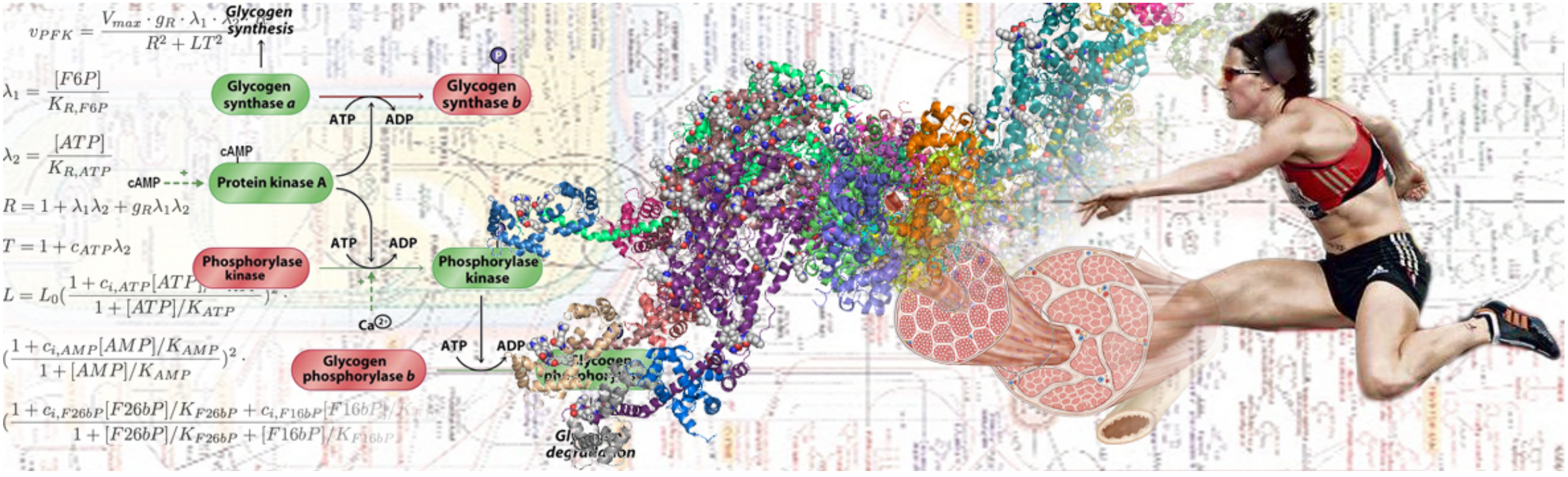
Mini-course: Molecular Systems Biology



Profs Jacky Snoep and Johann Rohwer

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Thus far

- First Lecture: Chemical kinetics
- Direction of reaction: dG, Gamma/Keq
- How far: Keq, dG^0; How fast: mass action kinetics
- Second Lecture: Enzyme kinetics
- Derivation of rate equations: equilibrium binding, steady state approximation
- Vmax, Km, saturation, cooperativity, allostery, reversibility, product inhibition
- Third Lecture: Coupled reactions
- Parameter estimation; initial rates, progress curves
- Closed, open systems; equilibrium, steady state, rate characteristics
- Fourth Lecture: Structural network analysis
- N, K, L matrix
- Steady state flux constraints, Flux analysis, Flux modes

