Constraint-Based Workshops

7. Robustness & Phase Planes
February 6th, 2008

No Meeting Next Week!!
Constraint-Based Methods

Optimal Solutions
1. FBA
2. Flux Variability

Flux Dependencies
1. Robustness
2. Phase Planes
3. Flux Coupling

All Allowable Solutions
1. Extreme Pathways
2. Elementary Modes
3. Sampling

Altering Phenotypes
1. Genetic Mutations
2. Strain Design

Application of Additional Constraints
1. Regulation
2. Energy Balance

Price, Reed, and Palsson
Nat. Reviews Microbiol. 2004
Robustness Analysis

Used to calculate how the objective function changes to incremental changes in a particular flux.

Curves are piecewise linear w/slope equal to Shadow Price
Oxygen Limitations and By-product Secretion

Restriction to a finite capacity

Uptake Limits
- Enzymatic limits
- Mass Transfer limits
- Supply Restrictions

Partially Aerobic (By-product Secretion)

Aerobic

Growth

\[ \text{Oxygen Demand (} q_{O_2} X \text{)} \text{ [mol/L-h]} \]

\[ \text{Oxygen Supply Capacity (} k_{a*c*} \text{) [mol/L-h]} \]

20 mmol/g DW-hr

(Respiratory chain limitation)

Anderson and Meyenburg (1986)
J. Bacteriol. 148: 114-23
Example

In this example we vary the maximum allowable uptake rate of oxygen. The whole range of oxygenation is shown, from fully aerobic conditions to fully anaerobic conditions.

The growth rate is graphed in the upper panel and the by-product secretion rates in the lower.
Review of Shadow Prices & Reduced Costs

• Shadow Prices (SP):
  - One for each constraint or metabolite
  - \( \frac{dZ}{db_i} \)
  - SP<0 means adding metabolite (ie. change \( b=0 \) to \( b<0 \)) would increase Z.
  - SP>0 means removing metabolite (ie. change \( b=0 \) to \( b>0 \)) would increase Z.

• Reduced Costs (RC):
  - One for each variable or flux.
  - \( \frac{dZ}{dv_j} \) (for zero fluxes)
  - RC < 0 means increasing flux (\( v_j \)) would reduce Z.
Shadow prices:
Interpret changes in optimal solutions

**PHASE I**
(Complete Anaerobiosis)

- Glc → G6P
  - 10.0 → 9.95
  - 9.74
  
- G6P → F6P
  - 0.047
  - 9.74
  - 9.74
  
- E4P → T3P
  - 0.142
  - 19.3
  - 3PG
  - 18.9
  
- PEP → OA
  - 0.75
  - 8.0
  - 5.39
  - 13.9

- For → Eth
  - 17.3
  - 7.9
  - 8.2

- AcCoA → Cit
  - 0.28
  - 0.28
  - 0.58

- IKG
  - 0.28
  - 0.58

**Shadow Prices**

- O2: 0.040
- NADPH: 0.0018
- Acetate: 0.0
- NADH: -0.0054
- Ethanol: 0.0
- ATP: 0.011
- FADH: -0.013
- Formate: 0.0
- Hexp: 0.0036
- QH2: -0.013
- Lactate: 0.0054
- Succinate: 0.011

**PHASE II**

- Glc → G6P
  - 10.0 → 8.23
  - 8.91
  
- G6P → F6P
  - 1.66
  - 8.23
  - 8.91
  
- E4P → T3P
  - 0.46
  - 18.0
  - 17.2
  
- PEP → OA
  - 1.54
  - 5.39
  - 13.9

- For → Eth
  - 17.3
  - 7.9

- AcCoA → Cit
  - 0.58
  - 0.58

- IKG
  - 0.58

**Shadow Prices**

- O2: 0.038
- NADPH: 0.0029
- Acetate: 0.0
- NADH: -0.0043
- Ethanol: 0.0021
- ATP: 0.011
- FADH: -0.011
- Formate: 0.0
- Hexp: 0.0036
- QH2: -0.011
- Lactate: 0.0064
- Succinate: 0.013

Formate, Acetate, Ethanol are Secreted ($0 shadow prices); Ethanol is not ($0.002)
Flux distributions for different levels (or phases) of oxygenation

Phase IV

Phase V
(Adequate Oxygen)

Acetate is Secreted ($0 shadow prices)

