Flux Balance Analysis (FBA)

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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

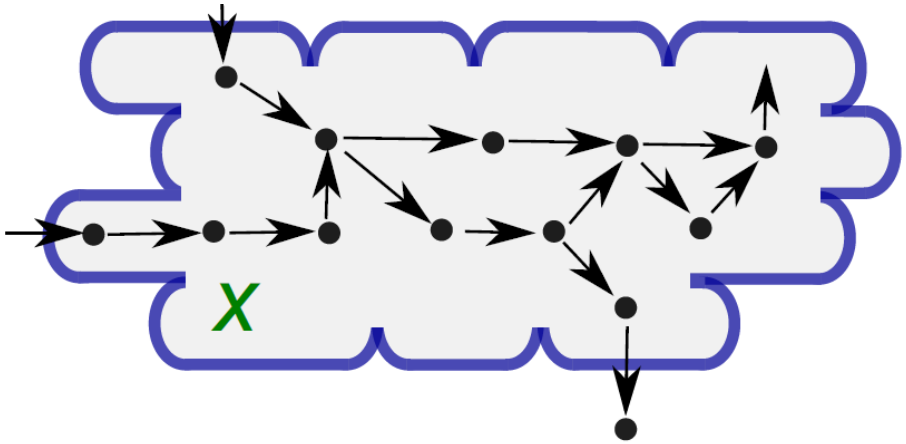
- Part 1. Systems biology and kinetic modeling
  - Introduction
  - Kinetic modeling of cellular reaction networks
- Part 2. Metabolic network modeling
  - Kinetic modeling of metabolism
  - Metabolic control analysis (MCA)
  - **Flux balance analysis (FBA)**
  - Practical on flux balance analysis (COBRA)
- Part 3. Gene regulatory network modeling

Biochemical reaction networks

- ODE model for growth of microbial populations:

$$\dot{x} = N \cdot v(x) - \mu \cdot x,$$

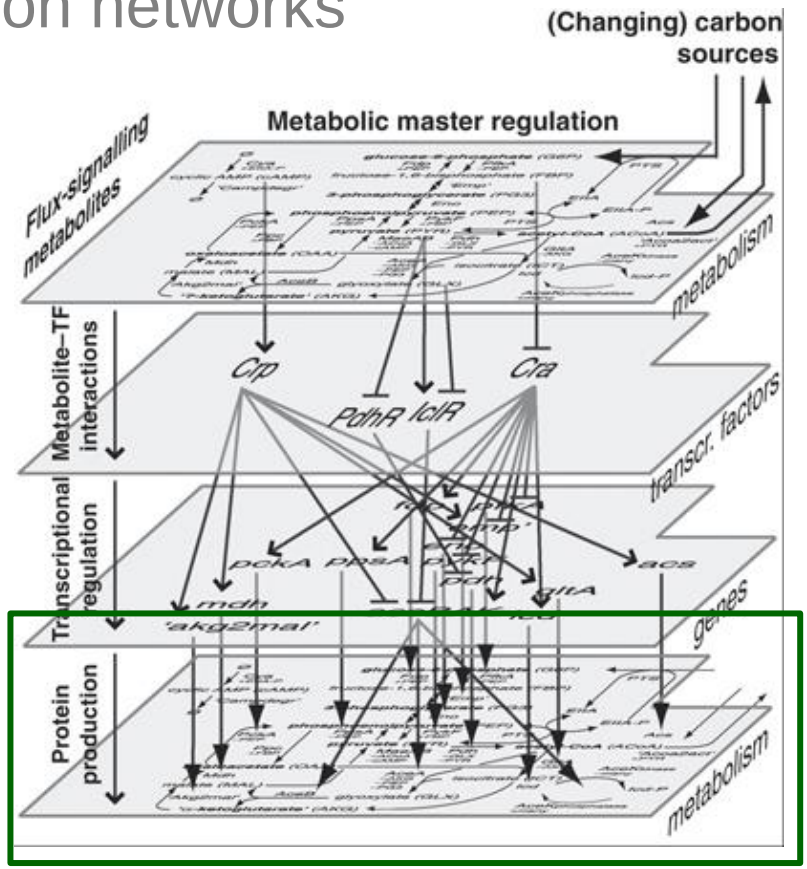
$$\mu = \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x).$$



- Reaction rates depend on concentrations x of substrates, products, effectors

Metabolic networks

- Focus on **subsystems** that can be studied in isolation due to **modular structure** of reaction networks
  - Time-scale hierarchies
  - Connectivity structure
- **Metabolic networks**
  - Metabolites and enzymatic reactions
  - Short turn-over times of metabolite pools in comparison with enzyme pools



Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6: 355

Metabolic networks

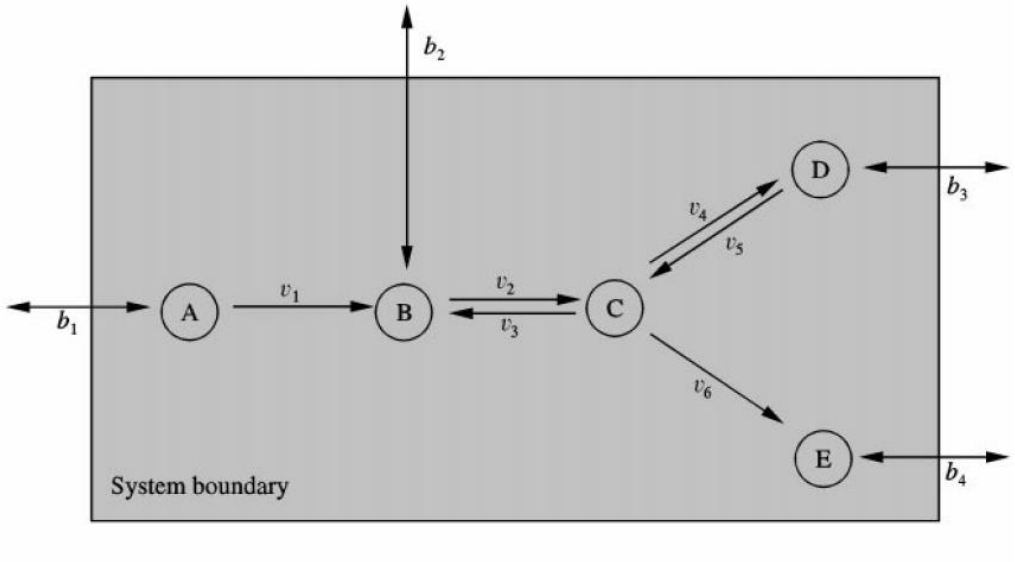
- Models describing dynamics of metabolism
  - Effect of growth dilution can often be ignored
  - Variables are metabolites and rates of enzyme-catalyzed reactions
  - Enzyme concentrations constant on time-scale of metabolic dynamics

$$\dot{x} = N\, v(x)$$

Stoichiometry matrix

- Stoichiometry matrix N describes structure of reaction network

  Internal reactions and exchange reactions, reversible and irreversible



$$N \qquad v$$

$$\begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix}$$

Schilling *et al.* (2000), *J. Theor. Biol.*, 203(3):229-48

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

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

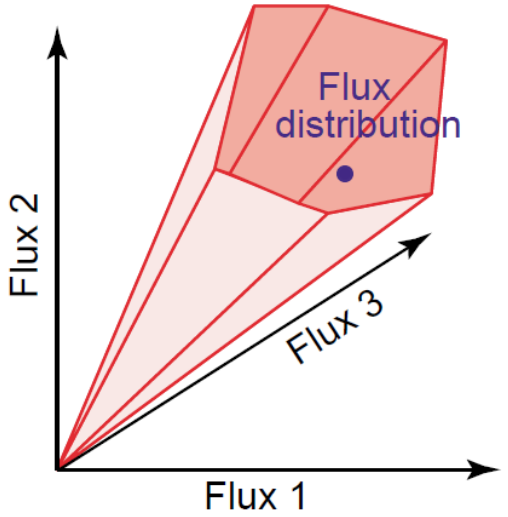
  - Bounds on fluxes derived from available information in literature, bounds may be infinite
  - For mathematical convenience, all fluxes must be positive $v \geq 0$
  - Reversible reaction modeled as pair of irreversible, positive fluxes

