Constraints and regulation of metabolic networks

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Network approaches to cell biology

• Understand the organization of cellular functions (molecular, modular, organellar) in novel ways and in the context of the system as a whole.

• Develop modeling capabilities to predict cellular functions on all hierarchical levels (i.e., from the molecular to the phenotypic level) in response to environmental and/or genomic/epigenomic changes.
Cellular metabolism is realized through the activity of a complex molecular interaction network.
The first constraint: the conservation of mass of intermediary metabolites

Flux balance analysis (FBA) of metabolic networks

1. System identification
2. Mass balance equations
3. Creation of Stoichiometric matrix
4. Linear optimization with respect to certain objective

Balance Equations:
A: \(-v_1 - b_1 = 0\)
B: \(v_1 + v_2 - v_3 = 0\)
C: \(v_2 - v_3 - v_4 - b_2 = 0\)
D: \(v_3 + v_4 - v_5 - b_3 = 0\)
E: \(v_5 + v_7 - b_4 = 0\)

Schilling & Palsson, P.N.A.S., 1998
Global and local flux organization in the *Escherichia coli* metabolic network

Almaas et al, Nature, 2004
Reorganization of the high-flux backbone upon shifting *E. coli* from glutamate- to succinate-limited growth media involves a limited number of reactions
Individual reactions with high flux display mono- or multi-modal flux distribution under different growth conditions.
Hypothetical effect of molecular crowding on intracellular metabolic enzyme levels

Scenario: substrate uptake at a given uptake rate

Beg et al, PNAS, 2007; Vazquez et al., BMC Systems Biol., 2008
How far is the cell density from its maximum value?

Proteins specific volume

\[ \nu_{\text{spec}} \approx 0.73 \text{ ml/g} \]

Maximum protein density,

spheres, densest packing: \( \rho_{\text{dens}} \approx 0.74/\nu_{\text{spec}} \approx 1.0 \text{ g/ml} \)

spheres, random packing: \( \rho_{\text{rand}} \approx 0.64/\nu_{\text{spec}} \approx 0.88 \text{ g/ml} \)

Macromolecules (DNA, RNA, protein) density (\textit{E. coli})

\[ \rho \approx 0.34 \text{ g/ml} \approx 0.34 \rho_{\text{dens}} \approx 0.39 \rho_{\text{rand}} \]
**E. coli:** crowding coefficients

- *E. coli* metabolic reactions and biomass vector - Palsson (UCSD).
- Carbon limited, oxygen abundance.
- Crowding coefficients $a_i$:
  - Enzyme’s turnover rates, about 100 enzymes - BRENDA.
E. coli 5-dilution rate steady-state growth

*for flux analysis 10% of the glucose was labeled tracer glucose [1,2-\textsuperscript{13}C]

- Enzyme activities
- Microarrays
- Flux analysis

Vazquez et al., BMC Systems Biol, 2008
Predicted vs. measured metabolic fluxes in the *E. coli* central metabolism

Model

Experiment
Comparison of measured metabolic fluxes and *in-vitro* enzyme activities

Flux rates

Enzyme activities
Mixed-substrate utilization

Substrate utilization and acetate production by *E. coli* MG1655 in M9 with 0.4 g/L of 5 carbon sources each

Predicted substrate utilization by FBAwMC

Beg et al, PNAS 2007
Gene expression and pO$_2$ signatures indicate metabolic pauses & changes

Select stress response genes in cluster B

Blue curve indicate oxygen level in media
The mixed- and single-substrate fermenter experiments

A. OD600nm

B. Relative Growth Rate

C. Acetic Acid

D. Mixed Carbon Source Consumption

E. Single Carbon Source Consumption

F. Predicted Single Substrate uptake
Promoter-GFP constructs of maltose regulon

**Transformation**

pCS21 → **MG1655**

In M9 solution with carbon source and 30µg/ml kanamycin

Only sample wells are added with 4mM cAMP and 200µM maltotriose

Quantify OD$_{600nm}$ and GFP with fluorescence plate reader

**Graphical Representation**

- **malEKSTPZ**
- pCS21 ➔ Transformation ➔ MG1655
- Quantify OD$_{600nm}$ and GFP with fluorescence plate reader

**Legend**

- Green lines indicate activation
- Orange lines indicate inhibition
Conclusions

1. Molecular crowding represent an important constraint on the metabolic capabilities of a cell, allowing an even higher level predictive capability for flux-balance based modeling.

2. There is a need to search for additional biologically-relevant physicochemical constraints that impact metabolic capabilities.

3. Generative models are not able to fully explain the structure and expression level distribution of cellular networks without modeling population-level evolutionary selection.
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