Dynamic optimization of metabolic networks coupled with gene expression

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Joint Work with S. Waldherr and D. Oyarzún

Research Center MATHEON

Mathematics for key technologies

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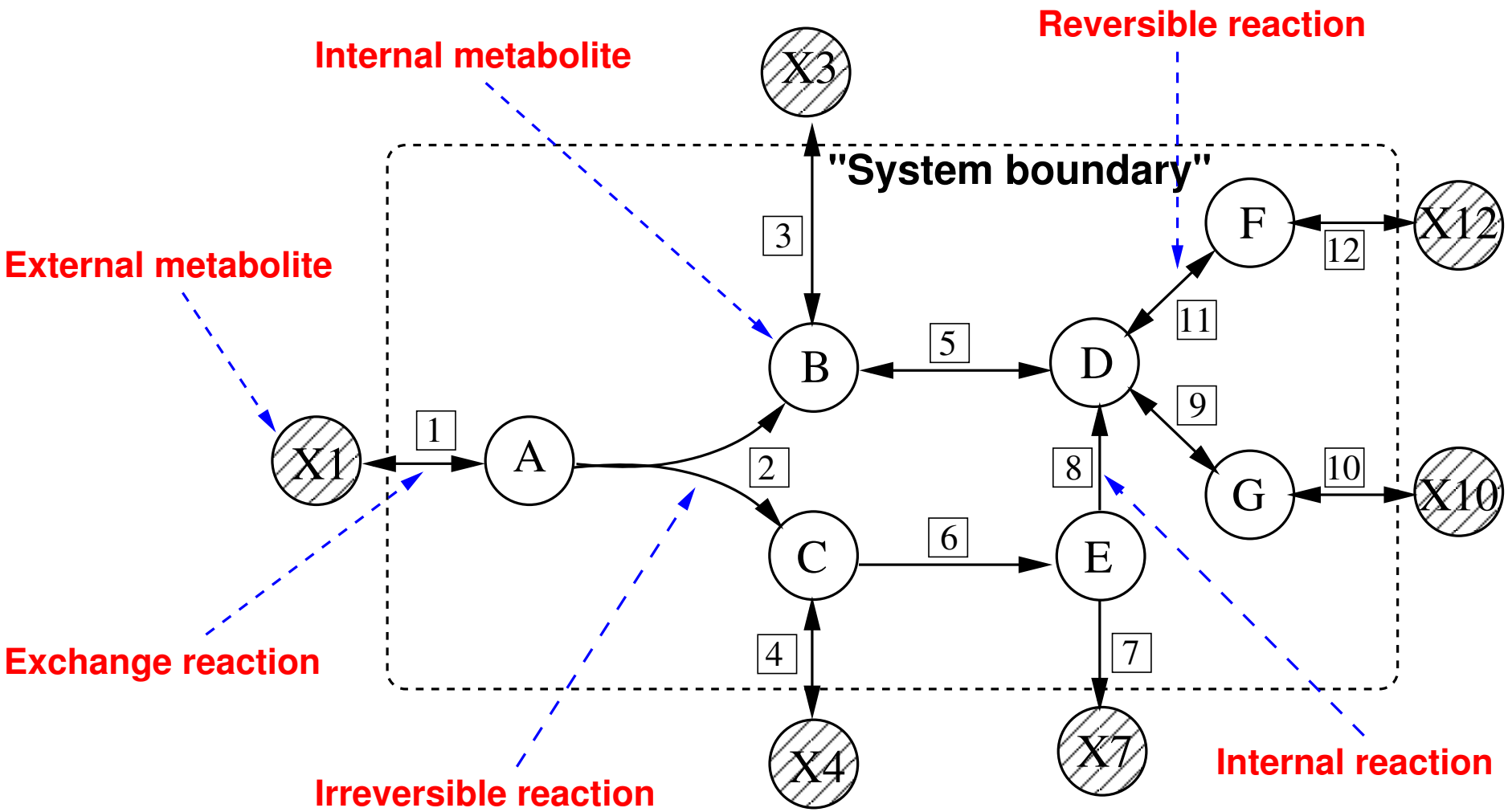
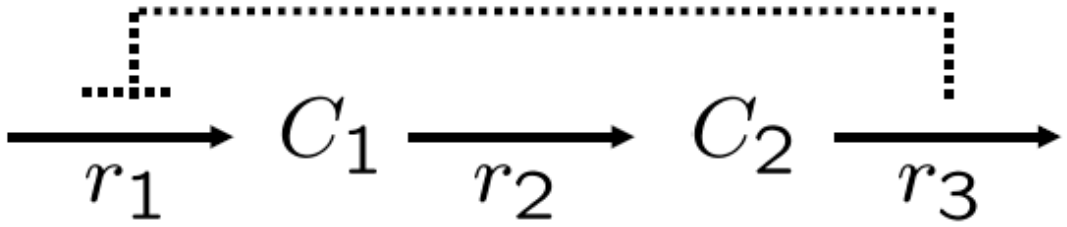

