Lecture 9:

• Metabolic Control Analysis (MCA), or, Distribution of Kinetic Control
Flux Control Coefficients (FCC)

• In a bioreaction network, how does the flux of a reaction J depend on the concentration of an enzyme E_i?

• Define: Flux Control Coefficient of flux J wrt enzyme E_i at (e,j)

\[
C_{E_i} = \left( \frac{\partial J}{\partial E_i} \right)_{e,j} = \frac{\partial (\ln J)}{\partial (\ln E_i)}_{e,j} = \frac{\delta J}{\delta E_i} \frac{1}{E_i}
\]
Flux Control Coefficients (FCC)

- FCCs extend the concept of the Rate Limiting Step
  - Obtained in the limit as $\delta E \rightarrow 0$
  - Independent of units
  - Defined at a steady state (different at different conditions, such as enzymes, environments)

Summation theorem
\[
\sum_i C^J_{E_i} = 1
\]

- Distribution of Kinetic Control vs. RLS
  - Kinetic control is shared but it is not shared evenly
**Interpretation of FCC**

- Think of a change in enzyme concentration small enough so that $C_{Ei}^J$ is effectively constant:

$$C_{Ei}^J = \frac{\partial (\ln J)}{\partial (\ln E_i)} \approx \frac{\Delta \ln J}{\Delta \ln E_i} \Rightarrow \Delta \ln J = C_{Ei}^J \Delta \ln E_i$$

$$\therefore J = aE^c$$

**Local Power Law.** Better approximation than:

$$\frac{\Delta J}{J} = C_{Ei}^J \frac{\Delta E_i}{E_i}$$

- FCC may take positive or negative values (what pathways?)
- FCC may take values greater than 1
- Most enzymes have small FCC. Diploids
- Interpretation of dominant vs. recessive genes in heterozygotes
Sensitivity of flux to enzymatic activity is not a measure of whether that enzyme is a **control** or a **regulatory** enzyme.

E is a regulatory enzyme but usually has small FCC. Why?

The implication that a regulatory enzyme is also the “rate limiting step” is nonsense.

- Compare FCCs of enzymatic steps in the same pathway
- Never compare FCCs of different pathways
Predicting Flux Changes (cont’d)

C = FCC, r = amplification factor, f = flux amplification
Predicting Flux Changes (cont’d)

C = FCC, r=amplification factor, f=flux amplification
Aromatic aminoacid biosynthesis: (P. Niederberger et al., *Biochem J.* 287:473 (1992))

**Distribution of kinetic control: Motivation**

- **Prephenic acid** (PA)
- **Tyr1** → **Tyr**
- **Phe2** → **Phe**
- **Chorismate** (CA)
- **Trp2** → **AAS**
- **Uptake of External AA**
- **Anthranilic acid** (AA)
- **AAS**: Anthranilate Synthase
- **PRT**: AA Phosphorybosl transferase
- **PRAI**: Phosphorybosl anthranilate isomerase
- **InGPS**: Indoleglycerol Phosphate Synthase
- **TRPS**: Tryptophan Synthase
- **AAT1,2**: Aromatic Amino Transferase
- **Degradation** to tryptophol
## Case Study (cont’d.)

**Trp accumulation and flux upon single TRP gene up-modulation**

<table>
<thead>
<tr>
<th>Up-modulated genes</th>
<th>Relative enzyme levels</th>
<th>Flux to Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>PR- synthase</td>
</tr>
<tr>
<td>(TRP2)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>(TRP4)</td>
<td>1.2</td>
<td>19.9</td>
</tr>
<tr>
<td>(TRP1)</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>(TRP3)</td>
<td>1.5</td>
<td>0.8</td>
</tr>
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</table>
Tetraploid yeast strains with 4, 3, 2, 1 and 0 doses of a TRP gene. Effect of Trp on $\mu$ (protein synthesis). Above effects are abolished in Trp-supplemented medium.
Case Study (cont’d.)

\[ C_a^\mu = 0.018, \quad C_b^\mu = 0.174, \quad C_c^\mu = 0.013 \quad C_d^\mu = 0.013 \]
\[ C_e^\mu = 0.040 \quad \rightarrow \text{All very small} \]

(Flux control coefficient evaluated at wild-type activity)
Suggestion: Trp flux relatively insensitive to TRP1-5

Note: \[ \sum_{i=a-e} C_i^\mu = 0.258 \quad \leftarrow \text{equal to a group coefficient} \]

\[ \sum_{i=a-e} C_i^\mu = 0.258 \quad \Rightarrow \text{Many enzymes contribute to flux control} \]

• Large fraction of control residues outside the above chain of 5 reactions.
# Case Study (cont’d.)