Flux balance analysis (FBA)

- Steady state of metabolic network

$$N\,v = 0$$

- Stoichiometry matrix and constraints define convex space of possible solutions: **flux cone**
  - System of steady-state equations underdetermined: more reactions than concentrations variables.
  - Flux cone represents **metabolic capabilities** of network (possible flux distributions)



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

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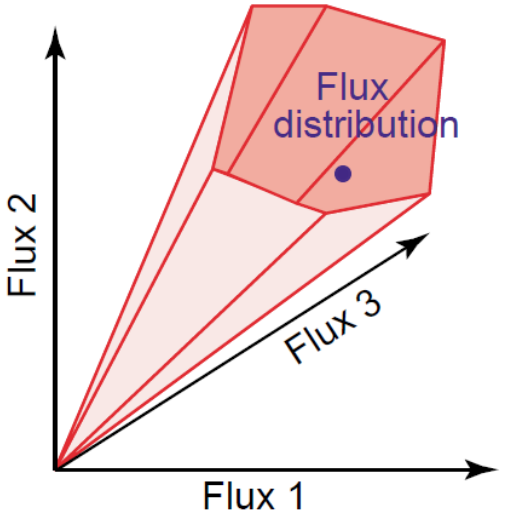
  - System of steady-state equations underdetermined: more reactions than concentrations variables.
  - Every solution can be written as linear combination of rays of flux cone (**extreme pathways**)

$$C = \{v \mid v = \sum_{i=1}^{k} w_i\, p^i,\ w_i \geq 0,\ i = 1, \ldots, k\}$$

p^i : extreme pathway *i*

w_i : weigth of *i*th pathway



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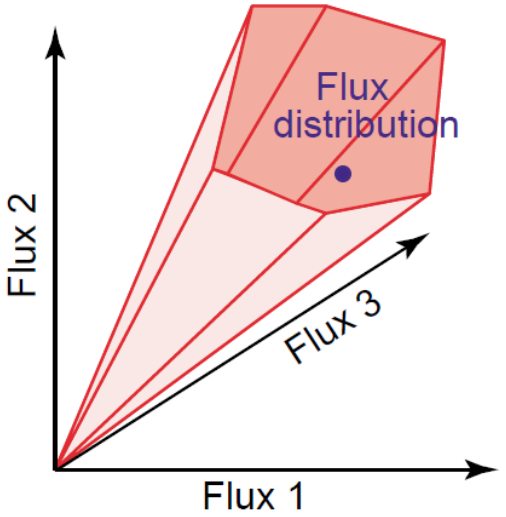
$$C = \{v \mid v = \sum_{i=1}^{k} w_i\, p^i,\ w_i \geq 0,\ i = 1, \ldots, k\}$$

  - Set of extreme pathways unique, but solutions not uniquely defined by extreme pathways



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

Flux balance analysis (FBA)

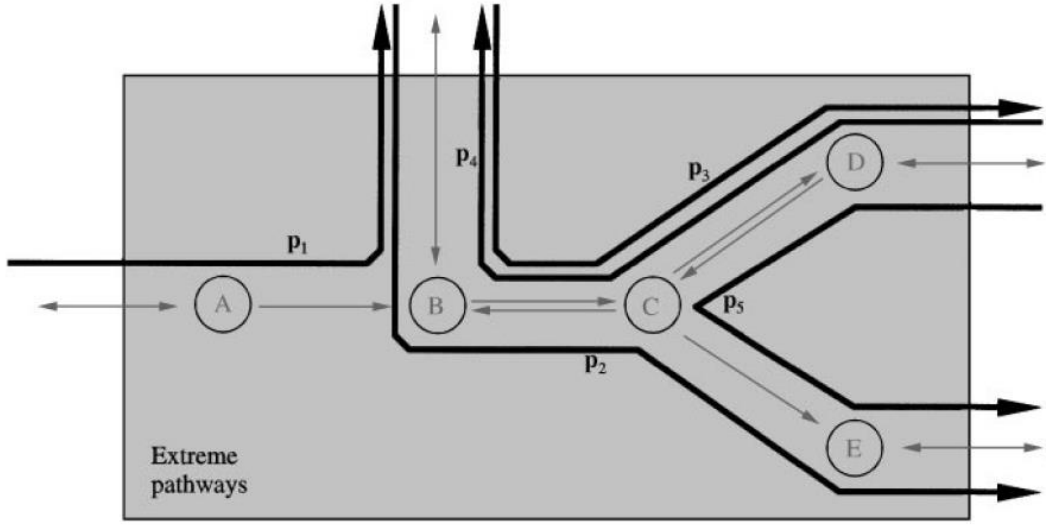
- Extreme pathways in example network

  Schilling *et al.* (2000), *J. Theor. Biol.*, 203(3):229-48

- Extreme pathways provide pathway-based view of network
- Related concept of elementary modes

  Schilling *et al.* (1999), *Biotechnol. Prog.*, 15(3):296-303

(a)



(b)

$$\mathbf{P} = \begin{array}{c} \begin{matrix} \mathbf{p}_1 & \mathbf{p}_2 & \mathbf{p}_3 & \mathbf{p}_4 & \mathbf{p}_5 & \mathbf{p}_6 & \mathbf{p}_7 \end{matrix} \\ \left[\begin{array}{ccccccc} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 1 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ \hdashline -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 \end{array}\right] \begin{array}{l} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ \hdashline b_1 \\ b_2 \\ b_3 \\ b_4 \end{array} \end{array}$$

(c)

Flux distribution:

$$\mathbf{v}^T = [4\ 2\ 0\ 1\ 0\ 1 - 4\ 2\ 1\ \ 1]$$

Subset pathways of **v**:

$$\{\mathbf{p}_1, \mathbf{p}_2, \mathbf{p}_3\}$$

Null space dimensions of $\mathbf{S}_{mod}$:

$$d(\mathbf{S}_{mod}) = n - r(\mathbf{S}_{mod}) = 8 - 5 = 3$$

Unique decomposition of **v**:

$$\mathbf{w}^T = [4\ 1\ 1\ 0\ 0\ 0\ 0]$$

or

$$\mathbf{v} = (4)\cdot\mathbf{p}_1 + (1)\cdot\mathbf{p}_2 + (1)\cdot\mathbf{p}_3$$

Flux balance analysis (FBA)