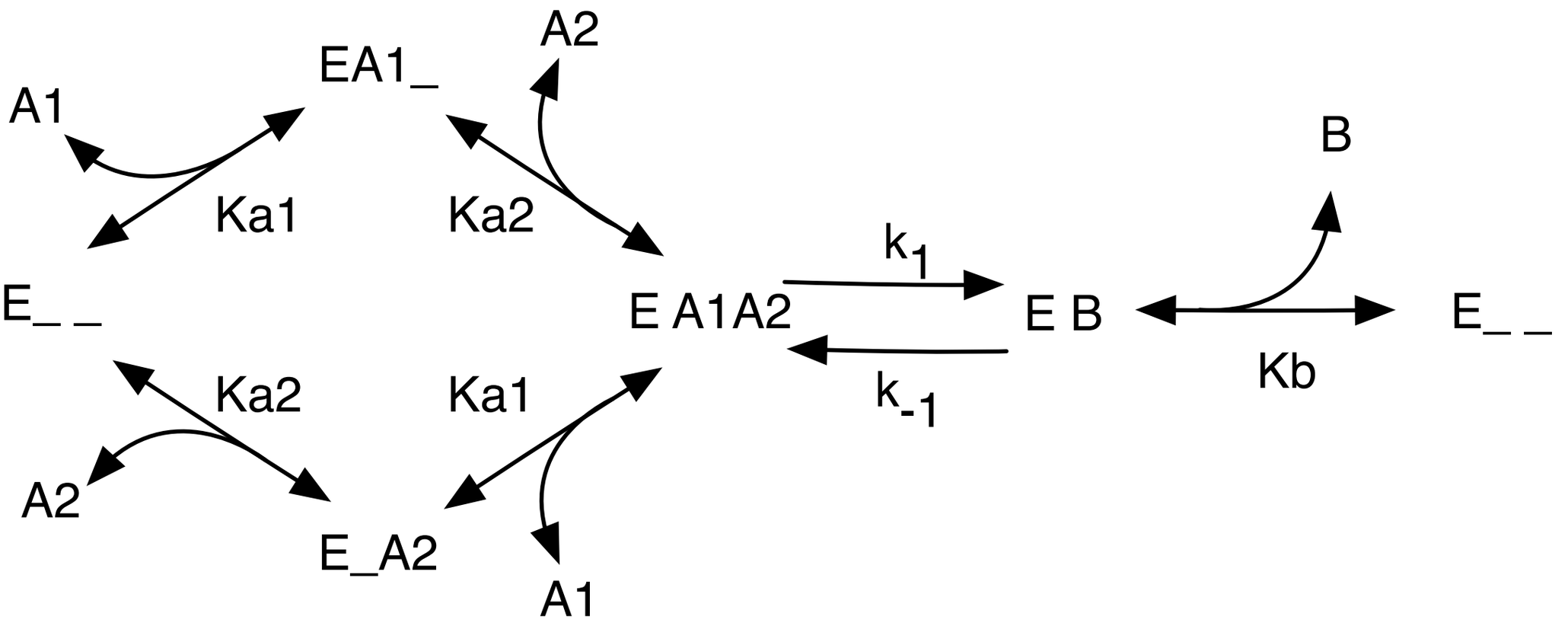
Exercise

Given an open system consisting of two enzymes that catalyze the conversion of substrate S (fixed at 10 mM) to product P (fixed at 1 mM), with common intermediate X.

The enzymes obey rev MM kinetics with identical parameter values:
Vm =1 mM/s, Keq = 10, Km substrate =1mM, Km product =10 mM

Calculate the steady state flux and the steady state concentration of the intermediate X.

