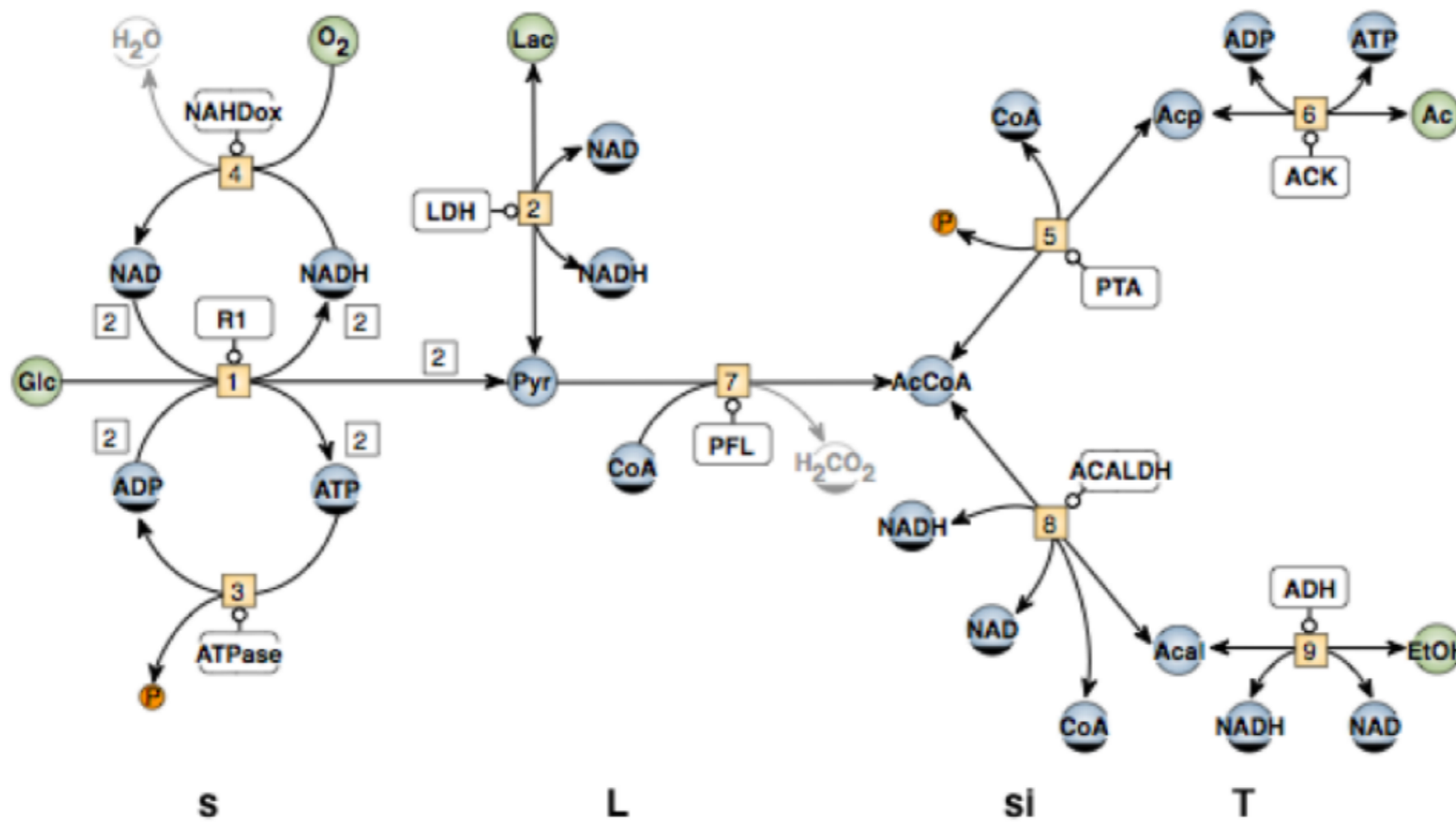


$$\begin{array}{l}
 \mathbf{d(s)/dt} \\
 \left(\begin{array}{l}
 d(\text{ACAL})/dt \\
 d(\text{ACCOA})/dt \\
 d(\text{ACP})/dt \\
 d(\text{ADP})/dt \\
 d(\text{ATP})/dt \\
 d(\text{COA})/dt \\
 d(\text{NAD})/dt \\
 d(\text{NADH})/dt \\
 d(\text{PYR})/dt
 \end{array} \right)
 \end{array}
 =
 \begin{array}{l}
 \mathbf{N} \\
 \left(\begin{array}{ccccccccc}
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1. & -1. \\
 0 & 0 & 0 & 0 & -1. & 0 & 1. & -1. & 0 \\
 0 & 0 & 0 & 0 & 1. & -1. & 0 & 0 & 0 \\
 -2. & 0 & 1. & 0 & 0 & -1. & 0 & 0 & 0 \\
 2. & 0 & -1. & 0 & 0 & 1. & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 1. & 0 & -1. & 1. & 0 \\
 -2. & 1. & 0 & 1. & 0 & 0 & 0 & 1. & 1. \\
 2. & -1. & 0 & -1. & 0 & 0 & 0 & -1. & -1. \\
 2. & -1. & 0 & 0 & 0 & 0 & -1. & 0 & 0
 \end{array} \right)
 \end{array}
 *
 \begin{array}{l}
 \mathbf{v} \\
 \left(\begin{array}{l}
 v1 \\
 v2 \\
 v3 \\
 v4 \\
 v5 \\
 v6 \\
 v7 \\
 v8 \\
 v9
 \end{array} \right)
 \end{array}$$

N stoichiometry matrix

v reaction rates

Reaction network: L matrix (link)



$$\begin{pmatrix} \text{ACAL} \\ \text{ACCOA} \\ \text{ACP} \\ \text{ADP} \\ \text{NAD} \\ \text{PYR} \\ \text{ATP} \\ \text{COA} \\ \text{NADH} \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 \end{pmatrix} * \begin{pmatrix} \text{ACAL} \\ \text{ACCOA} \\ \text{ACP} \\ \text{ADP} \\ \text{NAD} \\ \text{PYR} \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 5 \\ 1 \\ 10 \end{pmatrix}$$