Internal metabolite
Reversible reaction
X3
"System boundary"
3
F
12
X12
External metabolite
11
B
5
D
9
1
X1
A
2
8
G
10
X10
C
6
E
4
7
Exchange reaction
X4
X7
Irreversible reaction
Internal reaction

- Stoichiometric matrix $S \in^{m \times n}$
  - Rows $\rightsquigarrow$ internal metabolites $i = 1, \ldots, m$
  - Columns $\rightsquigarrow$ internal and exchange reactions $j = 1, \ldots, n$
  - S_{ij}: stoichiometric coefficient of reactant i in reaction j
- Set of irreversible reactions Irr
- Metabolic model $\mathcal{M} = (S, Irr)$

- Metabolites i and reactions j
- $C_i(t)$: metabolite concentrations at time t
- $v_j(t) = v_j(C(t), k)$: reaction rates, depending on kinetic law and kinetic parameters k
- S_{ij}: stoichiometric coefficient

$$\frac{dC_i}{dt} = \sum_{j=1}^{n} S_{ij} v_j \quad \text{or} \quad \frac{dC}{dt} = S \cdot v(C, k)$$

- System of ordinary differential equations (ODEs)



$$\begin{pmatrix} dC_1/dt \\ dC_2/dt \end{pmatrix} = \begin{pmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix} \cdot \begin{pmatrix} v_1(C,k) \\ v_2(C,k) \\ v_3(C,k) \end{pmatrix}$$

$$\begin{aligned} v_1(C,k) &= v_{m1}/(1+(C_2/k_i)^p) \\ v_2(C,k) &= v_{m2} \cdot C_1/(k_1+C_1) \\ v_3(C,k) &= v_{m3} \cdot C_2/(k_2+C_2) \end{aligned}$$

Which kinetic laws?

Which kinetic parameters?

- Steady-state assumption
  Assume metabolite concentrations C_i and reaction rates v_j are constant (over some time interval)
  $\rightsquigarrow$ steady-state flux vector $v \in \mathbb{R}^n$
- Stoichiometric constraints (mass balance):

$$\sum_{j=1}^{n} S_{ij} v_j = 0, \text{ for all } i = 1, \ldots, m$$

- Thermodynamic irreversibility constraints:

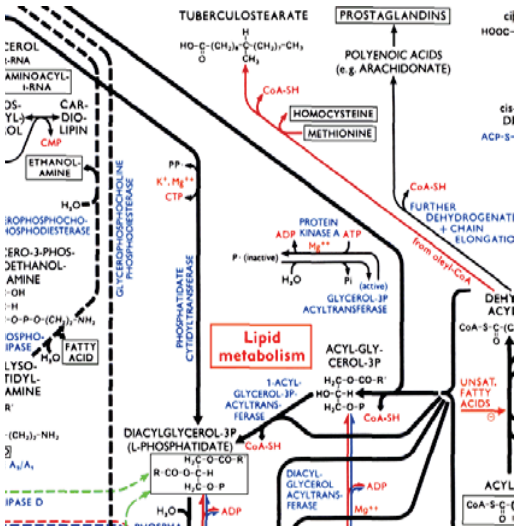
$$v_j \geq 0, \text{ if } j \text{ is irreversible}$$

