Dynamic optimization of metabolic networks coupled with gene expression

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Mathematics for key technologies

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Outline

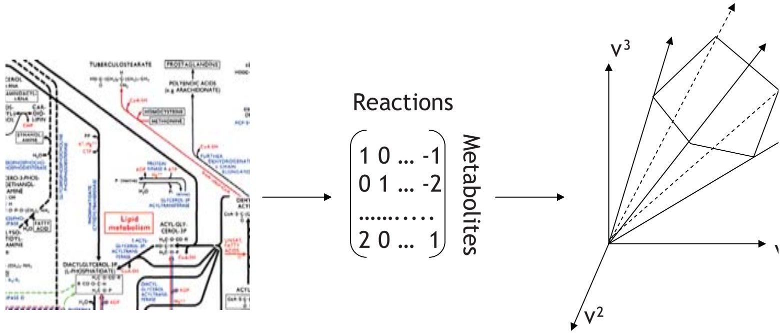
- ▷ Constraint-based modeling of metabolic networks
- ▷ Metabolism and enzyme production
- ▷ Dynamic model
- ▷ Time-scale separation
- ▷ Steady-state and dynamic optimisation: FBA, dFBA, RBA
- ▷ Dynamic enzyme-cost FBA: deFBA
- ▷ Applications

Constraint-based modeling

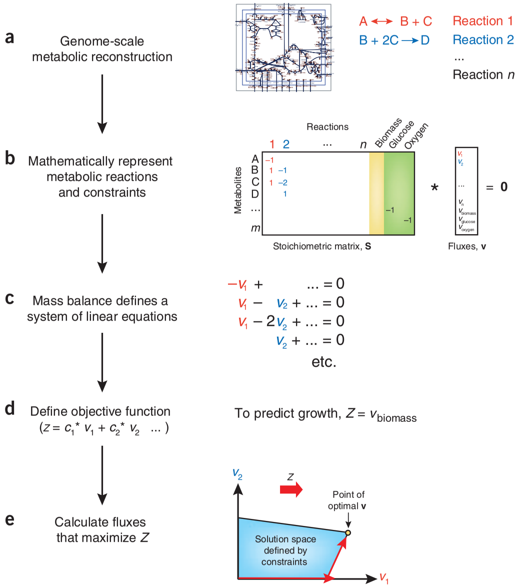
Set of possible behaviors of a metabolic network at steady state

$$C = \{v \in \mathbb{R}^n \mid Sv = 0,\ v_i \geq 0,\ i \in Irr\}$$

$\rightsquigarrow$ steady-state flux cone



Flux balance analysis (FBA)


a Genome-scale metabolic reconstruction

b Mathematically represent metabolic reactions and constraints

c Mass balance defines a system of linear equations

d Define objective function ($z = c_1^* v_1 + c_2^* v_2 \ \dots$)

e Calculate fluxes that maximize Z

- ▷ *E. coli* metabolism
- ▷ Genome-scale reconstruction (*i*JO1366)
- ▷ 1336 metabolites, 2251 reactions
- ▷ Objective function: biomass
- ▷ Glucose and oxygen uptake reactions
- ▷ Aerobic and anaerobic growth
- ▷ Software: e.g. COBRA Toolbox 2.0

Orth/Thiele/Palsson 10

Metabolism and enzyme production

$$\begin{array}{ccc} Y \xleftrightarrow{V_y} X, & X \xrightarrow{V_x} \tilde{X}, & X \xrightarrow{V_p} P \\ \uparrow P & \uparrow P & \uparrow P \end{array}$$

- ▷ Molecular species
  - Extracellular nutrients and/or waste Y
  - Intracellular metabolites X
  - Macromolecules/enzymes P
- ▷ Reaction fluxes
  - Exchange reactions V_y
  - Internal metabolic reactions V_x
  - Biomass reactions V_p
- ▷ Stoichiometric matrices S_j^i (species i, reactions j)

Dynamic model

- ▷ Mass balance

$$\begin{aligned} \dot{Y} &= -S_y^y V_y \\ \dot{P} &= S_p^p V_p \\ \dot{X} &= S_y^x V_y + S_x^x V_x - S_p^x V_p \end{aligned}$$

