



Flux Balance Analysis (FBA)

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INRIA Grenoble - Rhône-Alpes and IBIS



- IBIS: systems biology group at INRIA/Université Grenoble-Alpes
 - Analysis of bacterial regulatory networks by means of models and experiments
 - Biologists, computer scientists, mathematicians, physicists, ...

<http://team.inria.fr/ibis>



Overview

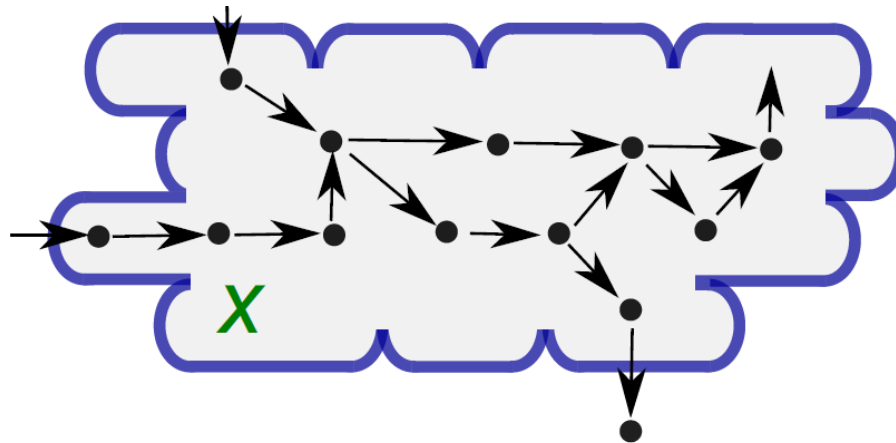
- Part 1. Systems biology and kinetic modeling
 - Introduction
 - Kinetic modeling of cellular reaction networks
- Part 2. Metabolic network modeling
 - Kinetic modeling of metabolism
 - Metabolic control analysis (MCA)
 - **Flux balance analysis (FBA)**
 - Practical on flux balance analysis (COBRA)
- Part 3. Gene regulatory network modeling
- Part 4. Models and data

Biochemical reaction networks

- ODE model for growth of microbial populations:

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

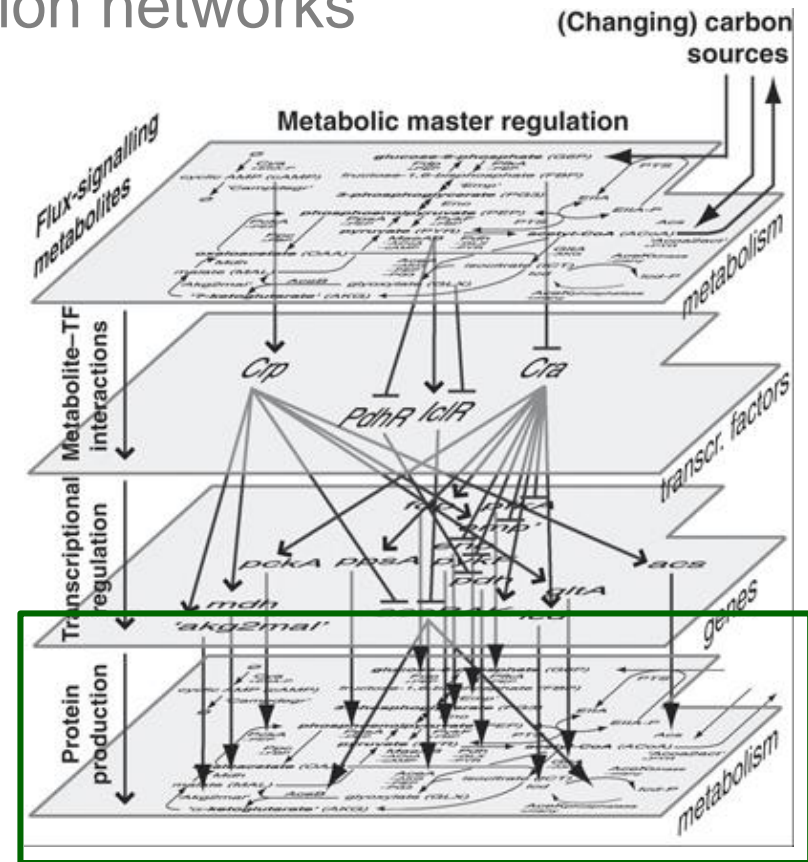
$$\mu = \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x).$$