Shadow Prices

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<th>Shadow Prices</th>
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<tr>
<td>O₂</td>
<td>0.026</td>
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<td>ATP</td>
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<td>HexP</td>
<td>0.0035</td>
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<tbody>
<tr>
<td>O₂</td>
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<tr>
<td>ATP</td>
<td>0.0049</td>
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<tr>
<td>HexP</td>
<td>0.0016</td>
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</tbody>
</table>

Partially anaerobic ➔ aerobic
Define Number of Steps

```plaintext
steps /step1*step25/;
```

Define Flux to Vary

```plaintext
*Select the flux that you want to vary
pick flux('TET1')-1;
```
Robustness Analysis Calculations

• Calculate the sensitivity of the objective function to changes in, use glucose uptake rate of 5 and aerobic conditions:
  - PGL (pentose phosphate flux)
  - GAPD (glycolytic flux)
  - ICDHyr (TCA cycle flux)

Graph results in excel!
Robustness Analysis

![Graph showing growth rate vs. flux value for PGL, ICDHy, and GAPD]

- **PGL**
- **ICDHy**
- **GAPD**

**Flux Value (mmol/gDW/hr)**

**Growth Rate (1/hr)**

0 0.1 0.2 0.3 0.4 0.5 0.6 30 35

0 5 10 15 20 25 30 35
What Does this Mean?

• Which reaction(s) are essential (note that FBA, MOMA, and ROOM would all predict the same lethal phenotype)?
Phase Plane Analysis:
Varying multiple fluxes simultaneously
Parameter Variation

Robustness Analysis: Projection of PhPP for Maximum Growth rate vs. O₂ uptake

Phenotypic Phase Plane (PhPP)

Robustness Analysis: Projection of PhPP for Maximum Growth rate vs. Succinate uptake
Phenotype Phase Plane

• 2-dimensional region
  - Spanned by 2 metabolic fluxes
    • Typically uptake rates
  - lines to demarcate phase of constant shadow price
  - By definition, metabolic pathway utilization is different in each region of the phase plane
Shadow Prices and Isoclines

Shadow Price

$$\gamma_i = \left. \frac{\partial Z}{\partial b_i} \right|_{\text{boundary}}$$

Relative shadow prices

$$\alpha = -\frac{\gamma_A}{\gamma_B} = -\frac{dZ/db_A}{dZ/db_B} = -\frac{db_B}{db_A}$$
Isoclines:
Lines w/ Constant Objective Values

\[ \alpha = -\frac{\gamma_A}{\gamma_B} = -\frac{dZ/db_A}{dZ/db_B} = \frac{db_B}{db_A} \]
Characteristics of Phase Planes

- Infeasible regions: fluxes don’t balance
- Regions of single substrate limitations ($\alpha = 0$ or infinity)
- Regions of dual substrate limitations ($\alpha < 0$)
- Futile regions ($\alpha > 0$)
- Isoclines (like constant height in topography maps)
- Line of optimality: corresponds to maximal biomass yield (g cells/mmole carbon source)
  - You find this by fixing carbon uptake rate and optimize for biomass using FBA, this will give you one point on the LO
Line of Optimality: Max. $Y_{X/s}$

Oxygen Uptake $B$

Infeasible Steady State

Carbon Source Uptake Rate

Metabolic Phenotype 1

Metabolic Phenotype 2

Infeasible Steady State

LO
Acetate Phase Plane for E. coli

Line of Optimality
**Hypothesis:**
Metabolic regulation will drive the operation of the metabolic network toward the line of optimality
Acetate PhPP & Experimental Data

\[ y = 0.9975x + 1.8663 \]
\[ R^2 = 0.8423 \]
Growth on Acetate
3D Phase Plane:

The Phase Plane
Succinate Phenotype Phase Plane

Succinate Uptake Rate vs. Oxygen Uptake Rate

- also works for: malate, glucose, fumarate
- does not work for glycerol

Also works for: malate, glucose, fumarate.
Growth on Succinate

Dual substrate limited region

LO

Growth Rate (1/hr)

Oxygen Uptake Rate (mmole/g-DW/hr)

Succinate Uptake Rate (mmole/g-DW/hr)
Application: Predicting complex biology; adaptive evolution and picking optimal growth states
Methods – adaptive evolution

- Cultures grown in 250ml minimal medium supplemented with 2g/L carbon source
- Serial passage during exponential growth
- Stable growth rate achieved at end of evolution
- Cells frozen throughout evolution for phenotype testing
Cellular Evolution: Growth rates on Glycerol