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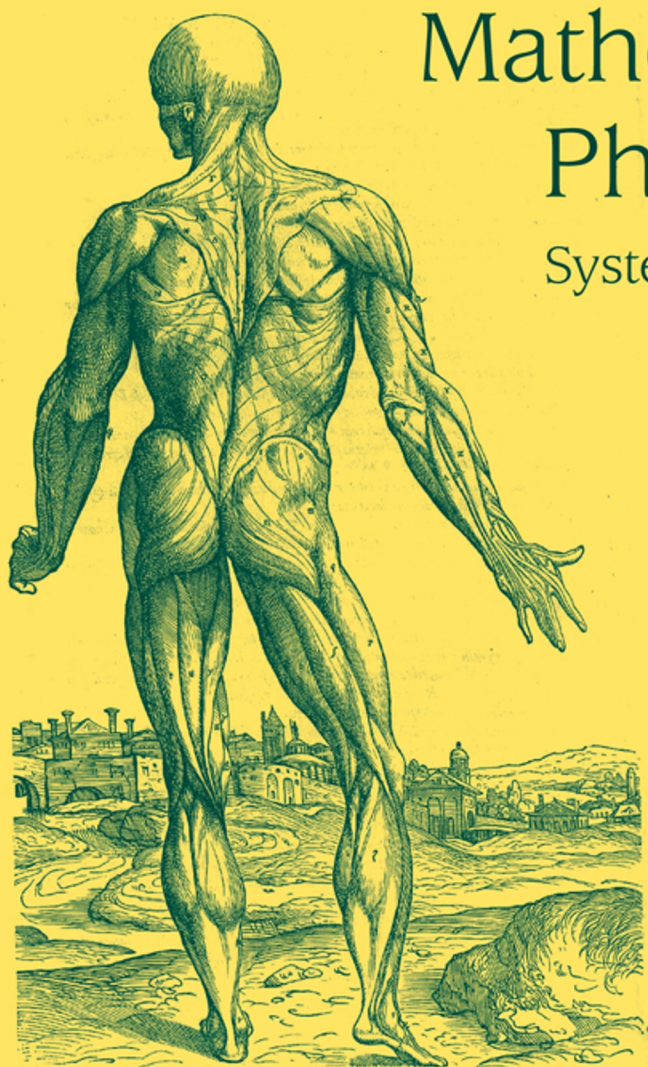
MATHEMATICAL BIOLOGY

Mathematical Physiology

Systems Physiology

James Keener
James Sneyd

Second Edition



 Springer

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Mathematical Physiology

I: Cellular Physiology

Second Edition

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Biochemical Reactions

Cells can do lots of wonderful things. Individually they can move, contract, excrete, reproduce, signal or respond to signals, and carry out the energy transactions necessary for this activity. Collectively they perform all of the numerous functions of any living organism necessary to sustain life. Yet, remarkably, all of what cells do can be described in terms of a few basic natural laws. The fascination with cells is that although the rules of behavior are relatively simple, they are applied to an enormously complex network of interacting chemicals and substrates. The effort of many lifetimes has been consumed in unraveling just a few of these reaction schemes, and there are many more mysteries yet to be uncovered.

1.1 The Law of Mass Action

The fundamental “law” of a chemical reaction is the law of mass action. This law describes the rate at which chemicals, whether large macromolecules or simple ions, collide and interact to form different chemical combinations. Suppose that two chemicals, say A and B, react upon collision with each other to form product C,



The rate of this reaction is the rate of accumulation of product, $\frac{d[C]}{dt}$. This rate is the product of the number of collisions per unit time between the two reactants and the probability that a collision is sufficiently energetic to overcome the free energy of activation of the reaction. The number of collisions per unit time is taken to be proportional

to the product of the concentrations of A and B with a factor of proportionality that depends on the geometrical shapes and sizes of the reactant molecules and on the temperature of the mixture. Combining these factors, we have

$$\frac{d[C]}{dt} = k[A][B]. \quad (1.2)$$

The identification of (1.2) with the reaction (1.1) is called the *law of mass action*, and the constant k is called the *rate constant* for the reaction. However, the law of mass action is not a law in the sense that it is inviolable, but rather it is a useful model, much like Ohm's law or Newton's law of cooling. As a model, there are situations in which it is not valid. For example, at high concentrations, doubling the concentration of one reactant need not double the overall reaction rate, and at extremely low concentrations, it may not be appropriate to represent concentration as a continuous variable.