**Trp Accumulation & Flux upon up-modulation of multiple TRP genes**

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<td>1.5</td>
<td>0.8</td>
</tr>
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<td>TRP5</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>TRP1 TRP3</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>TRP2 TRP4 TRP1d TRP3</td>
<td>25.0</td>
<td>34.0</td>
</tr>
<tr>
<td>TRP2f TRP1 TRP3</td>
<td>29.4</td>
<td>1.0</td>
</tr>
<tr>
<td>TRP2f TRP4 TRP1 TRP3</td>
<td>26.5</td>
<td>26.4</td>
</tr>
<tr>
<td>TRP2 TRP4 TRP1d TRP5</td>
<td>20.0</td>
<td>15.2</td>
</tr>
<tr>
<td>TRP2f TRP4 TRP1 TRP3 TRP5</td>
<td>24.0</td>
<td>19.6</td>
</tr>
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Multiple up-modulations

- Coordination theorem: If a system consisting of many enzymes sustains a flux $J$, then, if all enzyme concentrations are multiplied by $r$ the resulting flux will be $rJ$ (any $r$)

- OR:
  \[
  \frac{\Delta J}{J} \div \frac{\Delta E_i}{E_i} = 1
  \]
  Stands for the simultaneous and equal elevation of all $E_i$

- Implications:
  - Chromosomal integration, multi-copy plasmid effects must be analyzed in terms of large-finite-change systems. These have the above property. Individual contribution of smaller group of enzymes are not additive.
  - Identify the group of enzymes whose control coefficients sum up to a substantial part.
  - The small groups must be modulated simultaneously
Universal Method of Flux Amplification
(Henrik Kacser)

- Initial Flux \( J + J_S \)
- Final Flux \( rJ + J_S \)

\[
\frac{\Delta J}{J_{\text{initial}}} = \frac{rJ + J_s - J - J_s}{J + J_s} = \frac{r-1}{1 + (J_s / J)} = \lambda
\]

- Initial Flux \( J \)
- Final Flux \( rJ \)

\[
\frac{\Delta J}{J_{\text{initial}}} = r - 1
\]
Metabolism is Complex:

\[ \text{Event (} \Delta E_0 \text{)} \rightarrow \Delta S_i, \Delta p_i \]

\[ (\Delta E_j) \]

The net result of an event (such as a genetic modification) is a **Systemic** property and **not** an isolated change.
Elasticities

Definition:
Consider a small change \( \delta M \) is made in the amount of metabolite M affecting the rate of reaction \( v \) catalyzed by enzyme E and producing a change \( \delta v \). All other metabolites affecting \( v \) are kept constant at the steady state values. (We use \( v \) instead of \( J \) to indicate that we are considering the response of an isolated enzyme, not the pathway).

\[
\varepsilon_M^v = \left( \frac{\partial v}{\partial M} \right)_{\text{st.state}} \quad \frac{M}{v} = \left( \frac{\partial \ln v}{\partial \ln M} \right)_{\text{st.state}}
\]

Elasticity:

Note:
- \( \partial \) : partial derivate, evaluated at steady state
- \( \varepsilon \) : depends on metabolite concentrations and rate parameters
- \( \varepsilon_E^v \quad = 1 \) if E catalyzes reaction
- \( \varepsilon_E^v \quad = 0 \) if E does not participate in reaction
Connectivity Theorem

- How are FCC related to the kinetic properties of enzymes?

Connectivity theorem (one for each metabolite)

\[ C^J_{e_1} \cdot \varepsilon^1_{M_k} + C^J_{e_2} \cdot \varepsilon^2_{M_k} + \ldots + C^J_{e_n} \cdot \varepsilon^n_{M_k} = 0 \]

\( (M_k \) is any independent variable, metabolite, T, etc.)

For 2 enzymes

\[ C^J_{e_1} \cdot \varepsilon^1_{M} + C^J_{e_2} \cdot \varepsilon^2_{M} \Rightarrow \frac{C^J_{e_1}}{C^J_{e_2}} = -\frac{\varepsilon^2_{M}}{\varepsilon^1_{M}} \]

Unresponsive (rigid) enzymes exert strong control on fluxes!

Enzymes whose rates do not change greatly when the concentrations of their reactants or products vary. Elasticities wrt a common metabolite are the correct measure.
Illustration of Connectivity Theorem

- After addition of the inhibitor (at the arrow) and the resulting instantaneous drop in $v_2$ (dashed line), the concentration of the intermediary metabolite $M$ (blue) begins to rise. Depending on whether $v_1$ (red line) is less (left part of the figure) or more (right part) inhibited than $v_2$ is stimulated by this increase in $[M]$, the point at which the steady-state condition $v_1 = v_2$ is reattained will be at high (left part) or low (right part) reaction rates (flux), respectively. Consequently, the flux control exerted by enzyme increases with an increase in the Elasticity Coefficient of $v_2$ towards $M$.
Define:

Response Coefficient $R^J_X$ of flux $J$ to an effector $X$:

$$R^J_X = \frac{X}{J} \frac{dJ}{dX}$$

If the effect of $X$ is mediated by enzyme $E_i$

$$R^J_X = C^J_i \varepsilon^i_X$$

If an effecter acts upon more than one enzyme, the total response is the sum of responses from all individual enzymes

$$R^J_X = \sum_i C^J_i \varepsilon^i_X$$