$\rightsquigarrow$ system of linear equations and inequalities in $\mathbb{R}^n$

Set of all possible steady-state flux distributions

$$C = \{v \in^n | \; Sv = 0, \; v_i \geq 0, \; i \in Irr\}$$

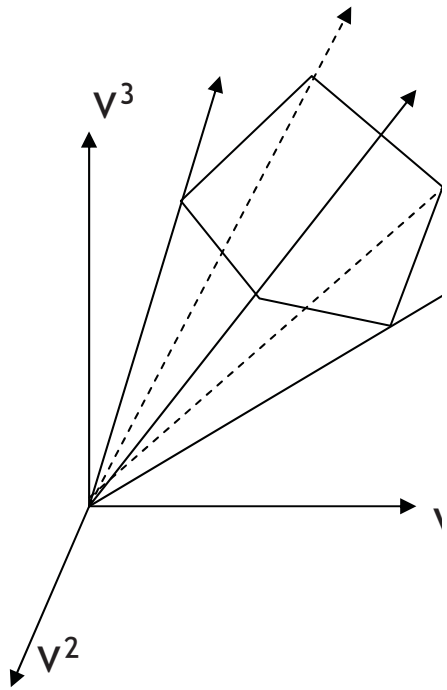
$\rightsquigarrow$ polyhedral cone



Reactions

$$\begin{pmatrix} 1 & 0 & \dots & -1 \\ 0 & 1 & \dots & -2 \\ \dots & \dots & \dots & \dots \\ 2 & 0 & \dots & 1 \end{pmatrix}$$

Metabolites



- Assume cellular behavior is determined by a certain biological objective.
- Determine a corresponding "best" flux distribution.
- Use mathematical optimization to predict phenotype.

- Simplest case: Linear programming (LP)

$$\max\{c^T x \mid Ax \leq b, x \in^n\}$$

- Flux balance problem (FBA) Varma/Palsson 94

$$\max\{c^T v \mid Sv = 0,\ l \leq v \leq u\}$$

Orth/Thiele/Palsson 10

- *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

Waldherr/Oyarzún/Bockmayr 15

$$Y \underset{\uparrow P}{\overset{V_y}{\longleftrightarrow}} X, \quad X \underset{\uparrow P}{\overset{V_x}{\longrightarrow}} \tilde{X}, \quad X \underset{\uparrow P}{\overset{V_p}{\longrightarrow}} P$$

- 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^i_j (species i, reactions j)

- Mass balance

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

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

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

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

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

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

	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 –

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

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

- Constraints: Bounds on fluxes

$$v_{i,min} \leq v_i \leq v_{i,max}$$

- Optimisation: Linear programming (LP)

$$\max_v v_{bm}$$

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

$$\dot{y}(t) = -S_y^y v_y(t)\, P(t)$$
$$\dot{x}(t) = S_y^x v_y(t)\, P(t) + S_x^x v_x(t)\, P(t) - S_{bm}^x v_{bm}(t)\, P(t)$$
$$\dot{P}(t) = v_{bm}(t)\, P(t)$$

- Constraints: Bounds on fluxes & flux changes

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

- Optimisation: Non-linear dynamic

$$\max_{v(t)} P(t_{end})$$

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

$$0 = S_y^x v_y + S_x^x v_x - \alpha\epsilon S_p^x v_p$$
$$0 = \varepsilon S_p^p v_p - \mu p$$

- Constraints: Enzyme capacity & cellular composition

$$\sum_{j\in\mathcal{V}_i} |v_j(t)/k_j| \leq p_i, \qquad \sum_i c_i p_i \leq 1$$

- Optimization: Iteratively solving LPs

$$\max_{v,p} \mu$$

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

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

- Constraints: Enzyme capacity & cellular composition

$$\sum_{j \in \mathcal{V}_i} |V_j(t)/k_j| \leq P_i(t), \qquad \sum_i c_i P_i(t) \leq 1$$