For thermodynamic reasons all reactions proceed in both directions. Thus, the reaction scheme for A, B, and C should have been written as



with k_+ and k_- denoting, respectively, the forward and reverse rate constants of reaction. If the reverse reaction is slow compared to the forward reaction, it is often ignored, and only the primary direction is displayed. Since the quantity A is consumed by the forward reaction and produced by the reverse reaction, the rate of change of [A] for this bidirectional reaction is

$$\frac{d[A]}{dt} = k_-[C] - k_+[A][B]. \quad (1.4)$$

At equilibrium, concentrations are not changing, so that

$$\frac{k_-}{k_+} \equiv K_{\text{eq}} = \frac{[A]_{\text{eq}}[B]_{\text{eq}}}{[C]_{\text{eq}}}. \quad (1.5)$$

The ratio k_-/k_+ , denoted by K_{eq} , is called the *equilibrium constant* of the reaction. It describes the relative preference for the chemicals to be in the combined state C compared to the dissociated state. If K_{eq} is small, then at steady state most of A and B are combined to give C.

If there are no other reactions involving A and C, then $[A] + [C] = A_0$ is constant, and

$$[C]_{\text{eq}} = A_0 \frac{[B]_{\text{eq}}}{K_{\text{eq}} + [B]_{\text{eq}}}, \quad [A]_{\text{eq}} = A_0 \frac{K_{\text{eq}}}{K_{\text{eq}} + [B]_{\text{eq}}}. \quad (1.6)$$

Thus, when $[B]_{\text{eq}} = K_{\text{eq}}$, half of A is in the bound state at equilibrium.

There are several other features of the law of mass action that need to be mentioned. Suppose that the reaction involves the dimerization of two monomers of the same species A to produce species C,



For every C that is made, two of A are used, and every time C degrades, two copies of A are produced. As a result, the rate of reaction for A is

$$\frac{d[A]}{dt} = 2k_-[C] - 2k_+[A]^2. \quad (1.8)$$

However, the rate of production of C is half that of A,

$$\frac{d[C]}{dt} = -\frac{1}{2} \frac{d[A]}{dt}, \quad (1.9)$$

and the quantity $[A]+2[C]$ is conserved (provided there are no other reactions).

In a similar way, with a trimolecular reaction, the rate at which the reaction takes place is proportional to the product of three concentrations, and three molecules are consumed in the process, or released in the degradation of product. In real life, there are probably no truly trimolecular reactions. Nevertheless, there are some situations in which a reaction might be effectively modeled as trimolecular (Exercise 2).

Unfortunately, the law of mass action cannot be used in all situations, because not all chemical reaction mechanisms are known with sufficient detail. In fact, a vast number of chemical reactions cannot be described by mass action kinetics. Those reactions that follow mass action kinetics are called *elementary reactions* because presumably, they proceed directly from collision of the reactants. Reactions that do not follow mass action kinetics usually proceed by a complex mechanism consisting of several elementary reaction steps. It is often the case with biochemical reactions that the elementary reaction steps are not known or are very complicated to write down.

1.2 Thermodynamics and Rate Constants

There is a close relationship between the rate constants of a reaction and thermodynamics. The fundamental concept is that of *chemical potential*, which is the Gibbs free energy, G , per mole of a substance. Often, the Gibbs free energy per mole is denoted by μ rather than G . However, because μ has many other uses in this text, we retain the notation G for the Gibbs free energy.

