Driving natural systems: Enzymes and metabolic control analysis

Stoichiometry is not always the full picture

- An example: we observe the phenomenological reaction description

$$NO_2 + CO \rightarrow NO + CO_2$$

- From our previous work, we might expect

$$\nu = \nu_+ = k_+[NO_2][CO]$$

- Instead we observe

$$\nu = k[NO_2]^2$$

- What's going on?

Stoichiometry is not always the full picture

- For $NO_2 + CO \rightarrow NO + CO_2$, we observe $\nu = k[NO_2]^2$ rather than $\nu = \nu_+ = k_+[NO_2][CO]$
- Leading to the hypothesis that the system is really

$$\begin{aligned} NO_2 + NO_2 &\rightarrow NO + NO_3 \\ NO_3 + CO &\rightarrow NO_2 + CO_2 \end{aligned}$$

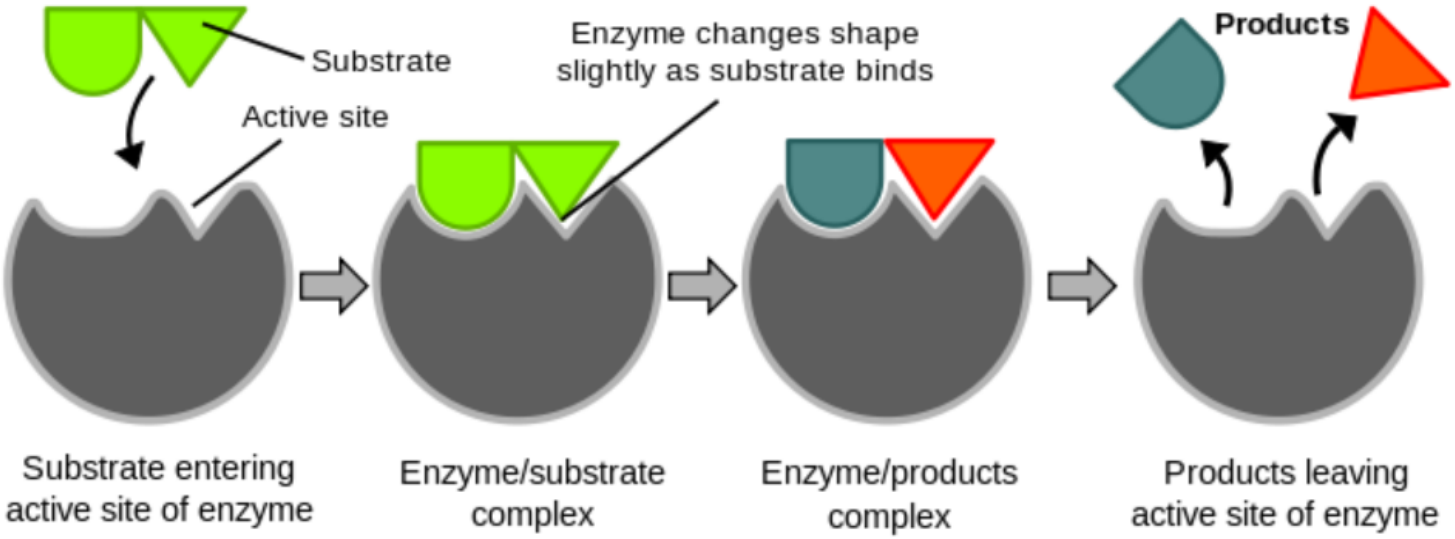
  and that the first reaction is much slower
- In this case, there's a 'rate-limiting step', the flux through which largely controls the flux through the whole pathway
- This picture leads us into a finer-grained representation of enzymatic reactions

Enzyme kinetics

- Simple enzyme kinetics described by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- Enzyme reversibly binds to substrate to form complex, reacts, and produces enzyme and product
- We'll assume that the reaction is irreversible for now



