Practical on integrated models of bacterial growth

Hidde de Jong
MICROCOSME
INRIA and UGA
Hidde.de-Jong@inria.fr

November 23, 2022

MICROCOSME: bacterial systems biology

- **MICROCOSME**: systems biology group at INRIA/Université Grenoble Alpes in Grenoble

  Microbiologists, computer scientists, mathematicians, physicists, …

https://team.inria.fr/microcosme

- **Objective:** analysis, engineering, and control of the growth of bacteria
  - Specific research problems shaped by **biological questions**
  - Problems often addressed by combination of **models and experiments**

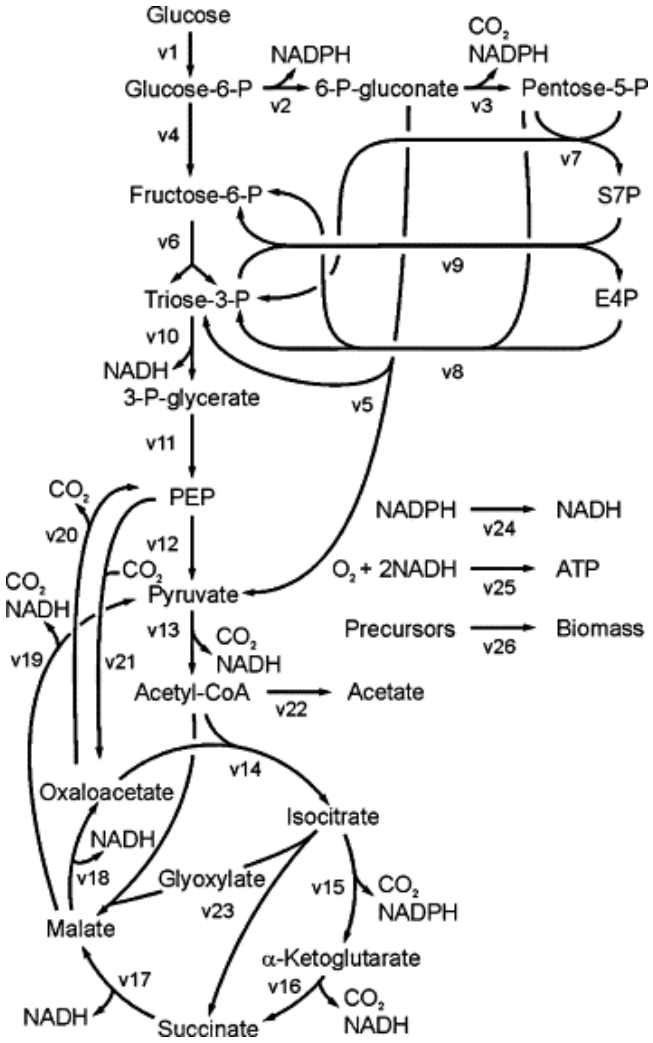
Overview

- Part 1. Systems biology and kinetic modeling
- Part 2. Metabolic network modeling
- Part 3. Gene regulatory network modeling
  - Quantitative modeling of gene regulatory networks
  - Qualitative modeling of gene regulatory networks
  - Stochastic modeling of gene regulatory networks
  - **Practical on integrated models of bacterial growth (Matlab)**
- Part 4. Models and data

Bacterial growth and metabolism

- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis

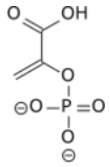
  Glucose, acetate, lactose, …

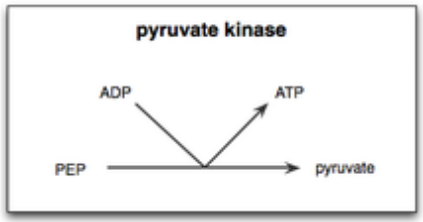


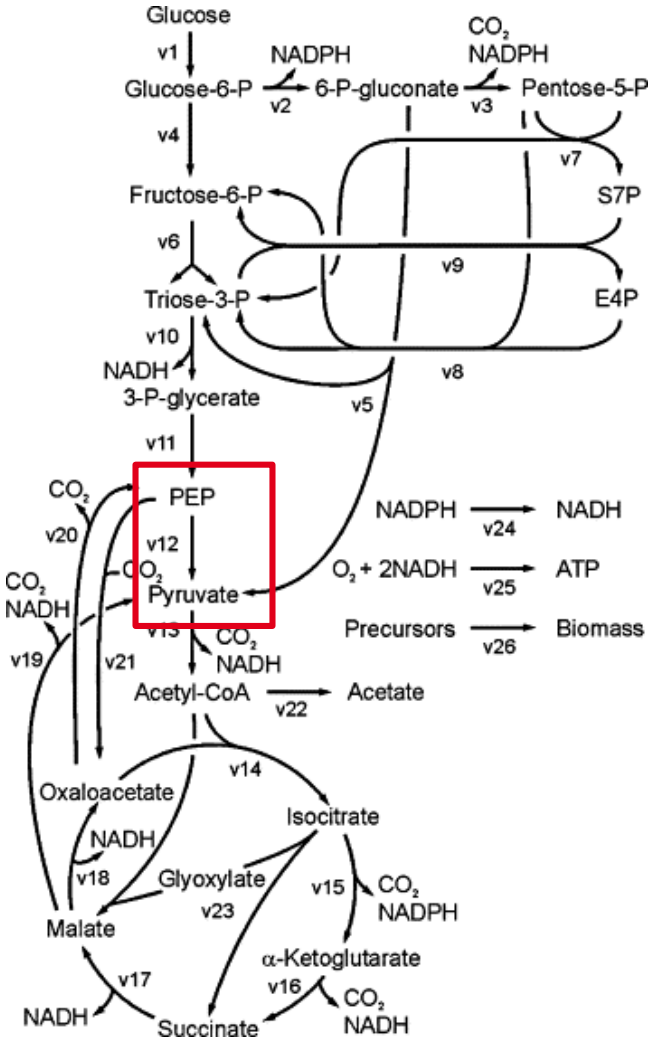
Fischer *et al.* (2004), *Anal. Biochem.*, 325(2):308–16

