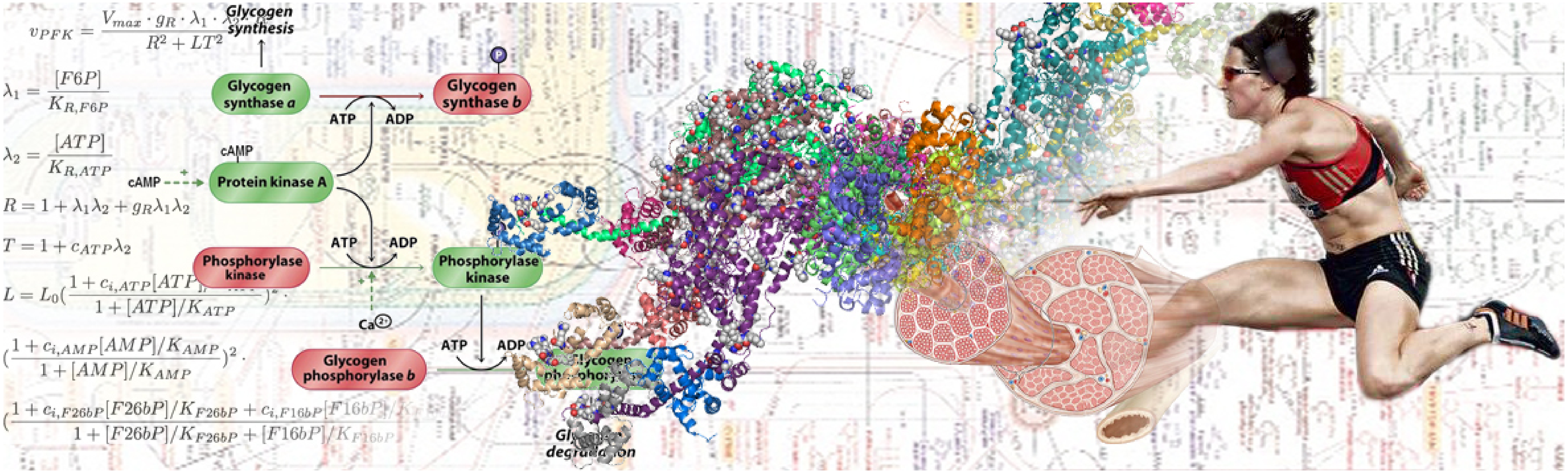
Biochemistry 714 Mini-course: Molecular Systems Biology



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

March – April 2025

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

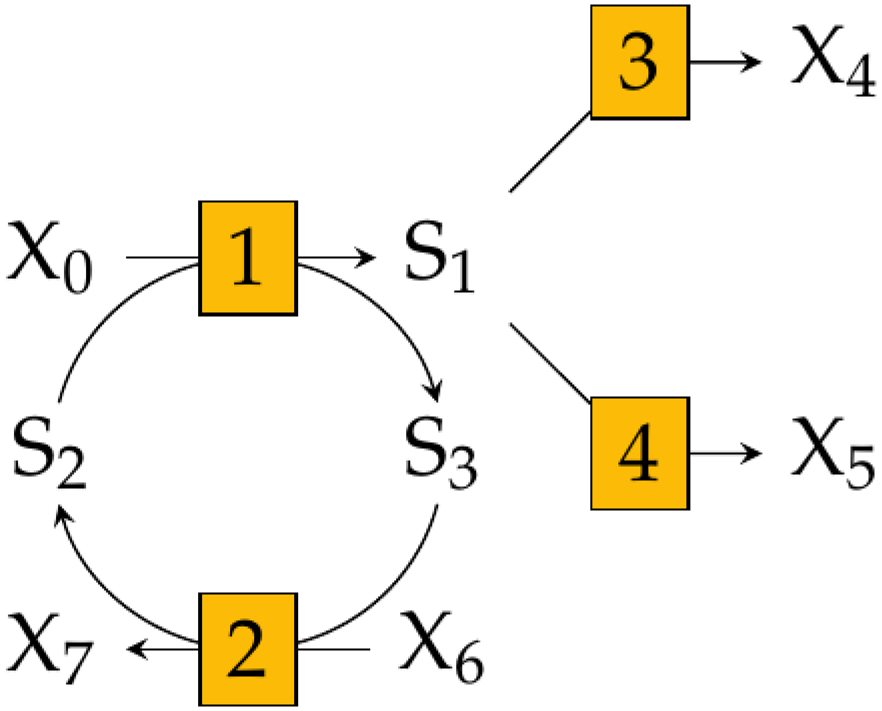
Kinetic model of a coupled reaction network