Enzyme kinetics

- Enzyme kinetics described by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- Descriptive equations

$$\begin{aligned}
\frac{d[S]}{dt} &= -k_1[E][S] + k_{-1}[ES] \\
\frac{d[E]}{dt} &= -k_1[E][S] + k_{-1}[ES] + k_2[ES] \\
\frac{d[ES]}{dt} &= k_1[E][S] - k_{-1}[ES] - k_2[ES] \\
\frac{d[P]}{dt} &= k_2[ES]
\end{aligned}$$

Enzyme kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- Quasi-steady-state approximation: separation of timescales
- Assume that concentration of complex doesn't change on the timescale of product formation

$$\begin{aligned} \frac{d[ES]}{dt} &= k_1[E][S] - k_{-1}[ES] - k_2[ES] \simeq 0 \\ &\rightarrow \quad k_1[E][S] = k_{-1}[ES] + k_2[ES] \end{aligned}$$

- Enzyme conservation law: $[E] + [ES] = [E]_0 = \text{const}$

Enzyme kinetics

- $k_1[E][S] = k_{-1}[ES] + k_2[ES]$
- $[E] + [ES] = [E]_0 = \text{const}$

$$\begin{aligned} k_1\left([E]_0 - [ES]\right)[S] &= k_{-1}[ES] + k_2[ES] \\ k_1[E]_0[S] &= \left(k_{-1} + k_2 + k_1[S]\right)[ES] \\ [ES] &= \frac{k_1[E]_0[S]}{k_{-1} + k_2 + k_1[S]} \\ [ES] &= \frac{[E]_0[S]}{k_m + [S]} \end{aligned}$$

- Where $k_m = \frac{k_{-1}+k_2}{k_1}$
- Assuming that reaction is fast:

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E]_0[S]}{k_m + [S]} \equiv \frac{v_{max}[S]}{k_m + [S]}$$

- 'Michaelis-Menten kinetics'

Beyond the ‘road map’ picture

- Metabolic maps, flux balance analysis, and simple enzyme models are useful but don’t provide the full picture
- Metabolic control analysis offers a way to explore how concentrations and enzymatic properties influence fluxes through a system
- MCA does *not* explicitly identify controlling or regulating enzymes – but finds the species that have the strongest effect on metabolic flux
- A mathematical framework describing how fluxes and concentrations are coupled to metabolic network parameters
- In particular, metabolic fluxes are global system-wide properties and depend on the set of local reaction parameters
- Can be thought of as how the architecture of a metabolic network applies control to biological quantities

Rate-limiting steps

- Reaction pathways are often thought of as having a single ‘rate-limiting step’: the slowest step in the pathway
- But in biology we usually observe that the net flux through a pathway depends on many if not all reaction constants
- We could consider the step least able to go faster – but how can we identify this?
- We need a refined picture: rather than identifying a single rate-limiting step, explore how metabolic flux varies as enzyme activities and concentrations change

Metabolic control theory

- We are interested in things like J_i (flux through a particular set i of reactions) and X_i (concentration of chemical i), and the control that features of the metabolic network exert on these quantities
- v_i (steady-state rate of reaction i) is a local parameter (describing one particular reaction)
- e.g. $v_i = \frac{k_2[E]_0[S]}{k_m+[S]}$ (or more complicated forms)
- Flux control coefficient, how *relative steady state* change in flux depends on *relative* change in steady-state value of a parameter (e.g. a reaction rate):

$$C^J_{v_i} = \lim_{\delta v_i \to 0} \frac{\delta J/J}{\delta v_i/v_i}$$

- 'Relative' here means relative to a particular steady-state realisation of the metabolic system: we're studying multiplicative perturbations

$$C^J_{v_i} = \frac{\partial J}{\partial v_i}\frac{v_i}{J} = \frac{\partial \ln J}{\partial \ln v_i}$$

- Encodes how much the flux through a pathway depends on the rate of a particular reaction

