Dynamic optimization of metabolic networks coupled with gene expression

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Cellular metabolism

Metabolism and enzyme production

\[
\begin{align*}
Y & \xleftarrow{V_y} X, \\
X & \xrightarrow{V_x} \bar{X}, \\
X & \xrightarrow{V_p} P
\end{align*}
\]

▷ 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

\[
\begin{align*}
\dot{Y} &= -S^y_y V_y \\
\dot{P} &= S^p_p V_p \\
\dot{X} &= S^x_y V_y + S^x_x V_x - S^x_p V_p
\end{align*}
\]

▷ Macromolecule production is slow: small \( \varepsilon \)

▷ Macromolecules are made from many components: large \( \alpha \)

\[
\begin{align*}
\dot{Y} &= -S^y_y V_y \\
\dot{P} &= \varepsilon S^p_p V_p \\
\dot{X} &= S^x_y V_y + S^x_x V_x - \varepsilon \alpha S^x_p V_p
\end{align*}
\]
Quasi steady state approximation

- Time-scale separation (using Tikhonov’s theorem)
  \[ \dot{Y} = -S^T_y V_y, \]
  \[ \dot{P} = \varepsilon S^T_p V_p, \]
  \[ 0 = S^T_y V_y + S^T_x V_x - \alpha \varepsilon S^T_p V_p. \]

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

- Model reduction

Tikhonov’s theorem

\[ \dot{z} = f(x, z, \varepsilon), \]
\[ \dot{x} = g(x, z, \varepsilon), \]
\[ (z(0), x(0)) = (z_0, x_0), \]

with \( f, g \) sufficiently smooth, under the following hypotheses:

(H1) There exists a unique solution \( x = g(z) \), sufficiently smooth, of \( g(x, z, 0) = 0 \); the matrix \( \frac{\partial g}{\partial x}(g(z), z, 0) \) has all eigenvalues with strictly negative real part;

(H2) The reduced system
\[ \dot{z} = f(g(z), z, 0), \]
\[ z(0) = z_0, \]
has a solution \( z^0(t) \) on an interval \([0, T]\), for some \( T > 0\);

(H3) \( x_0 \) is in the basin of attraction of the steady state \( g(z_0) \) 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
\[ \lim_{\varepsilon \to 0^+} x^\varepsilon(t) = x^0(t) = g(z_0)(t), \]
uniformly on any closed interval contained in \((0, T]\).

Steady-state vs. dynamic optimisation

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Flux balance analysis (FBA)

- **Goal:** Determine metabolic fluxes maximizing a cellular objective.
- **Network model:** Metabolism & steady state
  \[ 0 = S_y v_y + S_x v_x - S_{bm} v_{bm} \]
- **Constraints:** Bounds on fluxes
  \[ v_{i,min} \leq v_i \leq v_{i,max} \]
- **Optimization:** Linear programming (LP)
  \[ \max v_{bm} \text{ such that (1) and (2)} \]

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
  \[
  \dot{y}(t) = -S_y^x v_y(t) P(t) \\
  \dot{x}(t) = S_x^y v_x(t) P(t) + S_x^x v_x(t) P(t) - S_{bm}^x v_{bm}(t) P(t) \\
  \dot{P}(t) = v_{bm}(t) P(t)
  \]
- **Constraints:** Bounds on fluxes & flux changes
  \[ v_{i,min}(y,P) \leq v_i(t) \leq v_{i,max}(y,P), \quad |\dot{v}_i(t)| \leq \dot{v}_{i,max} \]
- **Optimization:** 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 \( \mu \).
- **Network model:** Metabolism+enzyme production & steady st.
  \[ 0 = S_y^x v_y + S_x^x v_x - \alpha \varepsilon S_p^x v_p \]
  \[ 0 = \varepsilon S_p^p v_p - \mu p \]
- **Constraints:** Enzyme capacity & cellular composition
  \[ \sum_{j \in V_i} \left| v_j(t)/k_j \right| \leq p_i, \quad \sum_i c_i p_i \leq 1 \]
- **Optimization:** Iteratively solving LPs
  \[ \max_{\mu,p} \mu \text{ such that (5) and (6)} \]

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
  \[
  \dot{Y} = -S_y^x V_y, \quad \dot{P} = \varepsilon S_p^p V_p, \\
  0 = S_x^y V_y + S_x^x V_x - \alpha \varepsilon S_p^x V_p
  \]
- **Constraints:** Enzyme capacity & cellular composition
  \[ \sum_{j \in V_i} \left| V_j(t)/k_j \right| \leq P_i(t), \quad \sum_i c_i P_i(t) \leq 1 \]
- **Optimization:** Linear dynamic
  \[ \max_{V,Y,P} \int_{0}^{t_{end}} c^T P(t)dt \text{ such that (7) and (8)} \]
Constraints

- Enzyme capacity constraints
  \[ \left| \frac{v_1}{c_1} \right| + \cdots + \left| \frac{v_m}{c_m} \right| \leq P_E \]
- 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 \( z = (Y, P) \).

\[
\max_{y(t), z_0} \int_0^{t_{end}} \Phi(z(t), v(t)) \, dt \quad + \quad \psi(z(t_{end}))
\]

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

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
  \[ \frac{V_y}{k_y} + \frac{\epsilon V_p}{k_p} \leq P \]
- Approximate model (on long time-scales)
  \[ \dot{Y} = -V_y, \quad \dot{P} = \epsilon V_p, \quad V_y = \alpha \epsilon V_p \]

Objective functionals

- Maximization of terminal biomass
  \[ J_1 = P(t_{end}) \]
- Maximization of discounted biomass integral
  \[ J_2 = \int_0^{t_{end}} P(t) e^{-\mu \tau} \, d\tau \]
- 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?

Core cellular network

Network specification

Minimal network yields Monod model

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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

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

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.