Bacterial growth and metabolism

- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis
  - Glucose, acetate, lactose, …
- **Enzymes** catalyse individual steps in metabolic network
  - Pyruvate kinase transforms phosphoenolpyruvate (PEP) into pyruvate





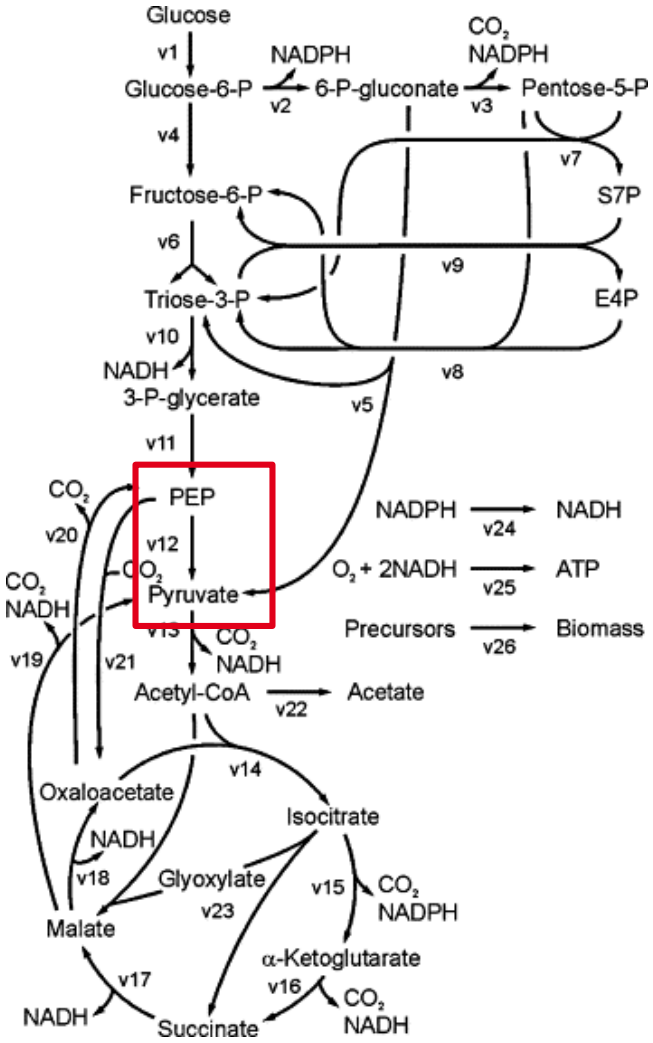


Bacterial growth and metabolism

- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis

  Glucose, acetate, lactose, …

- Enzymes produced from information encoded in **genes**
  - *pykF* is gene encoding pyruvate kinase

