

Modelling Biochemical Reaction Networks

Lecture 4: Simplifying biochemical systems

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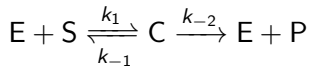
Recommended reading

- ▶ Fall, Marland, Wagner and Tyson, sections 4.1, 4.2 and 4.7

Enzyme kinetics

- ▶ Almost all enzymes catalyze reactions in a variation on the Michaelis-Menten mechanism.

For the conversion of a substrate S to a product P by an enzyme E, the mechanism is



where C is an enzyme-substrate complex.

Notation: Use $E = [E]$, etc.

Observation: $E + C = E_0$ is a constant.

Rate equations from mass action + enzyme conservation:

$$\begin{aligned}\frac{dS}{dt} &= -k_1 S(E_0 - C) + k_{-1} C \\ \frac{dC}{dt} &= k_1 S(E_0 - C) - (k_{-1} + k_{-2})C\end{aligned}$$

Modeling enzyme kinetics

- ▶ If we want to model an enzyme-catalyzed reaction using the law of mass action, we need at least three rate constants and the enzyme concentration.
- ▶ Need special experiments to get full set of rate constants
- ▶ Would give us concentration of C for which data is not often collected in experiments
- ▶ Can we simplify the rate equations?

The steady-state approximation

Observation: Most enzymes are very efficient catalysts present in low concentrations in cells.

Consequence 1: Concentration of C will remain low

Consequence 2: After an initial rise, we would expect C to change only slowly with time since it will be used up as fast as it is made.

Mathematically, this implies $dC/dt \approx 0$.

This is known as the steady-state approximation.

As a general rule, the SSA is applied to species that react quickly once formed, e.g. low-abundance intermediates.

The steady-state approximation

$$\begin{aligned}\frac{dC}{dt} &= k_1 S(E_0 - C) - (k_{-1} + k_{-2})C \approx 0 \\ \therefore C &\approx \frac{k_1 E_0 S}{k_1 S + k_{-1} + k_{-2}} \\ \therefore v &= \frac{dP}{dt} = k_{-2} C = \frac{k_1 k_{-2} E_0 S}{k_1 S + k_{-1} + k_{-2}}\end{aligned}$$

or

$$v = \frac{v_{\max} S}{S + K_S} \quad (\text{Michaelis-Menten equation})$$

where

$$\begin{aligned}v_{\max} &= k_{-2} E_0 \quad (\text{maximal velocity}) \\ K_S &= (k_{-1} + k_{-2})/k_1 \quad (\text{Michaelis constant})\end{aligned}$$

Michaelis-Menten rate law

$$v = \frac{v_{\max} S}{S + K_S}$$

- ▶ Depends on just two parameters, v_{\max} and K_S
 - ▶ Easily measurable
- ▶ Reduces the description in terms of elementary reactions to the single reaction $S \xrightarrow{E} P$

But is it OK to assume $\frac{dC}{dt} \approx 0$?

- ▶ Steady-state approximation based on smallness of $\frac{dC}{dt}$, in turn due to rapid degradation of C
- ▶ How do we know if C is degraded quickly?
What numbers should we be comparing?
Note: k_1 has different units than k_{-1} and k_{-2} .

Idea: Get rid of the units in all quantities in our equations.

Objective: Try to balance the terms so that the variables (S , C and t) are all of unit magnitude.
Then, small quantities will become apparent.

Scaling analysis

- ▶ Define $s = S/\tilde{S}$, $c = C/\tilde{C}$, and $\tau = t/\tilde{t}$, then try to pick \tilde{S} , \tilde{C} and \tilde{t} such that s , c and τ are all $O(1)$.
- ▶ Pick $\tilde{S} = S_0$.
- ▶ C rises from zero, hits a maximum, then starts to fall. $\tilde{C} = C_{\max}$ (or some estimate thereof) would be a good scaling factor.

$C(t)$ reaches a maximum when $dC/dt = 0$, i.e. when

$$C(t_{\max}) = \frac{k_1 E_0 S(t_{\max})}{k_1 S(t_{\max}) + k_{-1} + k_{-2}}$$

- ▶ $S \leq S_0$ and $C(t_{\max})$ is a strictly increasing function of $S(t_{\max})$, so pick

$$\tilde{C} = \frac{k_1 E_0 S_0}{k_1 S_0 + k_{-1} + k_{-2}} = \frac{E_0 S_0}{S_0 + K_S} \geq C(t_{\max}).$$

Scaling analysis

- ▶ Still need to find \tilde{t}
- ▶ Substitute $S = s\tilde{S}$, $C = c\tilde{C}$ and $t = \tau\tilde{t}$ into the rate equations:

$$\begin{aligned}\frac{dS}{dt} &= \frac{d(sS_0)}{d(\tau\tilde{t})} = \frac{S_0}{\tilde{t}} \frac{ds}{d\tau} \\ &= -k_1 s S_0 \left(E_0 - c \frac{E_0 S_0}{S_0 + K_S} \right) + k_{-1} c \frac{E_0 S_0}{S_0 + K_S} \\ \therefore \frac{ds}{d\tau} &= \tilde{t} \left\{ -k_1 E_0 s \left(1 - c \frac{S_0}{S_0 + K_S} \right) + k_{-1} c \frac{E_0}{S_0 + K_S} \right\}\end{aligned}$$

Similarly,

$$\frac{dc}{d\tau} = \tilde{t} k_1 (S_0 + K_S) \left\{ s \left(1 - c \frac{S_0}{S_0 + K_S} \right) - c \frac{K_S}{S_0 + K_S} \right\}$$

Scaling analysis

- ▶ Need to pick \tilde{t} such that $\tau = O(1)$

Principle: We are viewing C as a variable that changes slowly after the initial transient.