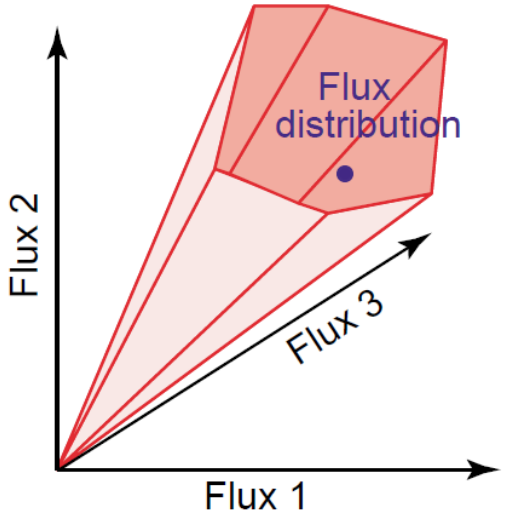
- Steady state of metabolic network

$$N\,v = 0$$

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

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

- Typical objective function: biomass production
- Optimal solution: maximum growth rate under constraints on uptake rates



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Flux balance analysis (FBA)

- Steady state of metabolic network

$$N\,v = 0$$

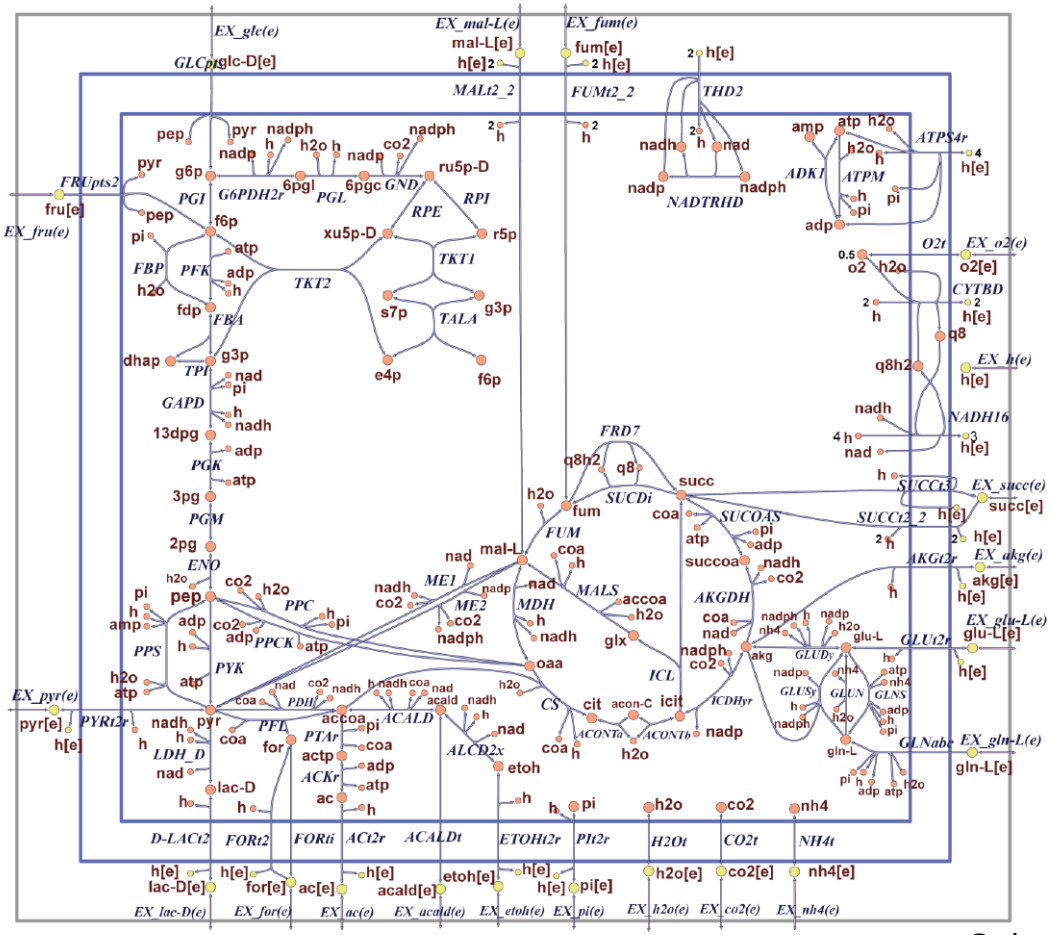
- Stoichiometry matrix and constraints define convex space of possible solutions: **flux cone**
- FBA aims at finding solutions maximising or minimising linear combination of fluxes: **objective function**
- Constrained optimisation problem in mathematics
  - Use of LP (linear programming) for solving optimisation problem
  - COBRA toolbox for building and analysing FBA models

Palsson (2006), *Systems Biology: Properties of Reconstructed Networks*, Cambridge University Press

Orth *et al.* (2010), *Nat. Biotechnol.*, 28(3):245-8

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism

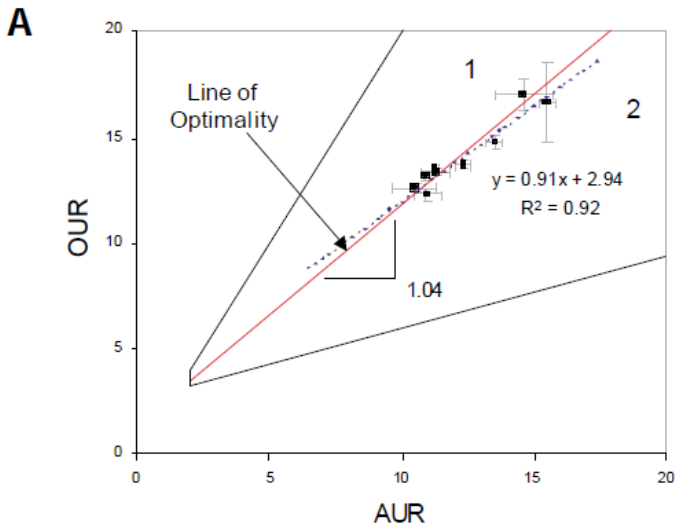


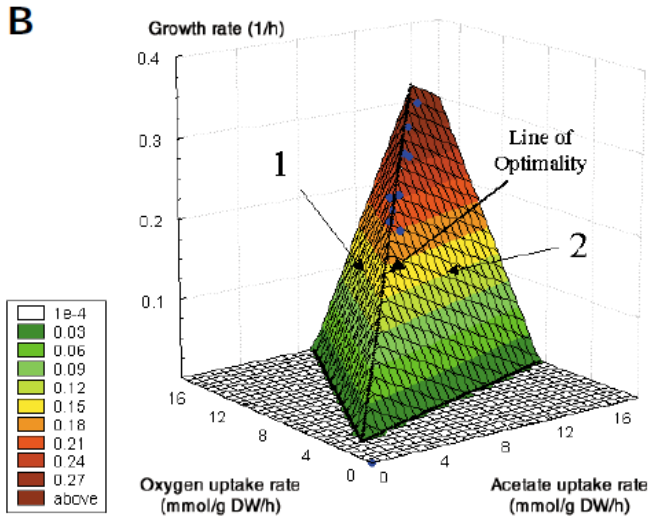
Core model

Orth *et al.* (2010), *Nat. Biotechnol.*, 28(3):245-8

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximizing growth rate with acetate as carbon source
  - Given acetate and oxygen uptake rates, compute optimal growth rate
  - Experimental test of predicted line of optimality: control of acetate uptake rate → measurement of growth rate and oxygen uptake rate