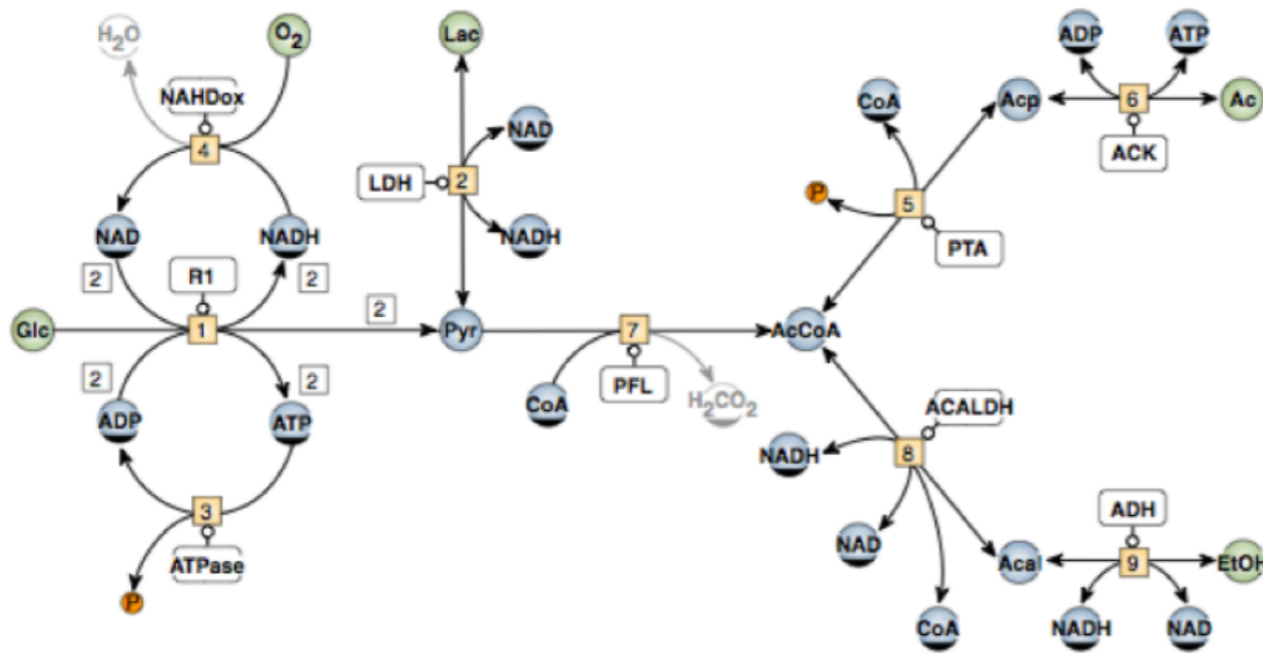
s vector of metabolite concentrations

L link matrix

si vector of independent metabolite concentrations

T vector of the sum of conserved moiety concentrations

Reaction network: steady-state constraint



Independent fluxes:
 J_3, J_7, J_9

$$\dot{A}cAl = 0 = v_8 - v_9; v_8 = v_9$$

$$\dot{A}cCoA = 0 = -v_5 + v_7 - v_8; v_5 = v_7 - v_8 = v_7 - v_9$$

$$\dot{A}cP = 0 = v_5 - v_6; v_6 = v_5 = v_7 - v_9$$

$$\dot{A}DP = 0 = -2 \cdot v_1 + v_3 - v_6; 2 \cdot v_1 = v_3 - v_6 = v_3 - v_7 + v_9$$

