Chapter 2

Enzyme kinetics

Biochemical reactions are extremely important for biological function. For example, they are involved in metabolism and its control, immunological responses, and cell-signalling processes. Biochemical processes are often controlled by enzymes (Figure 2.1, left). Enzymes are proteins that catalyse biochemical reactions by lowering the activation energy. Even when present in very small amounts, enzymes can have a dramatic effect (Figure 2.1, right). Table 2.1 illustrates how effective enzymes can be at accelerating reactions in biochemical systems.

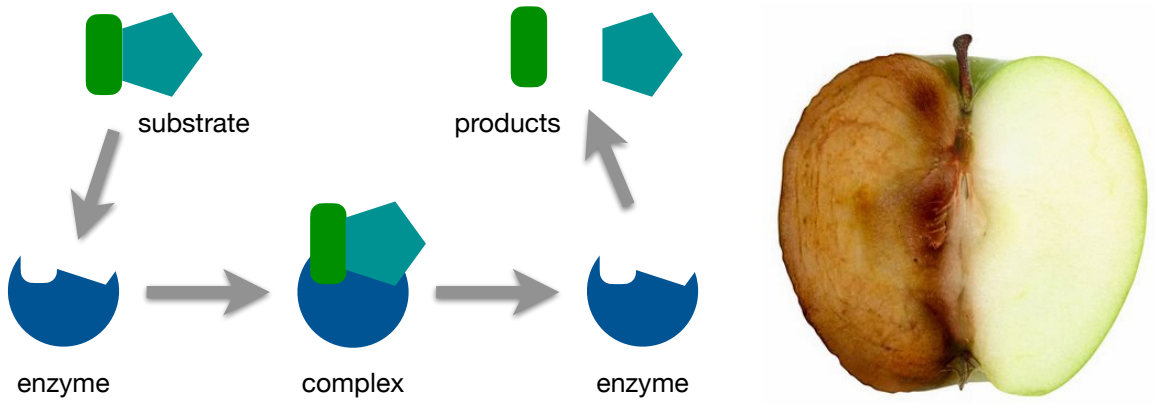

Figure 2.1: Left: How do enzymes work? An enzyme has an active site where the substrate and enzyme fit together so that the substrate reacts. After the reaction, the products are released and the enzyme assumes its original shape. Right: The enzyme catecholase catalyses a reaction between the molecule catechol and oxygen. The product of this reaction is polyphenol, the brown substance that accumulate when apples are exposed to air.

In this chapter we will focus on developing and analysing models for enzyme kinetics. These can be thought of as a special case of an interacting species model. In all cases we will neglect spatial variation, assuming the systems are well-mixed. As such, the models will consist of systems of ordinary differential equations that describe how the concentrations of the reactants evolve over time.

Enzyme	Substrate	Product	Rate without enzyme	Rate with enzyme	Acceleration due to enzyme
Hexokinase	Glucose	Glucose 6-Phosphate	< 0.0000001	1300	> 13 billion
Phosphorylase	–	–	< 0.000000005	1600	> 320 billion
Alcohol Dehydrogenase	Ethanol	Acetaldehyde	< 0.000006	2700	> 450 million
Creatine Kinase	Creatine	Creatine Phosphate	< 0.003	40	$> 13,000$

Table 2.1: Examples illustrating the dramatic effect that enzymes can have on reaction rates.

2.1 The Law of Mass Action

Throughout this chapter, we will consider reactions involving m chemical species $C_1, \ldots, C_m$.

- The concentration of C_i, denoted c_i, is defined to be the number of molecules of C_i per unit volume.
- A standard unit of concentration is moles m^{-3}, often abbreviated to mol m^{-3}. Recall that 1 mole $= 6.023 \times 10^{23}$ molecules.

We will use the Law of Mass Action to construct the reaction rates.

The Law of Mass Action. A chemical reaction proceeds at a rate proportional to the concentrations of the participating reactants. The constant of proportionality is called the rate constant.

Suppose $C_1, \ldots, C_m$ undergo the reaction

$$\lambda_1 C_1 + \lambda_2 C_2 + \ldots + \lambda_m C_m \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \nu_1 C_1 + \nu_2 C_2 + \ldots + \nu_m C_m. \tag{2.1}$$

The Law of Mass Action states that the forward reaction proceeds at rate

$$k_f c_1^{\lambda_1} c_2^{\lambda_2} \ldots c_m^{\lambda_m}, \tag{2.2}$$

while the backward (or reverse) reaction proceeds at rate

$$k_b c_1^{\nu_1} c_2^{\nu_2} \ldots c_m^{\nu_m}, \tag{2.3}$$

where k_f and k_b are (non-negative) dimensional constants.