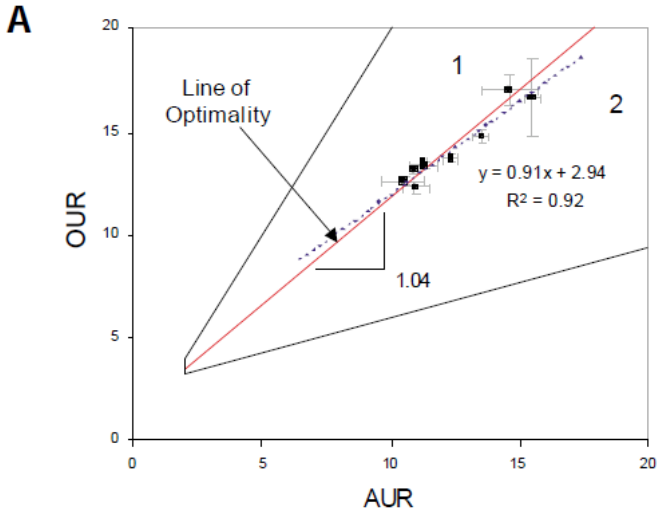


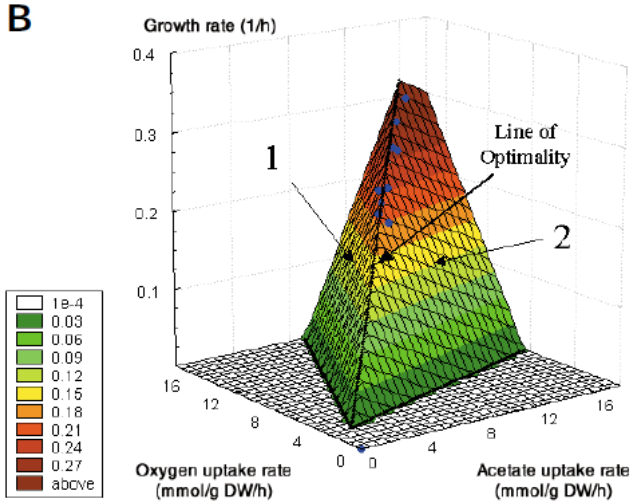


Edwards *et al.* (2001), *Nat. Biotechnol*, 19(2):125-30

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximising growth rate with acetate as carbon source
- Good correspondence of FBA predictions and experimental data suggests that *E. coli* metabolic network is optimised to maximise growth rate on acetate



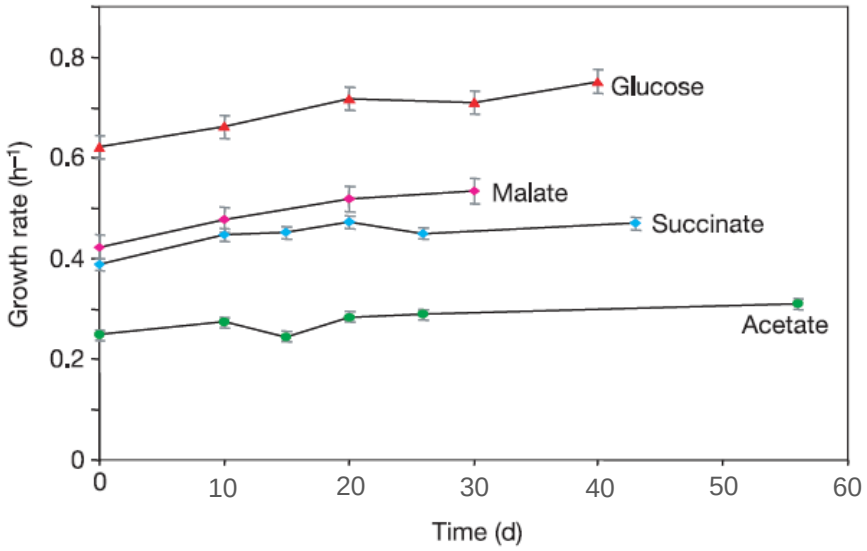


Edwards *et al.* (2001), *Nat. Biotechnol*, 19(2):125-30

Genome-scale models of *E. coli* metabolism

- In other cases, predicted optimal growth rate larger than observed growth rate
- However, experiments show that *E. coli* mutant undergoes **adaptive evolution** to achieve predicted optimal growth rate

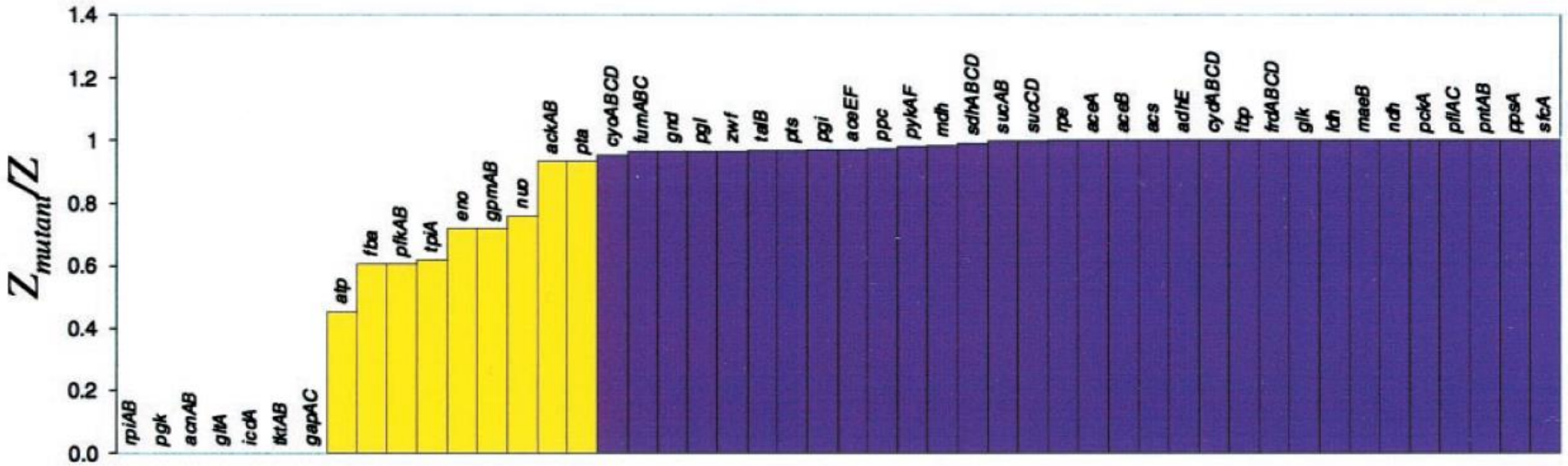
Growth rate increases on different substrates



Ibarra *et al.* (2002), *Nature*, 420(6912):186-9

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximising growth rate with glucose as carbon source and fixed oxygen uptake rate
- Effect on growth rate when deleting genes in central carbon metabolism: **essential genes** and **robustness**



Edwards *et al.* (2000), *Proc. Natl. Acad. Sci. USA*, 97(10):5528-33

Genome-scale models of *E. coli* metabolism

- Genome-scale reconstruction of *E. coli* metabolism
- FBA predictions of flux distributions maximising growth rate with glucose as carbon source and fixed oxygen uptake rate
- Good correspondence with data for gene deletions examined (86%), but less so for broader range of conditions (60%)
  - Observed growth rate lower than predicted growth rate
- Not surprising: **regulatory network** of wild-type cells may not be optimal in mutant backgrounds!
  - Regulatory network selects actual flux distribution from possible flux distributions in flux cone