Control coefficients

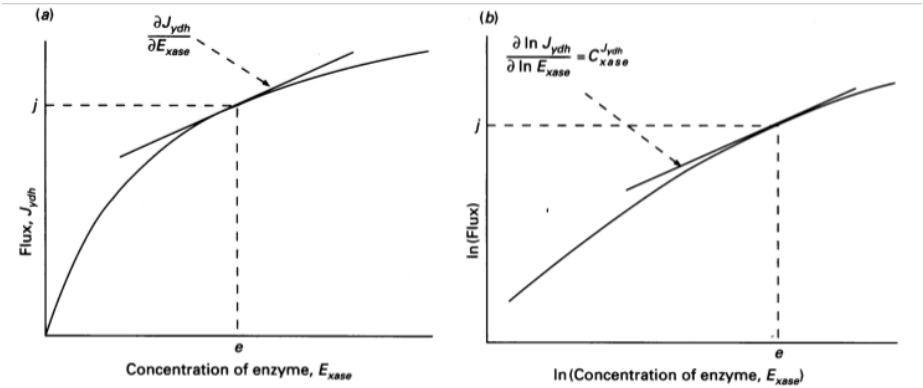
- Flux control coefficient

$$C^J_{v_i} = \frac{\partial J}{\partial v_i}\frac{v_i}{J} = \frac{\partial \ln J}{\partial \ln v_i}$$

- We also have concentration control coefficients

$$C^X_{v_i} = \frac{\partial \ln X}{\partial \ln v_i}$$

- Absence of dimensionality is useful when comparing different reactions



Elasticities

- Elasticity measures how relative change in a property (for example, concentration X_i) provokes relative change in a local reaction rate
- Again, 'relative' here means relative to a particular steady-state realisation of the metabolic system

$$\epsilon_X^v = \lim_{\delta S \to 0} \frac{\delta v / v}{\delta X / X}$$

- For example, consider ... $\xrightarrow{eno}$ PEP $\xrightarrow{pk}$ ...
- (enolase; phosphoenolpyruvate; pyruvate kinase)
- ϵ_{PEP}^{pk} tells us how changes in the concentration of *PEP* provoke change in the rate of *pk*

$$\epsilon_X^v = \frac{\partial v}{\partial X} \frac{X}{v} = \frac{\partial \ln v}{\partial \ln X}$$

- Encodes kinetic details of the enzyme's behaviour (how reaction rate depends on reactant concentration); resembles the order of reaction

Coefficients in MCA

- Control coefficients: how flux J or concentration X depends on a reaction rate v
- Elasticities: how reaction rate v depends on concentration X

The summation theorem

- Flux control summation theorem

$$\sum_i C^J_{v_i} = 1$$

- So for a rate-limiting step i, $C^J_{v_i} = 1$ and $C^J_{v_j} = 0$ for all $j \neq i$
- The rate v_i of step i thus has total control over the flux J
- More realistic: the control over J is spread across several steps
- The flux control coefficient of each enzyme is a system property – if we change one enzyme's contribution, the others change to compensate

The summation theorem

- System-wide effects of perturbations

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C$$

- Increasing k_2 affects A despite its lack of direct connection
- More B is used up; flux through reaction 1 increases due to lack of product inhibition; A decreases
- An analogy – a perhaps more familiar system in which a local perturbation has global consequences – food webs, where e.g. removal of a predator impacts prey, prey's prey, prey's competitors, and so on

The connectivity theorem

- For every enzyme that responds to the concentration of metabolite X:

$$\sum_i C^J_{v_i} \epsilon^{v_i}_X = 0$$

- $\epsilon^{v_i}_X$ is the elasticity with which concentration X affects rate v_i
- $C^J_{v_i}$ is the control that rate v_i has on flux J
- Quantifies how the kinetics of the enzymes (represented by elasticities) affects flux control coefficients