- Optimization: Linear dynamic

$$\max_{V,Y,P} \int_0^{t_{end}} c^{\mathrm{T}} P(t) dt$$

Dynamic optimization problem [with $z = (Y, P)$]

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

s.t. $\dot{Y} = -S_y^y V_y, \quad \dot{P} = \varepsilon S_p^p V_p,$

$S_y^x V_y + S_x^x V_x - \alpha \varepsilon S_p^x V_p = 0,$ Quasi steady-state

$z(0) = z_0, z(t) \geq 0,$

$v_{min} \leq v(t) \leq v_{max},$

$H_C v(t) \leq H_E P(t),$ Enzyme capacity

$H_B P(t) \leq h_B$ Cellular composition

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

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

- Enzymatic constraint

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

- Quasi steady-state approximation

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

- Maximization of terminal biomass

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

- Maximization of discounted biomass integral

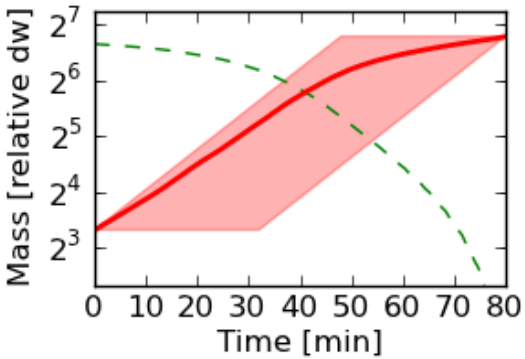
$$J_2 = \int_0^{t_{end}} P(\tau) e^{-\mu\tau} d\tau$$

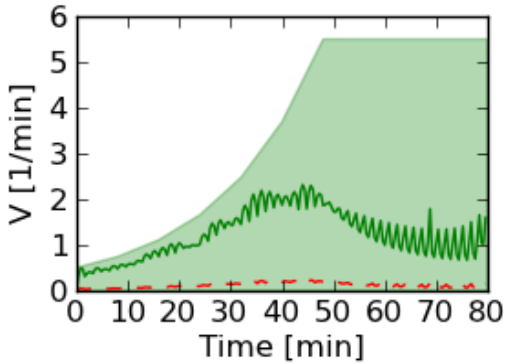
- Minimization of time to consume nutrients

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

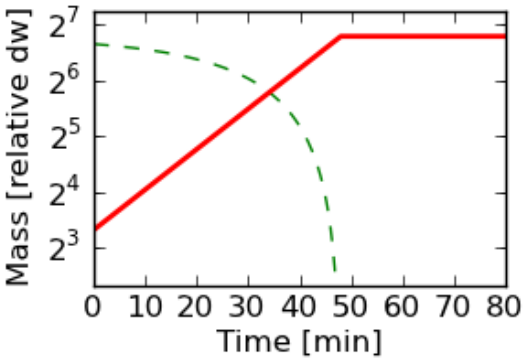
with $Y(t_{end}) = 0$.