- 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

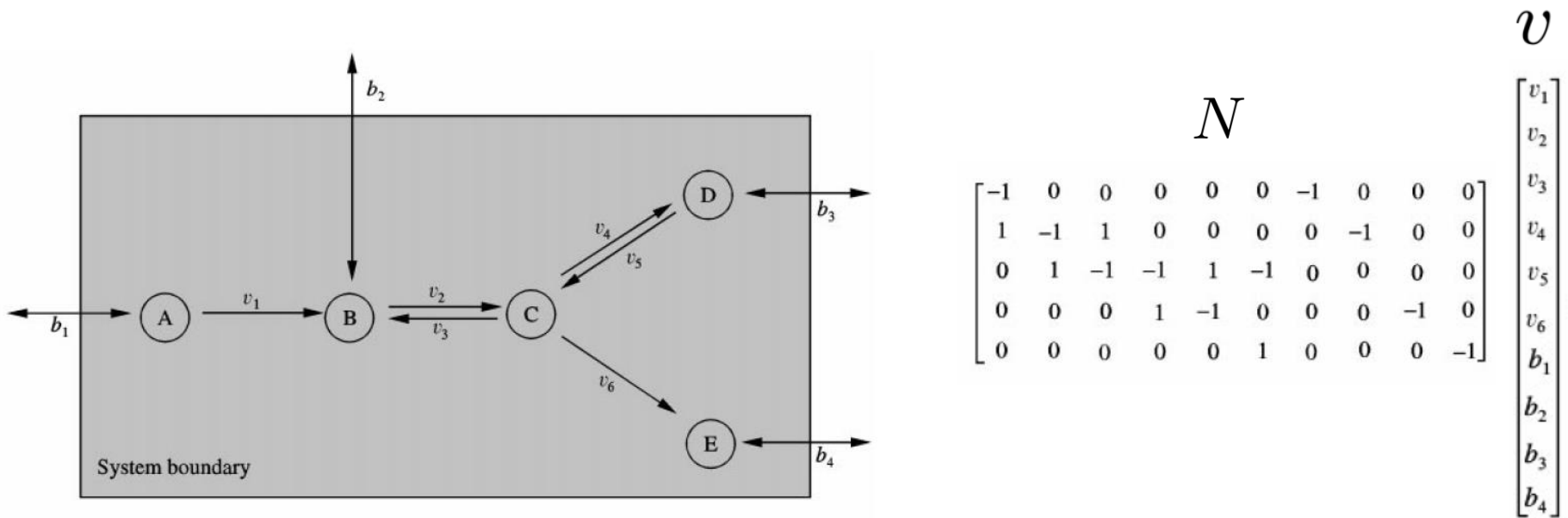
$$\dot{x} = N v(x)$$

p

Stoichiometry matrix

- Stoichiometry matrix N describes structure of reaction network

Internal reactions and exchange reactions, reversible and irreversible



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

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

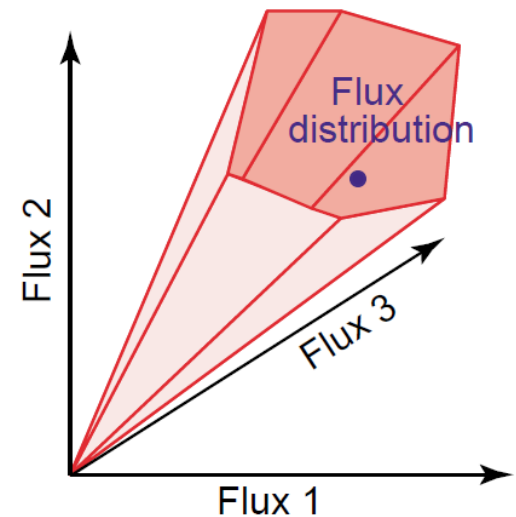
- Bounds on fluxes derived from available information in literature, bounds may be infinite
- For mathematical convenience, all fluxes must be positive $v \geq 0$
- Reversible reaction modeled as pair of irreversible, positive fluxes

Flux balance analysis (FBA)

- Steady-state dynamics 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

Flux balance analysis (FBA)

- Steady-state dynamics of metabolic network

$$N v = 0$$

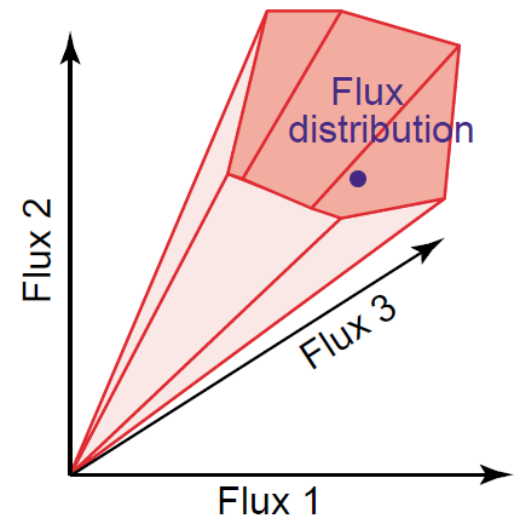
- Stoichiometry matrix and constraints define convex space of possible solutions: **flux cone**

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

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

p^i : extreme pathway i

w_i : weight of i th pathway



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

Flux balance analysis (FBA)

- Steady-state dynamics of metabolic network

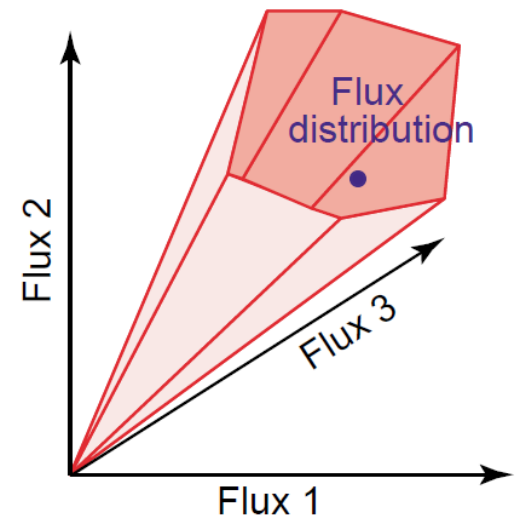
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$$C = \left\{ v \mid v = \sum_{i=1}^k w_i p^i, w_i \geq 0, i = 1, \dots, k \right\}$$

- 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)

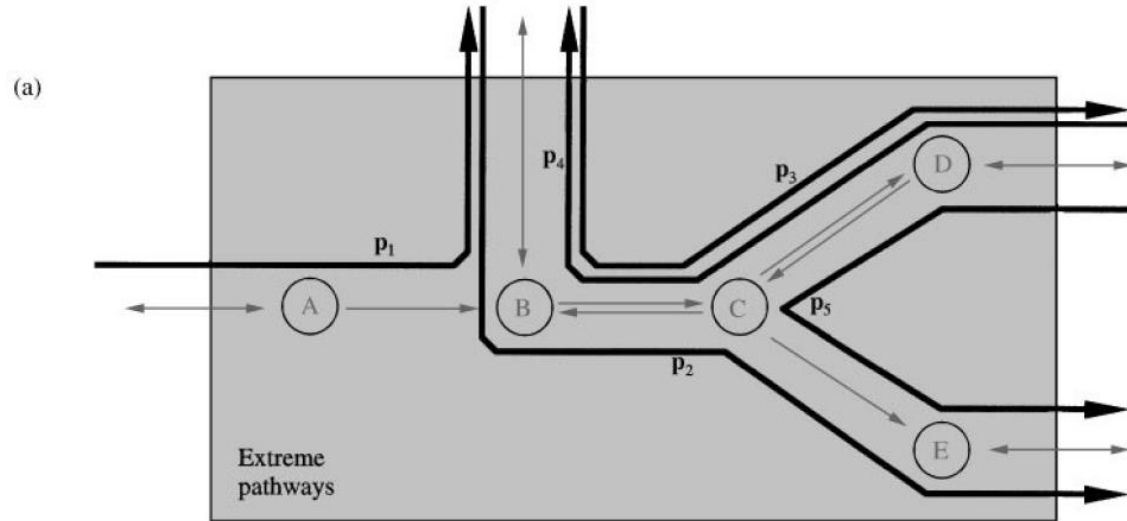
- Extreme pathways in example network

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



(b)