Connectivity theorem

$$\sum_i C^J_{v_i} \epsilon^{v_i}_X = 0$$

- Consider ... $\xrightarrow{eno}$ PEP $\xrightarrow{pk}$ ...
- (enolase; phosphoenolpyruvate; pyruvate kinase)

$$C^J_{eno} \epsilon^{eno}_{PEP} + C^J_{pk} \epsilon^{pk}_{PEP} = 0$$

$$\frac{C^J_{eno}}{C^J_{pk}} = -\frac{\epsilon^{pk}_{PEP}}{\epsilon^{eno}_{PEP}}$$

- Relative values of control coefficients depend on *PEP* elasticities
- Links local properties (elasticities: functions of enzyme behaviour) to global properties (control of flux through pathways in the system)
- With corrections due to branching and other structures, can solve for control coefficients given elasticities

Summary

- Elasticities encode dependence of reaction rate on substrate concentration

$$\epsilon_X^v = \frac{\partial v}{\partial X}\frac{X}{v} = \frac{\partial \ln v}{\partial \ln X}$$

- (Concentration—flux) control coefficients encode dependence of (concentration—flux) on individual reaction rates

$$C_{v_i}^J = \frac{d \ln J}{d \ln v_i};\ C_{v_i}^X = \frac{d \ln X}{d \ln v_i};$$

- Summation theorem demonstrates the coupled, system-wide sharing of pathway flux control between reaction

$$\sum_i C_{v_i}^J = 1$$

- Connectivity theorem links local elasticities with global control coefficients (large elasticity $\rightarrow$ small control coefficient)

$$\sum_i C_{v_i}^J \epsilon_X^{v_i} = 0$$

Summary

- Elasticities provide a more complete way of describing enzymatic functionality than single-substrate Michaelis-Menten (multiple substrates, allostery, competition and so on can be included)
- We have a way, via the connectivity theorem, of computing global control coefficients from local enzymatic properties (elasticities)
- These control coefficients tell us the degree of control that different steps in reaction pathways have on flux through the pathway (or subsets of the pathway)
- There are also control coefficients and theorems associated with control of concentration (as opposed to flux) – these are an extension exercise

Metabolic control picture

- Let's write the dynamics of a metabolic network as

$$\dot{\underline{s}}(t) = \underline{\underline{N}}\underline{\nu}(\underline{s}(t), \underline{p}(t))$$

- $\underline{\underline{N}}$ is stoichiometry matrix, $\underline{\nu}$ are reaction rates, which are generally functions of state $\underline{s}$ and parameters $\underline{p}$
- Assume that concentrations are able to vary independently (though this can be relaxed)
- Change variables to represent difference from a steady state of interest: $\underline{x} = \underline{s} - \underline{s}_0$; $\underline{u} = \underline{p} - \underline{p}_0$
- If we depart from (s_0, p_0):

$$\Delta\underline{\nu} = \frac{\partial \underline{\nu}}{\partial \underline{s}}\Delta\underline{s} + \frac{\partial \underline{\nu}}{\partial \underline{p}}\Delta\underline{p}$$

Metabolic control picture

- Our metabolic system

$$\dot{\underline{s}}(t) = \underline{\underline{N}}\underline{\nu}(\underline{s}(t), \underline{p}(t))$$

- With change of variables $\underline{x} = \underline{s} - \underline{s}_0$; $\underline{u} = \underline{p} - \underline{p}_0$ as we depart from (s_0, p_0)

$$\begin{aligned}
\frac{d\underline{x}}{dt} &= \frac{d\underline{s}}{dt} \\
&= \underline{\underline{N}}\Delta\underline{\nu} \\
&= \underline{\underline{N}}\frac{\partial\underline{\nu}}{\partial\underline{s}}\Delta\underline{s} + \underline{\underline{N}}\frac{\partial\underline{\nu}}{\partial\underline{p}}\Delta\underline{p} \\
&\equiv \underline{\underline{N}}\frac{\partial\underline{\nu}}{\partial\underline{s}}\underline{x} + \underline{\underline{N}}\frac{\partial\underline{\nu}}{\partial\underline{p}}\underline{u}
\end{aligned}$$

