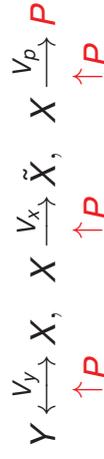## 12. Dynamic enzyme-cost FBA (deFBA)

Dynamic optimization of metabolic networks coupled with gene expression

Waldherr S, Oyarzún DA, Bockmayr A.

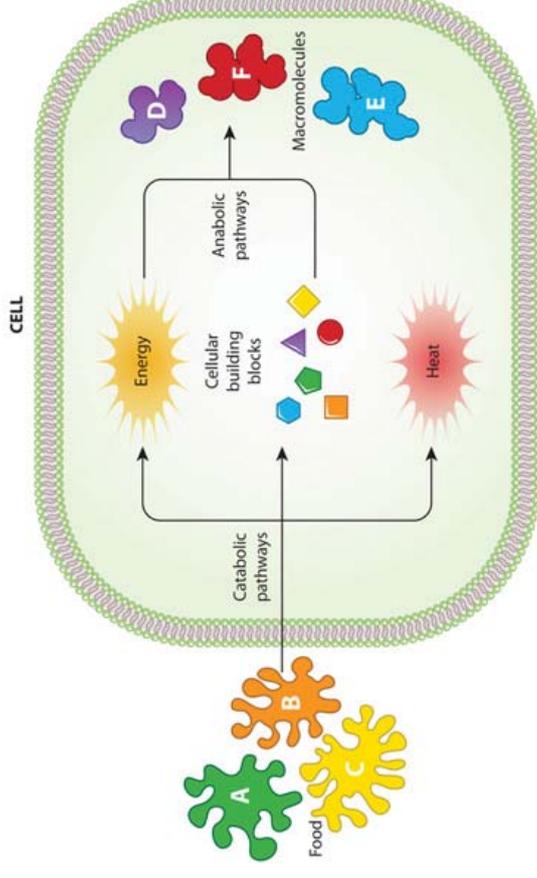Journal of Theoretical Biology, 365, 469-485, 2015

Cellular metabolism

Metabolism and enzyme production

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

△ 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_j (species i, reactions j)

Dynamic model

△ 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

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

$$\dot z = f(x, z, \varepsilon),$$
$$\varepsilon \dot x = g(x, z, \varepsilon), \qquad (*)$$
$$(z(0), x(0)) = (z_0, x_0),$$

with f, g sufficiently smooth, under the following hypotheses:

(H1) there exists a unique solution $x = \bar g(z)$, sufficiently smooth, of $g(x, z, 0) = 0$; the matrix $\partial g/\partial x(\bar g(z), z, 0)$ has all eigenvalues with strictly negative real part;

(H2) the reduced system

$$\dot z = f(\bar g(z), z, 0),$$
$$z(0) = z_0, \qquad (**)$$

has a solution $z^0(t)$ on an interval $[0, T]$, for some $T > 0$;

	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) – TODAY –

▷ 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

(H3) x_0 is in the basin of attraction of the steady state $\bar g(z_0)$ of the fast system $\dot \xi = g(\xi, z_0, 0)$.

If these hypotheses are satisfied, system (*) admits a solution $(x^\varepsilon(t), z^\varepsilon(t))$ on $[0, T]$. In addition,

$$\lim_{\varepsilon \to 0+} z^\varepsilon(t) = z^0(t)$$

and

$$\lim_{\varepsilon \to 0+} x^\varepsilon(t) = x^0(t) = \bar g(z_0)(t),$$

uniformly on any closed interval contained in $(0, T]$.

Flux balance analysis (FBA)

△ Goal: Determine metabolic fluxes maximizing a cellular objective.