For a mixture of ideal gases, X_i , the chemical potential of gas i is a function of temperature, pressure, and concentration,

$$G_i = G_i^0(T, P) + RT \ln(x_i), \quad (1.10)$$

where x_i is the mole fraction of X_i , R is the universal gas constant, T is the absolute temperature, and P is the pressure of the gas (in atmospheres); values of these constants, and their units, are given in the appendix. The quantity $G_i^0(T, P)$ is the standard free energy per mole of the pure ideal gas, i.e., when the mole fraction of the gas is 1. Note

that, since $x_i \leq 1$, the free energy of an ideal gas in a mixture is always less than that of the pure ideal gas. The total Gibbs free energy of the mixture is

$$G = \sum_i n_i G_i, \quad (1.11)$$

where n_i is the number of moles of gas i .

The theory of Gibbs free energy in ideal gases can be extended to ideal dilute solutions. By redefining the standard Gibbs free energy to be the free energy at a concentration of 1 M, i.e., 1 mole per liter, we obtain

$$G = G^0 + RT \ln(c), \quad (1.12)$$

where the concentration, c , is in units of moles per liter. The standard free energy, G^0 , is obtained by measuring the free energy for a dilute solution and then extrapolating to $c = 1$ M. For biochemical applications, the dependence of free energy on pressure is ignored, and the pressure is assumed to be 1 atm, while the temperature is taken to be 25°C. Derivations of these formulas can be found in physical chemistry textbooks such as Levine (2002) and Castellan (1971).

For nonideal solutions, such as are typical in cells, the free energy formula (1.12) should use the chemical activity of the solute rather than its concentration. The relationship between chemical activity a and concentration is nontrivial. However, for dilute concentrations, they are approximately equal.

Since the free energy is a potential, it denotes the preference of one state compared to another. Consider, for example, the simple reaction



The change in chemical potential ΔG is defined as the difference between the chemical potential for state B (the product), denoted by G_B , and the chemical potential for state A (the reactant), denoted by G_A ,

$$\begin{aligned} \Delta G &= G_B - G_A \\ &= G_B^0 - G_A^0 + RT \ln([B]) - RT \ln([A]) \\ &= \Delta G^0 + RT \ln([B]/[A]). \end{aligned} \quad (1.14)$$

The sign of ΔG is important, which is why it is defined with only one reaction direction shown, even though we know that the back reaction also occurs. In fact, there is a wonderful opportunity for confusion here, since there is no obvious way to decide which is the forward and which is the backward direction for a given reaction. If $\Delta G < 0$, then state B is preferred to state A, and the reaction tends to convert A into B, whereas, if $\Delta G > 0$, then state A is preferred to state B, and the reaction tends to convert B into A. Equilibrium occurs when neither state is preferred, so that $\Delta G = 0$, in which case

$$\frac{[B]_{\text{eq}}}{[A]_{\text{eq}}} = e^{-\frac{\Delta G^0}{RT}}. \quad (1.15)$$

Expressing this reaction in terms of forward and backward reaction rates,



we find that in steady state, $k_+[A]_{\text{eq}} = k_-[B]_{\text{eq}}$, so that

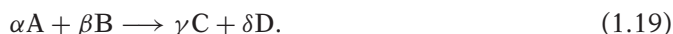
$$\frac{[A]_{\text{eq}}}{[B]_{\text{eq}}} = \frac{k_-}{k_+} = K_{\text{eq}}. \quad (1.17)$$

Combining this with (1.15), we observe that

$$K_{\text{eq}} = e^{\frac{\Delta G^0}{RT}}. \quad (1.18)$$

In other words, the more negative the difference in standard free energy, the greater the propensity for the reaction to proceed from left to right, and the smaller is K_{eq} . Notice, however, that this gives only the ratio of rate constants, and not their individual amplitudes. We learn nothing about whether a reaction is fast or slow from the change in free energy.