- ▷ Macromolecule production is slow: small ε
- ▷ Macromolecules are made from many components: large α

$$\begin{aligned} \dot{Y} &= -S_y^y V_y \\ \dot{P} &= \varepsilon\, S_p^p V_p \\ \dot{X} &= S_y^x V_y + S_x^x V_x - \varepsilon \alpha\, S_p^x V_p \end{aligned}$$

Quasi steady state approximation

- ▷ Time-scale separation (using Tikhonov's theorem)

$$\begin{aligned} \dot{Y} &= -S_y^y V_y, \\ \dot{P} &= \varepsilon\, S_p^p V_p, \\ 0 &= S_y^x V_y + S_x^x V_x - \alpha\, \varepsilon\, S_p^x V_p. \end{aligned}$$

- ▷ Exchange reactions and biomass production coupled via quasi steady-state constraint for intracellular metabolism.
- ▷ Model reduction

Steady-state vs. dynamic optimisation

	Metabolism	+ Enzyme production
Steady state	Flux Balance Analysis (FBA) Varma/Palsson 94	Resource Balance Analysis (RBA) Goelzer et al. 11
Dynamic	Dynamic FBA (dFBA) Mahadevan et al. 02	Dynamic Enzyme Cost Analysis (deFBA) – THIS TALK –

Flux balance analysis (FBA)

- Goal: Determine metabolic fluxes maximizing a cellular objective.
- Network model: Metabolism & steady state

$$0 = S_y v_y + S_x v_x - S_{bm} v_{bm} \tag{1}$$

- Constraints: Bounds on fluxes

$$v_{i,min} \le v_i \le v_{i,max} \tag{2}$$

- Optimisation: Linear programming (LP)

$$\max_{v} v_{bm} \;\text{ such that }\; (1) \text{ and } (2)$$

Dynamic flux balance analysis (dFBA)

- Goal: Determine metabolic fluxes maximizing a cellular objective over time (based on biomass concentration $P(t)$).
- Network model: Metabolism & dynamic

$$\begin{aligned}\dot{y}(t) &= -S^y_y v_y(t)\, P(t)\\ \dot{x}(t) &= S^x_y v_y(t)\, P(t) + S^x_x v_x(t)\, P(t) - S^x_{bm} v_{bm}(t)\, P(t)\\ \dot{P}(t) &= v_{bm}(t)\, P(t)\end{aligned} \tag{3}$$

- Constraints: Bounds on fluxes & flux changes

$$v_{i,min}(y,P) \le v_i(t) \le v_{i,max}(y,P), \qquad |\dot{v}_i(t)| \le \dot{v}_{i,max} \tag{4}$$

- Optimisation: Non-linear dynamic

$$\max_{v(t)} P(t_{end}) \;\text{ such that }\; (3) \text{ and } (4)$$

Resource balance analysis (RBA)

- Goal: Determine cell composition (protein concentrations p) and metabolic fluxes v maximizing the growth rate μ.
- Network model: Metabolism+enzyme production & steady st.

$$\begin{aligned}0 &= S^x_y v_y + S^x_x v_x - \alpha\varepsilon S^x_p v_p\\ 0 &= \varepsilon S^p_p v_p - \mu p\end{aligned} \tag{5}$$

- Constraints: Enzyme capacity & cellular composition

$$\sum_{j \in \mathscr{V}_i} |v_j(t)/k_j| \le p_i, \qquad \sum_i c_i p_i \le 1 \tag{6}$$

- Optimization: Iteratively solving LPs

$$\max_{v,p} \mu \;\text{ such that }\; (5) \text{ and } (6)$$

Dynamic enzyme-cost FBA (deFBA)

- Goal: Determine the dynamic cell composition and metabolic fluxes to maximize a cellular objective over a time interval
- Network model: Metabolism+enzyme production & dynamic

$$\begin{aligned}\dot{Y} = -S^y_y V_y, \qquad \dot{P} &= \varepsilon\, S^p_p V_p,\\ 0 &= S^x_y V_y + S^x_x V_x - \alpha\, \varepsilon\, S^x_p V_p\end{aligned} \tag{7}$$

- Constraints: Enzyme capacity & cellular composition

$$\sum_{j \in \mathscr{V}_i} |V_j(t)/k_j| \le P_i(t), \qquad \sum_i c_i P_i(t) \le 1 \tag{8}$$

- Optimization: Linear dynamic

$$\max_{V,Y,P} \int_0^{t_{end}} c^{\mathrm{T}} P(t) dt \;\text{ such that }\; (7) \text{ and } (8)$$