Note 1. Strictly speaking, to treat k_f, k_b as constant we must assume that the temperature is constant. This is a good approximation for most biochemical reactions occurring in, for example, physiological systems. However, if one wanted to model reactions that produce significant amounts of heat (for example, burning petrol), one must include temperature dependence in k_f and k_b and, subsequently, keep track of how the temperature of the system changes as the reaction proceeds. This typically makes the modelling more difficult. To keep things simple here, we will assume that we are dealing with systems where the temperature remains approximately constant as the reaction proceeds.

Note 2. The Law of Mass Action for chemical reactions can be derived from statistical mechanics under quite general conditions (see, for example [12]).

Note 3. The Law of Mass Action is used in a variety of biological scenarios. For example, we use it to write down equations describing interactions between people infected with, and people susceptible to, a pathogen during an epidemic. In such circumstances the validity of the Law of Mass Action must be taken as a modelling assumption as one cannot rely on thermodynamic/statistical mechanical arguments to justify the Law of Mass Action.

2.1.1 Example: stoichiometry

Suppose m molecules of A react reversibly with n molecules of B to create C:

$$mA + nB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C. \tag{2.4}$$

Then the Law of Mass Action takes the form

$$\frac{\mathrm{d}a}{\mathrm{d}t} = -mk_1a^mb^n + mk_{-1}c, \tag{2.5}$$

$$\frac{\mathrm{d}b}{\mathrm{d}t} = -nk_1a^mb^n + nk_{-1}c, \tag{2.6}$$

$$\frac{\mathrm{d}c}{\mathrm{d}t} = k_1a^mb^n - k_{-1}c, \tag{2.7}$$

as m molecules of A and n molecules of B must collide to produce one molecule of C. Note that mass conservation supplies

$$a + mc = \text{constant}, \quad b + nc = \text{constant}. \tag{2.8}$$

2.2 Michaelis-Menten kinetics

Michaelis-Menten kinetics approximately describe the dynamics of a number of enzyme systems. The reactions are

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \qquad \text{and} \qquad C \xrightarrow{k_2} P + E, \tag{2.9}$$

where C represents the complex SE, and s, e, p and c denote the concentrations of S, E, P and C, respectively. From the Law of Mass Action, we can derive the following ordinary differential equations for s, e, p and c:

$$\frac{\mathrm{d}s}{\mathrm{d}t} = -k_1 se + k_{-1}c; \tag{2.10}$$

$$\frac{\mathrm{d}c}{\mathrm{d}t} = k_1 se - k_{-1}c - k_2 c; \tag{2.11}$$

$$\frac{\mathrm{d}e}{\mathrm{d}t} = -k_1 se + k_{-1}c + k_2 c; \tag{2.12}$$

$$\frac{\mathrm{d}p}{\mathrm{d}t} = k_2 c. \tag{2.13}$$

Note that the equation for p decouples and, hence, we can neglect it (at least initially).

The initial conditions are

$$s(0) = s_0, \quad e(0) = e_0 \ll s_0, \quad c(0) = 0, \quad p(0) = 0. \tag{2.14}$$

Key Point: Conservation laws. In systems described by the Law of Mass Action, linear combinations of the variables are often conserved. In this example we have

$$\frac{\mathrm{d}}{\mathrm{d}t}(e + c) = 0 \quad \Longrightarrow \quad e(t) = e_0 - c(t), \tag{2.15}$$

and, hence, the equations simplify to

$$\frac{\mathrm{d}s}{\mathrm{d}t} = -k_1(e_0 - c)s + k_{-1}c, \tag{2.16}$$

$$\frac{\mathrm{d}c}{\mathrm{d}t} = k_1(e_0 - c)s - (k_{-1} + k_2)c. \tag{2.17}$$

The dynamics of p and e are readily achievable once the dynamics of s and c are known.