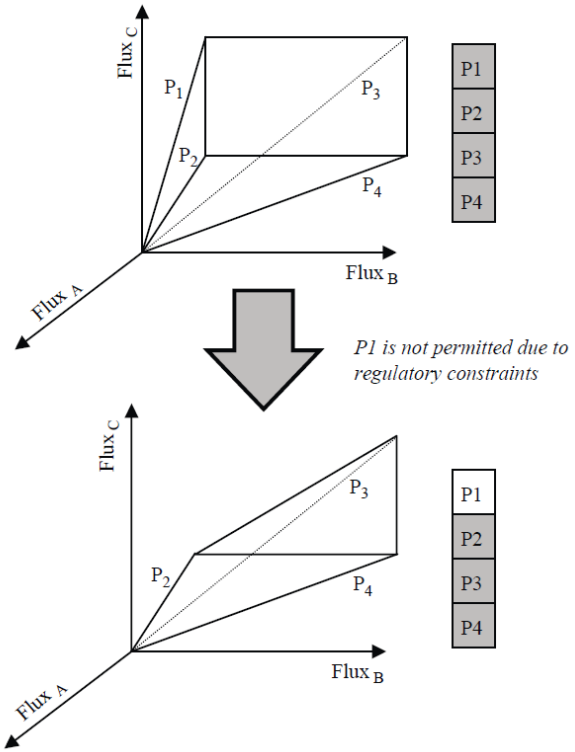
Regulatory flux balance analysis

- Steady-state dynamics of metabolic network

$$N\,v = 0$$

- Stoichiometry matrix and constraints define convex space of possible solutions: **flux cone**
- Refinement of flux cone using additional constraints

  Regulation of enzyme activity or expression, switching on/off extreme pathways

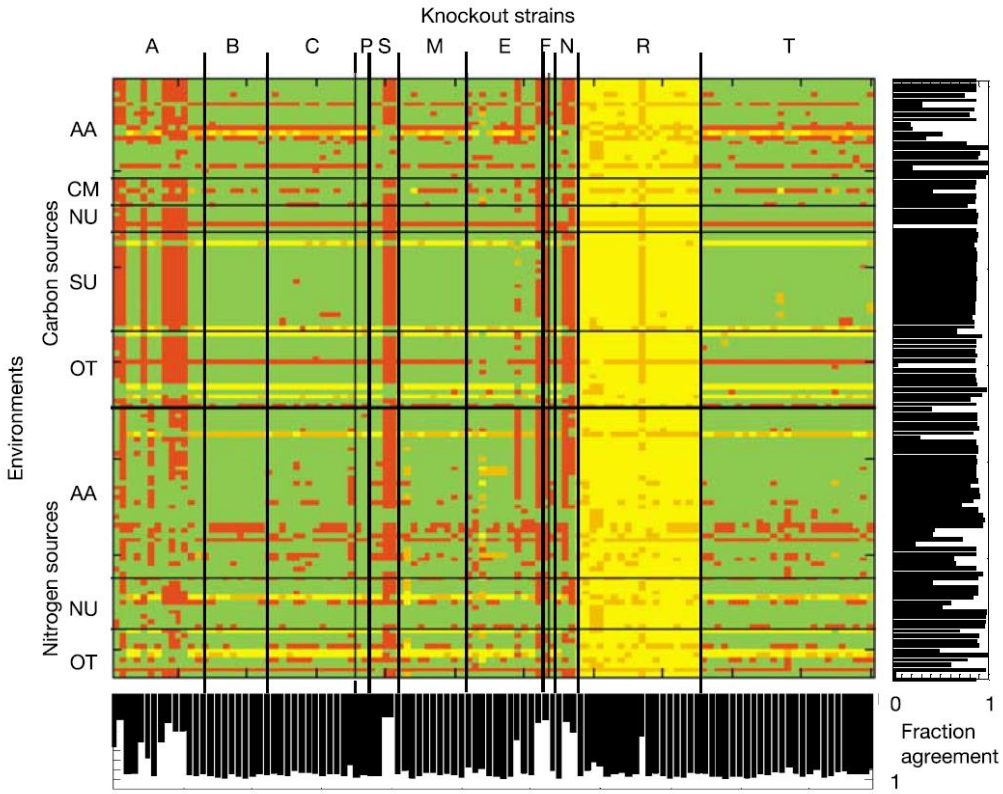


Covert *et al.* (2003), *J. Theor. Biol.*, 221(3):309-25

Genome-scale models of *E. coli* metabolism

- Regulatory network of wild-type cells may not be optimal in mutant backgrounds
- How do predictions change when **including regulatory network**?
- Genome-scale model of *E. coli* metabolism, including regulation of enzymatic genes

  Boolean models relating expression of enzymatic genes to growth conditions

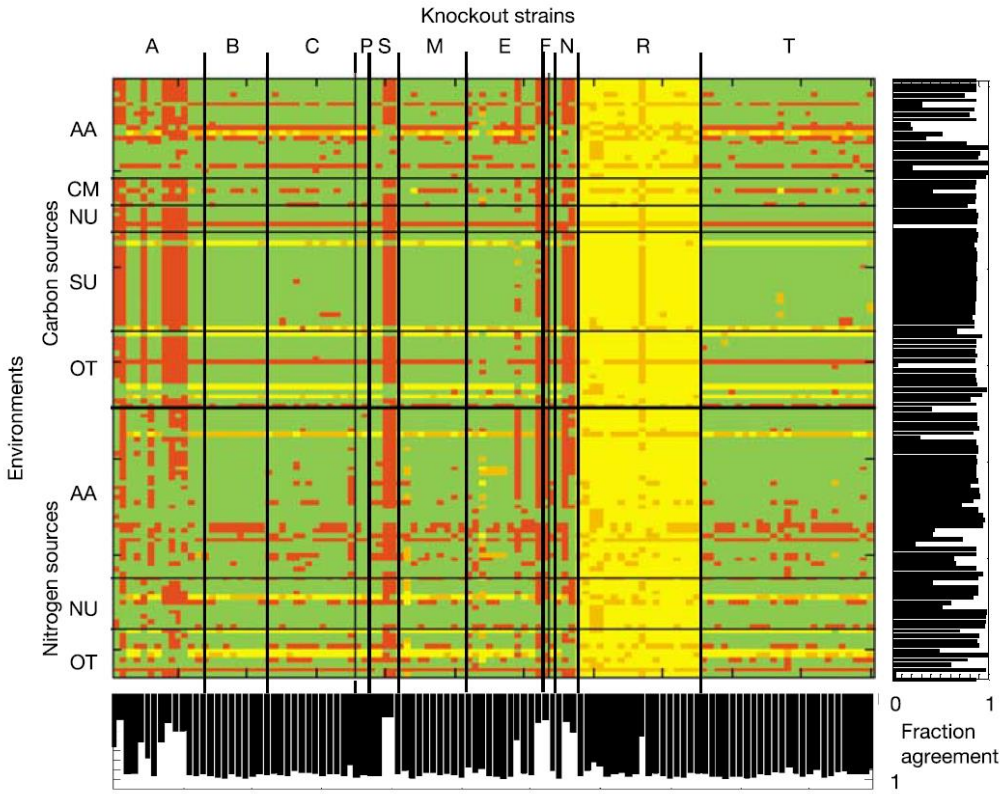


Covert *et al.* (2004), *Nature*, 429(6987):92-6

Genome-scale models of *E. coli* metabolism

- Regulatory network of wild-type cells may not be optimal in mutant backgrounds
- Genome-scale model of *E. coli* metabolism, including regulation of enzymatic genes
- Prediction of growth rate in different mutants and growth conditions improved