△ Network model: Metabolism & steady state

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

△ Constraints: Bounds on fluxes

$$v_{i,min} \leq v_i \leq v_{i,max} \qquad (2)$$

△ Optimisation: Linear programming (LP)

$$\max_v v_{bm} \text{ such that (1) and (2)}$$

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

$$\dot{y}(t) = -S_y^y v_y(t)\,P(t)$$
$$\dot{x}(t) = S_y^x v_y(t)\,P(t) + S_x^x v_x(t)\,P(t) - S_{bm}^x v_{bm}(t)\,P(t) \qquad (3)$$
$$\dot{P}(t) = v_{bm}(t)\,P(t)$$

△ Constraints: Bounds on fluxes & flux changes

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

△ Optimisation: Non-linear dynamic

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

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.

$$0 = S_y^x v_y + S_x^x v_x - \alpha\varepsilon S_p^x v_p$$
$$0 = \varepsilon S_p^p v_p - \mu p \qquad (5)$$

△ Constraints: Enzyme capacity & cellular composition

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

△ Optimization: Iteratively solving LPs

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

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

$$\dot{Y} = -S_y^y V_y, \qquad \dot{P} = \varepsilon S_p^p V_p,$$
$$0 = S_y^x V_y + S_x^x V_x - \alpha\varepsilon S_p^x V_p \qquad (7)$$

△ Constraints: Enzyme capacity & cellular composition

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

△ Optimization: Linear dynamic

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

Constraints

▷ Enzyme capacity constraints

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

▷ Biomass-independent flux bounds

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

▷ Non-negativity of molecular species

$$Y \ge 0, \quad P \ge 0$$

▷ Biomass composition constraints

$$H_B P \le h_B$$

Dynamic optimization problem

Let $z = (Y, P)$.

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

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

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

$\qquad z(0) = z_0$,

$\qquad z(t) \ge 0$,

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

$\qquad H_C v(t) \le H_E P(t), \quad H_B P(t) \le h_B.$

Minimal example

▷ 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

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

▷ Approximate model (on long time-scales)

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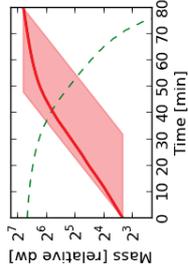
Objective functionals

▷ 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

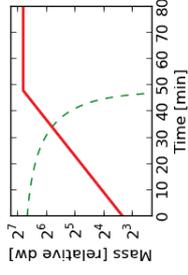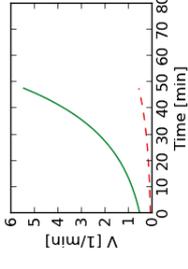$$J_3 = -\int_0^{t_{end}} d\tau = -t_{end}$$

with $Y(t_{end}) = 0.$
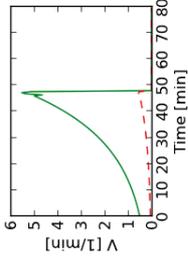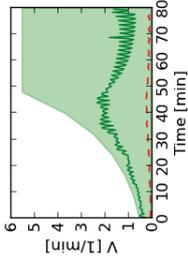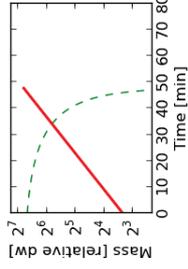
Optimization results for minimal network

Terminal biomass   Discounted biomass   Minimal Time

Analytical proof of existence and uniqueness for J_2 and J_3

↪ mathematical optimum biologically meaningful?