Constraints

- Enzyme capacity constraints

$$\left|\frac{v_1}{c_1}\right| + \cdots + \left|\frac{v_m}{c_m}\right| \;\leq\; P_E$$

- Biomass-independent flux bounds

$$v_{min} \leq v \leq v_{max}$$

- Non-negativity of molecular species

$$Y \geq 0, \quad P \geq 0$$

- Biomass composition constraints

$$H_B P \leq h_B$$

Dynamic optimization problem

Let $z = (Y, P)$.

$$\max_{\mathscr{V}(\mathscr{Z}, z_0)} \int_0^{t_{end}} \Phi(z(t), v(t))dt \;+\; \Psi(z(t_{end}))$$

$$\begin{aligned}
\text{s.t. } \dot{Y} &= -S_y^y V_y, \quad \dot{P} = \varepsilon\, S_p^p V_p,\\
& S_y^x V_y + S_x^x V_x - \alpha\,\varepsilon\, S_p^x V_p = 0,\\
& z(0) = z_0,\\
& z(t) \geq 0,\\
& v_{min} \leq v(t) \leq v_{max},\\
& H_C v(t) \leq H_E P(t), \quad H_B P(t) \leq h_B.
\end{aligned}$$

Minimal example

- Components: Nutrient Y, metabolite X, generic enzyme P
- Reactions

$$V_y : Y \rightarrow X \;\text{ (uptake)}, \quad V_p : \alpha X \rightarrow P \;\text{ (biomass)}$$

- Enzymatic constraint

$$\frac{V_y}{k_y} + \frac{\varepsilon V_p}{k_p} \leq P$$

- Approximate model (on long time-scales)

$$\dot{Y} = -V_y, \quad \dot{P} = \varepsilon\, V_p, \qquad V_y = \alpha\,\varepsilon\, V_p$$

Objective functionals

- Maximization of terminal biomass

$$J_1 = P(t_{end})$$

- Maximization of discounted biomass integral

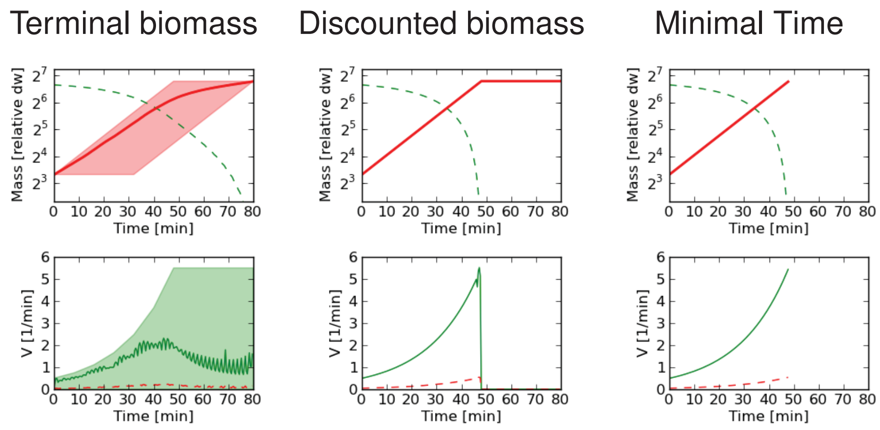
$$J_2 = \int_0^{t_{end}} P(\tau) e^{-\mu\tau} d\tau$$

- Minimization of time to consume nutrients

$$J_3 = -\int_0^{t_{end}} d\tau = -t_{end}$$

with $Y(t_{end}) = 0$.

Optimization results for minimal network



Analytical proof of existence and uniqueness for J_2 and J_3
⇝ mathematical optimum biologically meaningful?