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

(c) Flux distribution:
 $\mathbf{v}^T = [4 \ 2 \ 0 \ 1 \ 0 \ 1 \ -4 \ 2 \ 1 \ 1]$

Subset pathways of \mathbf{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 \mathbf{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)

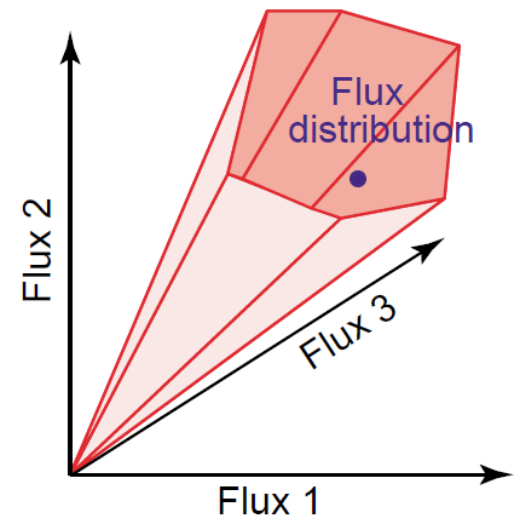
- Steady state of metabolic network

$$N v = 0$$

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

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

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



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

Flux balance analysis (FBA)

- Steady-state dynamics of metabolic network

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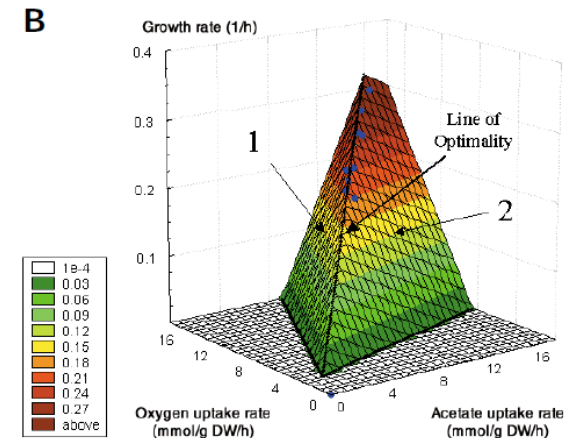
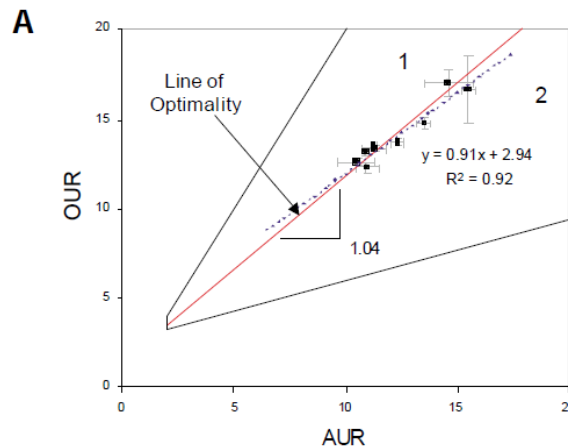
- Stoichiometry matrix and constraints define convex space of possible solutions: **flux cone**
- FBA aims at finding solution(s) maximising or minimising linear combination of fluxes: **objective function**
- Constrained optimisation problem in mathematics
 - Use of LP (linear programming) for solving 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
- FBA predictions of flux distributions maximising growth rate with acetate as carbon source
 - Given acetate and oxygen uptake rates, compute optimal growth rate
 - Line of optimality indicates combinations of acetate and oxygen uptake rates with maximal growth rate
 - Experimental test of predicted line of optimality: control of acetate uptake rate and measurement of growth and oxygen uptake rate

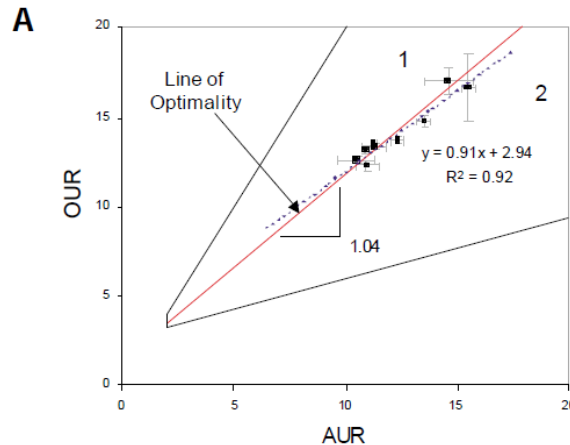


Edwards *et al.* (2001), *Nat. Biotechnol.*, 19(2):125-30

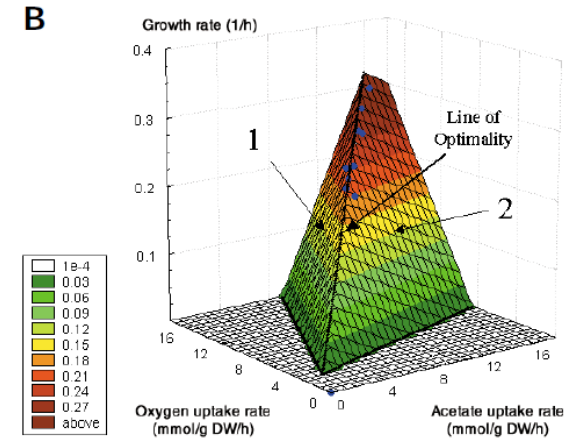
Genome-scale models of *E. coli* metabolism