  60% vs 78%



Covert *et al.* (2004), *Nature*, 429(6987):92-6

Dynamic flux balance analysis

- Dynamics of metabolic network through interactions with environment

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

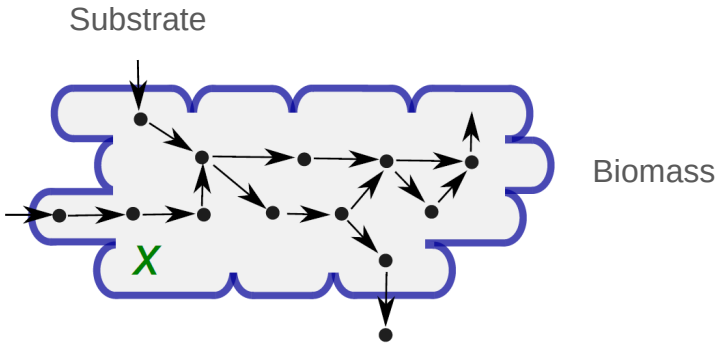
$$\dot{B} = \mu(t) \cdot B, \quad B(0) = B_0$$

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 by means of FBA at each time-point *t*
  - Change in substrate concentrations puts bounds on uptake rates

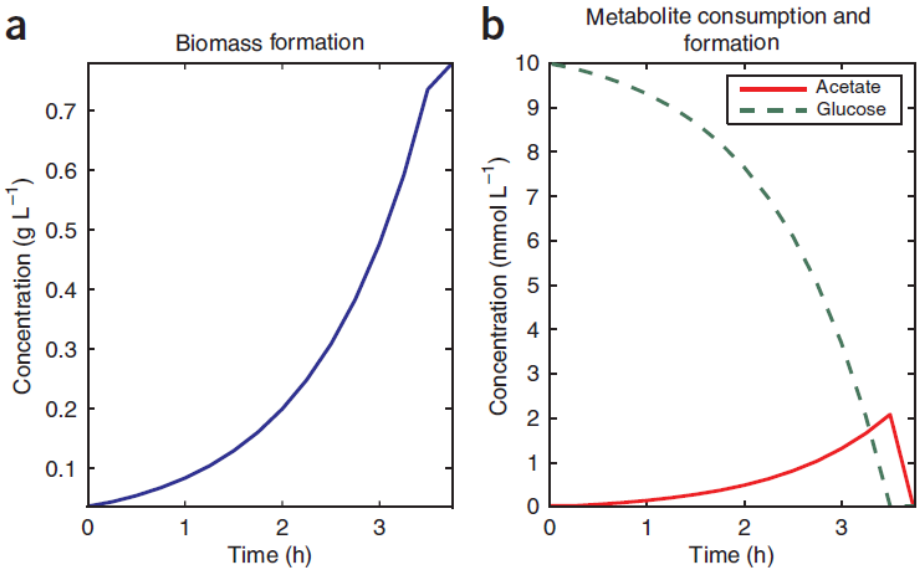
Mahadevan *et al.* (2002), *Biophys. J.*, 83(3):1331-40

Dynamic flux balance analysis

- Dynamics predicted by means of **dynamic FBA**

  Sequential growth of *E. coli* on different carbon sources (glucose, acetate)

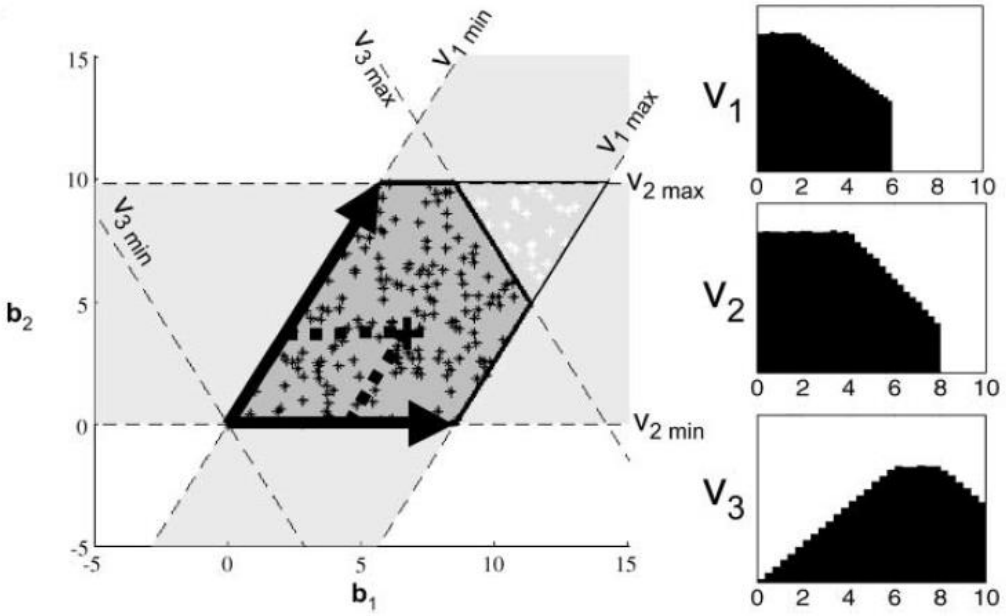
Orth *et al.* (2002), *Nat. Protocols*, 2(3):727-38



Monte-Carlo sampling of FBA solutions

- Stoichiometry matrix and constraints define convex space of possible solutions: **flux cone**
- FBA selects solutions from flux cone optimizing objective function, but no single solution
- Alternative approach: Monte-Carlo sampling of optimal solutions

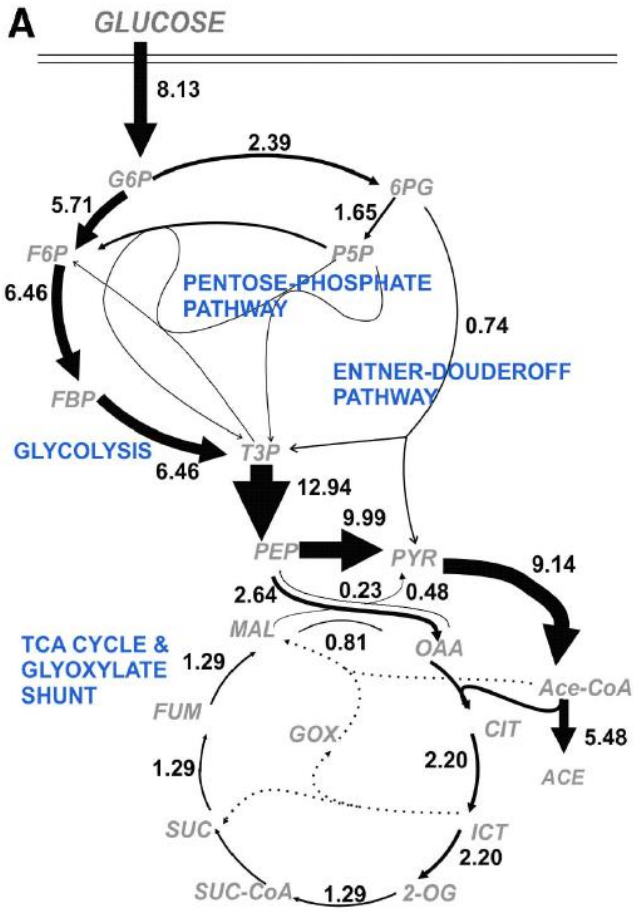
  Distributions for individual fluxes in network