Exercise

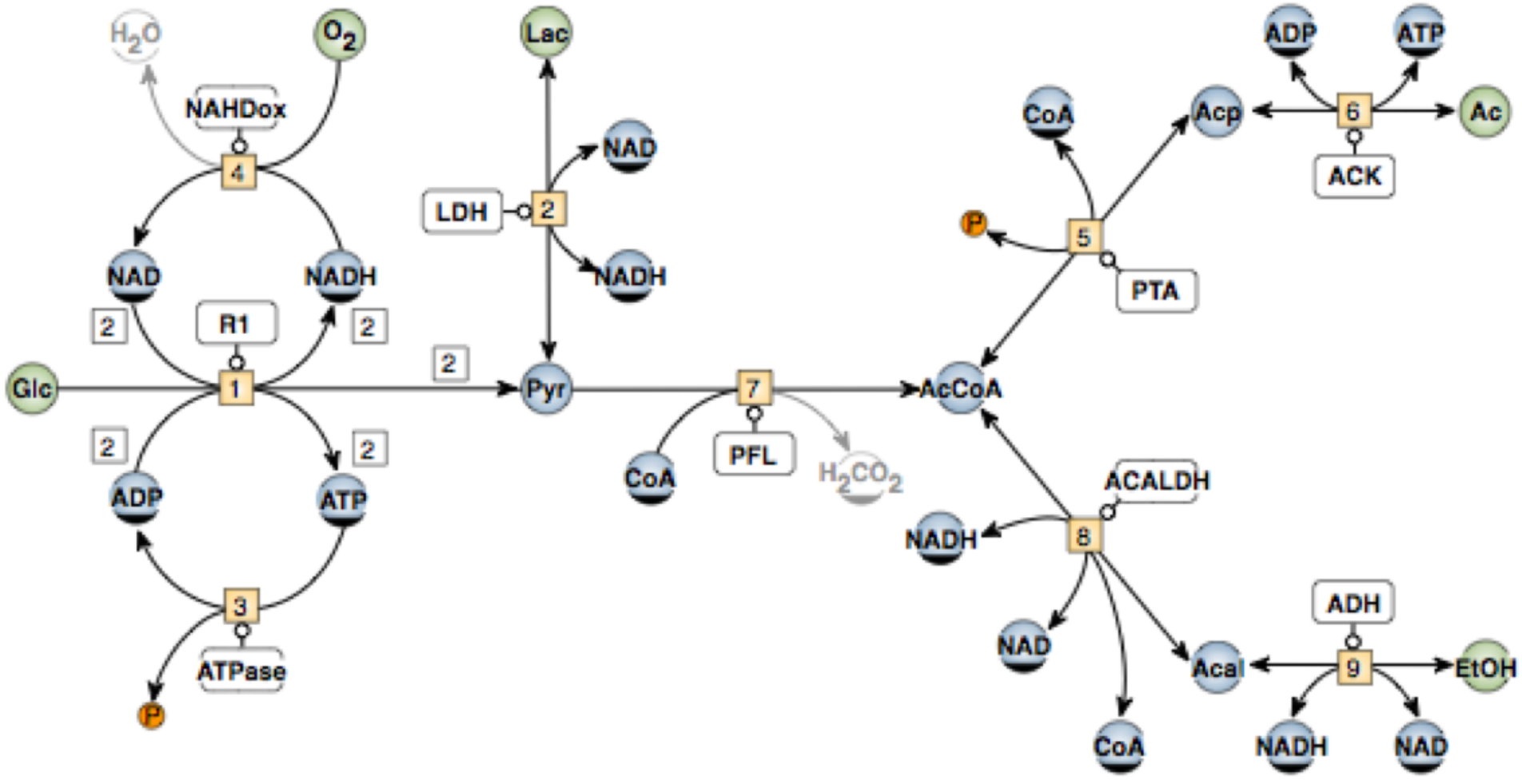


Derive a rate equation for the above, reversible, 2 substrate, 1 product, random order mechanism, using the rapid equilibrium binding assumption.

