Modelling Biochemical Reaction Networks

Lecture 11:
Metabolic control analysis of glycerol metabolism

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Model
Questions, revisited

- Flux through pathway: rate of formation of pyruvate
- When we started developing our glycerol metabolism model, we had two questions:
  1. What factor(s) limit the flux through this pathway?
  2. Can we engineer a strain of *Saccharomyces cerevisiae* that is capable of a higher flux through this pathway?
- Easier to answer 2 if you know the answer to 1
- 1 can be addressed using Metabolic Control Analysis (MCA)
Metabolic Control Analysis

- Imagine an experiment in which we change a parameter ($p$) and measure the resulting change in the flux ($J$).

- Rate of change of flux with respect to changes in $p = \frac{\partial J}{\partial p} \approx \frac{\Delta J}{\Delta p}$

**Problem:** size of rate of change is difficult to interpret because a change of (e.g.) 1 $\mu$M/min in $J$ can be a small change if $J \sim 1000$ $\mu$M/min or a very large change if $J \sim 1$ $\mu$M/min.

**Solution:** Use relative changes $\Delta J/J$ and $\Delta p/p$.

Control coefficient:

$$C_p^J = \frac{\partial J/J}{\partial p/p} = \frac{\partial \ln J}{\partial \ln p} \approx \frac{\Delta J/J}{\Delta p/p} \approx \frac{\Delta \ln J}{\Delta \ln p}$$
**Metabolic Control Analysis**

**Control coefficients**

\[ C_p^J = \frac{\partial \ln J}{\partial \ln p} \]

- Control coefficients can be positive or negative.
- A very small control coefficient would imply that a particular parameter has little effect on the flux.
- Special case: If \( p = E \) is the concentration of an enzyme (or transporter), then \( C_p^J \) is called a flux control coefficient.
- The classical idea of a rate-limiting step would correspond to \( C_E^J \sim 1 \), i.e. doubling the enzyme concentration doubles the flux.
- Because of the logarithms, any quantity proportional to \( E \) (e.g. \( v_{\text{max}} \)) will give the same value for the flux control coefficient.
For irreversible steps, increase \( v_{\text{max}} \) (or equivalent parameter) by a small amount (say, 5%). Then decrease it by the same amount (to check for consistency).

Calculate

\[
C_E^J \approx \frac{\Delta \ln J}{\Delta \ln v_{\text{max}}} = \frac{\ln J(v_{\text{max}} + \delta) - \ln J(v_{\text{max}} - \delta)}{\ln(v_{\text{max}} + \delta) - \ln(v_{\text{max}} - \delta)} = \frac{\ln \left( \frac{J(v_{\text{max}} + \delta)}{J(v_{\text{max}} - \delta)} \right)}{\ln \left( \frac{v_{\text{max}} + \delta}{v_{\text{max}} - \delta} \right)}
\]
Metabolic control analysis
Measurement of flux control coefficients

For reversible steps, the rate for both directions is proportional to $E$.
Introduce a “dummy” parameter that scales both the forward and reverse $v_{\text{max}}$ in proportion, e.g.

$$v = e \frac{v_{\text{max}}^+ S / K_S - v_{\text{max}}^- P / K_P}{1 + \frac{S}{K_S} + \frac{P}{K_P}}$$

$e = 1$: original enzyme concentration
$e = 2$: doubling of enzyme concentration

Calculate $C_E^J$ using (e.g.) $J(e = 1.05)$ and $J(e = 0.95)$. 
Steady-state flux

- We need to get steady-state fluxes.
- If we run our model, we find that it does not reach a steady state: The glycerol 3-phosphate concentration just keeps rising.
- This is a common problem when we extract a set of reactions from a metabolic system. What we’re leaving out might be important for homeostasis.
- In our case, the model includes an arbitrary external glycerol concentration. We can adjust this downward to avoid overwhelming glycerol 3-phosphate dehydrogenase. A steady-state is reached if \([\text{Glyc} \text{(ext)}] = 5 \times 10^{-5} \text{ mM}\).
- \([\text{Glyc} \text{(ext)}]\) is really tiny: Probably should reconsider model instead.
- Use the corresponding steady-state concentrations to accelerate simulations.
Metabolic control analysis of glycerol metabolism

Example: Control coefficient with respect to glycerol diffusion

- We need to consider all steps from source (external glycerol) to sink (pyruvate), including transport.
- The model contains a rate law for diffusive transport of glycerol (Glyc) through the cell membrane:

\[
v_{\text{diff}, \text{Glyc}} = \frac{k_{16}}{Y_{\text{vol}}} ([\text{Glyc}] - [\text{Glyc}_{\text{ext}}])
\]

- Here, the diffusive rate constant \( k_{16} \) acts as the equivalent of an enzyme concentration.
Metabolic control analysis of glycerol metabolism

Example: Control coefficient with respect to glycerol diffusion

- Data collected from simulations:

<table>
<thead>
<tr>
<th>$k_{16}/s^{-1}$</th>
<th>$J/mM,s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>$8.9677 \times 10^{-5}$</td>
</tr>
<tr>
<td>1.9</td>
<td>$9.4640 \times 10^{-5}$ (default)</td>
</tr>
<tr>
<td>2.0</td>
<td>$9.9602 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

- Control coefficient:

$$C_{\text{diff,Glyc}}^J = \frac{\ln \left( \frac{9.9602 \times 10^{-5}}{8.9677 \times 10^{-5}} \right)}{\ln \left( \frac{2.0}{1.8} \right)} = 0.9963$$
**Metabolic control analysis of glycerol metabolism**

**Flux control coefficients**

<table>
<thead>
<tr>
<th>Enzyme/process</th>
<th>Parameter</th>
<th>$C_J^E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol diffusion</td>
<td>$k_{16}$</td>
<td>0.9963</td>
</tr>
<tr>
<td>Glycerol kinase</td>
<td>$v_{\text{max,}gk}$</td>
<td>0.0038</td>
</tr>
<tr>
<td>Glycerol 3-phosphate dehydrogenase</td>
<td>$e_{\text{g3pd}}$</td>
<td>0</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>$e_{\text{tpi}}$</td>
<td>0</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
<td>$e_{\text{GAPDP}}$</td>
<td>0</td>
</tr>
<tr>
<td>PEP synthesis</td>
<td>$e_{\text{PEPsynth}}$</td>
<td>0</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>$V_{10m}$</td>
<td>1.0001</td>
</tr>
</tbody>
</table>
Conclusions

- Under the conditions considered here, the flux through the glycerol to pyruvate pathway is mostly controlled by transport into the cell.
- Overexpressing a transporter alone is not sufficient because the glycerol 3-phosphate dehydrogenase becomes saturated at higher concentrations of glycerol 3-phosphate resulting from higher levels of glycerol.
- Might be worth investigating the addition of a gene for a more-efficient glycerol 3-phosphate dehydrogenase (perhaps from another organism)