2.2.1 Non-dimensionalisation

We non-dimensionalise as follows

$$\tau = k_1 e_0 t, \quad u = \frac{s}{s_0}, \quad v = \frac{c}{e_0}, \quad \lambda = \frac{k_2}{k_1 s_0}, \quad \epsilon \stackrel{\text{def}}{=} \frac{e_0}{s_0} \ll 1, \quad K \stackrel{\text{def}}{=} \frac{k_{-1} + k_2}{k_1 s_0}, \tag{2.18}$$

which yields

$$\frac{\mathrm{d}u}{\mathrm{d}\tau} = -u + (u + K - \lambda)v, \tag{2.19}$$

$$\epsilon \frac{\mathrm{d}v}{\mathrm{d}\tau} = u - (u + K)v, \tag{2.20}$$

where $u(0) = 1$, $v(0) = 0$ and $\epsilon \ll 1$.

A typical value for ϵ is $\epsilon \sim 10^{-6}$. It is tempting to set $\epsilon = 0$ and analyse the system of ordinary differential equations. However, this gives

$$v = \frac{u}{u + K}, \tag{2.21}$$

which is inconsistent with the initial conditions since

$$v(0) = 0 \neq \frac{1}{1 + K} = \frac{u(0)}{u(0) + K}. \tag{2.22}$$

We have a singular perturbation problem; there must be a (boundary) region with respect to the time variable around $t = 0$ where $v' \nsim \mathcal{O}(1)$. Indeed, for the stated initial conditions we find $v'(0) \sim \mathcal{O}(1/\epsilon)$, with $u(0)$, $v(0) \leq \mathcal{O}(1)$. This gives us the scaling we need to perform a singular perturbation analysis.

2.2.2 Singular perturbation investigation

We first re-scale time to consider dynamics in the very early stages of the reaction. We let

$$\sigma = \frac{\tau}{\epsilon}, \tag{2.23}$$

with

$$u(\tau, \epsilon) = \tilde{u}(\sigma, \epsilon) = \tilde{u}_0(\sigma) + \epsilon \tilde{u}_1(\sigma) + \ldots, \tag{2.24}$$

$$v(\tau, \epsilon) = \tilde{v}(\sigma, \epsilon) = \tilde{v}_0(\sigma) + \epsilon \tilde{v}_1(\sigma) + \ldots. \tag{2.25}$$

Proceeding in the usual way — substituting into Equations (2.19)-(2.20) and collecting terms — we find that $\tilde{u}_0$, $\tilde{v}_0$ satisfy

$$\frac{\mathrm{d}\tilde{u}_0}{\mathrm{d}\sigma} = 0 \quad \Longrightarrow \quad \tilde{u}_0 = \text{constant} = 1, \tag{2.26}$$

and

$$\frac{\mathrm{d}\tilde{v}_0}{\mathrm{d}\sigma} = \tilde{u}_0 - (1+K)\tilde{v}_0 = 1 - (1+K)\tilde{v}_0 \quad \Longrightarrow \quad \tilde{v}_0 = \frac{1 - e^{-(1+K)\sigma}}{1+K}, \tag{2.27}$$

which gives us the *inner* solution.

To find the *outer* solution, which describes how the reaction proceeds on longer time scales, we expand in the original, non-dimensional time variable

$$u(\tau, \epsilon) = u_0(\tau) + \epsilon u_1(\tau) + \ldots, \tag{2.28}$$

$$v(\tau, \epsilon) = v_0(\tau) + \epsilon v_1(\tau) + \ldots. \tag{2.29}$$

Again, substituting into Equations (2.19)-(2.20) and collecting terms, we find that u_0 and v_0 satisfy

$$\frac{\mathrm{d}u_0}{\mathrm{d}\tau} = -u_0 + (u_0 + K - \lambda)v_0, \tag{2.30}$$

$$0 = u_0 - (u_0 + K)v_0. \tag{2.31}$$

This gives

$$v_0 = \frac{u_0}{u_0 + K} \quad \text{and} \quad \frac{\mathrm{d}u_0}{\mathrm{d}\tau} = -\frac{\lambda u_0}{u_0 + K}. \tag{2.32}$$

In order to match the solutions as $\sigma \to \infty$ and $\tau \to 0$ we require

$$\lim_{\sigma\to\infty} \tilde{u}_0 = \lim_{\tau\to 0} u_0 = 1 \quad \text{and} \quad \lim_{\sigma\to\infty} \tilde{v}_0 = \lim_{\tau\to 0} v_0 = \frac{1}{1+K}. \tag{2.33}$$

The resulting solution looks like that shown in Figure 2.2. The left-hand plot shows the solution over long times scales, whilst the right-hand plot shows the initial, transient dynamics.