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

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, **N**



Stoichiometry matrix, **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

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

$$\begin{aligned} ds_1/dt &= v_1 - v_3 - v_4 \\ ds_2/dt &= v_2 - v_1 \\ ds_3/dt &= v_1 - v_2 \end{aligned}$$

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

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

In steady state $\frac{d\mathbf{s}}{dt} = \mathbf{N}\mathbf{v} = 0$ 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

$$\mathbf{J} = \mathbf{K}\mathbf{J_i} \qquad \text{or} \qquad \begin{bmatrix} \mathbf{J_i} \\ \mathbf{J_d} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{K_0} \end{bmatrix} \mathbf{J_i}$$

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

The link-matrix, **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

$$\frac{d\mathbf{s}}{dt} = \mathbf{L} \frac{d\mathbf{s_i}}{dt} \qquad \text{or} \qquad \frac{d}{dt} \begin{bmatrix} \mathbf{s_i} \\ \mathbf{s_d} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{L_0} \end{bmatrix} \frac{d\mathbf{s_i}}{dt}$$

where **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 **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

$$\mathbf{N} = \mathbf{L}\mathbf{N}_{\mathbf{R}}$$

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

Kinetic model of a coupled reaction network

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

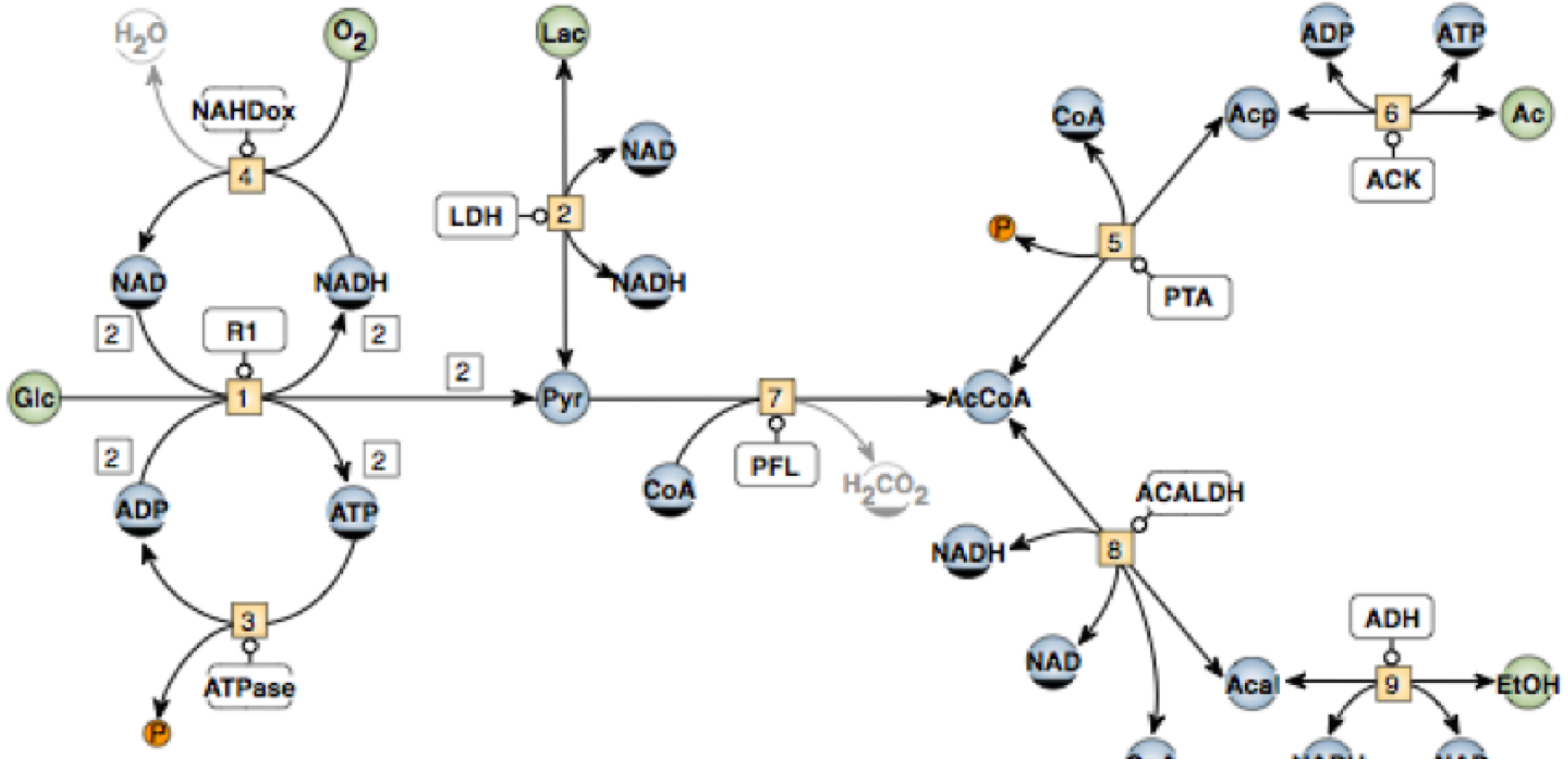
For independent metabolites:

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

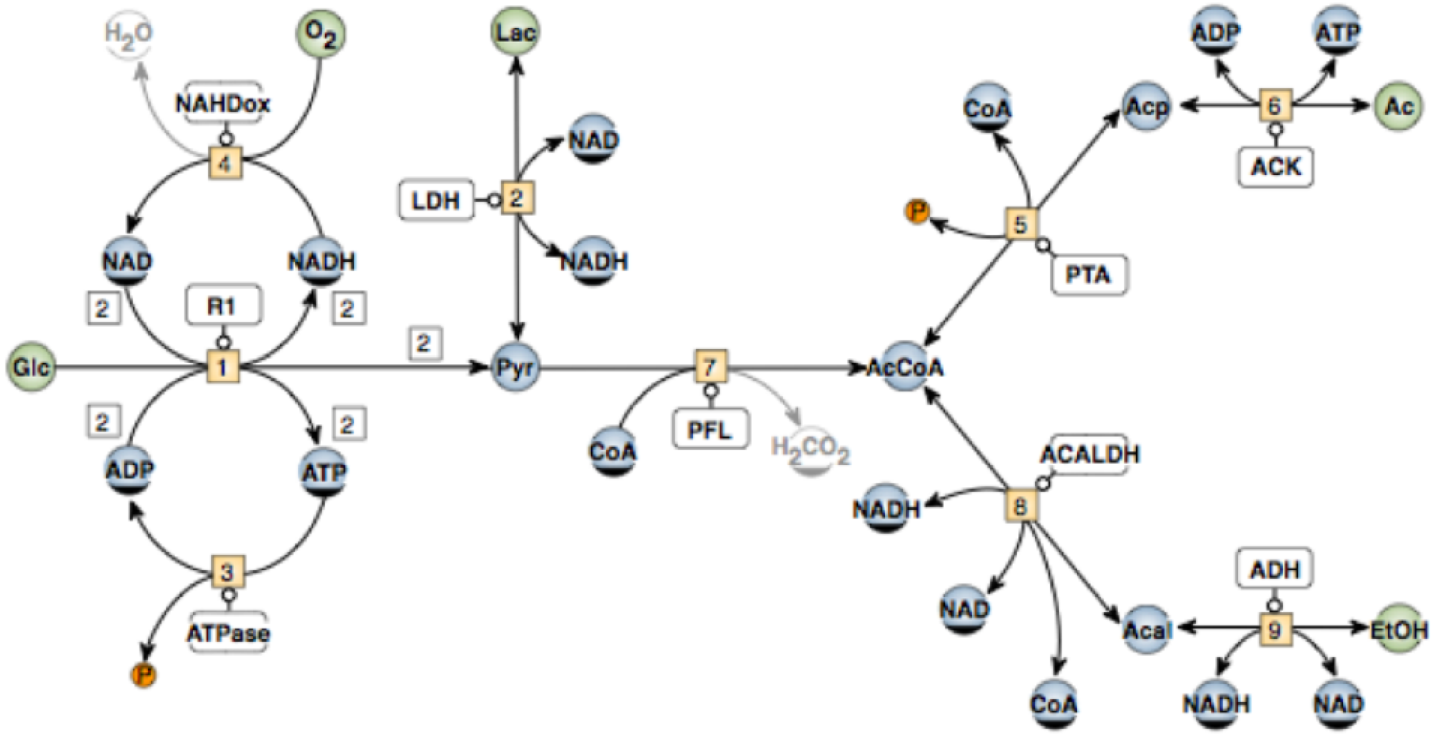
Combine

$$\frac{d\mathbf{s}}{dt} = \mathbf{L}\frac{d\mathbf{s_i}}{dt} = \mathbf{L}\mathbf{N_R}\mathbf{v}[\mathbf{s}, \mathbf{p}]$$

Reaction network



Reaction network: N matrix (stoichiometry)