Similar relationships hold when there are multiple components in the reaction. Consider, for example, the more complex reaction



The change of free energy for this reaction is defined as

$$\begin{aligned} \Delta G &= \gamma G_C + \delta G_D - \alpha G_A - \beta G_B \\ &= \gamma G_C^0 + \delta G_D^0 - \alpha G_A^0 - \beta G_B^0 + RT \ln \left(\frac{[C]^\gamma [D]^\delta}{[A]^\alpha [B]^\beta} \right) \\ &= \Delta G^0 + RT \ln \left(\frac{[C]^\gamma [D]^\delta}{[A]^\alpha [B]^\beta} \right), \end{aligned} \quad (1.20)$$

and at equilibrium,

$$\Delta G^0 = RT \ln \left(\frac{[A]_{\text{eq}}^\alpha [B]_{\text{eq}}^\beta}{[C]_{\text{eq}}^\gamma [D]_{\text{eq}}^\delta} \right) = RT \ln(K_{\text{eq}}). \quad (1.21)$$

An important example of such a reaction is the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate P_i , represented by the reaction



The standard free energy change for this reaction is

$$\Delta G^0 = G_{\text{ADP}}^0 + G_{P_i}^0 - G_{\text{ATP}}^0 = -31.0 \text{ kJ mol}^{-1}, \quad (1.23)$$

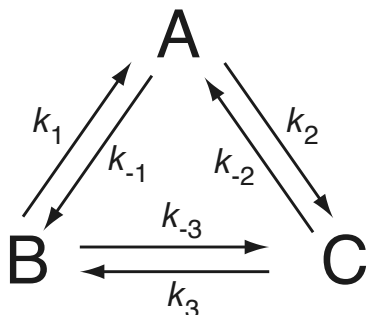


Figure 1.1 Schematic diagram of a reaction loop.

and from this we could calculate the equilibrium constant for this reaction. However, the primary significance of this is not the size of the equilibrium constant, but rather the fact that ATP has free energy that can be used to drive other less favorable reactions. For example, in all living cells, ATP is used to pump ions against their concentration gradient, a process called free energy transduction. In fact, if the equilibrium constant of this reaction is achieved, then one can confidently assert that the system is dead. In living systems, the ratio of $[ATP]$ to $[ADP][P_i]$ is held well above the equilibrium value.

1.3 Detailed Balance

Suppose that a set of reactions forms a loop, as shown in Fig. 1.1. By applying the law of mass action and setting the derivatives to zero we can find the steady-state concentrations of A, B and C. However, for the system to be in thermodynamic equilibrium a stronger condition must hold. Thermodynamic equilibrium requires that the free energy of each state be the same so that each individual reaction is in equilibrium. In other words, at equilibrium there is not only, say, no net change in $[B]$, there is also no net conversion of B to C or B to A. This condition means that, at equilibrium, $k_1[B] = k_{-1}[A]$, $k_2[A] = k_{-2}[C]$ and $k_3[C] = k_{-3}[B]$. Thus, it must be that

$$k_1 k_2 k_3 = k_{-1} k_{-2} k_{-3}, \quad (1.24)$$

or

$$K_1 K_2 K_3 = 1, \quad (1.25)$$

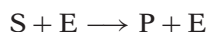
where $K_i = k_{-i}/k_i$. Since this condition does not depend on the concentrations of A, B or C, it must hold in general, not only at equilibrium.

For a more general reaction loop, the principle of detailed balance requires that the product of rates in one direction around the loop must equal the product of rates in the other direction. If any of the rates are dependent on concentrations of other chemicals, those concentrations must also be included.