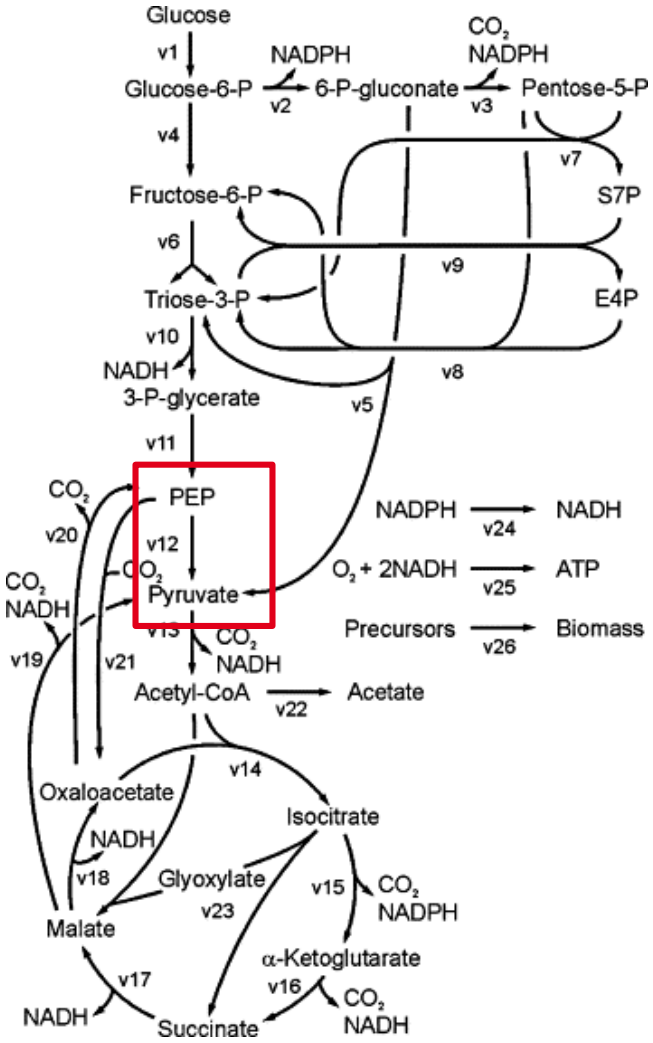


Bacterial growth and metabolism

- Central **carbon metabolism** breaks down carbon sources for energy production and macromolecular synthesis

  Glucose, acetate, lactose, …

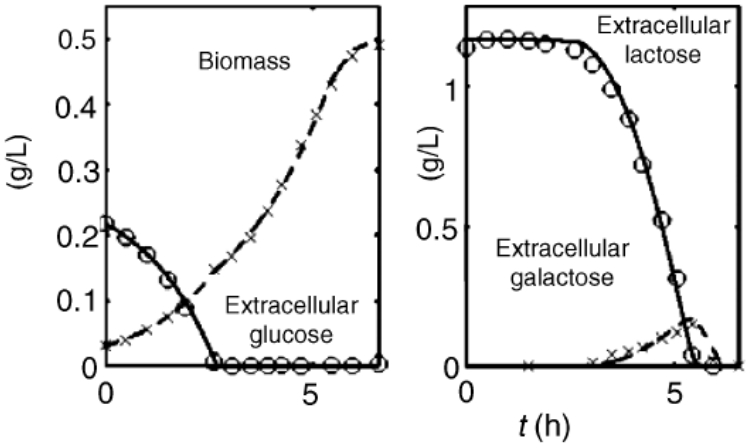
- Enzymes produced from information encoded in **genes**
  - *pykF* is gene encoding pyruvate kinase
  - Expression of *pykF* regulated by transcription factor Cra



Carbon catabolite repression (CCR)

- Many bacteria have evolved strategy consisting of **sequential utilization** of carbon sources
  - In order of decreasing growth rate supported by carbon source
- In presence of preferred substrate, **repression of enzymes** necessary for utilization of less favourable carbon sources
- **Carbon catabolite repression (CCR)**

  Best-known manifestation of CCR: **diauxic growth**

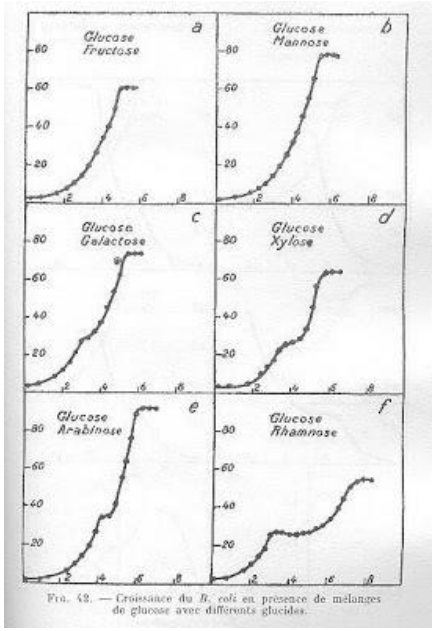


Bettenbrock *et al.* (2006), *J. Biol. Chem.*, 281(5):2578-84

Carbon catabolite repression (CCR)

- Many bacteria have evolved strategy consisting of **sequential utilization** of carbon sources
  - In order of decreasing growth rate supported by carbon source
- In presence of preferred substrate, **repression of enzymes** necessary for utilization of less favourable carbon sources
- **Carbon catabolite repression (CCR)**