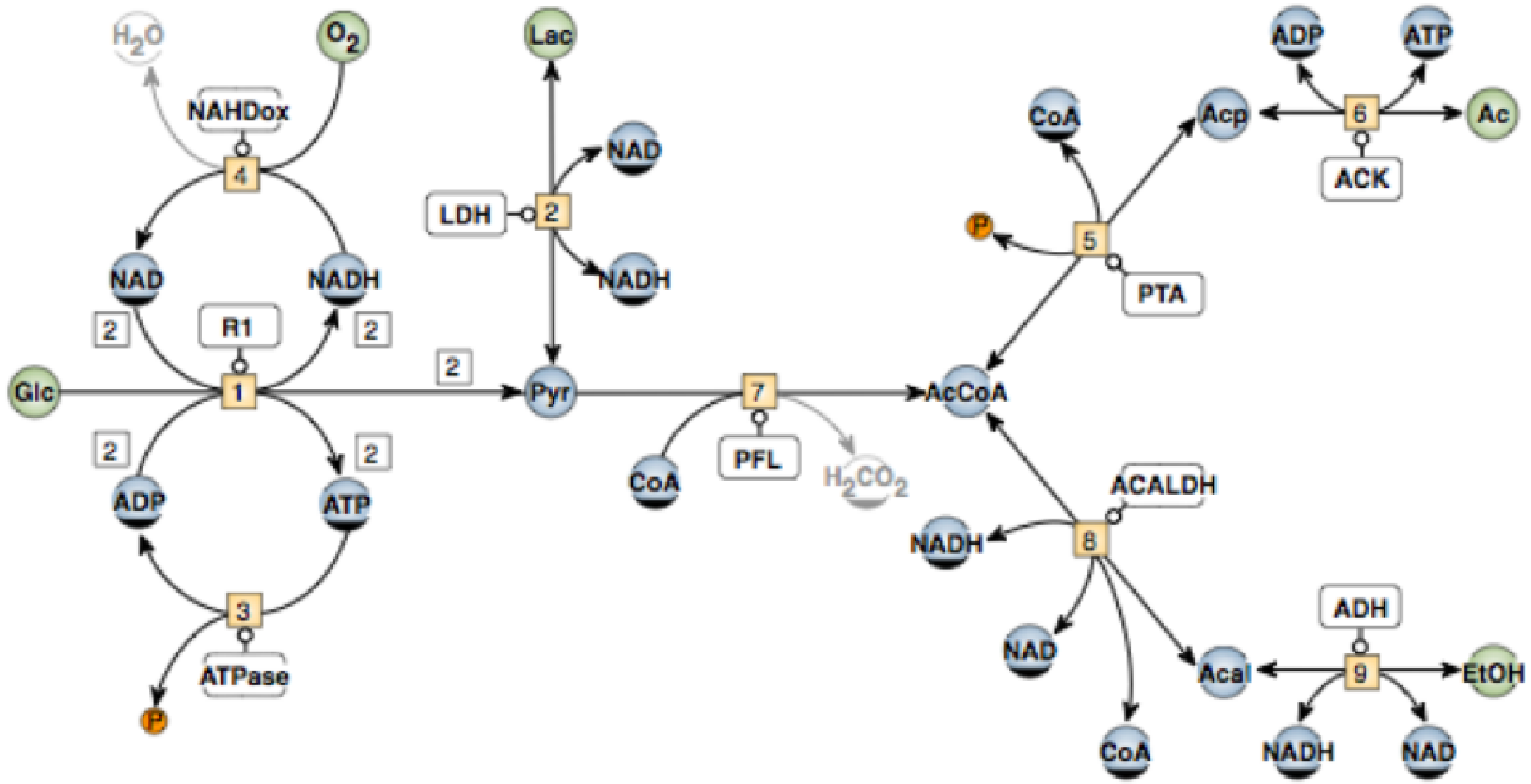
d(s)/dt **N** **v**

$$\begin{pmatrix} d(ACAL)/dt \\ d(ACCOA)/dt \\ d(ACP)/dt \\ d(ADP)/dt \\ d(ATP)/dt \\ d(COA)/dt \\ d(NAD)/dt \\ d(NADH)/dt \\ d(PYR)/dt \end{pmatrix} = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1. & -1. \\ 0 & 0 & 0 & 0 & -1. & 0 & 1. & -1. & 0 \\ 0 & 0 & 0 & 0 & 1. & -1. & 0 & 0 & 0 \\ -2. & 0 & 1. & 0 & 0 & -1. & 0 & 0 & 0 \\ 2. & 0 & -1. & 0 & 0 & 1. & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1. & 0 & -1. & 1. & 0 \\ -2. & 1. & 0 & 1. & 0 & 0 & 0 & 1. & 1. \\ 2. & -1. & 0 & -1. & 0 & 0 & 0 & -1. & -1. \\ 2. & -1. & 0 & 0 & 0 & 0 & -1. & 0 & 0 \end{pmatrix} * \begin{pmatrix} v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \end{pmatrix}$$

N stoichiometry matrix

v reaction rates

Reaction network: L matrix (link)



$$\begin{array}{ccccccc} \mathbf{s} & & \mathbf{L} & & \mathbf{si} & & \mathbf{T} \\ \begin{pmatrix} \text{ACAL} \\ \text{ACCOA} \\ \text{ACP} \\ \text{ADP} \\ \text{NAD} \\ \text{PYR} \\ \text{ATP} \\ \text{COA} \\ \text{NADH} \end{pmatrix} & = & \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0. & 0. & 0. & -1. & 0. & 0. \\ 0. & -1. & 0. & 0. & 0. & 0. \\ 0. & 0. & 0. & 0. & -1. & 0. \end{pmatrix} & * & \begin{pmatrix} \text{ACAL} \\ \text{ACCOA} \\ \text{ACP} \\ \text{ADP} \\ \text{NAD} \\ \text{PYR} \end{pmatrix} & + & \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 5. \\ 1. \\ 10. \end{pmatrix} \end{array}$$