2.2.3 The pseudo steady state hypothesis

Often the initial, fast, transient is not seen or modelled: we consider only the outer equations, with a suitably adjusted initial condition (ultimately determined from consistency/matching with the inner solution). In particular, we often use *Michaelis-Menten kinetics* where the equations

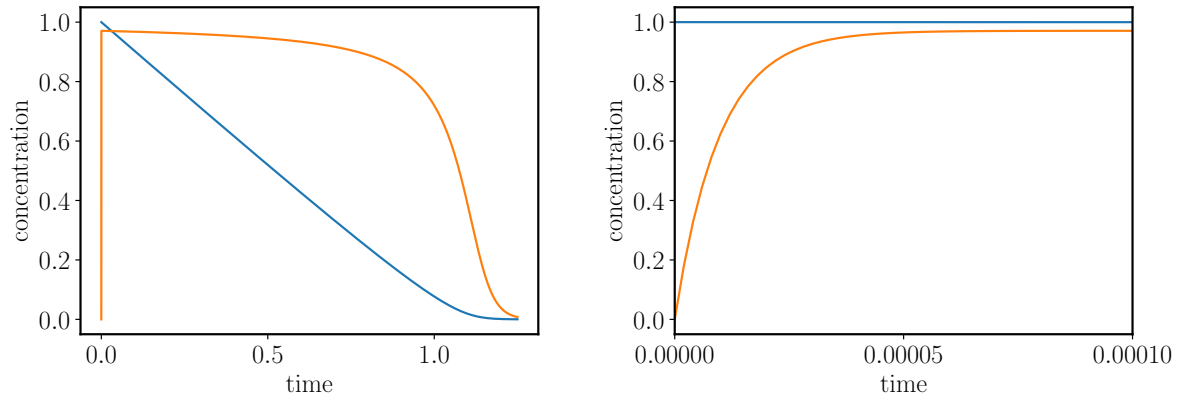

Figure 2.2: Numerical solution of the non-dimensional Michaelis-Menten equations clearly illustrating the two different time scales on which the system evolves: at short times, v increases rapidly while u remains approximately constant; at long times, u decreases to zero and the dynamics of v are slave to those of u. The u dynamics are indicated by the blue line and the v dynamics by the orange line. Parameters are $\epsilon = 10^{-5}$, $K = 0.03$ and $\lambda = 1.0$.

are simply

$$\frac{\mathrm{d}u}{\mathrm{d}t} = -\frac{\lambda u}{u+K} \quad \text{with} \quad u(0) = 1 \quad \text{and} \quad v = \frac{u}{u+K}. \tag{2.34}$$

Definition. When the time derivative is fast, *i.e.* of the form

$$\epsilon \frac{\mathrm{d}v}{\mathrm{d}\tau} = g(u, v), \tag{2.35}$$

where $\epsilon \ll 1$ and $g(u, v) \sim \mathcal{O}(1)$, taking the temporal dynamics to be trivial,

$$\frac{\mathrm{d}v}{\mathrm{d}\tau} \simeq 0, \tag{2.36}$$

is known as the *pseudo-steady state hypothesis*. This is a common assumption in the literature. We have seen its validity for enzyme kinetics, at least away from the inner region.

Note. While the Michaelis-Menten kinetics derived above are a useful approximation, they hinge on the validity of the Law of Mass Action. Even in simple biological systems the Law of Mass Action may break down. One (of many) reasons, and one that is potentially relevant at the sub-cellular level, is that the system in question has too few reactant molecules to justify the statistical mechanical assumptions underlying the Lass of Mass Action. Another reason is that the reactants are not well-mixed, but vary spatially as well as temporally. We will see what happens in this case later in the course.

2.3 More complex systems

Here we consider a number of other simple systems involving enzymatic reactions. In each case the Law of Mass Action is used to write down a system of ordinary differential equations describing the dynamics of the various reactants.

2.3.1 Allosteric enzymes

Here the binding of one substrate molecule at one site affects the binding of another substrate molecules at other sites. A typical reaction scheme is:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} P + E; \tag{2.37}$$

$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} C_1 + P. \tag{2.38}$$

2.3.2 Several enzyme reactions and the pseudo-steady state hypothesis

We can have multiple enzymes. For example, consider an enzymatic reaction in which an enzyme can be activated or inactivated by a chemical X as follows:

$$E + X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_1, \qquad E_1 + X \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E_2, \qquad E_1 + S \xrightarrow{k_3} P + Q + E. \tag{2.39}$$

Suppose further that X is supplied at a constant rate, and removed at a rate proportional to its concentration. As before, we can use the Law of Mass Action to write down a system of ordinary differential equations describing the evolution of the chemical concentrations.