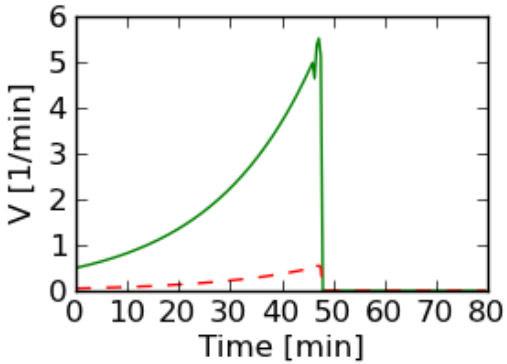
Terminal biomass



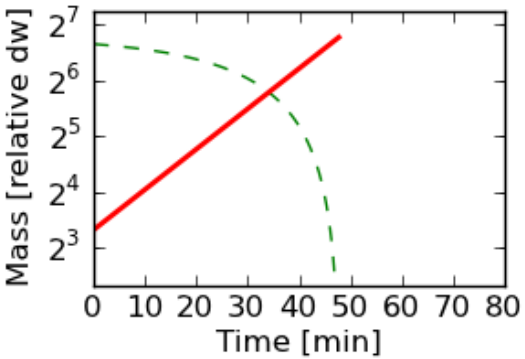


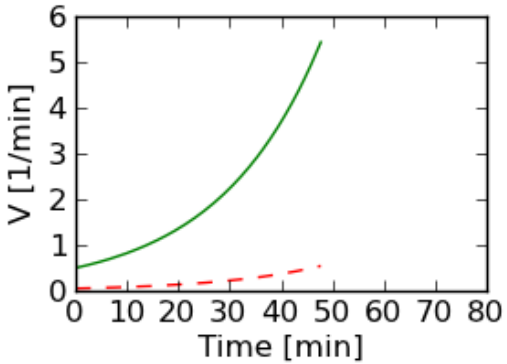
Discounted biomass





Minimal Time





Analytical proof of existence and uniqueness for J_2 and J_3

$\rightsquigarrow$ mathematical optimum biologically meaningful?