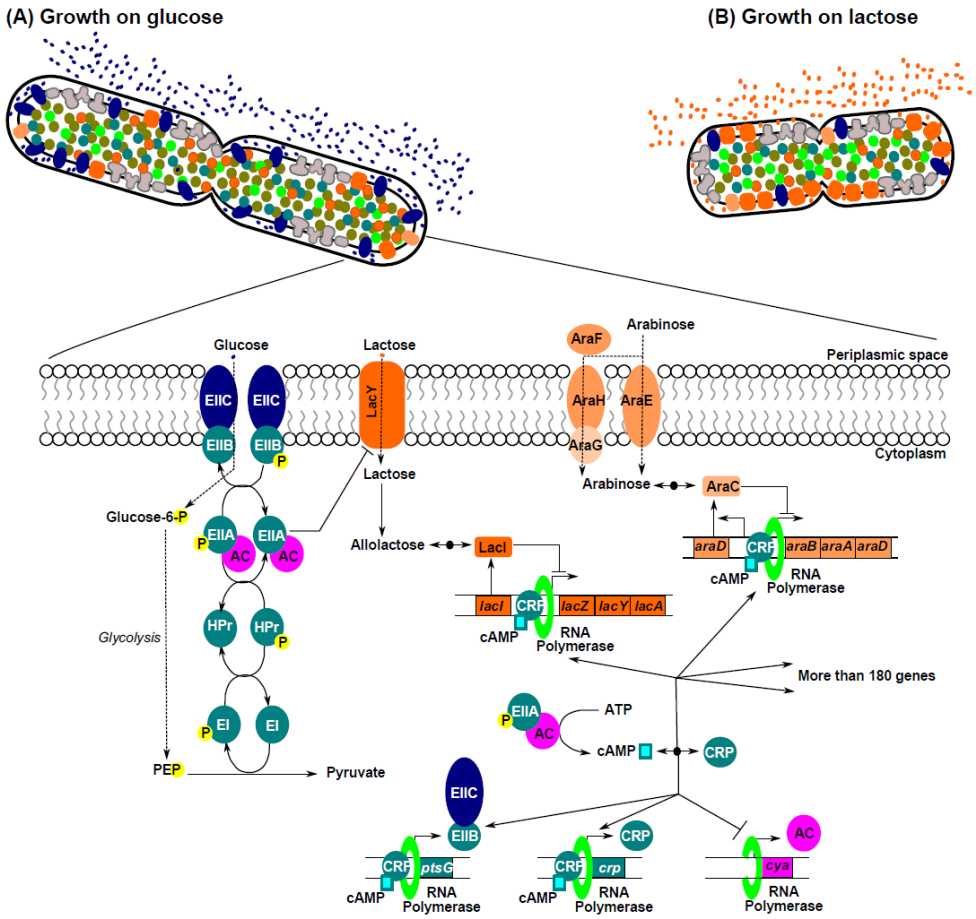
  Best-known manifestation of CCR: **diauxic growth**



Monod, *Recherches sur la croissance des cultures bactériennes*, Hermann, Paris, 1958

Regulation of carbon catabolite repression

- Molecular mechanisms involved in CCR
  - Enzyme induction
  - Inducer exclusion
  - Global regulation by metabolism (cAMP)
- Global changes in cellular physiology
  - Macromolecular composition
  - Activity of transcriptional and translational machinery
  - Stability of mRNA/protein



Kremling *et al.* (2015), *Trends Microbiol.*, 23(2):99-109

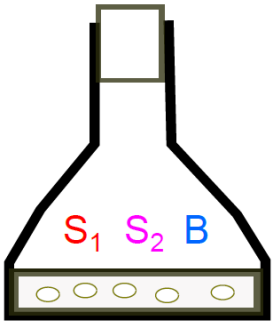
Carbon catabolite repression as case study

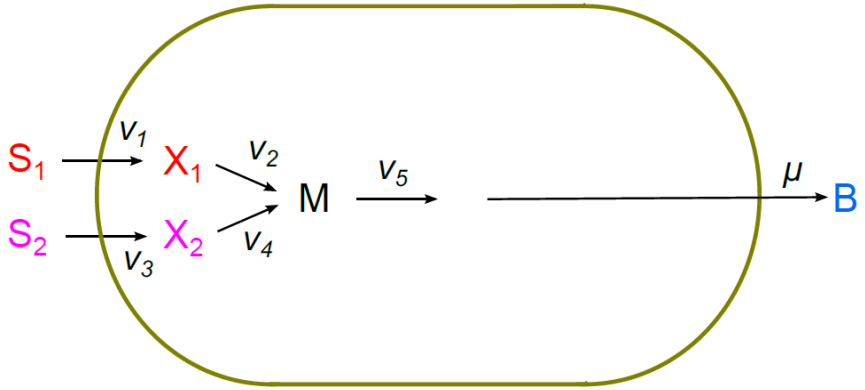
- CCR is **complex system** cutting across metabolism, signalling, gene expression