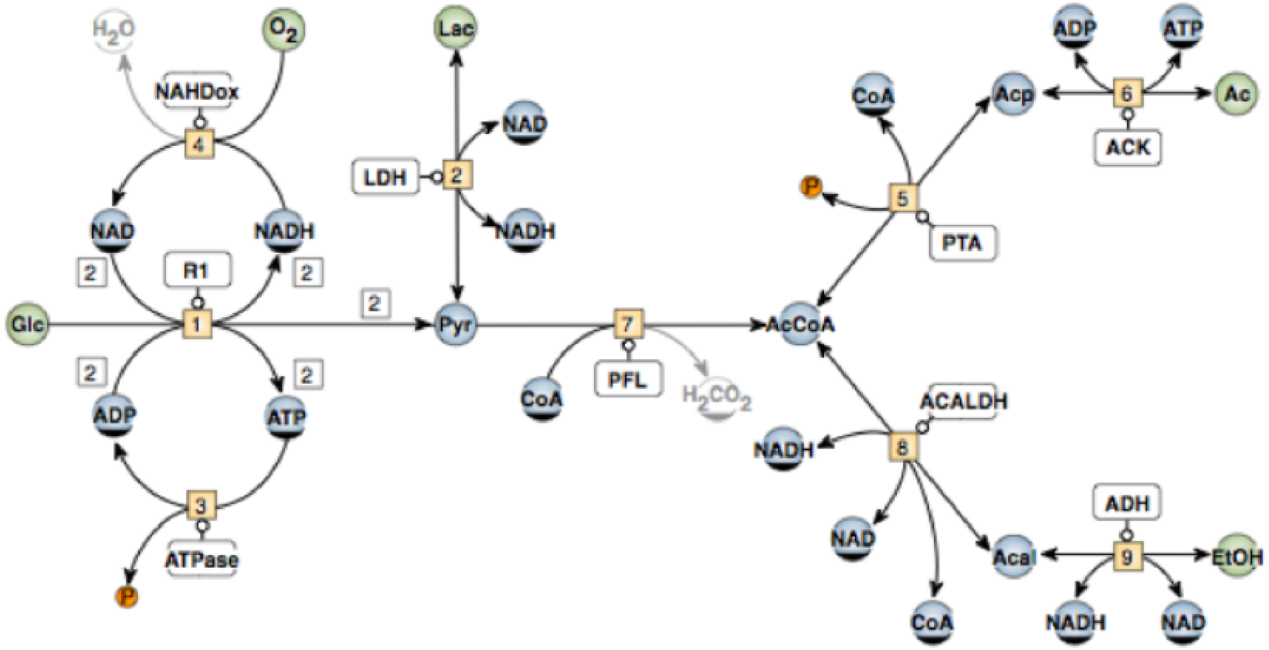
s vector of metabolite concentrations

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

$$A\dot{c}al = 0 = v8 - v9; v8 = v9$$

$$Ac\dot{C}oA = 0 = -v5 + v7 - v8; v5 = v7 - v8 = v7 - v9$$

$$A\dot{c}P = 0 = v5 - v6; v6 = v5 = v7 - v9$$

$$A\dot{D}P = 0 = -2 \cdot v1 + v3 - v6; 2 \cdot v1 = v3 - v6 = v3 - v7 + v9$$

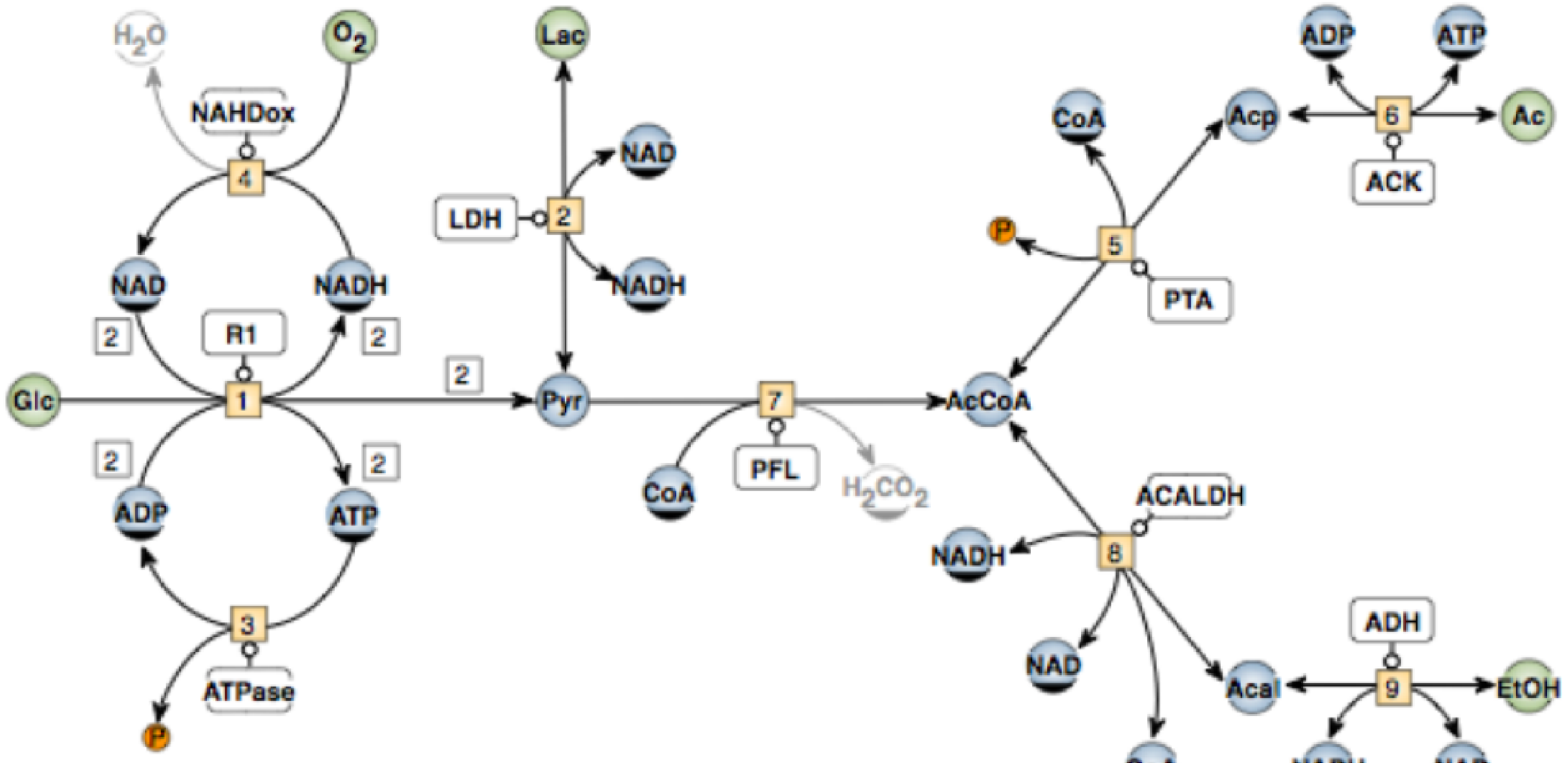
$$A\dot{T}P = -A\dot{D}P$$

$$C\dot{o}A = -Ac\dot{C}oA$$

$$P\dot{y}r = 0 = 2 \cdot v1 - v2 - v7; v2 = 2 \cdot v1 - v7 =$$
$$v3 - v7 + v9 - v7 = v3 - 2 \cdot v7 + v9$$

$$N\dot{A}D = 0 = -v1 + v2 + v4 + v8 + v9; v4 = 2 \cdot v1 - v2 - v8 - v9 =$$
$$v3 - v7 + v9 - v2 - 2 \cdot v9 = v3 - v7 - v9 - v2 = v3 - v7 - v9 - v3 + 2 \cdot v7 - v9 = v7 - 2 \cdot v9$$

Reaction network: K matrix (kernel)