- 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

Idem succinate

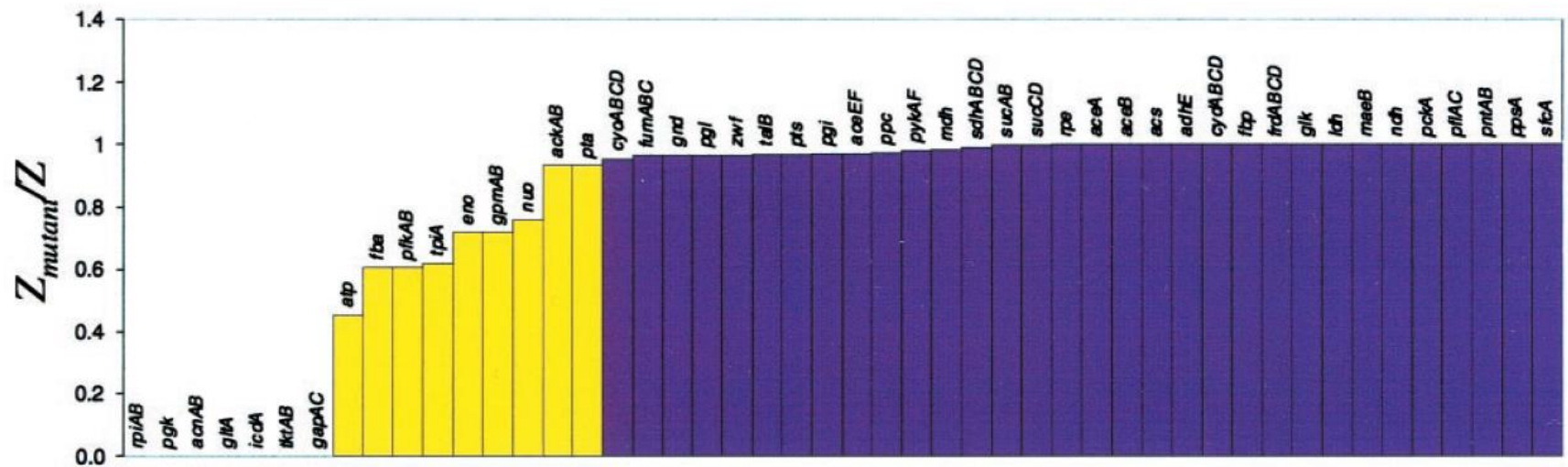


Edwards *et al.* (2001), *Nat. Biotechnol.*, 19(2):125-30



Genome-scale models of *E. coli* metabolism

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

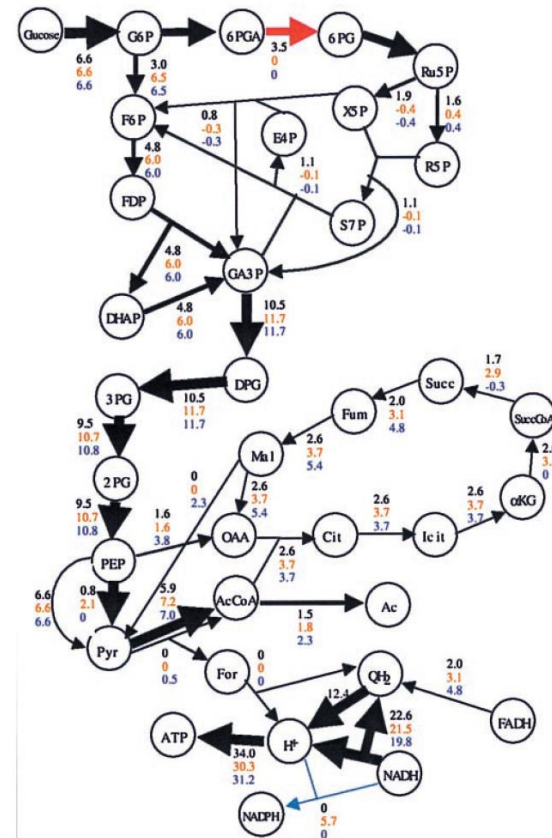


Edwards *et al.* (2000), *Proc. Natl. Acad. Sci. USA*, 97(10):5528-33

Genome-scale models of *E. coli* metabolism

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- Effect on flux distribution when deleting genes in central carbon metabolism

Deletion of *zwf* (red) and *zwf/pnt* (blue)



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

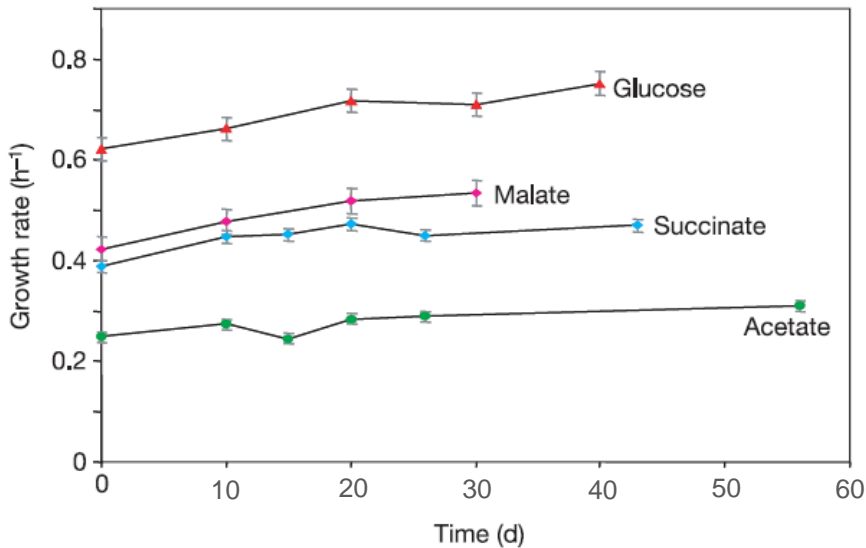
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- However, experiments show that *E. coli* mutant undergoes **adaptive evolution** to achieve predicted optimal growth rate