  Görke and Stülke (2008), *Nat. Rev. Microbiol.*, 6:613-24

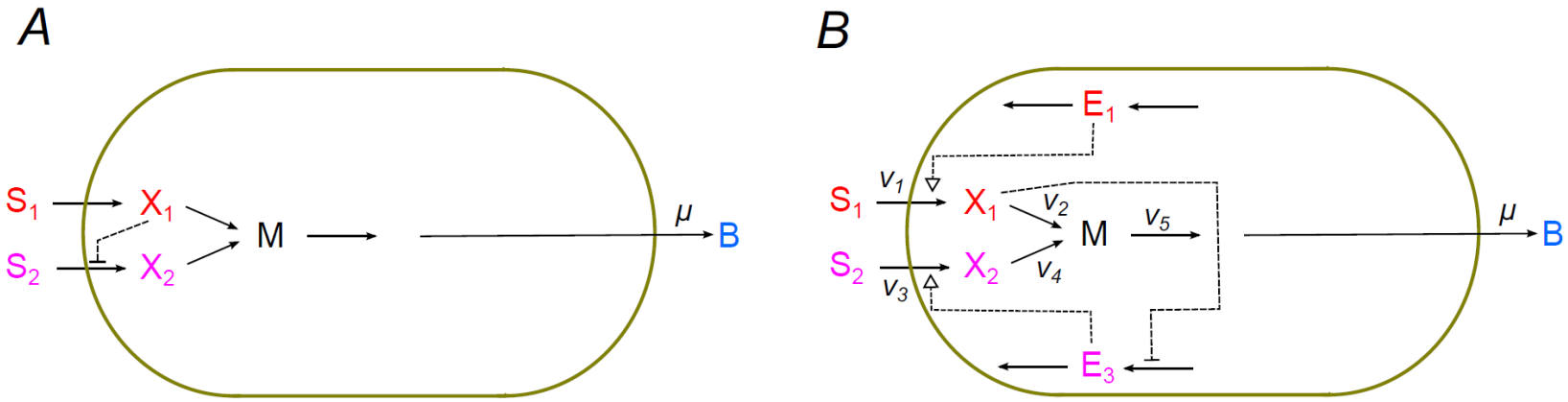
- Case-study in systems biology!

