Flux Balance Analysis (FBA)
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

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

\[
N = \begin{bmatrix}
-1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
1 & -1 & 1 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\
\end{bmatrix}
\]

Flux balance analysis (FBA)

- Steady state of metabolic network
  \[ N \nu = 0 \]
  Steady-state reaction rates are called **fluxes**

- **Constraints** on fluxes: upper and lower bounds
  \[ v^l \leq \nu \leq v^u \]
  - 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
  \[ N \nu = 0 \]

- 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)

Flux balance analysis (FBA)

• Steady state of metabolic network

\[ Nv = 0 \]

• 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)

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

\( p^i \) : extreme pathway \( i \)

\( w_i \) : weight of \( i^{th} \) pathway

Flux balance analysis (FBA)

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  - Every solution can be written as linear combination of rays of flux cone (extreme pathways)
    \[ C = \{ \nu | \nu = \sum_{i=1}^{k} w_i p^i, \ w_i \geq 0, \ i = 1, \ldots, k \} \]
  - Set of extreme pathways unique, but solutions not uniquely defined by extreme pathways

Flux balance analysis (FBA)

- Extreme pathways in example network
- Extreme pathways provide pathway-based view of network
- Related concept of elementary modes
  Schilling et al. (1999), Biotechnol. Prog., 15(3):296-303
Flux balance analysis (FBA)

• Steady state of metabolic network

\[ \mathbf{N} \mathbf{v} = 0 \]

• 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**

\[ \mathbf{Z} = \mathbf{c}^T \mathbf{v} \quad \mathbf{c} \in \mathbb{R}^n \]

• Typical objective function: biomass production

• Optimal solution: maximum growth rate under constraints on uptake rates

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

Genome-scale models of *E. coli* metabolism

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

Core model

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

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

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

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**

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
  \[ N \nu = 0 \]

- 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

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

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%

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
- \( \mu \): 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

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

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

Conclusion FBA

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