Link to control theory

- Recall the description of a control problem:

$$\begin{aligned} \dot{\underline{x}}(t) &= \underline{\underline{A}}\underline{x}(t) + \underline{\underline{B}}\underline{u}(t) \\ \underline{y}(t) &= \underline{\underline{C}}\underline{x}(t) + \underline{\underline{D}}\underline{u}(t) \end{aligned}$$

- $\underline{x}$ is system state; $\underline{y}$ is an output; $\underline{u}$ is a control
- Let's use the simplified picture where $\underline{y} = \underline{x}$; so $\underline{\underline{C}} = \underline{\underline{I}}$ and $\underline{\underline{D}} = \underline{\underline{0}}$

Metabolic control picture

- Compare

$$\dot{\underline{x}}(t) = \left(\underline{\underline{N}}\frac{\partial \underline{\nu}}{\partial \underline{s}}\right)\underline{x}(t) + \left(\underline{\underline{N}}\frac{\partial \underline{\nu}}{\partial \underline{p}}\right)\underline{u}(t)$$

- with

$$\dot{\underline{x}}(t) = \underline{\underline{A}}\underline{x}(t) + \underline{\underline{B}}\underline{u}(t)$$

- $\underline{\underline{A}} = \underline{\underline{N}}\left.\frac{\partial \underline{\nu}}{\partial \underline{s}}\right|_{\underline{s}_0,\underline{p}_0}$; $\underline{\underline{B}} = \underline{\underline{N}}\left.\frac{\partial \underline{\nu}}{\partial \underline{p}}\right|_{\underline{s}_0,\underline{p}_0}$
- $\underline{\underline{C}} = \underline{\underline{I}}$; $\underline{\underline{D}} = \underline{\underline{0}}$
- These derivatives describe changes in flux provoked by changes in concentrations and rate parameters, just as we saw previously

Metabolic control picture

- Transfer function for a single-input-single-output system

$$\underline{\underline{H}}(z) = \underline{\underline{C}} \left(z\underline{\underline{I}} - \underline{\underline{A}} \right)^{-1} \underline{\underline{B}} + \underline{\underline{D}}$$

- In our metabolic case we have

$$\underline{\underline{H}}(z) = \left(z\underline{\underline{I}} - \underline{\underline{N}} \frac{\partial \underline{\nu}}{\partial \underline{s}} \right)^{-1} \underline{\underline{N}} \frac{\partial \underline{\nu}}{\partial \underline{p}}$$

- A transfer function containing things that look like metabolic control coefficients – so we can explore the control behaviour of metabolic systems
- A little more work is needed to complete the mapping, but then equivalents of the two MCA theorems appear (see reference later)

Conclusions from these lectures

- First pair: introduction to metabolism; biochemistry of cellular energy production and use; ATP production; mitochondria; implementation of ODE modelling of mitochondrial physiology; tailoring ODE models to explore specific questions
- Second pair: flux balance analysis for simple metabolic modelling; linear programming and simplex algorithm; uses of flux balance analysis; evolution and metabolic optimisation; curation of metabolic information; implementation of linear programming for flux balance analysis; perturbation and responses in a metabolic model
- Third pair: metabolic reactions beyond stoichiometry; enzyme kinetics; metabolic control analysis; elasticities, control coefficients and theorems; link to classical control theory; implementation of metabolic control analysis in a simple model; theorem verification
- Metabolism is responsible for energy production in the natural world, providing the driving for inference, control, and a host of other processes
- Metabolism is itself controlled on a variety of levels and we have met and implemented several tools for quantitative investigation of metabolic networks

References for recommended reading

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