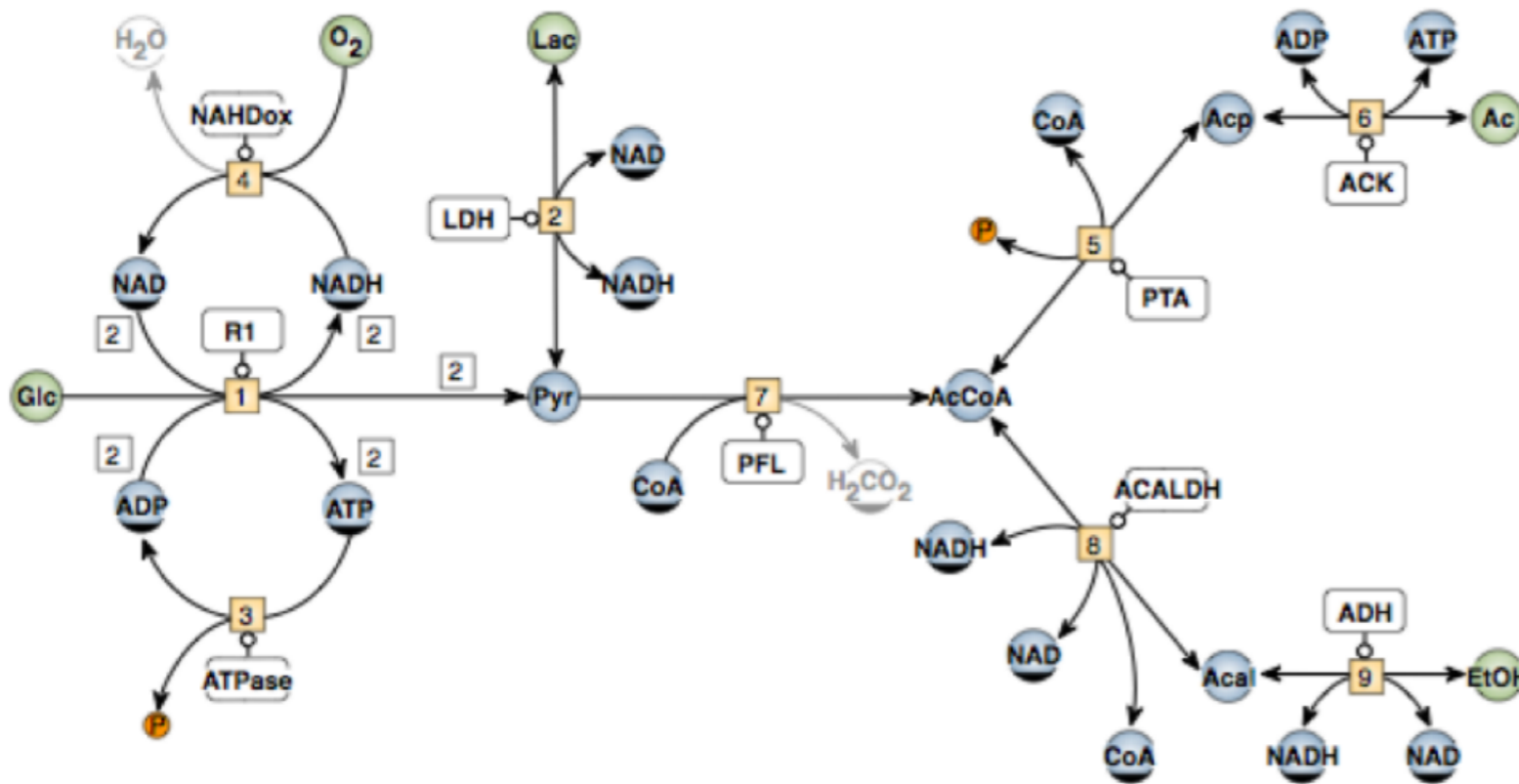
$$\dot{A}TP = -\dot{A}DP$$

$$\dot{C}oA = -\dot{A}cCoA$$

$$\begin{aligned} \dot{P}yr = 0 &= 2 \cdot v_1 - v_2 - v_7; v_2 = 2 \cdot v_1 - v_7 = \\ &v_3 - v_7 + v_9 - v_7 = v_3 - 2 \cdot v_7 + v_9 \end{aligned}$$

$$\begin{aligned} \dot{N}AD = 0 &= -v_1 + v_2 + v_4 + v_8 + v_9; v_4 = 2 \cdot v_1 - v_2 - v_8 - v_9 = \\ &v_3 - v_7 + v_9 - v_2 - 2 \cdot v_9 = v_3 - v_7 - v_9 - v_2 = v_3 - v_7 - v_9 - v_3 + 2 \cdot v_7 - v_9 = v_7 - 2 \cdot v_9 \end{aligned}$$