  Kremling *et al.* (2015), *Trends Microbiol.*, 23(2):99-109

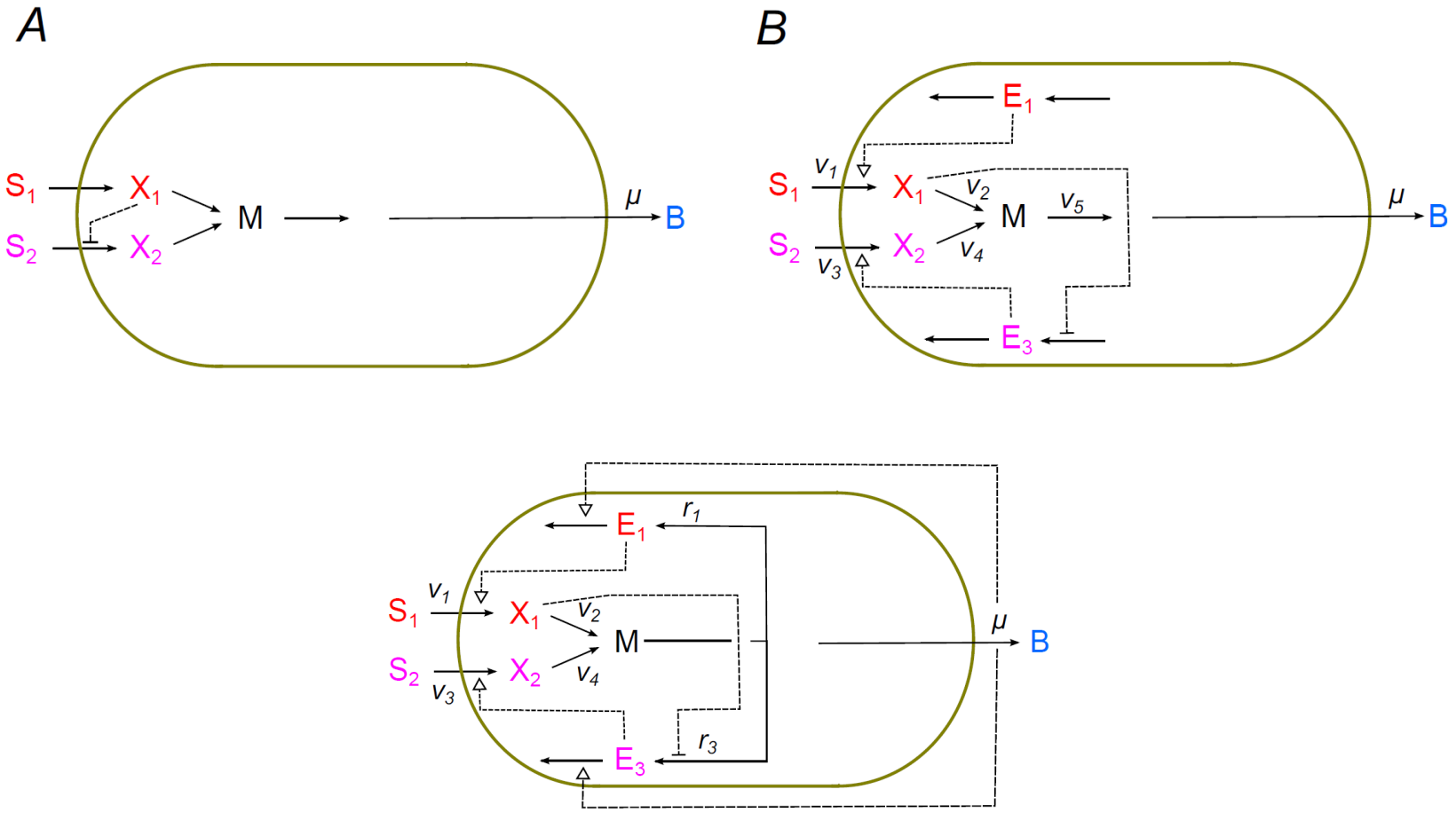




Carbon catabolite repression as case study



Carbon catabolite repression as case study



Basic equations for model

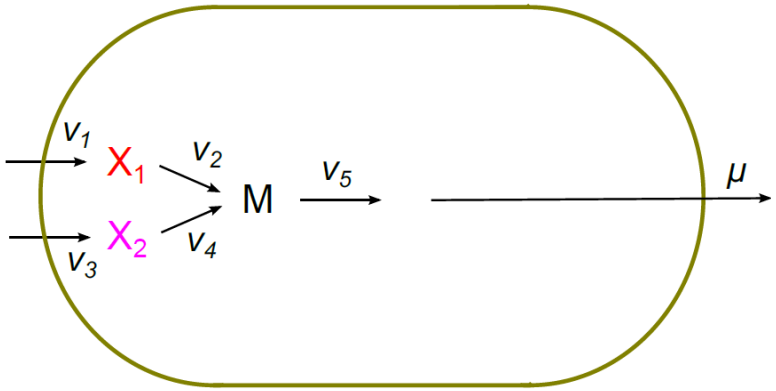
- Intracellular model

$$\dot{x} = N \cdot v, \qquad x(0) = x_0.$$

$$N = \begin{bmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 4 & 0 & 1 & -10 \end{bmatrix}$$

$x = [X_1, X_2, M]'$ [mmol gDW^{-1}]

$v = [v_1, v_2, v_3, v_4, v_5]'$ [mmol gDW^{-1} h^{-1}]