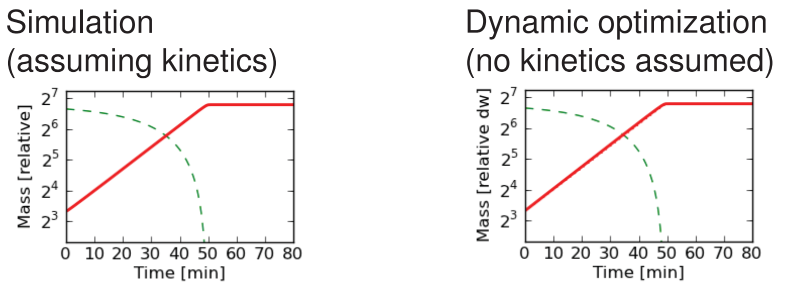
Minimal network yields Monod model

- ▷ Assume Michaelis-Menten kinetics

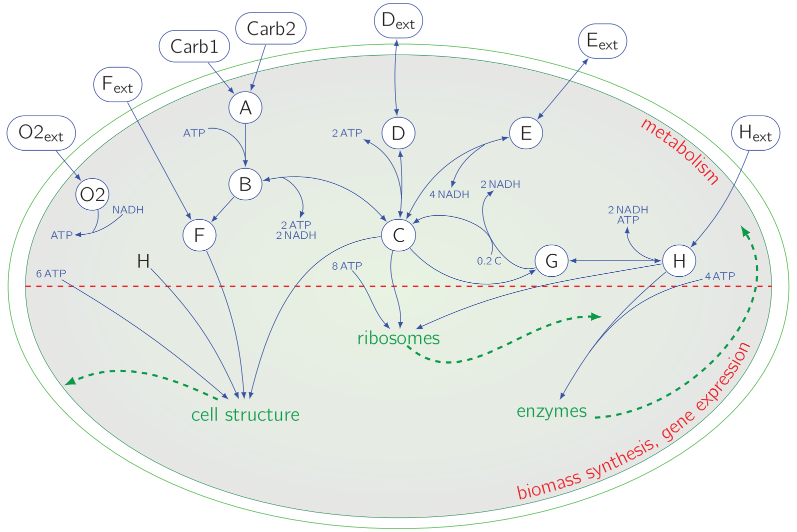
$$V_y = \frac{V_{m,y} P Y}{K_y \vartheta_e + Y}, \qquad \varepsilon V_p = \frac{V_{m,p} P X}{K_p + X}.$$

- ▷ Choose consistent parameters

$$V_{m,y} = \left(\frac{1}{k_y} + \frac{1}{\alpha k_p}\right)^{-1}, \qquad V_{m,p} = \left(\frac{\alpha}{k_y} + \frac{1}{k_p}\right)^{-1}.$$



Core cellular network



Covert et al. 2001 (for the metabolic part)

Network specification

Reaction	Enz	k_{cat}
Exchange reactions		
Carb1 → A	T_{C1}	$3000\ \frac{1}{\min}$
Carb2 → A	T_{C2}	$2000\ \frac{1}{\min}$
$F_{ext} \to F$	T_F	$3000\ \frac{1}{\min}$
$O2_{ext} \to O2$	S	$1000\ \frac{1}{\min}$
$D \leftrightarrow D_{ext}$	S	$1000\ \frac{1}{\min}$
$E \leftrightarrow E_{ext}$	S	$1000\ \frac{1}{\min}$
$H_{ext} \to A$	T_H	$3000\ \frac{1}{\min}$
Metabolic reactions		
$A + ATP \to B$	E_B	$1800\ \frac{1}{\min}$
$B \to C + 2\,ATP + 2\,NADH$	E_C	$1800\ \frac{1}{\min}$
$B \to F$	E_F	$1800\ \frac{1}{\min}$
$C \to G$	E_G	$1800\ \frac{1}{\min}$
$G \to 0.8\,C + 2\,NADH$	E_N	$1800\ \frac{1}{\min}$
$C \leftrightarrow 2\,ATP + 3\,D$	E_D	$1800\ \frac{1}{\min}$
$C + 4\,NADH \leftrightarrow 3\,E$	E_E	$1800\ \frac{1}{\min}$
$G + ATP + 2\,NADH \leftrightarrow H$	E_H	$1800\ \frac{1}{\min}$
$NADH + O \to ATP$	E_T	$1800\ \frac{1}{\min}$