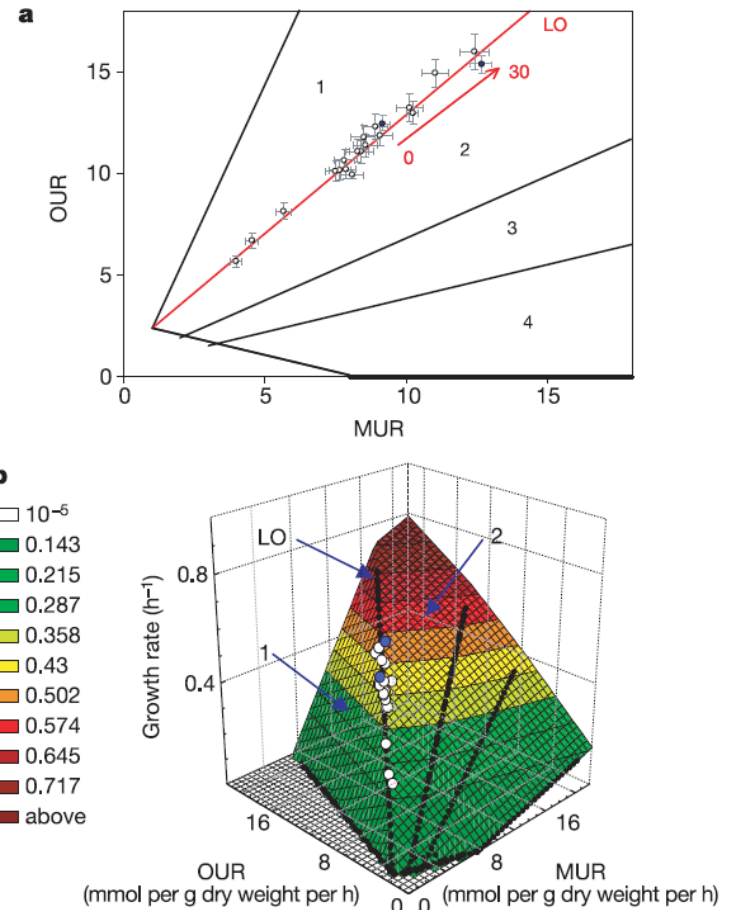
Ibarra *et al.* (2002), *Nature*, 420(6912):186-9

Genome-scale models of *E. coli* metabolism

- However, experiments show that *E. coli* mutant undergoes **adaptive evolution** to achieve predicted optimal growth rate
 - Growth on malate and other substrates
 - Measured substrate and oxygen uptake rates