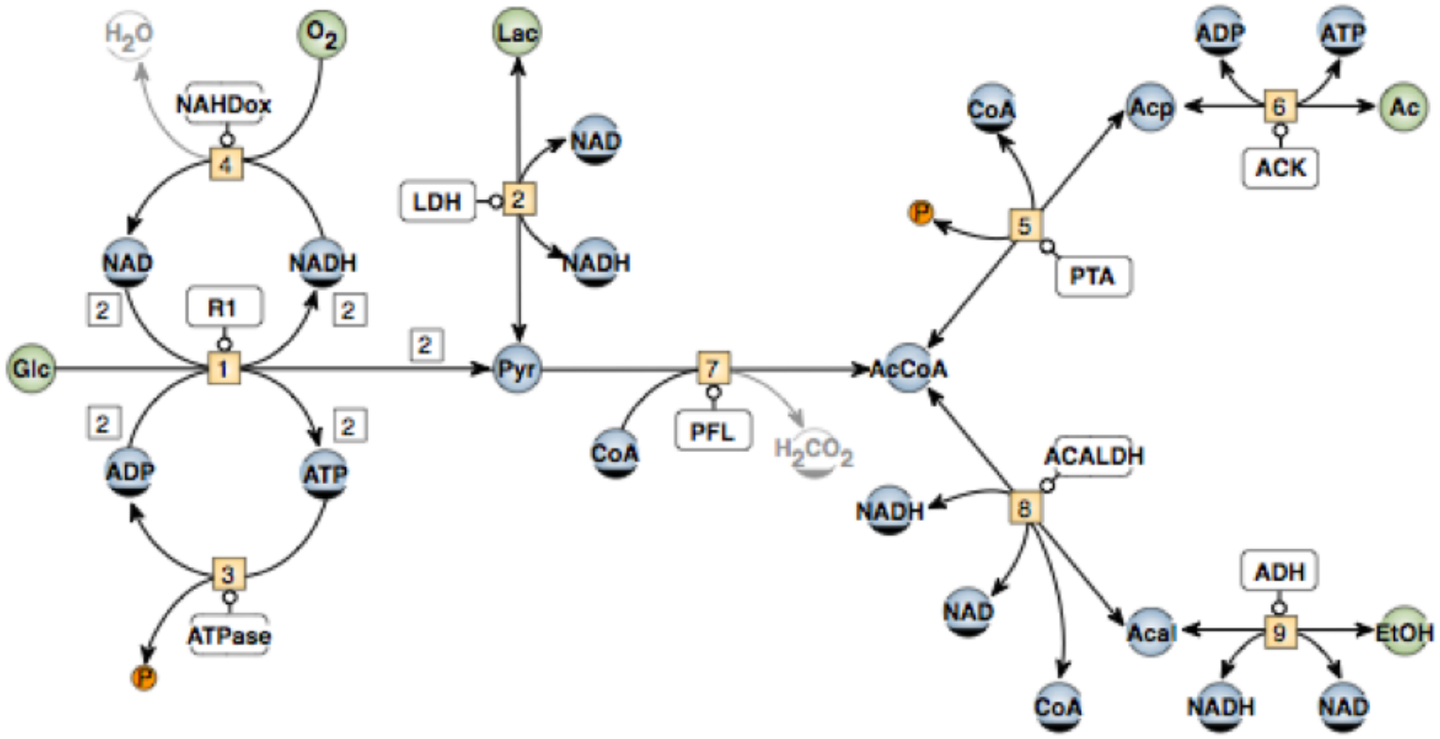
Network analysis

- For a given system, derive N, K, L, matrix, interpret
- Flux modes
- Solution space, FA
- Objective function, FBA

