Expression, Regulation, and Steady State Metabolic Modeling

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Quantitative Flux Prediction

Can models quantitatively predict fluxes and/or growth rate?

• Predict externally measurable fluxes or growth rate as function of
  – Controlled uptake rates
  – Environmental conditions
  – Quasi-steady state growth predictions

Growth vs Uptake Fluxes

Predict growth rate as function of oxygen and acetate (or succinate) uptake rates.

Compare to measurements of uptake and growth from batch reactors.

FBA Summary

Stoichiometric Matrix
Gene annotation
Enzyme and reaction catalyzing

Feasible Space
\[ \mathbf{S} \mathbf{v} = \mathbf{0} \]
Add constraints:
\[ v_i > 0 \]
\[ \alpha_i < v_i < \beta_i \]

Optimal Flux
Growth objective
\[ \mathbf{Z} = \mathbf{c} \mathbf{v} \]
Solve with linear programming

In silico predictions of Escherichia coli metabolic capabilities are consistent with experimental data

Jeremy S. Edwards, Rafael G. Barreto, and Samuel D. Pollock

A significant goal of the post-genome era is to study the unanticipated gene sequence and physiological function of a cell. Working from the annotated genome sequence, as well as biochemical and physiological information, it is possible to generate complete metabolic networks. Furthermore, complex cells can be grown under a range of growth conditions where the culture interacts with its environment. We have constructed a metabolic network for the E. coli model and reported metabolic pathways (based on this hypothesis), an advanced process that defines the quantitative relationship between autotrophy, its physical, chemical, and environmental conditions. We have integrated this metabolic network with mathematical optimization to obtain a quantitative description of the cell's metabolic parameters. The results have also been validated with measurements of the cell's metabolic fluxes under a range of growth conditions. We have developed a metabolic model of the E. coli network under a range of growth conditions. This has led to the hypothesis that a E. coli metabolic network is optimized to maintain growth under the environmental conditions considered. This study demonstrates how the combination of an in vitro and computational biology can be used to obtain a quantitative description of the cell's metabolic parameters. The results have also been validated with measurements of the cell's metabolic fluxes under a range of growth conditions.

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Growth vs Uptake Fluxes

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Uptake vs Growth

Glucose uptake rate was specified

Simulations predicted growth, oxygen uptake, and acetate secretion


Quasi Steady State Modeling

Can we use a steady state model of metabolism to predict the time-dependent changes in the cell or environments?

QSSA Assumptions

• Metabolism adjusts to changes in environment/cell more rapidly than the changes themselves
• Cell and environment concentrations may be changing, but metabolism operates as if concentration is static at each point in time (i.e. steady state)

Modeling Substrate/Growth Dynamics

Divide time into slices of $\Delta t$

For each time, $t$

1. Use FBA to predict substrate uptake ($S_u$) & growth ($g$) by cells during interval $\Delta t^*$

$$\frac{dB}{dt} = gB ightarrow B = B_0 \cdot e^{gt}$$

2. Integrate to obtain biomass ($B$) & substrate concentration at next time point $t + \Delta t$

$$\frac{dS_c}{dt} = -S_u \cdot B \rightarrow S_c = S_{c0} \cdot \left[ e^{gt} - 1 \right]$$

*quasi steady state assumption means these predictions are constant over $\Delta t$

E. Coli QSS Predictions

Limited initial glucose supply Continuous glucose feed

Regulation in Metabolic Models

• Metabolic flux controlled by many levels of regulation
  - Metabolic regulation
  - Transcriptional regulation
  - Translational regulation
  - Post-translational regulation
• Many errors in FBA predictions can be explained by known gene regulation
• How can we incorporate regulation into metabolic models?
Regulation as Boolean Logic

trans = IF (G) AND NOT (B)
rxn = IF (A) AND (E)  
(also must specify protein synthesis and degradation delays)

If an enzyme is not present the flux through corresponding reaction is zero (i.e. NOT rxn)

Not quantitative, but can we predict qualitative flux shifts?

Overview

Simple Regulometabolic Model


Simulate diauxic shift

(RpC1 senses Carbon1

RpC1 = IF (Carbon1)

T12 transports Carbon2

T12 is transcription of T2

T2= IF NOT (RpC1)

Thus, if Carbon 1, no uptake of Carbon 2


Complex Medium

Many different scenarios can be simulated

Coupling Expression Data with Flux Balance Analysis
Coupling Expression with Metabolism

Can We Algorithmically Interpret Expression Data in a Metabolic Context?

Caroline Colijn, Aaron Brandes, Jeremy Zucker, Brian Weiner, Desmond Lun

Interpreting Array Data in Metabolic Context

Modeling Metabolism with Expression Data

Applying Flux Balance Analysis (FBA), we use expression data to model the maximum flux through each reaction

E. coli

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Mycolic Acid Biosynthesis

• Major cell wall constituent
  – Antibiotic permeability
  – Intracellular growth

• Target of several first-line TB drugs

• Published FBA Model
  – 197 Metabolites
  – 219 Reactions

Caroline Colijn

Boshoff TB Expression Compendium

Can we predict the impact of each drug/condition on mycolic acid synthesis (capacity)?


Raman et al. (2005) PLoS Comp. Biol
Predicting the Environment using Metabolic Models

• Organisms likely adjust metabolic state to available nutrients
• Expression data gives us a readout of metabolic state
• Can we predict nutrient source from predictions of metabolic state from expression data?

Rank nutrients by how well they “match” a metabolic state
The Basic Idea

1. Determine flux cone that represents maximum metabolic capabilities of the system
2. Find optimal flux $v_k$ for each nutrient $k$
3. Determine expression-constrained flux cone
4. Calculate distance of each $v_k$ from the expression-constrained flux cone
5. Choose nutrient $k$ with smallest $v_k$

Predicting Nutrients: Method

Maximal flux cone
Expression-constrained flux cone

E. coli Central Metabolism Model

E. coli Nutrient Prediction

Color indicates distance from expression-constrained flux cone to optimal flux for that nutrient combination

Ranking Multiple Nutrients

How is gene expression regulated during metabolism?

Many questions remain, but one very interesting recent study…
Just-in-time transcription program in metabolic pathways

Amino Acid Biosynthesis Promoters

Baseline: no amino acids present
Add each AA one at a time
Measure promoter dynamics

Temporal Order of Transcription

Magnitude of Promoter Activity

Temporal order is correlated with promoter activation strength

Flux Goal with Minimal Enzyme?