Reaction	Enz	k_{cat}
Biomass reactions		
$400\,H + 1600\,ATP \to T_{C1}$	R	$2.5\ \frac{1}{\min}$
$1500\,H + 6000\,ATP \to T_{C2}$	R	$0.67\ \frac{1}{\min}$
$400\,H + 1600\,ATP \to T_F$	R	$2.5\ \frac{1}{\min}$
$400\,H + 1600\,ATP \to T_O$	R	$2.5\ \frac{1}{\min}$
$400\,H + 1600\,ATP \to T_D$	R	$2.5\ \frac{1}{\min}$
$400\,H + 1600\,ATP \to T_E$	R	$2.5\ \frac{1}{\min}$
$400\,H + 1600\,ATP \to T_H$	R	$2.5\ \frac{1}{\min}$
$500\,H + 2000\,ATP \to E_B$	R	$2\ \frac{1}{\min}$
$500\,H + 2000\,ATP \to E_C$	R	$2\ \frac{1}{\min}$
$1000\,H + 4000\,ATP \to E_D$	R	$1\ \frac{1}{\min}$
$1000\,H + 4000\,ATP \to E_E$	R	$1\ \frac{1}{\min}$
$1500\,H + 6000\,ATP \to E_F$	R	$0.67\ \frac{1}{\min}$
$500\,H + 2000\,ATP \to E_G$	R	$2\ \frac{1}{\min}$
$2500\,H + 10000\,ATP \to E_H$	R	$0.4\ \frac{1}{\min}$
$500\,H + 2000\,ATP \to E_N$	R	$2\ \frac{1}{\min}$
$500\,H + 2000\,ATP \to E_T$	R	$2\ \frac{1}{\min}$
$2000\,H + 4000\,C + 16000\,ATP \to R$	R	$0.2\ \frac{1}{\min}$
$250\,H + 250\,C + 250\,F + 1500\,ATP \to S$	R	$3\ \frac{1}{\min}$

⇝ preferred carbon source Carb1

Case study: Carbon switch

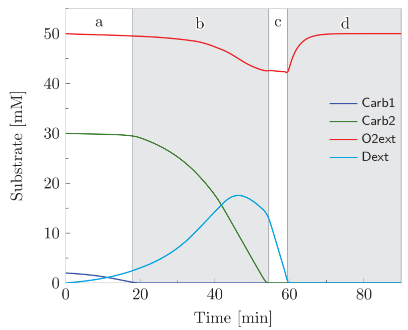
Growth scenario:
- Low amount of preferred carbon source C_1
- High amount of non-preferred carbon source C_2
- Ample oxygen supply

Objective: Discounted biomass

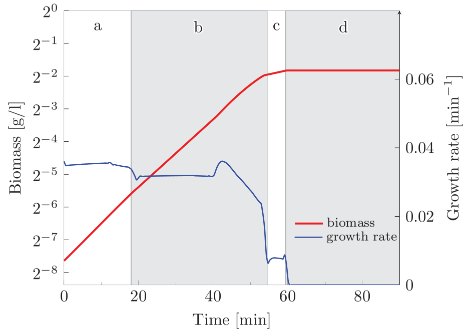
$$J = \int_0^{t_{end}} c_{bm}^{\mathrm{T}} P(t)\, e^{-\mu t} dt$$

Dynamic optimization results I

Substrates



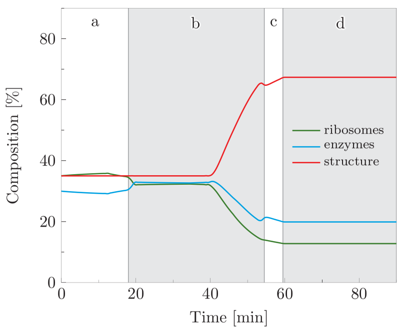
Biomass & Growth



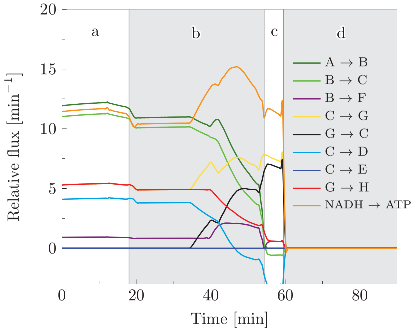
Four cellular growth phases

Dynamic optimization results II

Cell composition



Metabolic fluxes



Cellular reorganisation at the end of phase (b)

Discussion

Modeling metabolism including enzyme costs
- Mass balance ODE model for cellular metabolism and biomass production.
- Time-scale separation yields dynamic biomass model with quasi steady-state metabolic constraints.
- Dynamic optimisation framework: deFBA

Case studies
- Minimal network yields Monod growth model.
- Core network shows different exponential growth phases and pre-adaptation to impending nutrient depletion.
- Choice of the objective functional crucial for the results.