Reaction network



Reaction network: N matrix



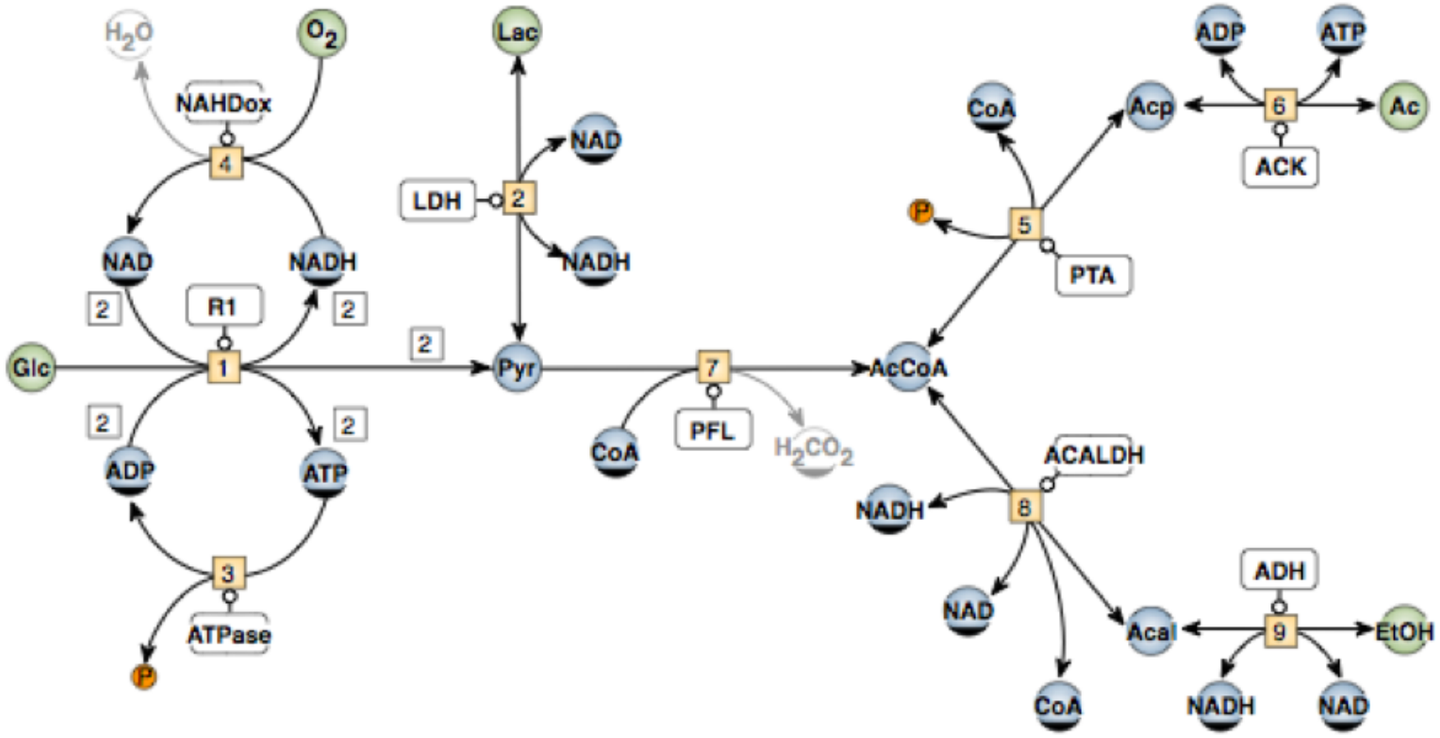
d(s)/dt **N** **v**

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

N stoichiometry matrix

v reaction rates

Reaction network: L matrix



s **L** **si** **T**

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

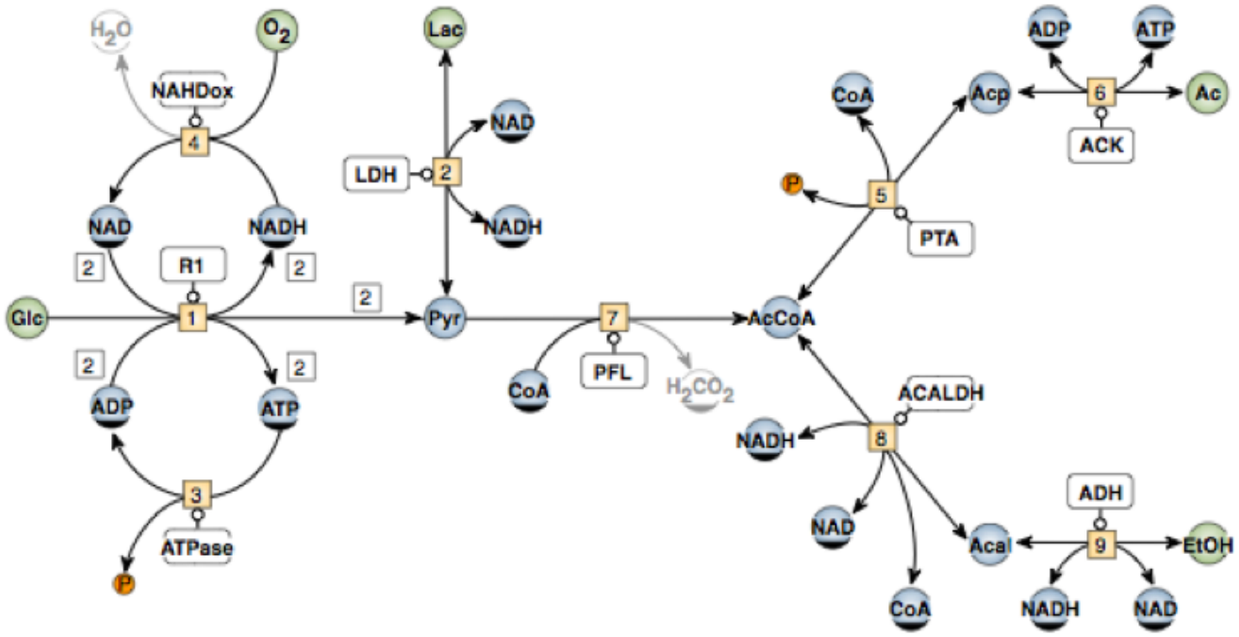
s vector of metabolite concentrations

L link matrix

si vector of independent metabolite concentrations

T vector of the sum of conserved moiety concentrations

Reaction network: st. st. constraint



$$\dot{Acal} = 0 = v8 - v9; v8 = v9$$