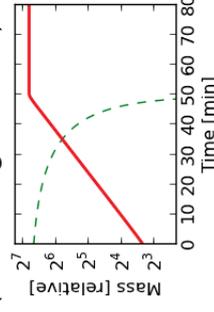## Minimal network yields Monod model

△ Assume Michaelis-Menten kinetics

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

△ Choose consistent parameters

$$V_{m,y} = \left(\frac{1}{k_y}+\frac{1}{\alpha k_p}\right)^{-1}, \qquad V_{m,p} = \left(\frac{\alpha}{k_y}+\frac{1}{k_p}\right)^{-1}.$$
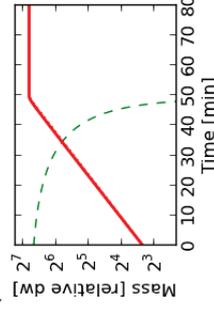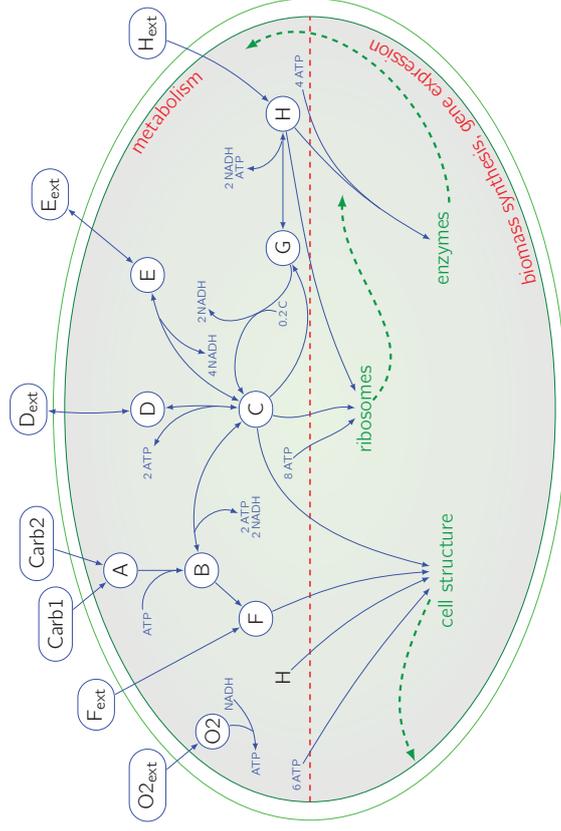
Simulation (assuming kinetics)   Dynamic optimization (no kinetics assumed)

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} → F	T_F	$3000\ \frac{1}{min}$
$O2_{ext}$ → O2	S	$1000\ \frac{1}{min}$
D ↔ D_{ext}	S	$1000\ \frac{1}{min}$
E ↔ E_{ext}	S	$1000\ \frac{1}{min}$
H_{ext} → A	T_H	$3000\ \frac{1}{min}$
Metabolic reactions		
A + ATP → B	E_B	$1800\ \frac{1}{min}$
B → C + 2ATP + 2NADH	E_C	$1800\ \frac{1}{min}$
B → F	E_F	$1800\ \frac{1}{min}$
C → G	E_G	$1800\ \frac{1}{min}$
G → 0.8C + 2NADH	E_N	$1800\ \frac{1}{min}$
C ↔ 2ATP + 3D	E_D	$1800\ \frac{1}{min}$
C + 4NADH ↔ 3E	E_E	$1800\ \frac{1}{min}$
G + ATP + 2NADH ↔ H	E_H	$1800\ \frac{1}{min}$
NADH + O → ATP	E_T	$1800\ \frac{1}{min}$

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

↝ preferred carbon source Carb1

Dynamic optimization results I

Biomass & Growth

Substrates

Four cellular growth phases

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.

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

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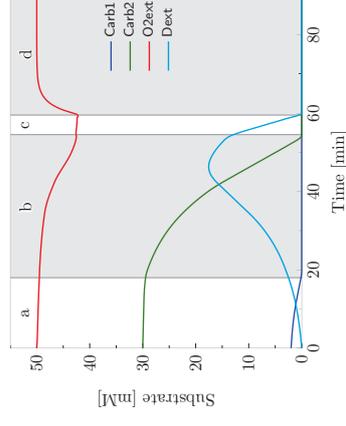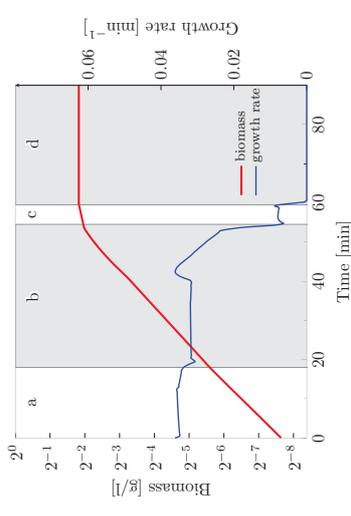

Dynamic optimization results II

Metabolic fluxes

Cell composition

Cellular reorganisation at the end of phase (b)