1.4 Enzyme Kinetics

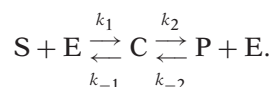
To see where some of the more complicated reaction schemes come from, we consider a reaction that is catalyzed by an enzyme. Enzymes are catalysts (generally proteins) that help convert other molecules called *substrates* into products, but they themselves are not changed by the reaction. Their most important features are catalytic power, specificity, and regulation. Enzymes accelerate the conversion of substrate into product by lowering the free energy of activation of the reaction. For example, enzymes may aid in overcoming charge repulsions and allowing reacting molecules to come into contact for the formation of new chemical bonds. Or, if the reaction requires breaking of an existing bond, the enzyme may exert a stress on a substrate molecule, rendering a particular bond more easily broken. Enzymes are particularly efficient at speeding up biological reactions, giving increases in speed of up to 10 million times or more. They are also highly specific, usually catalyzing the reaction of only one particular substrate or closely related substrates. Finally, they are typically regulated by an enormously complicated set of positive and negative feedbacks, thus allowing precise control over the rate of reaction. A detailed presentation of enzyme kinetics, including many different kinds of models, can be found in Dixon and Webb (1979), the encyclopedic Segel (1975) or Kernevez (1980). Here, we discuss only some of the simplest models.

One of the first things one learns about enzyme reactions is that they do not follow the law of mass action directly. For, as the concentration of substrate (S) is increased, the rate of the reaction increases only to a certain extent, reaching a maximal reaction velocity at high substrate concentrations. This is in contrast to the law of mass action, which, when applied directly to the reaction of S with the enzyme E

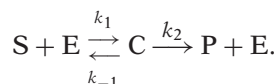


predicts that the reaction velocity increases linearly as [S] increases.

A model to explain the deviation from the law of mass action was first proposed by Michaelis and Menten (1913). In their reaction scheme, the enzyme E converts the substrate S into the product P through a two-step process. First E combines with S to form a complex C which then breaks down into the product P releasing E in the process. The reaction scheme is represented schematically by



Although all reactions must be reversible, as shown here, reaction rates are typically measured under conditions where P is continually removed, which effectively prevents the reverse reaction from occurring. Thus, it often suffices to assume that no reverse reaction occurs. For this reason, the reaction is usually written as



The reversible case is considered in Section 1.4.5.

There are two similar, but not identical, ways to analyze this equation; the equilibrium approximation, and the quasi-steady-state approximation. Because these methods give similar results it is easy to confuse them, so it is important to understand their differences.

We begin by defining $s = [S]$, $c = [C]$, $e = [E]$, and $p = [P]$. The law of mass action applied to this reaction mechanism gives four differential equations for the rates of change of s, c, e , and p ,

$$\frac{ds}{dt} = k_{-1}c - k_1se, \quad (1.26)$$

$$\frac{de}{dt} = (k_{-1} + k_2)c - k_1se, \quad (1.27)$$

$$\frac{dc}{dt} = k_1se - (k_2 + k_{-1})c, \quad (1.28)$$

$$\frac{dp}{dt} = k_2c. \quad (1.29)$$

Note that p can be found by direct integration, and that there is a conserved quantity since $\frac{de}{dt} + \frac{dc}{dt} = 0$, so that $e + c = e_0$, where e_0 is the total amount of available enzyme.

1.4.1 The Equilibrium Approximation

In their original analysis, Michaelis and Menten assumed that the substrate is in instantaneous equilibrium with the complex, and thus

$$k_1se = k_{-1}c. \quad (1.30)$$

Since $e + c = e_0$, we find that

$$c = \frac{e_0s}{K_1 + s}, \quad (1.31)$$

where $K_1 = k_{-1}/k_1$. Hence, the velocity, V , of the reaction, i.e., the rate at which the product is formed, is given by

$$V = \frac{dp}{dt} = k_2c = \frac{k_2e_0s}{K_1 + s} = \frac{V_{\max}s}{K_1 + s}, \quad (1.32)$$

where $V_{\max} = k_2e_0$ is the maximum reaction velocity, attained when all the enzyme is complexed with the substrate.

At small substrate concentrations, the reaction rate is linear, at a rate proportional to the amount of available enzyme e_0 . At large concentrations, however, the reaction rate saturates to V_{\max} , so that the maximum rate of the reaction is limited by the amount of enzyme present and the dissociation rate constant k_2 . For this reason, the dissociation reaction $C \xrightarrow{k_2} P + E$ is said to be *rate limiting* for this reaction. At $s = K_1$, the reaction rate is half that of the maximum.