$$\dot{AcCoA} = 0 = -v5 + v7 - v8; v5 = v7 - v8 = v7 - v9$$

$$\dot{AcP} = 0 = v5 - v6; v6 = v5 = v7 - v9$$

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

$$\dot{ATP} = -\dot{ADP}$$

$$\dot{CoA} = -\dot{AcCoA}$$

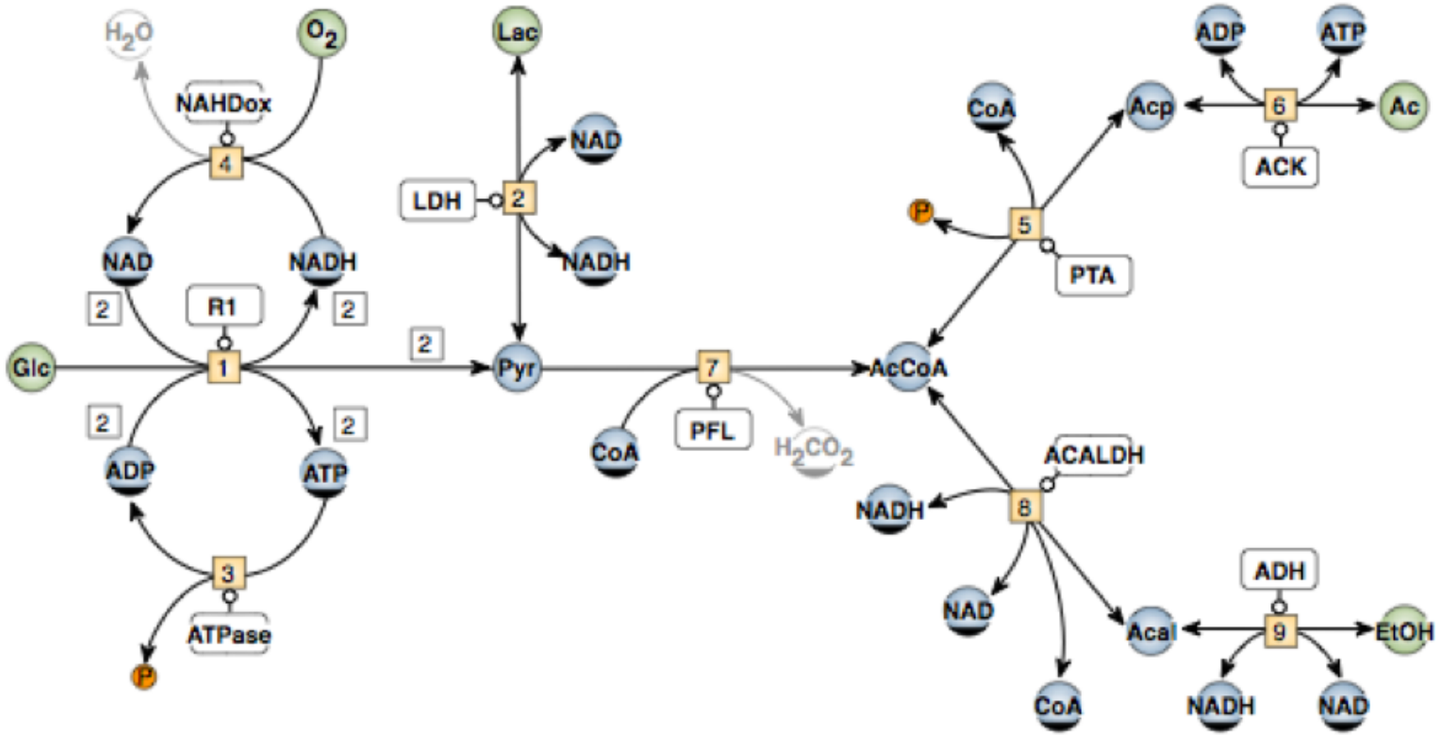
$$\dot{Pyr} = 0 = 2 \cdot v1 - v2 - v7; v2 = 2 \cdot v1 - v7 =$$

$$v3 - v7 + v9 - v7 = v3 - 2 \cdot v7 + v9$$

$$\dot{NAD} = 0 = -v1 + v2 + v4 + v8 + v9; v4 = 2 \cdot v1 - v2 - v8 - v9 =$$

$$v3 - v7 + v9 - v2 - 2 \cdot v9 = v3 - v7 - v9 - v2 = v3 - v7 - v9 - v3 + 2 \cdot v7 - v9 = v7 - 2 \cdot v9$$