Price *et al.* (2004), *Biophys. J.*, 87(4):2172-86

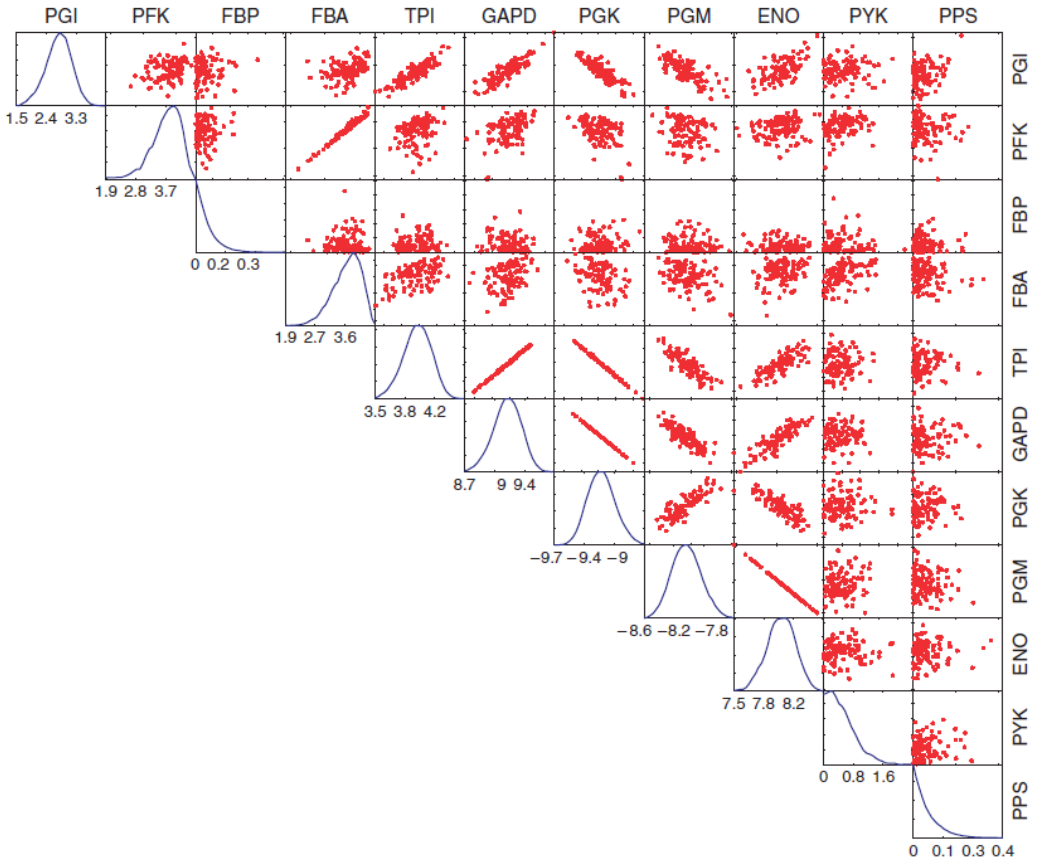
Monte-Carlo sampling of FBA solutions

- Analysis of glycolysis pathway in *E. coli* during growth on glucose



Monte-Carlo sampling of FBA solutions

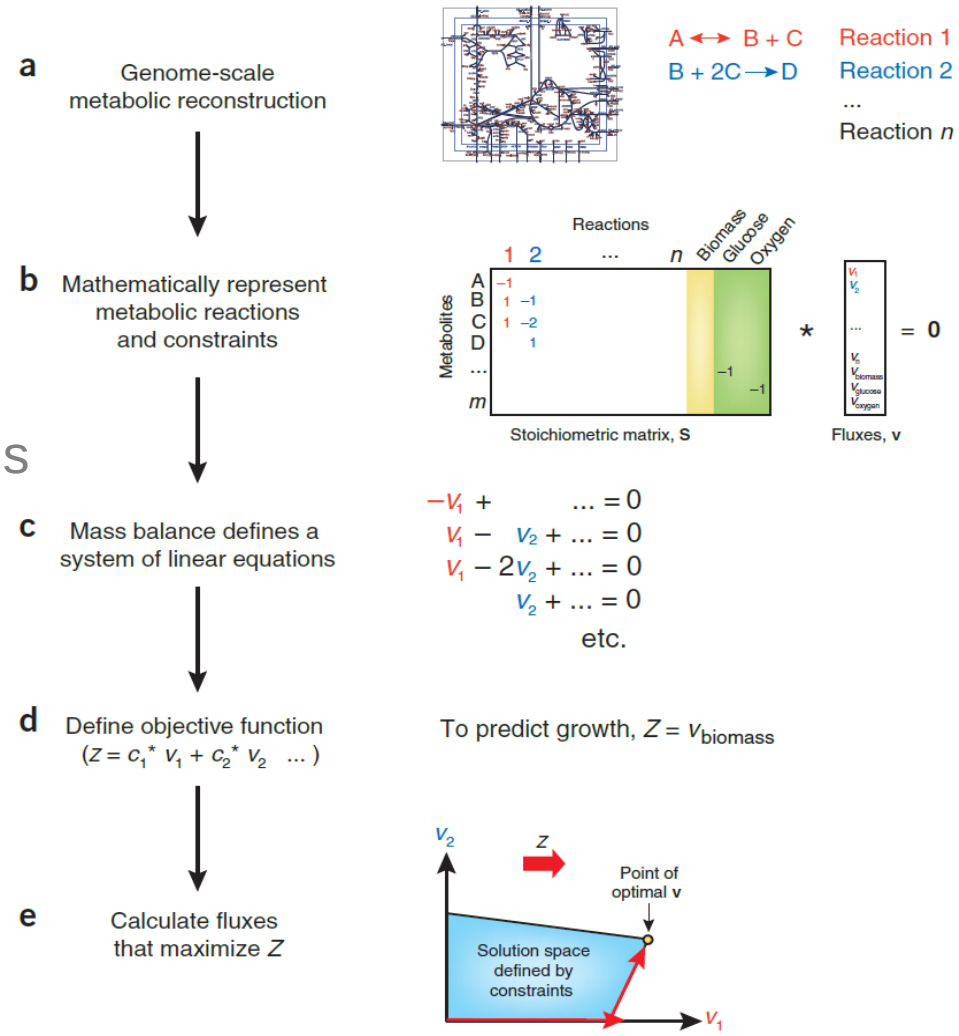
- Analysis of glycolysis pathway in *E. coli* during growth on glucose
  - Tight distributions
  - Correlations between fluxes