Therefore, the evolution of the reaction towards equilibrium is controlled by the rate of change of S , so look for the appropriate time scale in that equation.

$$\frac{ds}{d\tau} = \tilde{t} \left\{ -k_1 E_0 s \left(1 - c \frac{S_0}{S_0 + K_S} \right) + k_{-1} c \frac{E_0}{S_0 + K_S} \right\}$$

Note: The second term in $\frac{ds}{d\tau}$ is associated with $C \rightarrow E + S$, not typically a dominant process in enzyme kinetics.

Pick $\tilde{t} = (k_1 E_0)^{-1}$.

Scaling analysis

- ▶ Substitute \tilde{t} into the rate equations:

$$\begin{aligned}\frac{ds}{d\tau} &= -s \left(1 - c \frac{S_0}{S_0 + K_S} \right) + c \frac{k_{-1}}{k_1(S_0 + K_S)} \\ &= -s \left(1 - c \frac{S_0}{S_0 + K_S} \right) + c \frac{k_{-1}}{k_{-1} + k_{-2}} \frac{k_{-1} + k_{-2}}{k_1(S_0 + K_S)} \\ \frac{dc}{d\tau} &= \frac{S_0 + K_S}{E_0} \left[s \left(1 - c \frac{S_0}{S_0 + K_S} \right) - c \frac{K_S}{S_0 + K_S} \right]\end{aligned}$$

- ▶ Define

$$\mu = \frac{E_0}{S_0 + K_S}, \quad \alpha = \frac{S_0}{S_0 + K_S}, \quad \beta = \frac{k_{-1}}{k_{-1} + k_{-2}}$$

noting that

$$1 - \alpha = \frac{K_S}{S_0 + K_S}$$

Scaling analysis

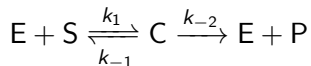
- ▶ Final equations:

$$\frac{ds}{d\tau} = -s(1 - \alpha c) + \beta c(1 - \alpha) \quad (1)$$

$$\text{and } \mu \frac{dc}{d\tau} = s(1 - \alpha c) - c(1 - \alpha) \quad (2)$$

- ▶ If μ is small (approaching zero), then the right-hand side of equation 2 must also be small.
This is the formal justification for the steady-state approximation.
- ▶ The steady-state approximation for the Michaelis-Menten mechanism will be valid if $E_0 \ll S_0 + K_S$.

The equilibrium approximation



- ▶ If k_{-2} is small, then we might expect the reversible step to approach equilibrium, with the formation of product being only a minor perturbation on this equilibrium.

Equilibrium approximation: $k_1ES = k_1S(E_0 - C) \approx k_{-1}C$

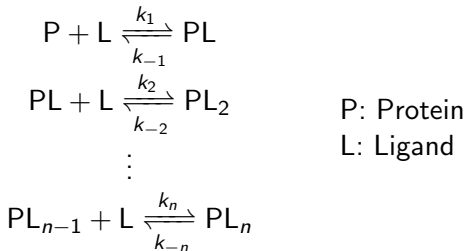
- ▶ Solving this equation for C and then calculating v , we get

$$v \approx \frac{v_{\max}S}{S + K_E}$$

with $K_E = k_{-1}/k_1$.

- ▶ This is of **exactly the same form** as the steady-state approximation.

Cooperative binding



- ▶ Equilibrium constants for the individual steps: $K_i = k_i/k_{-i}$
- ▶ We say that binding is **positively cooperative** if $K_n > K_{n-1} > \dots > K_1$ (often \gg).
- ▶ This implies $k_n > k_{n-1} > \dots > k_1$ or $k_{-n} < k_{-(n-1)} < \dots < k_{-1}$.

Cooperative binding

- ▶ Can often treat cooperative systems as if they are in quasi-equilibrium, even if (e.g.) PL_n goes on to other reactions:

$$k_i[PL_{i-1}][L] \approx k_{-i}[PL_i] \quad i = 1, 2, \dots, n$$

or

$$[PL_i] \approx K_i[PL_{i-1}][L]$$

- ▶ Start with $i = 1$:

$$\begin{aligned} [PL] &\approx K_1[P][L] &&= Q_1[P][L] \\ [PL_2] &\approx K_2[PL][L] &= K_1K_2[P][L]^2 &= Q_2[P][L]^2 \\ &\vdots &&\vdots \\ [PL_n] &\approx K_n[PL_{n-1}][L] = \left(\prod_{i=1}^n K_i \right) [P][L]^n = Q_n[P][L]^n \end{aligned}$$

Cooperative binding

- ▶ Assume strong cooperativity: $K_i \gg K_{i-1} \forall i$
- ▶ Then intermediate complexes are negligible.
- ▶ The total amount of protein (P_0) is conserved so, assuming an excess of ligand L,

$$P_0 = \sum_{i=0}^n [PL_i] \approx [P] + [PL_n] = [P] (1 + Q_n [L]^n)$$

$$\therefore [P] \approx \frac{P_0}{1 + Q_n [L]^n}$$

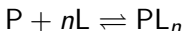
$$\text{and } [PL_n] \approx \frac{P_0 [L]^n}{Q_n^{-1} + [L]^n}$$

Cooperative binding

- ▶ The expression

$$[PL_n] \approx \frac{P_0[L]^n}{Q_n^{-1} + [L]^n}$$

is what we would get for a single reaction



with equilibrium constant Q_n (or dissociation/Michaelis constant Q_n^{-1}).

- ▶ Usually model cooperative interactions as a single step, even though these reactions are never elementary