It is important to note that (1.30) cannot be exactly correct at all times; if it were, then according to (1.26) substrate would not be used up, and product would not be

formed. This points out the fact that (1.30) is an approximation. It also illustrates the need for a systematic way to make approximate statements, so that one has an idea of the magnitude and nature of the errors introduced in making such an approximation.

It is a common mistake with the equilibrium approximation to conclude that since (1.30) holds, it must be that $\frac{ds}{dt} = 0$, which if this is true, implies that no substrate is being used up, nor product produced. Furthermore, it appears that if (1.30) holds, then it must be (from (1.28)) that $\frac{dc}{dt} = -k_2c$, which is also false. Where is the error here?

The answer lies with the fact that the equilibrium approximation is equivalent to the assumption that the reaction (1.26) is a very fast reaction, faster than others, or more precisely, that $k_{-1} \gg k_2$. Adding (1.26) and (1.28), we find that

$$\frac{ds}{dt} + \frac{dc}{dt} = -k_2c, \quad (1.33)$$

expressing the fact that the total quantity $s + c$ changes on a slower time scale. Now when we use that $c = \frac{e_0s}{K_1+s}$, we learn that

$$\frac{d}{dt} \left(s + \frac{e_0s}{K_1+s} \right) = -k_2 \frac{e_0s}{K_1+s}, \quad (1.34)$$

and thus,

$$\frac{ds}{dt} \left(1 + \frac{e_0K_1}{(K_1+s)^2} \right) = -k_2 \frac{e_0s}{K_1+s}, \quad (1.35)$$

which specifies the rate at which s is consumed.

This way of simplifying reactions by using an equilibrium approximation is used many times throughout this book, and is an extremely important technique, particularly in the analysis of Markov models of ion channels, pumps and exchangers (Chapters 2 and 3). A more mathematically systematic description of this approach is left for Exercise 20.

1.4.2 The Quasi-Steady-State Approximation

An alternative analysis of an enzymatic reaction was proposed by Briggs and Haldane (1925) who assumed that the rates of formation and breakdown of the complex were essentially equal at all times (except perhaps at the beginning of the reaction, as the complex is “filling up”). Thus, dc/dt should be approximately zero.

To give this approximation a systematic mathematical basis, it is useful to introduce dimensionless variables

$$\sigma = \frac{s}{s_0}, \quad x = \frac{c}{e_0}, \quad \tau = k_1 e_0 t, \quad \kappa = \frac{k_{-1} + k_2}{k_1 s_0}, \quad \epsilon = \frac{e_0}{s_0}, \quad \alpha = \frac{k_{-1}}{k_1 s_0}, \quad (1.36)$$

in terms of which we obtain the system of two differential equations

$$\frac{d\sigma}{d\tau} = -\sigma + x(\sigma + \alpha), \quad (1.37)$$

$$\epsilon \frac{dx}{d\tau} = \sigma - x(\sigma + \kappa). \quad (1.38)$$

There are usually a number of ways that a system of differential equations can be nondimensionalized. This nonuniqueness is often a source of great confusion, as it is often not obvious which choice of dimensionless variables and parameters is “best.” In Section 1.6 we discuss this difficult problem briefly.

The remarkable effectiveness of enzymes as catalysts of biochemical reactions is reflected by their small concentrations needed compared to the concentrations of the substrates. For this model, this means that ϵ is small, typically in the range of 10^{-2} to 10^{-7} . Therefore, the reaction (1.38) is fast, equilibrates rapidly and remains in near-equilibrium even as the variable σ changes. Thus, we take the *quasi-steady-state approximation* $\epsilon \frac{dx}{d\tau} = 0$. Notice that this is *not* the same as taking $\frac{dx}{d\tau} = 0$. However, because of the different scaling of x and c , it is equivalent to taking $\frac{dc}{dt} = 0$ as suggested in the introductory paragraph.