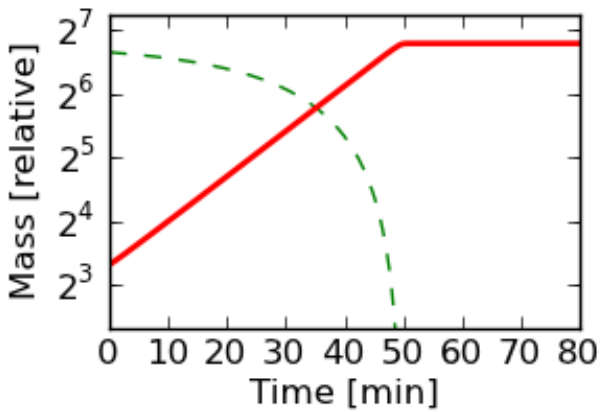
- Assume Michaelis-Menten kinetics

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

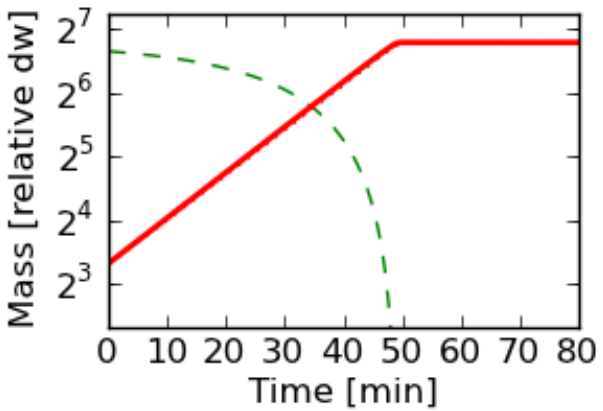
- Choose consistent parameters

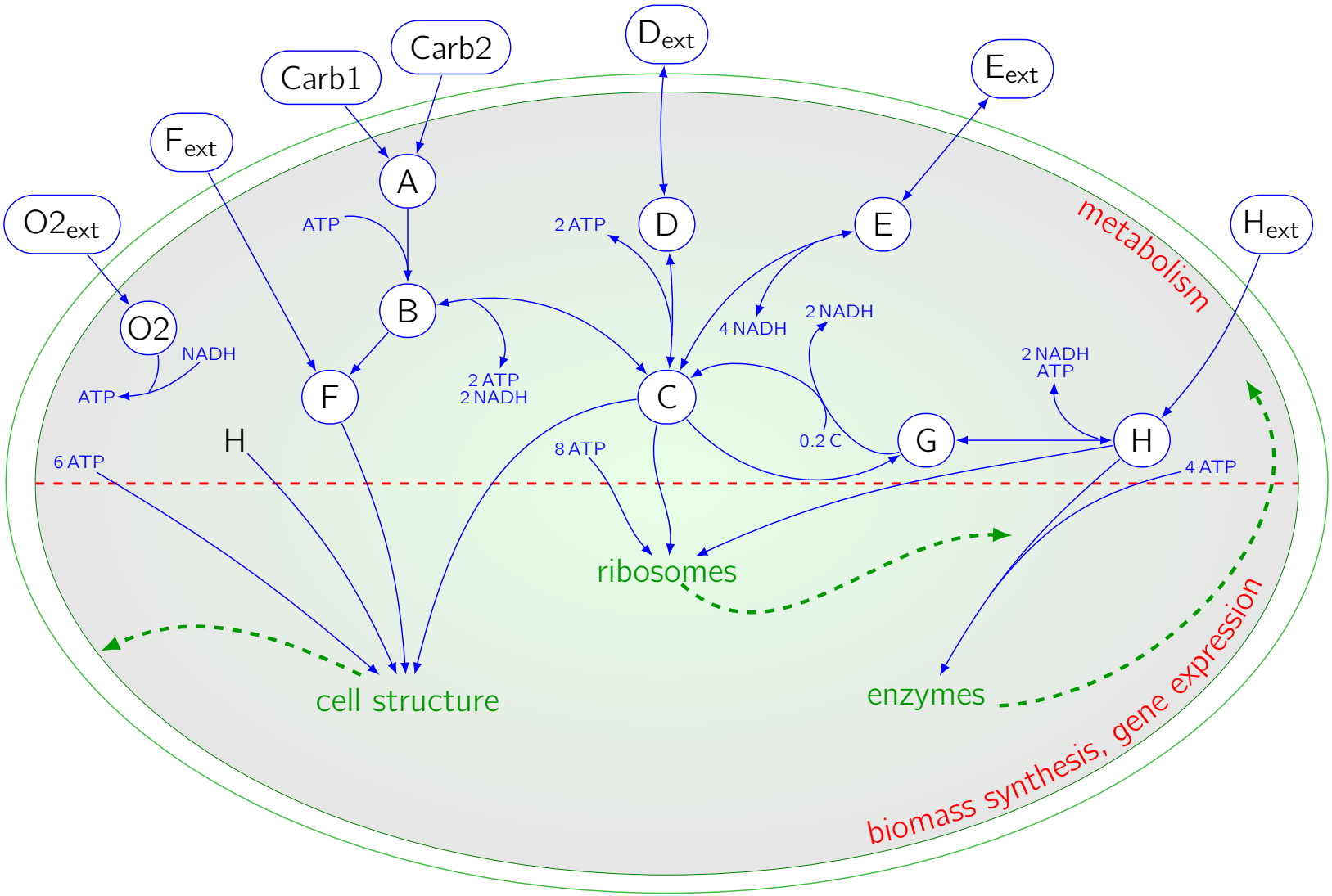
$$V_{m,y} = \left(\frac{1}{k_y} + \frac{1}{\alpha k_p}\right)^{-1}, \qquad V_{m,p} = \left(\frac{\alpha}{k_y} + \frac{1}{k_p}\right)^{-1}.$$

Simulation
(assuming kinetics)



Dynamic optimization
(no kinetics assumed)





Covert et al. 2001 [for the metabolic part]

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

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

$\rightsquigarrow$ preferred carbon source Carb1

- Carbon switch
  - Low amount of preferred carbon source C_1
  - High amount of non-preferred carbon source C_2
  - Ample oxygen supply