Reaction network: K matrix



J K Ji

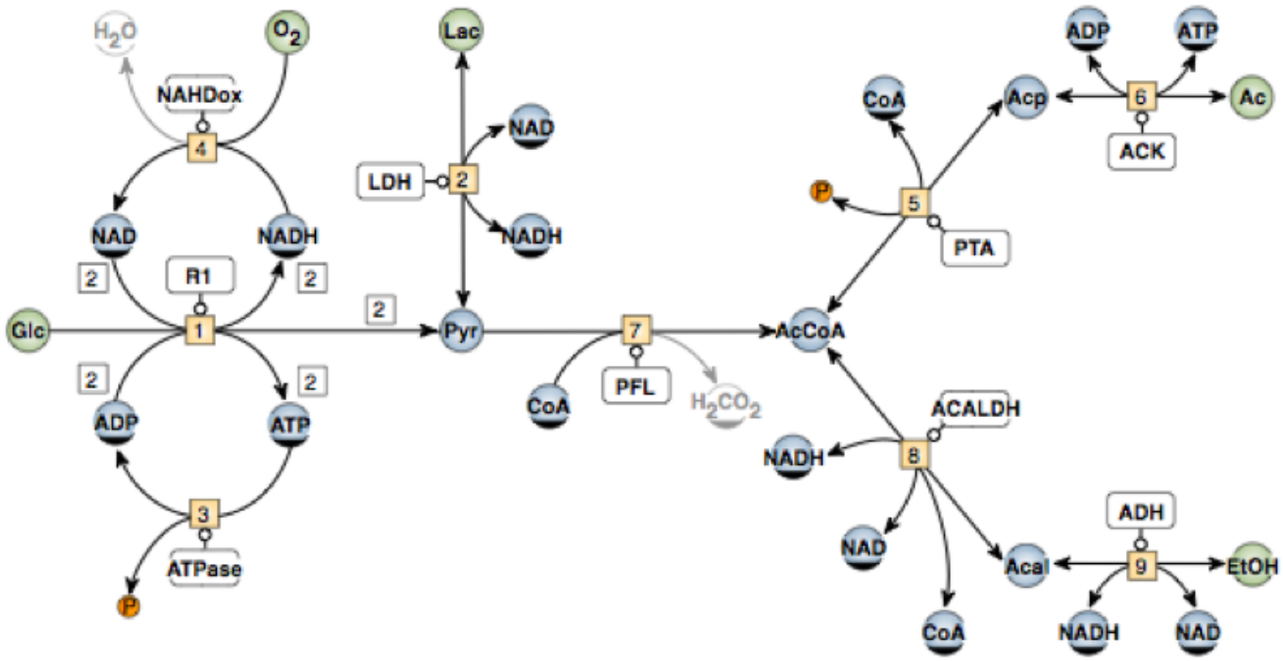
$$\begin{pmatrix} J3 \\ J7 \\ J9 \\ J1 \\ J2 \\ J4 \\ J5 \\ J6 \\ J8 \end{pmatrix} = \begin{pmatrix} 1. & 0. & 0. \\ 0. & 1. & 0. \\ 0. & 0. & 1. \\ 0.5 & -0.5 & 0.5 \\ 1. & -2. & 1. \\ 0. & 1. & -2. \\ 0. & 1. & -1. \\ 0. & 1. & -1. \\ 0. & 0. & 1. \end{pmatrix} * \begin{pmatrix} J3 \\ J7 \\ J9 \end{pmatrix}$$