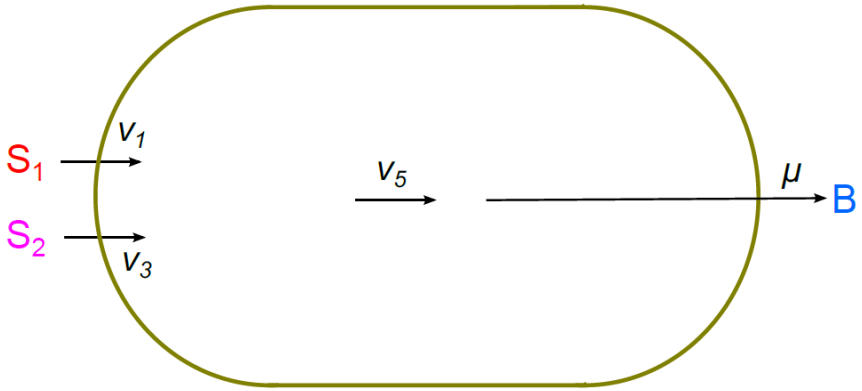


Basic equations for model

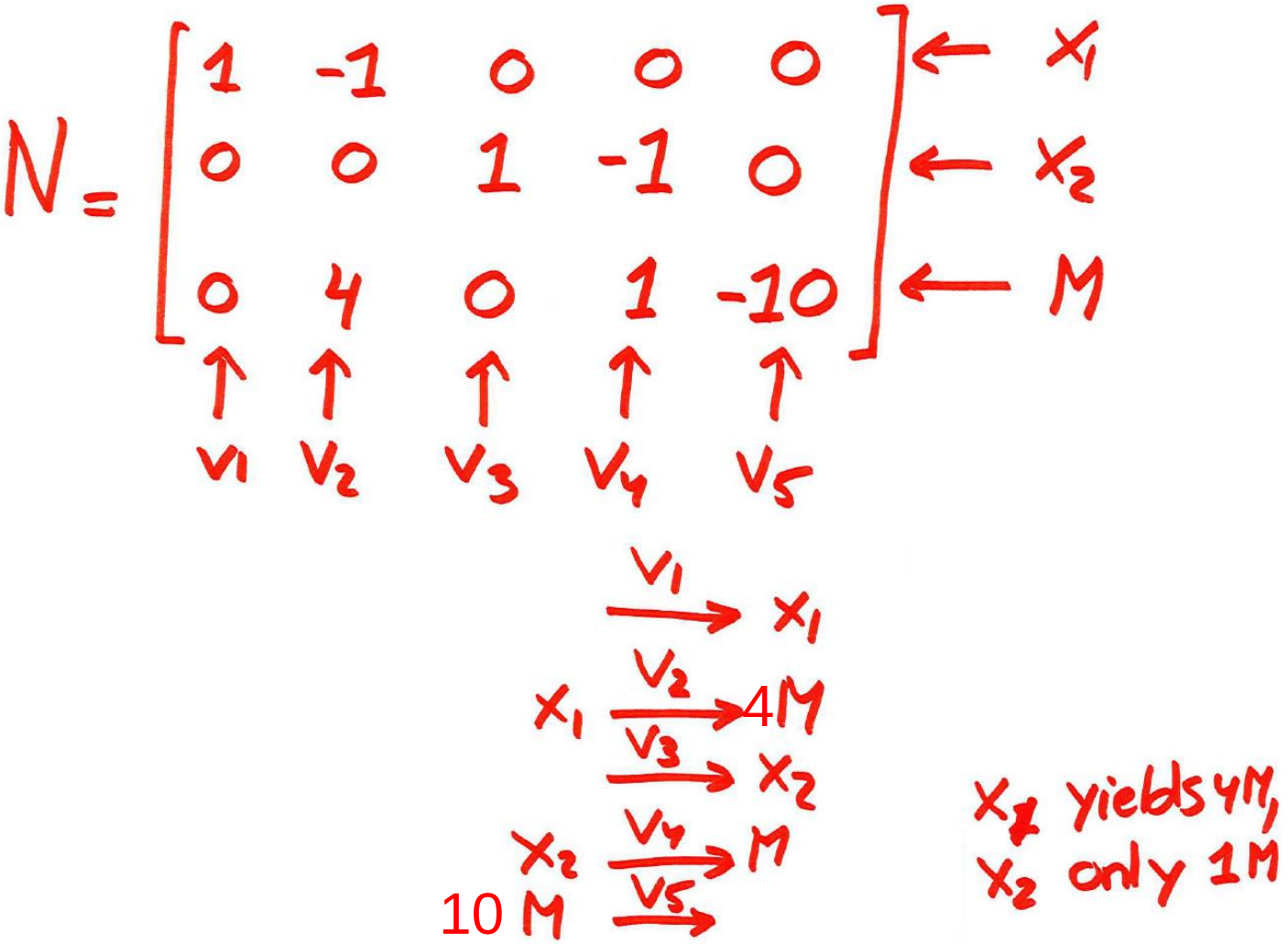
- Extracellular model

$$
\begin{aligned}
\dot{S}_1 &= -v_1 \cdot B, & S_1(0) &= S_{1,0}, \\
\dot{S}_2 &= -v_3 \cdot B, & S_2(0) &= S_{2,0}, \\
\dot{B} &= \beta \cdot v_5 \cdot B, & B(0) &= B_0,
\end{aligned}
$$

S_1 and S_2 [mmol L^{-1}]
B [gDW L^{-1}]



Exercise 1


N =
1 -1 0 0 0
0 0 1 -1 0
0 4 0 1 -10
v1 v2 v3 v4 v5
X1
X2
M
v1
x1
v2
4M
x1
v3
x2
v4
M
x2
v5
10 M
X1 yields 4M,
X2 only 1M

Exercise 2

$$\alpha \cdot V_{pop} = B \cdot V_{med}$$

V_{pop} : L

B : $gDW.L^{-1}$

V_{med} : L

$gDW.L^{-1}$: biomass density

$B \cdot V_{med}$: total amount of biomass

Exercise 3

$$\alpha \cdot V_{pop} = \dot{B} \cdot V_{med}$$

$$\alpha \cdot \dot{V}_{pop} = \dot{B} \cdot V_{med}$$

$$\mu = \frac{\dot{V}_{pop}}{V_{pop}} = \frac{\dot{B} \cdot (V_{med} / \alpha)}{B \cdot (V_{med} / \alpha)} = \frac{\dot{B}}{B} = \beta \cdot V_S$$

$$\Rightarrow \dot{B} = \mu \cdot B$$

Flux balance analysis (FBA)

- Steady state of metabolic network

$$N\,v = 0$$

  Steady-state reaction rates are called **fluxes**

- **Constraints** on fluxes: upper and lower bounds

$$v^l \leq v \leq v^u$$

  - Bounds on fluxes derived from available information in literature, bounds may be infinite
  - For mathematical convenience, all fluxes must be positive $v \geq 0$
  - Reversible reaction modeled as pair of irreversible, positive fluxes

Flux balance analysis (FBA)

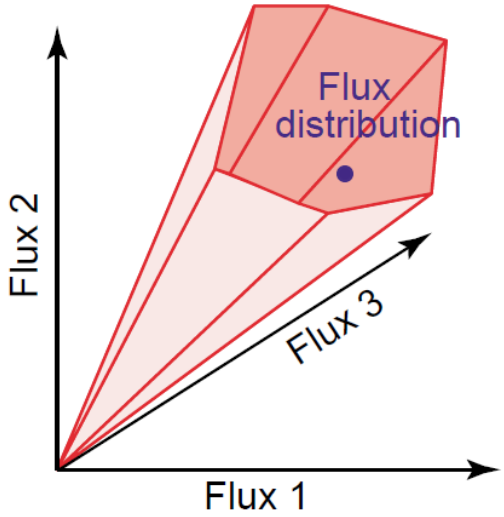
- Steady state of metabolic network

$$N\,v = 0$$

- Stoichiometry matrix and constraints define convex space of possible solutions: **flux cone**
- FBA aims at finding solutions(s) maximising or minimising linear combination of fluxes: **objective function**