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



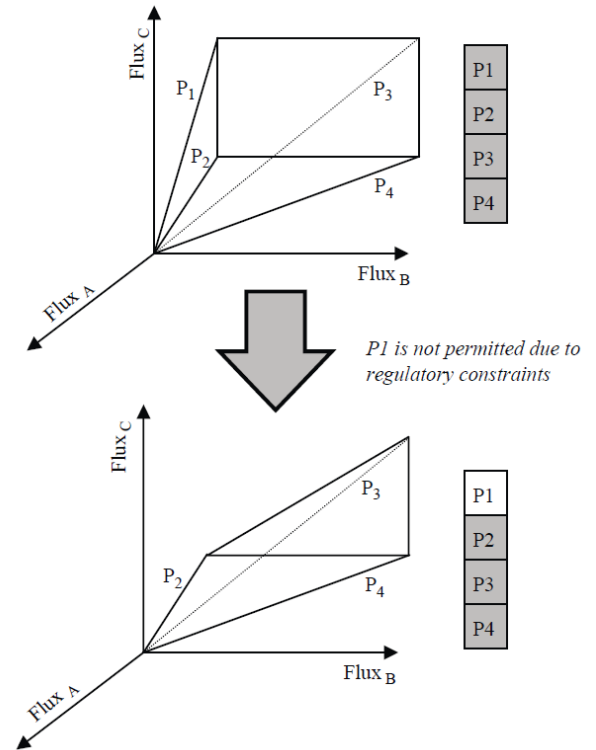
Regulatory flux balance analysis

- 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

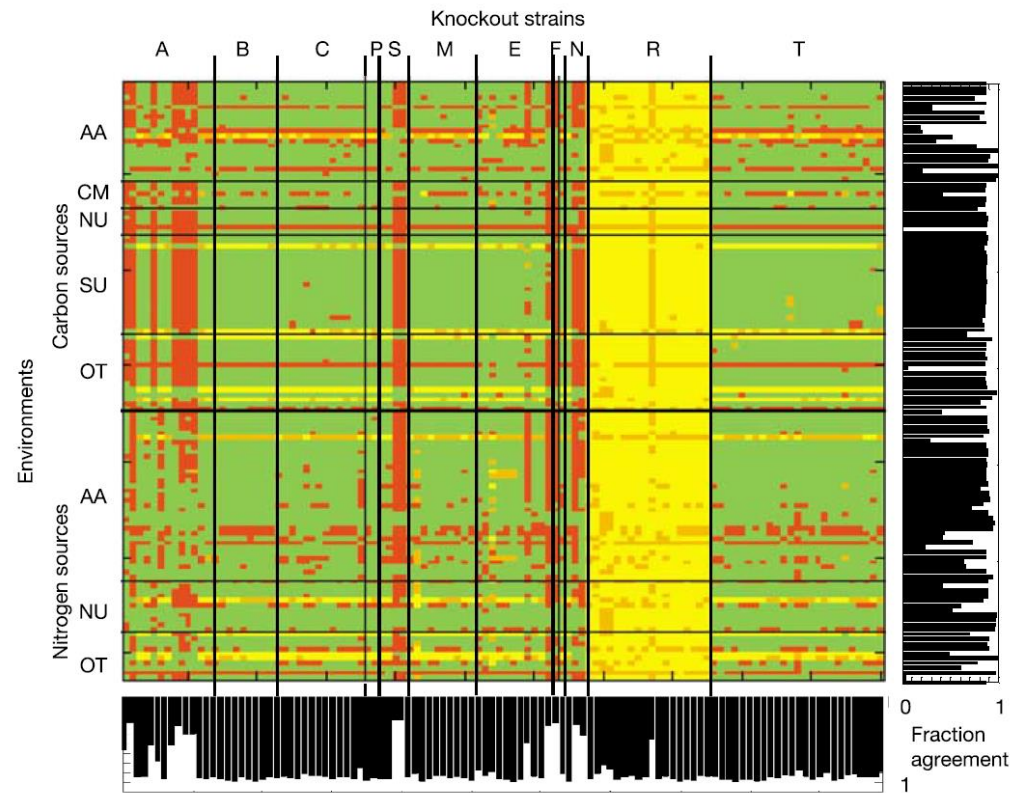


Covert *et al.* (2003), *J. Theor. Biol.*, 221(3):309-25

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

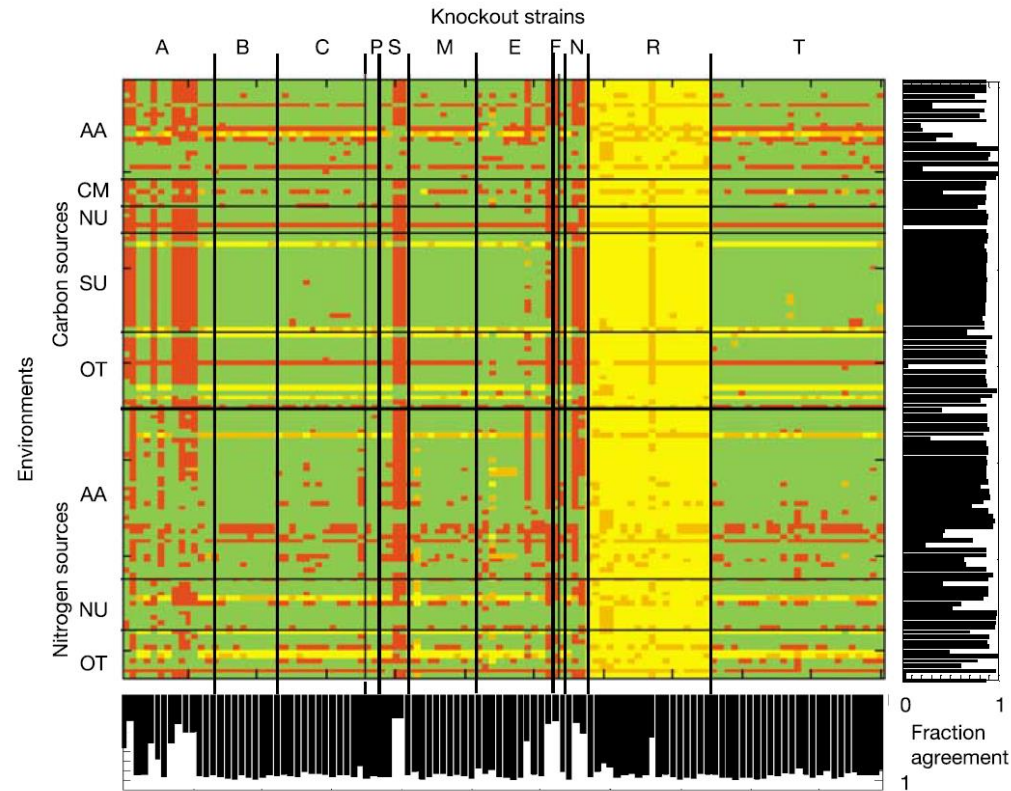


Covert *et al.* (2004), *Nature*, 429(6987):92-6

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

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

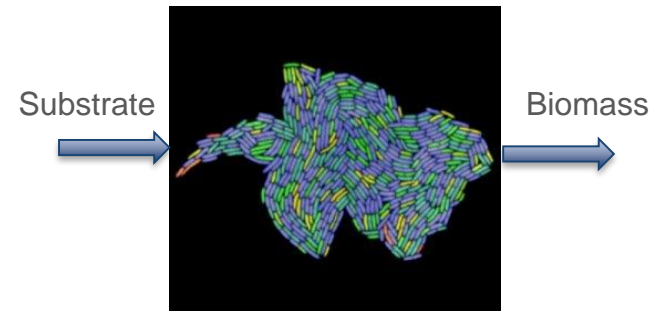
$$\dot{B} = \mu(t) \cdot B, \quad B(0) = B_0$$

B : biomass concentration in medium

s : substrate concentration in medium

μ : growth rate

v_{ext} : substrate uptake rate



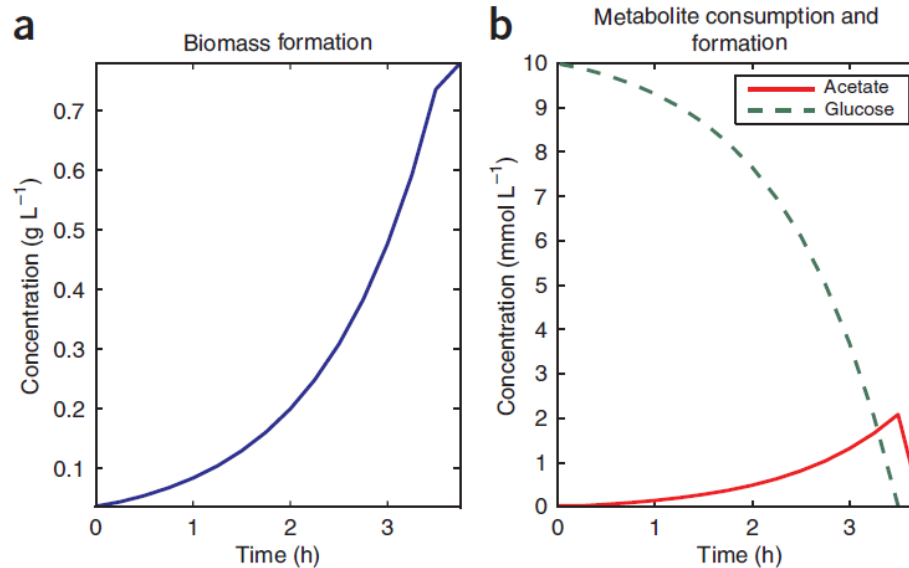
- Dynamics predicted by means of **dynamic FBA**
 - Metabolic network at quasi-steady state with respect to environment
 - Computation of exchange rates and growth rate by means of FBA at each time-point t
 - Change in substrate concentrations puts bounds on uptake rates

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)