J steady state reaction rate (flux) vector

K kernel, nullspace of stoichiometry matrix

Ji independent flux vector

Reaction network: K matrix; flux modes



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

J (J) steady state reaction rate (flux) vector

K (K) kernel, nullspace of stoichiometry matrix

Ji (Ji) independent flux vector

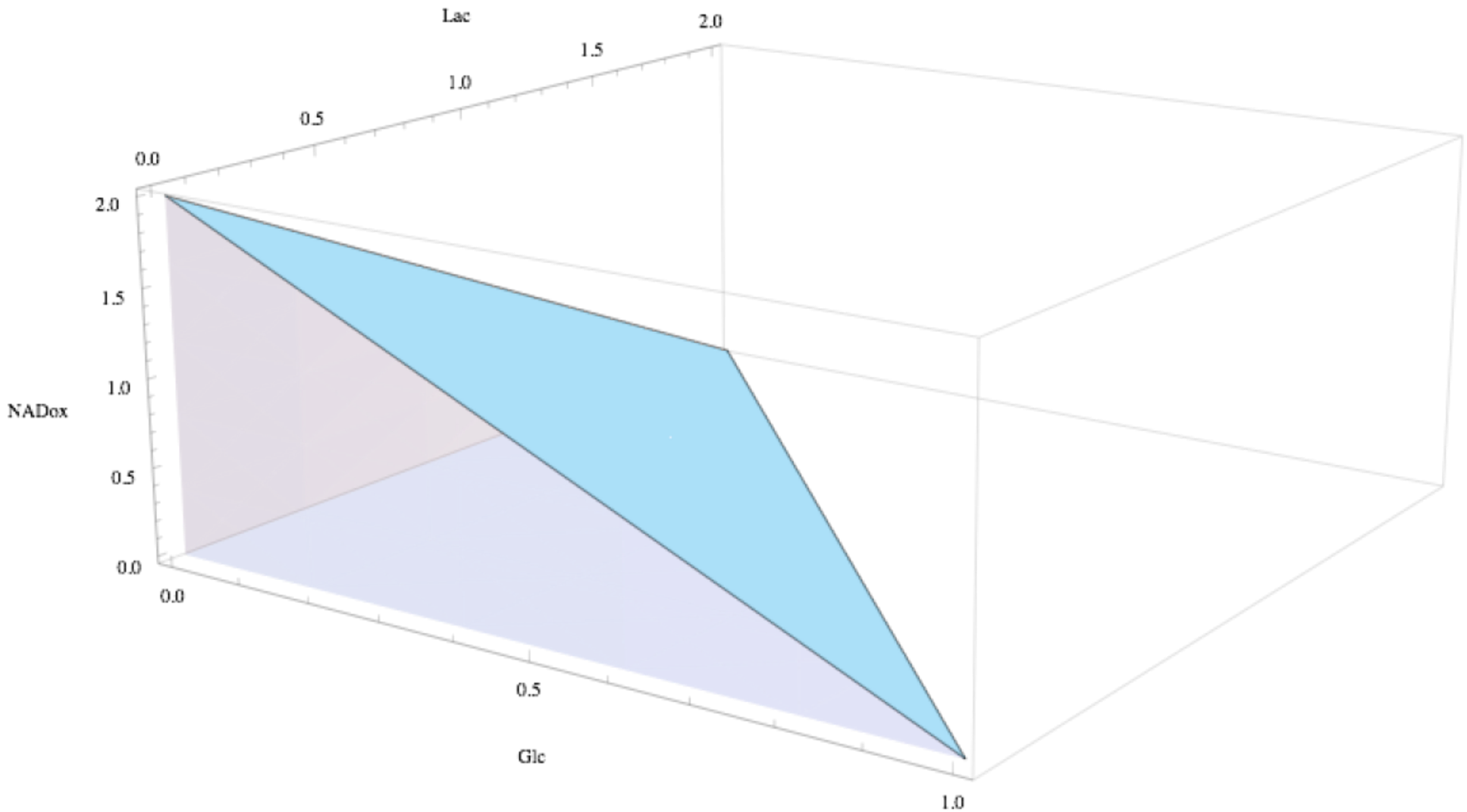
$$J3 + 0.5 \cdot J1 + J2 \text{ homolactic fermentation}$$

$$2 \cdot J3 + J1 + 2 \cdot J2 + J7 - 0.5 \cdot J1 - 2 \cdot J2 + J4 + J5 + J6$$

$$2 \cdot J3 + 0.5 \cdot J1 + J7 + J4 + J5 + J6 \text{ homo acetate formation}$$

$$J9 + 0.5 \cdot J1 + J2 - 2 \cdot J4 - J5 - J6 + J8 + 2 \cdot (\text{collumn } 2) + 3 \cdot (\text{collumn } 1)$$

$$3 \cdot J3 + J1 + 2 \cdot J7 + J5 + J6 + J8 + J9 \text{ mixed acid fermentation}$$


Lac
0.0
0.5
1.0
1.5
2.0
NADox
0.0
0.5
1.0
1.5
2.0
Glc
0.0
0.5
1.0