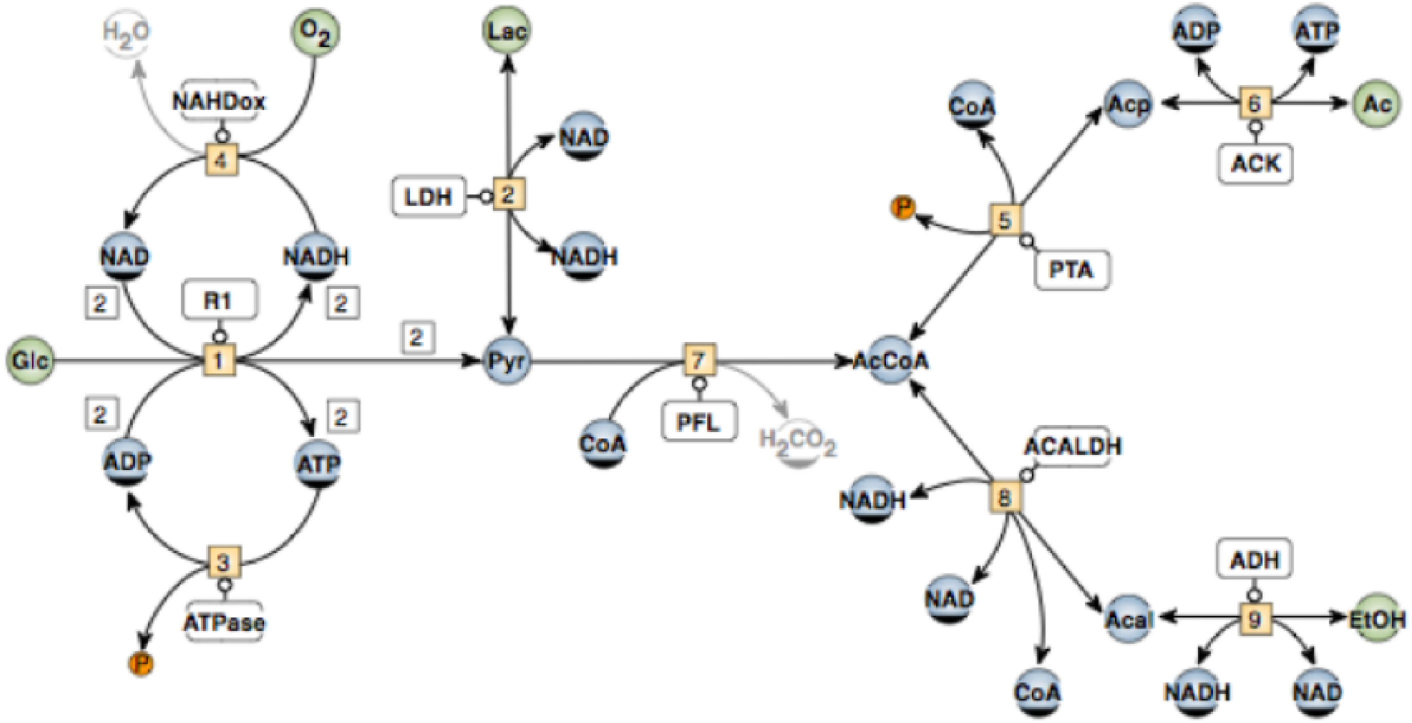
$$\overset{\textbf{J}}{\begin{pmatrix} J3 \\ J7 \\ J9 \\ J1 \\ J2 \\ J4 \\ J5 \\ J6 \\ J8 \end{pmatrix}} = \overset{\textbf{K}}{\begin{pmatrix} 1. & 0. & 0. \\ 0. & 1. & 0. \\ 0. & 0. & 1. \\ 0.5 & -0.5 & 0.5 \\ 1. & -2. & 1. \\ 0. & 1. & -2. \\ 0. & 1. & -1. \\ 0. & 1. & -1. \\ 0. & 0. & 1. \end{pmatrix}}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

J steady state reaction rate (flux) vector

K kernel, nullspace of stoichiometry matrix

Ji independent flux vector

Reaction network: K matrix; flux modes



$$\begin{array}{ccccc} \mathbf{J} & & \mathbf{K} & & \mathbf{Ji} \\ \begin{pmatrix} J3 \\ J7 \\ J9 \\ J1 \\ J2 \\ J4 \\ J5 \\ J6 \\ J8 \end{pmatrix} & = & \begin{pmatrix} 1. & 0. & 0. \\ 0. & 1. & 0. \\ 0. & 0. & 1. \\ 0.5 & -0.5 & 0.5 \\ 1. & -2. & 1. \\ 0. & 1. & -2. \\ 0. & 1. & -1. \\ 0. & 1. & -1. \\ 0. & 0. & 1. \end{pmatrix} & * & \begin{pmatrix} J3 \\ J7 \\ J9 \end{pmatrix} \end{array}$$

J steady state reaction rate (flux) vector

K kernel, nullspace of stoichiometry matrix

Ji independent flux vector

- col.1 = homolactic fermentation
- 2×col.1 + col.2 = homo-acetate fermentation
- col.3 + 2×col.2 + 3×col.1 = mixed acid fermentation