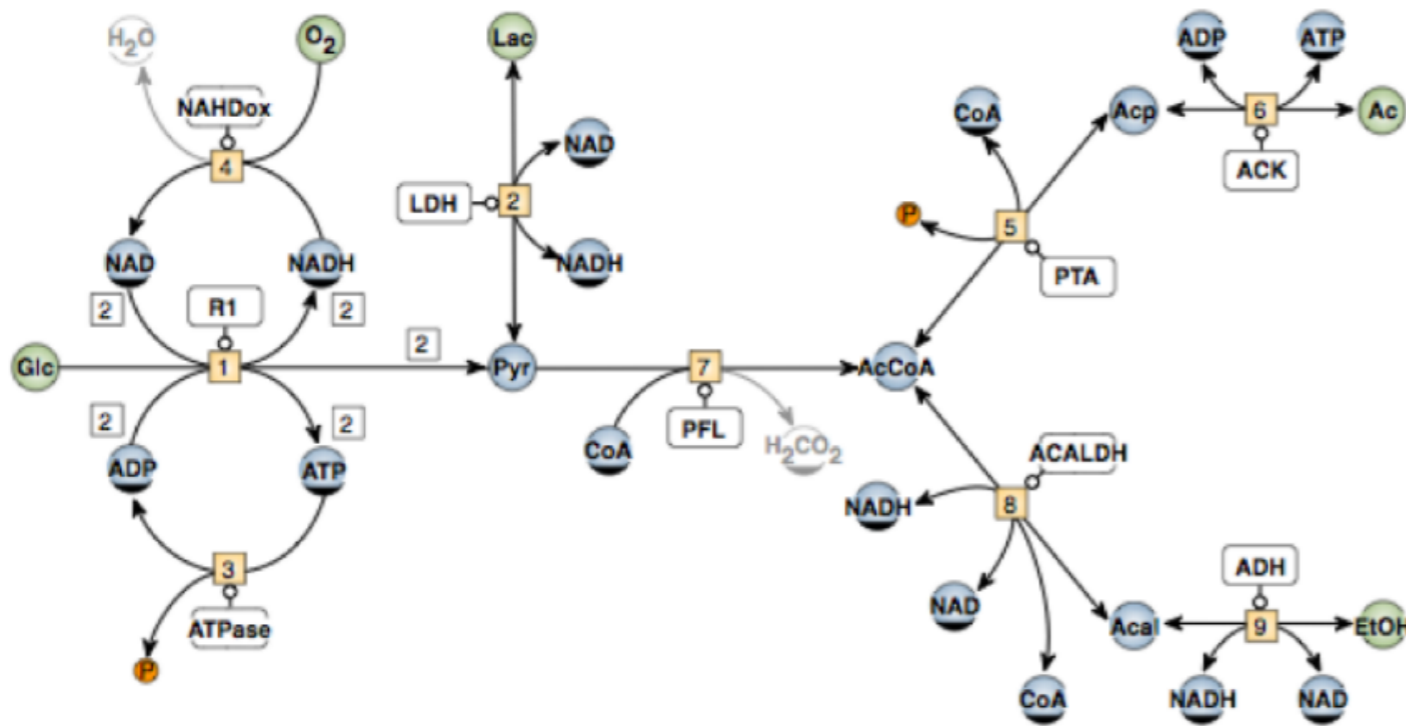
Reaction network: K matrix (kernel)



$$\begin{matrix} \mathbf{J} \\ \mathbf{J}_3 \\ \mathbf{J}_7 \\ \mathbf{J}_9 \\ \mathbf{J}_1 \\ \mathbf{J}_2 \\ \mathbf{J}_4 \\ \mathbf{J}_5 \\ \mathbf{J}_6 \\ \mathbf{J}_8 \end{matrix} = \begin{matrix} \mathbf{K} \\ \begin{pmatrix} 1. & 0. & 0. \\ 0. & 1. & 0. \\ 0. & 0. & 1. \\ 0.5 & -0.5 & 0.5 \\ 1. & -2. & 1. \\ 0. & 1. & -2. \\ 0. & 1. & -1. \\ 0. & 1. & -1. \\ 0. & 0. & 1. \end{pmatrix} \end{matrix} * \begin{matrix} \mathbf{J}_i \\ \begin{pmatrix} \mathbf{J}_3 \\ \mathbf{J}_7 \\ \mathbf{J}_9 \end{pmatrix} \end{matrix}$$

- J** steady state reaction rate (flux) vector
- K** kernel, nullspace of stoichiometry matrix
- J_i** independent flux vector

Reaction network: K matrix; flux modes



$$\begin{pmatrix} \text{J3} \\ \text{J7} \\ \text{J9} \\ \text{J1} \\ \text{J2} \\ \text{J4} \\ \text{J5} \\ \text{J6} \\ \text{J8} \end{pmatrix} = \begin{pmatrix} 1. & 0. & 0. \\ 0. & 1. & 0. \\ 0. & 0. & 1. \\ 0.5 & -0.5 & 0.5 \\ 1. & -2. & 1. \\ 0. & 1. & -2. \\ 0. & 1. & -1. \\ 0. & 1. & -1. \\ 0. & 0. & 1. \end{pmatrix} * \begin{pmatrix} \text{J3} \\ \text{J7} \\ \text{J9} \end{pmatrix}$$

J steady state reaction rate (flux) vector
K kernel, nullspace of stoichiometry matrix
Ji independent flux vector

- col.1 = homolactic fermentation
- $2 \times \text{col.1} + \text{col.2}$ = homo-acetate fermentation
- $\text{col.3} + 2 \times \text{col.2} + 3 \times \text{col.1}$ = mixed acid fermentation