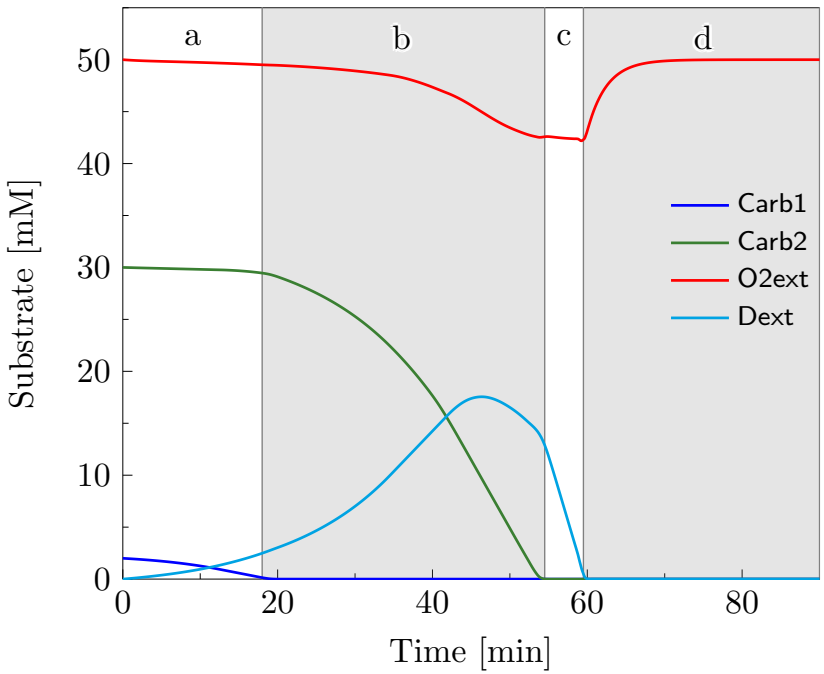
  $\rightsquigarrow$ first consume C_1, then C_2.

- Objective: Discounted biomass

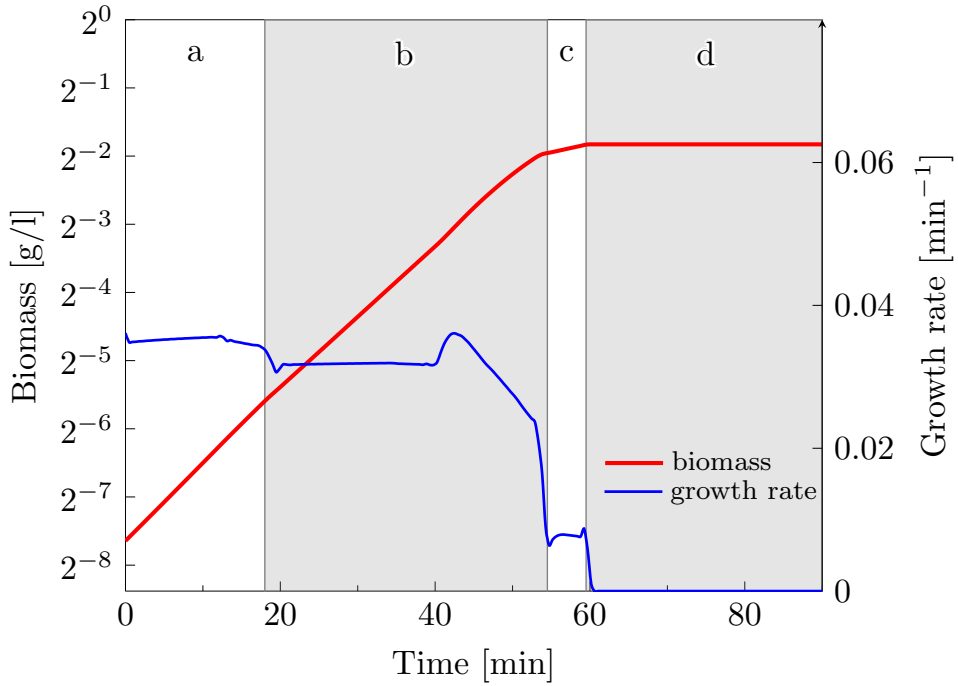
$$J = \int_0^{t_{end}} c_{bm}^{\mathrm{T}} P(t)\, e^{-\mu t}\, dt$$

- Information in the model: Stoichiometry, biomass composition, kcats.
- No information on enzyme activities

