Iterated flux balance analysis

Varma/Palsson 1994

Flux balance model

\[ S \cdot v = b \]

(b net metabolic uptake)

Objective

\[
\min \ Z = -v_{growth} \\
\sum_{all M} d_M \cdot M \quad v_{growth} \quad \text{biomass}
\]

Divide experimental time into small time steps \( \Delta t \).

Specify initial values for external concentrations.

Use flux balance model to predict concentrations for the next step (dynamic profiles).

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Iterative algorithm

1. Determine substrate concentration \( S_c \) from previous substrate concentration \( S_{co} \) and additional supply:

\[ S_c = S_{co} + \frac{\text{supply} \cdot \Delta t}{\text{vol}} \]

2. Scale substrate concentration

\[ \text{Substr_avail} = \frac{S_c}{X \cdot \Delta t} \]

(\( X \) is the cell density)

3. Use FBA to determine actual substrate uptake rate \( S_u \), growth rate \( \mu \), and potential by-product secretion.

4. Compute new concentrations

\[ \frac{dX}{dt} = \mu X \quad \Rightarrow \quad X = X_0 \cdot e^{\mu \Delta t} \]

\[ \frac{dS_c}{dt} = -S_u \cdot X \quad \Rightarrow \quad S_c = S_{co} + \frac{S_u}{\mu} X_0 (1 - e^{\mu \Delta t}) \]

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Regulatory constraints

Gene \( G \) is transcribed by a process \( \text{trans} \) to produce an enzyme \( E \).
This enzyme then catalyses a reaction \( \text{rxn} \) which converts substrate \( A \) into product \( B \).
Product \( B \) then represses transcription of \( G \), leading to depletion of \( E \).
Regulatory flux balance analysis (rFBA)

Covert/Schilling/Palsson 2001

Refinement of iterative FBA

Divide experimental time into small time steps $\Delta t$.

Reactions may happen in a given time interval $[t_1,t_2]$, if corresponding regulatory constraints are satisfied.

If a regulatory constraint for reaction $k$ does not hold in $[t_1,t_2]$, we impose the temporary constraint $v_k(t) = 0$ when $t \in [t_1,t_2]$.

Simplified core carbon metabolism

Covert/Schilling/Palsson 2001

Mathematical model I

Preferential carbon source uptake

Assume Carbon1 to be the preferred carbon source.

Presence of extracellular Carbon1 activates a regulatory protein which inhibits the transcription of the gene which encodes a protein for transport of Carbon2 into the cell, via a transport process $Tc2$.

$RPc1$ is the regulatory protein which senses extracellular Carbon1,

$tTc2$ is the occurrence of a transcription event (which will eventually result in the protein enabling transport process $Tc2$ and the relaxation of one regulatory constraint, $v_{Tc2} = 0$).

\[
RPc1 = IF (\text{Carbon1})
\]

\[
tTc2 = IF NOT (RPc1)
\]
Anaerobic growth

The transcription of many enzymes is regulated according to whether or not oxygen is available to the cell.

Here, the presence of Oxygen will inactivate regulatory protein RPO2, which inhibits transcription of the genes for Rres and R5a but induces transcription of the gene for R5b.

R5a and R5b are reactions catalyzed by isozymes.

\[
\begin{align*}
RPO2 & = \text{IF NOT (Oxygen)} \\
tRres & = \text{IF NOT (RPO2)} \\
tR5a & = \text{IF NOT (RPO2)} \\
tR5b & = \text{IF (RPO2)}.
\end{align*}
\]

Amino acid biosynthesis pathway repression

The transcription of amino acid biosynthesis genes is often induced by a low intracellular concentration.

Since intracellular concentrations cannot be determined by FBA, use fluxes to approximate the regulation.

Metabolite \( H \) represents the amino acid, and can be made by the cell via reaction R8a or transported from the extracellular media through transport process \( Th \).

For the regulatory structure, \( Th \) will be used to activate \( RPh \) which will repress transcription of the gene encoding R8a.

\[
\begin{align*}
RPh & = \text{IF } (v_{Th} > 0), \\
tR8a & = \text{IF NOT (RPh)}.
\end{align*}
\]

Maintain concentrations

Transcriptional regulation maintains concentration levels of important metabolites.

The activation or repression of these genes depends on the level of B in the cell.

Use a flux rather than concentration to turn off an enzyme.

Choose R2b as the determining factor; it will activate \( RPh \) which in turn will inactivate \( tR2a \) and \( tR7 \).

\[
\begin{align*}
RPh & = \text{IF } (v_{R2b} > 0), \\
tR2a & = \text{IF NOT (RPh)}, \\
tR7 & = \text{IF NOT (RPh)}.
\end{align*}
\]