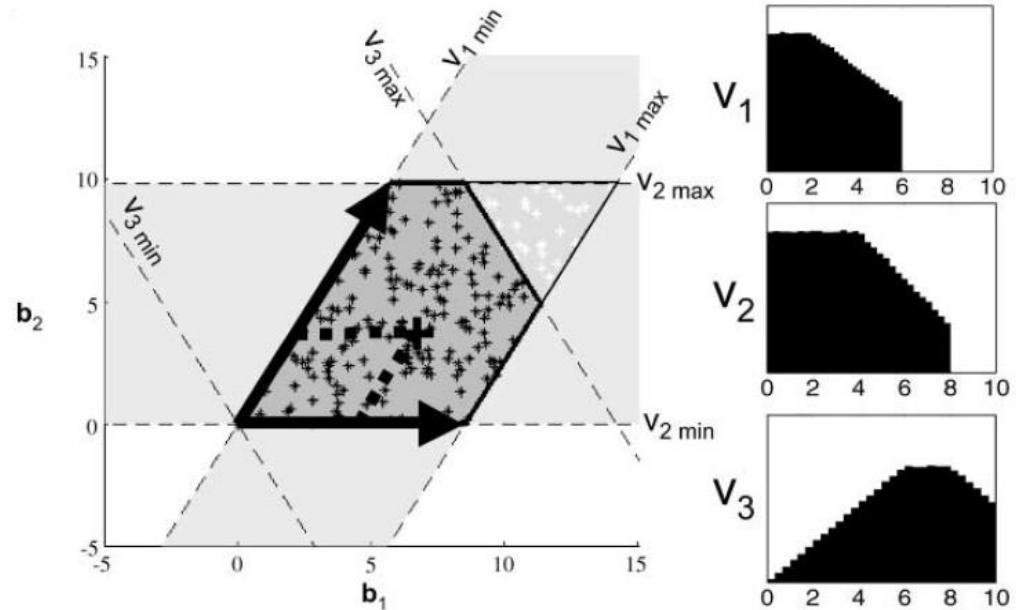
Orth *et al.* (2002), *Nat. Protocols*, 2(3):727-38



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

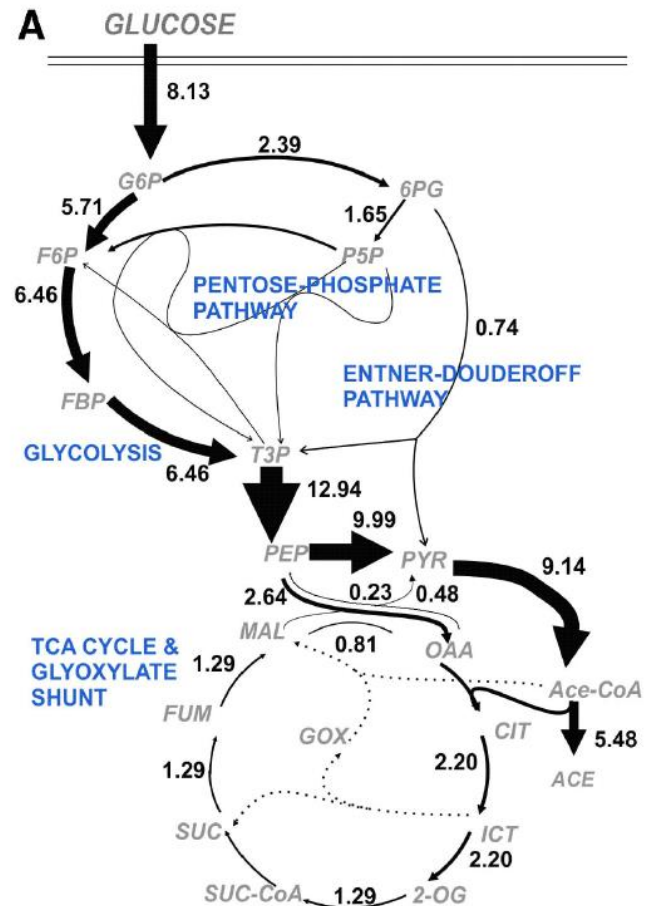
Distributions for individual fluxes in network