$$Z = c^T\,v \qquad\qquad c \in \mathbb{R}^n$$

- Typical objective functions: biomass production, ATP production, …



Stelling (2004), *Curr. Opin. Microbiol.*, 7:513-8

Dynamic flux balance analysis

- Dynamics of metabolic network through interactions with environment

$$\dot{s} = -v_{ext}(t) \cdot B, \quad s(0) = s_0$$

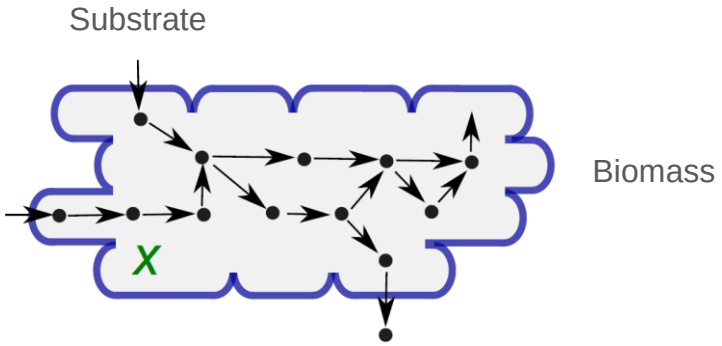
$$\dot{B} = \mu(t) \cdot B, \quad B(0) = B_0$$

B : biomass concentration in medium

s : substrate concentration in medium

μ : growth rate

v_{ext} : substrate uptake rate



- Dynamics predicted by means of **dynamic FBA**
  - Metabolic network at quasi-steady state with respect to environment
  - Computation of exchange rates and growth rate $_x$ by means of FBA at each time-point *t*
  - Change in substrate concentrations puts bounds on uptake rates

Mahadevan *et al.* (2002), *Biophys. J.*, 83(3):1331-40

Exercise 9

$$X = x \cdot B \qquad [\text{mmol } \cancel{gDW^{-1}}\ \cancel{gDW}\ L_{med}^{-1}]$$

$$V = v \cdot B \qquad [\text{mmol } \cancel{gDW^{-1}}\ h^{-1}\ \cancel{gDW}\ L_{med}^{-1}]$$

$$\dot{X} = N \cdot V \Rightarrow \dot{x}B + x\dot{B} = N \cdot v \cdot B$$

$$\dot{x} = Nv - \frac{\dot{B}}{B}x$$

$$= Nv - \mu x$$

Exercise 10

$$B = \underbrace{(\gamma_1 X_1 + \gamma_2 X_2 + \gamma_3 M + \gamma_4 E_1 + \gamma_5 E_3)}_{g \cdot gDW^{-1}} \alpha \cdot \frac{V_{pop}}{V_{med}}$$

$gDW\,L^{-1}_{med}$

$$g \cdot gDW^{-1}\, gDW\, L^{-1}_{pop}$$

$$g \cdot L^{-1}_{pop} \cdot L_{pop} \cdot L_{med}$$

Exercise 11

$$\mu = \frac{\dot{B}}{B} = \frac{(\gamma_1 \dot{X}_1 + \ldots + \gamma_5 \dot{E}_3)\alpha \frac{V_{pop}}{V_{med}} + (\gamma_1 X_1 + \ldots)\alpha \frac{\dot{V}_{pop}}{V_{med}}}{(\gamma_1 X_1 + \ldots + \gamma_5 E_3)\alpha \frac{V_{pop}}{V_{med}}}$$

$$= \frac{(\gamma_1 \dot{X}_1 + \ldots + \gamma_5 \dot{E}_3)}{(\gamma_1 X_1 + \ldots + \gamma_5 E_3)} + \frac{\dot{V}_{pop}}{V_{pop}}$$

$$\frac{\dot{V}_{pop}}{V_{pop}} = \mu$$

Exercise 11

$$\gamma_1 X_1 + \dots + \gamma_5 E_3 = 4 X_1 + \dots + 5 E_3$$

$$\gamma_1 \dot{X}_1 = 4 (V_1 - V_2 - \mu x_1)$$

$$\gamma_2 \dot{X}_2 = 1 (V_3 - V_4 - \mu x_2)$$

$$\gamma_3 \dot{M} = 1 \cdot (4 V_2 + V_4 - 5 r_1 - 5 r_3 - \mu M)$$

$$\gamma_4 \dot{E}_1 = 5 \cdot (r_1 - \mu E_1)$$

$$\gamma_5 \dot{E}_3 = 5 (r_3 - \mu E_3)$$

Exercise 11

$$\mu = \frac{(\gamma_1 \dot{X}_1 + \ldots + \gamma_5 \dot{E}_3)}{(\gamma_1 X_1 + \ldots + \gamma_5 E_3)} + \mu$$

$$= \frac{4V_1 - 4V_2 + V_3 - V_3 + 4V_2 + V_4 - 5r_1 - 5r_3 + 5r_1 + 5r_3}{4X_1 + \ldots + 5E_3}$$

$$- \frac{\mu(4X_1 + \ldots + 5E_3)}{4X_1 + \ldots + 5E_3} + \mu$$

$$= \frac{4V_1 + V_3}{4X_1 + \ldots + 5E_3}$$

Merci !

team.inria.fr/ibis