Mathematical model II

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Name</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic reactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1 ( A ) - 1 ATP + 1 ( B )</td>
<td>( R1 )</td>
<td>( \text{IF NOT (RPh)} )</td>
</tr>
<tr>
<td>- 1 ( B ) + 2 ATP + 2 NADH + 1 ( C )</td>
<td>( R2a )</td>
<td>( \text{IF NOT (RPO2)} )</td>
</tr>
<tr>
<td>- 1 ( C ) - 2 ATP + 2 NADH + 1 ( B )</td>
<td>( R2b )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( B ) + 1 ( F )</td>
<td>( R3 )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( C ) + 1 ( G )</td>
<td>( R4 )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( G ) + 0.5 ( C ) + 2 NADH</td>
<td>( R5a )</td>
<td>( \text{IF NOT (RPO2)} )</td>
</tr>
<tr>
<td>- 1 ( G ) + 0.5 ( C ) + 2 NADH</td>
<td>( R5b )</td>
<td>( \text{IF RPO2} )</td>
</tr>
<tr>
<td>- 1 ( C ) + 2 ATP + 3 ( D )</td>
<td>( R6 )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( C ) - 4 NADH + 3 ( E )</td>
<td>( R7 )</td>
<td>( \text{IF NOT (RPh)} )</td>
</tr>
<tr>
<td>- 1 ( G ) - 1 ATP - 2 NADH + 3 ( H )</td>
<td>( R8a )</td>
<td>( \text{IF NOT (RPh)} )</td>
</tr>
<tr>
<td>- 1 ( G ) + 1 ATP + 2 NADH - 3 ( H )</td>
<td>( R8b )</td>
<td></td>
</tr>
<tr>
<td>- 1 NADH - 1 ( O_2 ) + 1 ATP</td>
<td>( Rres )</td>
<td>( \text{IF NOT (RPO2)} )</td>
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<tr>
<td>Transport processes</td>
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<td></td>
</tr>
<tr>
<td>- 1 ( Carbon1 + 1 )</td>
<td>( T_{-1} )</td>
<td>( \text{IF NOT (RPh)} )</td>
</tr>
<tr>
<td>- 1 ( Carbon2 + 1 )</td>
<td>( T_{+1} )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( F_{ext} + 1 )</td>
<td>( T_{f} )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( B_{ext} + 1 )</td>
<td>( T_{b} )</td>
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<tr>
<td>- 1 ( E_{ext} + 1 )</td>
<td>( T_{e} )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( M_{ext} + 1 )</td>
<td>( T_{m} )</td>
<td></td>
</tr>
<tr>
<td>- 1 ( Oxygen + 1 )</td>
<td>( T_{o} )</td>
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<tr>
<td>Maintenance and growth processes</td>
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<td>Growth</td>
</tr>
<tr>
<td>- 1 ( C ) - 1 ( F ) - 1 ( H ) - 10 ATP + 1 Biomass</td>
<td></td>
<td></td>
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<tr>
<td>Regulatory proteins</td>
<td></td>
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</tr>
<tr>
<td>- ( RPO2 )</td>
<td>( \text{IF NOT (RPh)} )</td>
<td></td>
</tr>
<tr>
<td>- ( RPh )</td>
<td>( \text{IF Carbon1} )</td>
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</tr>
<tr>
<td>( tRPh )</td>
<td>( \text{IF (RPh)} )</td>
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<tr>
<td>( tRf )</td>
<td>( \text{IF (RPh)} )</td>
<td></td>
</tr>
<tr>
<td>( tRb )</td>
<td>( \text{IF (RPh)} )</td>
<td></td>
</tr>
</tbody>
</table>
Generating dynamic profiles (rFBA)

Covert/Schilling/Palsson 2001

Divide experimental time into small time steps $\Delta t$.

At a given time point, use linear programming to identify an optimal metabolic flux distribution (by maximizing the growth flux).

Using the resulting flux distribution and the conditions of the system in a previous time step, the conditions of the next time step are calculated to obtain biomass as well as extracellular substrate and by-product concentrations.

Not considered: Variability of the optimal flux distribution

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Numerical parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum transport rates (mmol g-DCW$^{-1}$ hr$^{-1}$)</td>
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<tr>
<td>Carbon1</td>
<td>10.5</td>
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<tr>
<td>Carbon2</td>
<td>10.5</td>
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<tr>
<td>$D$</td>
<td>12.0</td>
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<tr>
<td>$E$</td>
<td>12.0</td>
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<td>$F$</td>
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<tr>
<td>$H$</td>
<td>5.0</td>
</tr>
<tr>
<td>$O2$</td>
<td>15.0</td>
</tr>
<tr>
<td>Protein synthesis/decay delay (hr)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

five simulations to illustrate each regulatory element separately and in a complex medium

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1. Catabolite repression

2. Aerobic/anaerobic diauxie
3. Amino acid biosynthesis

4. Growth on carbon and amino acid

5. Growth on complex media

5. Growth on complex media (ctd)
Discussion

1. quantitative dynamic simulation of substrate uptake, cell growth and by-product secretion;
2. qualitative simulation of gene transcription events and the presence of proteins in the cell;
3. investigation of the systemic effects of imposing temporary regulatory constraints on the solution space.

Regulatory constraints and extreme pathways

Covert/Palsson 2003
- Split all internal reversible reactions in a metabolic network, the flux cone becomes pointed.
- Extreme rays of the flux cone are called extreme pathways.
- Certain extreme pathways may not be permitted due to regulatory or environmental constraints.

Impact of environment

25 = 32 possible environments
21 extreme pathways always impossible due to regulatory constraints
several environments show (near-)identical sets of extreme pathways.
Highest number of available pathways is 26, lowest number is 2.

Simplified core carbon metabolism

Covert/Schilling/Palsson 2001
- 80 extreme pathways (if neglecting regulatory constraints)
Growth on C1, C2 and O2

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</tr>
</tbody>
</table>

Four remaining extreme pathways

- 26 extreme pathways, four groups with high similarity
- Small degree of variation, once regulatory constraints are taken into account.

References

Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type Escherichia coli W3110.
Varma A, Palsson BO.

Regulation of gene expression in flux balance models of metabolism.
Covert MW, Schilling CH, Palsson B.

Transcriptional regulation in constraints-based metabolic models of Escherichia coli.
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Constraints-based models: regulation of gene expression reduces the steady-state solution space.
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