In general, for chemical reaction networks of this type, after non-dimensionalisation the system of ordinary differential equations reduces to

$$\frac{\mathrm{d}u}{\mathrm{d}t} = f(u, v_1, \ldots, v_n), \tag{2.40}$$

$$\epsilon_i \frac{\mathrm{d}v_i}{\mathrm{d}t} = g_i(u, v_1, \ldots, v_n), \tag{2.41}$$

for $i \in \{1, \ldots, n\}$.

In this case, the pseudo-steady state hypothesis gives a single ordinary differential equation

$$\frac{\mathrm{d}u}{\mathrm{d}t} = f(u, v_1(u), \ldots, v_n(u)), \tag{2.42}$$

where $v_1(u), \ldots, v_n(u)$ are the appropriate roots of the equations

$$g_i(u, v_1, \ldots, v_n) = 0, \quad i \in \{1, \ldots, n\}. \tag{2.43}$$

Exercise (hard – use for revision).

1. Write down ordinary differential equations for the evolution of the concentrations of E, E_1, E_2, X and S.

2. Show that $E + E_1 + E_2$ is a conserved quantity, E^*, say.

3. Nondimensionalise the system, scaling E, E_1 and E_2 with E^*, X and S with $X_0 = X(0)$, and time with $1/(k_1 E^*)$. Assuming that $\delta = E^*/X_0 \ll 1$, use the resulting 'quasi-steady' equations for the dimensionless quantities e, e_1, and e_2 to solve for these variables in terms of x and s, and hence obtain the following system of ordinary differential equations for x and s only:

$$\frac{\mathrm{d}x}{\mathrm{d}\tau} = \alpha_0 - \nu_4 x - \frac{\kappa_3 x s}{\mu_1 + \kappa_3 s + x + \kappa_2 x^2/\mu_2}; \tag{2.44}$$

$$\frac{\mathrm{d}s}{\mathrm{d}\tau} = -\frac{\kappa_3 x s}{\mu_1 + \kappa_3 s + x + \kappa_2 x^2/\mu_2}. \tag{2.45}$$

   Identify all parameters and variables in these equations.

2.3.3 Autocatalysis and activator-inhibitor systems

Here a molecule catalyses its own production. The simplest example is the reaction scheme

$$A + B \xrightarrow{k} 2B, \tag{2.46}$$

although the positive feedback in autocatalysis is usually ameliorated by inhibition from another molecule. This leads to an example of an activator-inhibitor system which can have a very rich behaviour. Other examples of these systems are given below.

Example 1. This model qualitatively incorporates activation and inhibition:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = \frac{a}{b + v} - cu; \tag{2.47}$$

$$\frac{\mathrm{d}v}{\mathrm{d}t} = du - ev. \tag{2.48}$$

Example 2. This model is commonly referred to as the Gierer-Meinhardt model and was proposed in 1972 [4]:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = a - bu + \frac{u^2}{v}; \tag{2.49}$$

$$\frac{\mathrm{d}v}{\mathrm{d}t} = u^2 - v. \tag{2.50}$$

Example 3. This model is commonly referred to as the Thomas model [13]. Proposed in 1975, it is an empirical model based on a specific reaction involving uric acid and oxygen:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = a - u - \rho R(u, v); \tag{2.51}$$

$$\frac{\mathrm{d}v}{\mathrm{d}t} = \alpha(b - v) - \rho R(u, v). \tag{2.52}$$

The function

$$R(u, v) = \frac{uv}{1 + u + Ku^2}, \tag{2.53}$$

represents the interactive uptake.

Suggested reading.

- J. D. Murray, *Mathematical Biology, Volume I: An Introduction* – Chapter 6.
- J. P. Keener and J. Sneyd, *Mathematical Physiology* – Chapter 1.
- K. A. Johnson and R. S. Goody (2011). The original Michaelis constant: translation of the 1913 Michaelis-Menten paper. *Biochem.* 50(39):8264–8269.
- J. Gunawardena (2011). Some lessons about models from Michaelis and Menten. *Mol. Biol. Cell* 23(4): 517–519.