Substrates

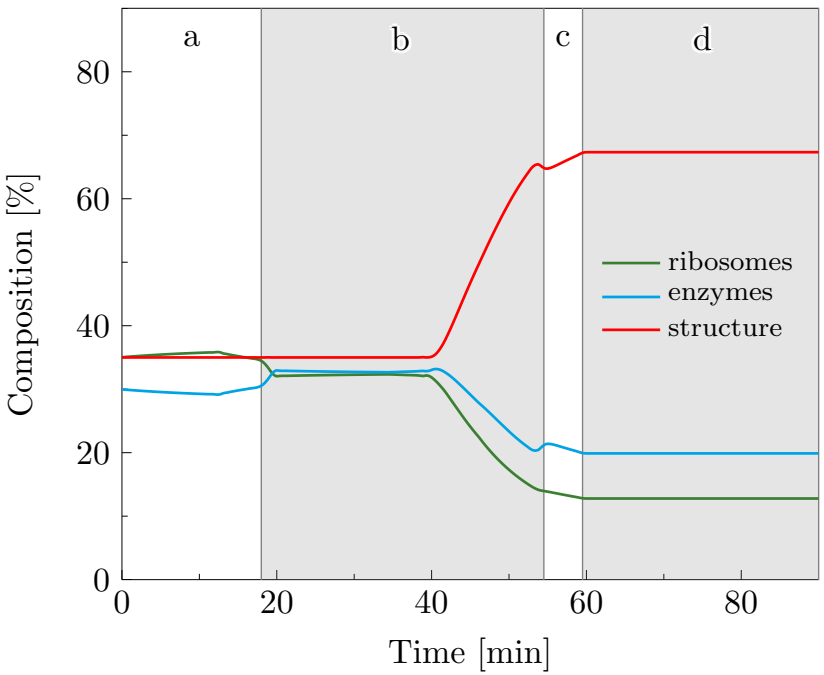


Biomass & Growth

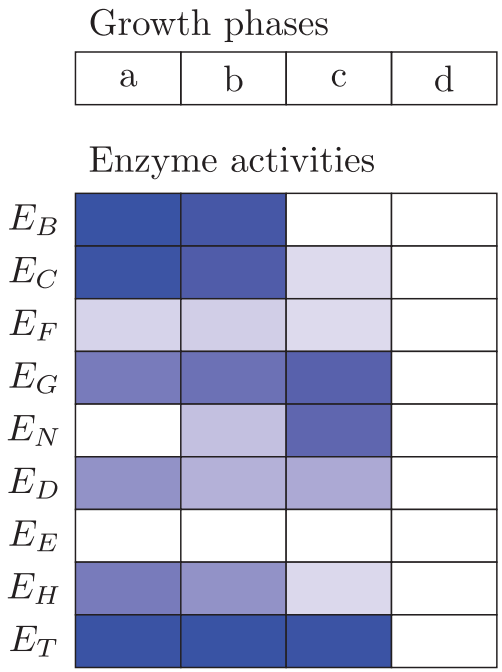


Four cellular growth phases

Cell composition



Enzyme activities



Cellular reorganisation at the end of phase (b)

Modeling metabolism including enzyme costs

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

Related work

- Model of core phototrophic metabolism of cyanobacteria under day/night conditions (with M. Rügen, R. Steuer)
- Model of genome-scale reconstruction of *Synechococcus elongatus 7942* (with A.-M. Reimers, R. Steuer)
- ERASysApp project ROBUSTYEAST (with S. Waldherr, F. Bruggeman, V. Hatzimanikatis)

- Orth JD, Thiele I, Palsson BO. What is flux balance analysis? Nat Biotechnol., 28(3):245-8, 2010.
- Lewis NE, Nagarajan H, Palsson BO. Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico methods. Nat Rev Microbiol., 10(4):291-305, 2012.
- Waldherr S, Oyarzún DA, Bockmayr A. Dynamic optimization of metabolic networks coupled with gene expression. J Theor Biol, 365, 469-485, Jan 2015
- Rügen M, Bockmayr A, Steuer R: Elucidating temporal resource allocation and diurnal dynamics in phototrophic metabolism using conditional FBA. Scientific Reports, 5:15247, Oct 2015