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

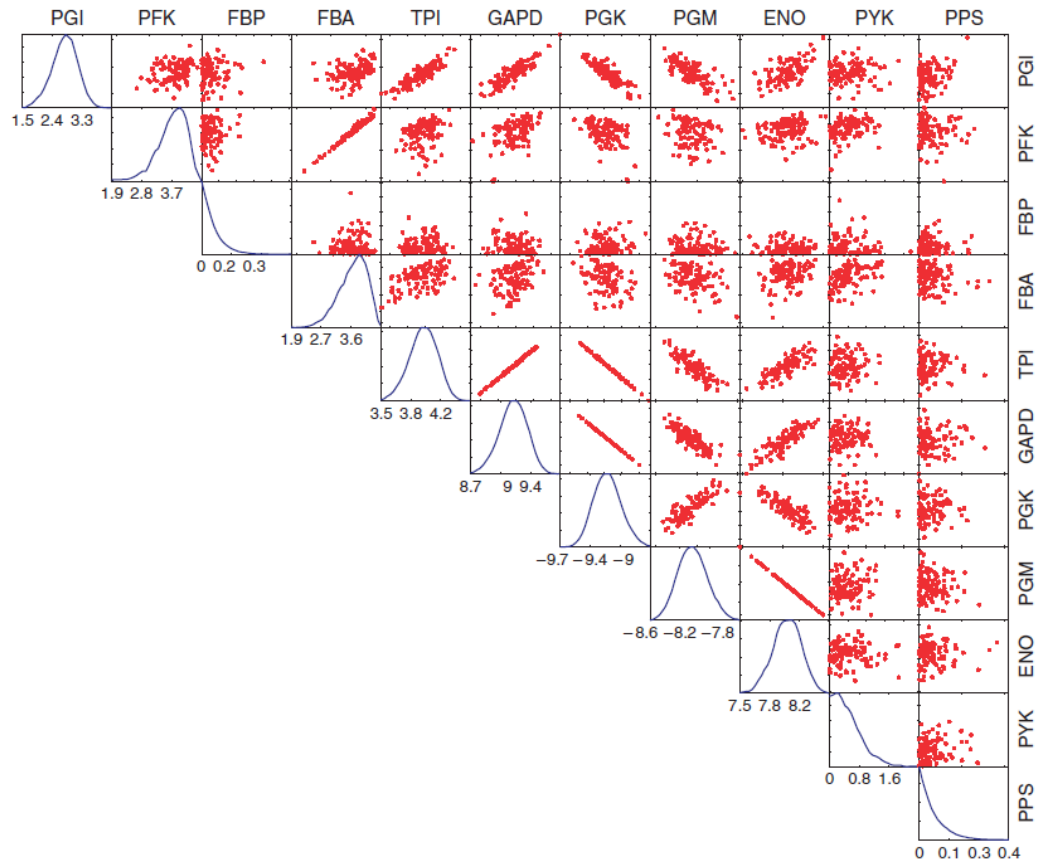
Monte-Carlo sampling of FBA solutions

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



Monte-Carlo sampling of FBA solutions

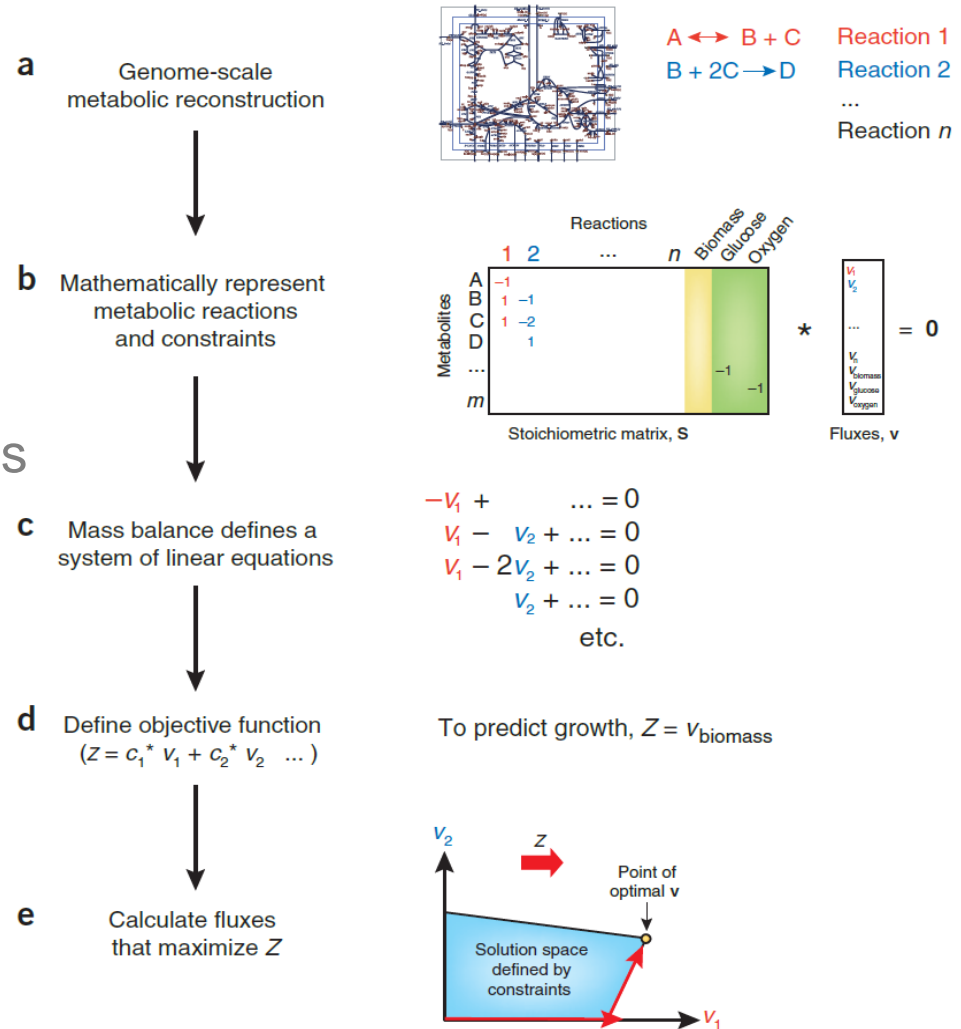
- Analysis of glycolysis pathway in *E. coli* during growth on glucose
 - Tight distributions
 - Correlations between fluxes



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

Conclusion FBA

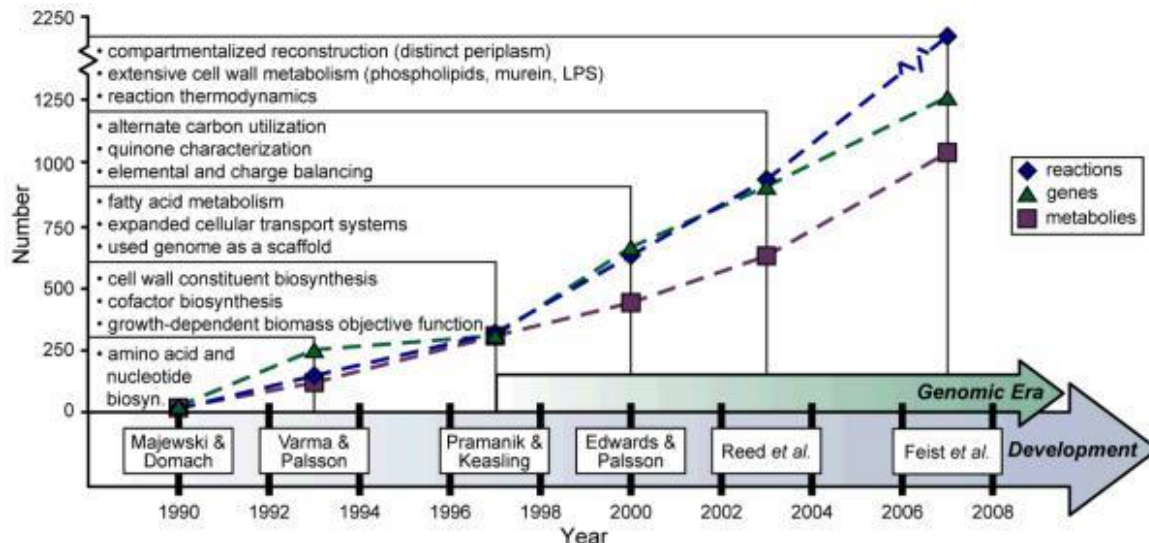
- FBA models provide genome-scale picture of metabolism and yield experimentally-testable predictions
 - Predictions of flux distributions in different growth conditions and genetic backgrounds



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 IBIS

- Challenging problems for biologists, physicists, computer scientists, mathematicians, ...
- ... in a multidisciplinary working environment
- Contact: Hidde.de-Jong@inria.fr and ibis.inrialpes.fr



Courtesy Guillaume Baptist (2008)

Merci !



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