Becker *et al.* (2007), *Nat. Protocols*, 2(3):727-38

Conclusion FBA

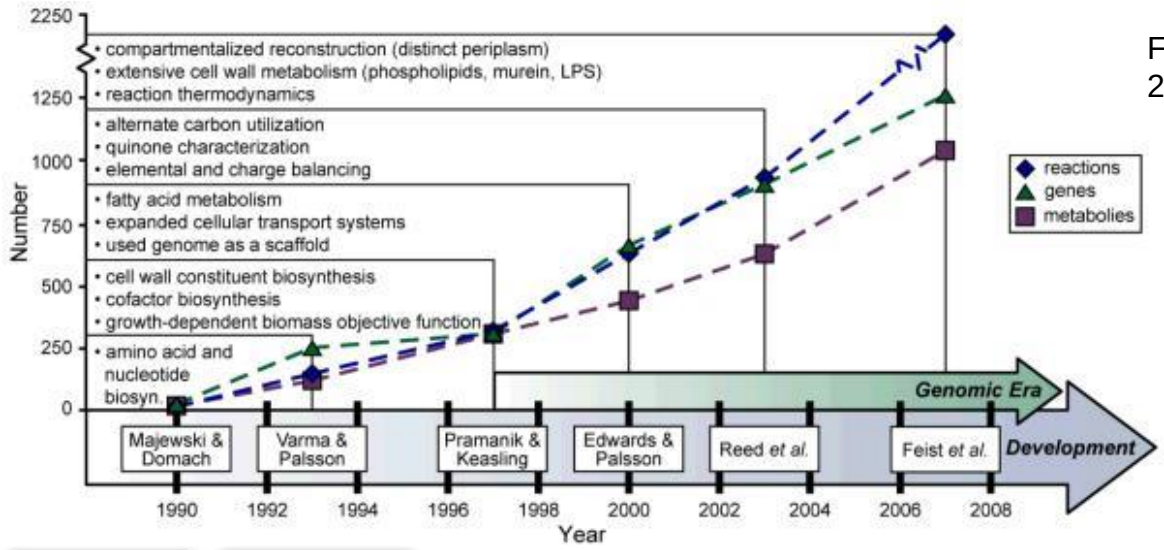
- FBA models provide genome-scale picture of metabolism and yield experimentally-testable predictions
  - Predictions of flux distributions in different growth conditions and genetic backgrounds



Orth *et al.* (2010), *Nat. Biotechnol.*, 28(3):245-8

Conclusion FBA

- FBA models provide genome-scale picture of metabolism and yield experimentally-testable predictions
  - Predictions of flux distributions in different growth conditions and genetic backgrounds
  - Tool for metabolic engineering
  - In *E. coli* and other (less well-characterised) organisms



Feist and Palsson (2008), *Nat. Biotechnol.*, 26(6):659-67

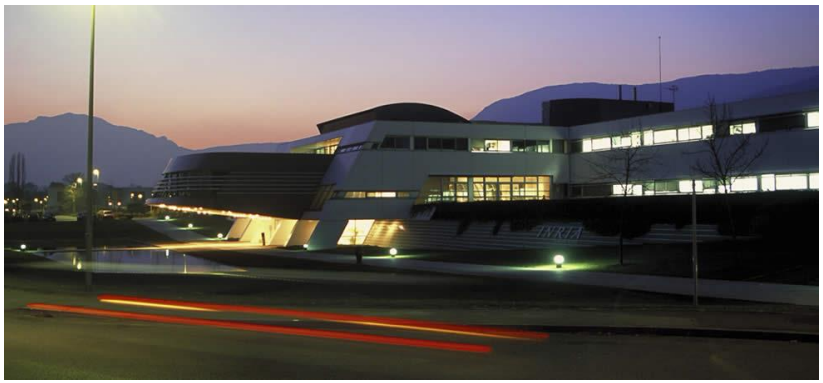
Conclusion FBA

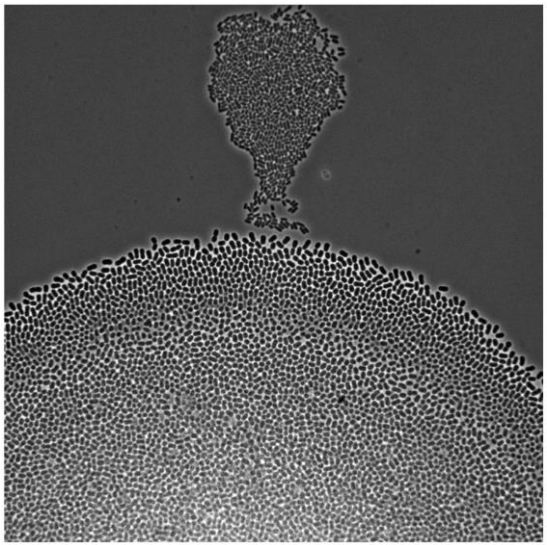
- But FBA has problems as well!
  - Practical question: which **objective function** works best for problem considered?
  - Fundamental question: what do microorganisms optimise?

    Schuetz *et al.* (2007), *Mol. Syst. Biol.*, 3:119
  - Integration of **regulatory mechanisms** on metabolic and genetic level is not easy to achieve in FBA formalism
  - No predictions on **dynamics** on time-scale of metabolism

Internships in MICROCOSME

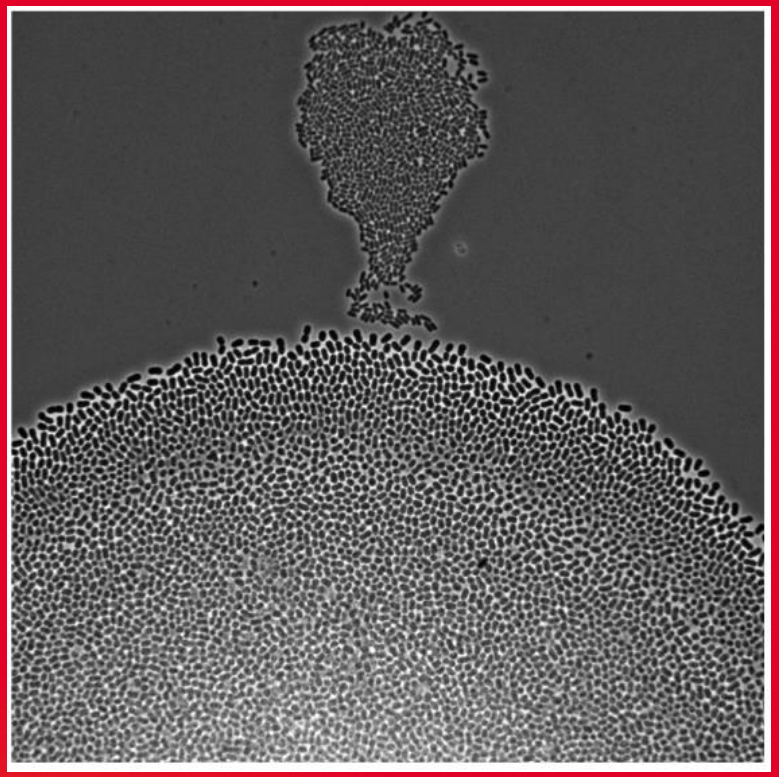
- Challenging problems for biologists, physicists, computer scientists, mathematicians, …
- … in a multidisciplinary working environment
- Contact: Hidde.de-Jong@inria.fr and https://team.inria.fr/microcosme





Courtesy Antrea Pavlou (2021)

Thanks!



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informatics mathematics

Université Grenoble Alpes