One useful way of looking at this system is as follows; since

$$\frac{dx}{d\tau} = \frac{\sigma - x(\sigma + \kappa)}{\epsilon}, \quad (1.39)$$

$dx/d\tau$ is large everywhere, except where $\sigma - x(\sigma + \kappa)$ is small, of approximately the same size as ϵ . Now, note that $\sigma - x(\sigma + \kappa) = 0$ defines a curve in the σ, x phase plane, called the *slow manifold* (as illustrated in the right panel of Fig. 1.14). If the solution starts away from the slow manifold, $dx/d\tau$ is initially large, and the solution moves rapidly to the vicinity of the slow manifold. The solution then moves along the slow manifold in the direction defined by the equation for σ ; in this case, σ is decreasing, and so the solution moves to the left along the slow manifold.

Another way of looking at this model is to notice that the reaction of x is an exponential process with time constant at least as large as $\frac{\epsilon}{\kappa}$. To see this we write (1.38) as

$$\epsilon \frac{dx}{d\tau} + \kappa x = \sigma(1 - x). \quad (1.40)$$

Thus, the variable x “tracks” the steady state with a short delay.

It follows from the quasi-steady-state approximation that

$$x = \frac{\sigma}{\sigma + \kappa}, \quad (1.41)$$

$$\frac{d\sigma}{d\tau} = -\frac{q\sigma}{\sigma + \kappa}, \quad (1.42)$$

where $q = \kappa - \alpha = \frac{k_2}{k_1 s_0}$. Equation (1.42) describes the rate of uptake of the substrate and is called a *Michaelis–Menten law*. In terms of the original variables, this law is

$$V = \frac{dp}{dt} = -\frac{ds}{dt} = \frac{k_2 e_0 s}{s + K_m} = \frac{V_{\max} s}{s + K_m}, \quad (1.43)$$

where $K_m = \frac{k_{-1} + k_2}{k_1}$. In quasi-steady state, the concentration of the complex satisfies

$$c = \frac{e_0 s}{s + K_m}. \quad (1.44)$$

Note the similarity between (1.32) and (1.43), the only difference being that the equilibrium approximation uses K_1 , while the quasi-steady-state approximation uses K_m . Despite this similarity of form, it is important to keep in mind that the two results are based on different approximations. The equilibrium approximation assumes that $k_{-1} \gg k_2$ whereas the quasi-steady-state approximation assumes that $\epsilon \ll 1$. Notice, that if $k_{-1} \gg k_2$, then $K_m \approx K_1$, so that the two approximations give similar results.

As with the law of mass action, the Michaelis–Menten law (1.43) is not universally applicable but is a useful approximation. It may be applicable even if $\epsilon = e_0/s_0$ is not small (see, for example, Exercise 14), and in model building it is often invoked without regard to the underlying assumptions.

While the individual rate constants are difficult to measure experimentally, the ratio K_m is relatively easy to measure because of the simple observation that (1.43) can be written in the form

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \frac{1}{s}. \quad (1.45)$$

In other words, $1/V$ is a linear function of $1/s$. Plots of this double reciprocal curve are called *Lineweaver–Burk plots*, and from such (experimentally determined) plots, V_{\max} and K_m can be estimated.

Although a Lineweaver–Burk plot makes it easy to determine V_{\max} and K_m from reaction rate measurements, it is not a simple matter to determine the reaction rate as a function of substrate concentration during the course of a single experiment. Substrate concentrations usually cannot be measured with sufficient accuracy or time resolution to permit the calculation of a reliable derivative. In practice, since it is more easily measured, the initial reaction rate is determined for a range of different initial substrate concentrations.

An alternative method to determine K_m and V_{\max} from experimental data is the direct linear plot (Eisenthal and Cornish-Bowden, 1974; Cornish-Bowden and Eisenthal, 1974). First we write (1.43) in the form

$$V_{\max} = V + \frac{V}{s} K_m, \quad (1.46)$$

and then treat V_{\max} and K_m as variables for each experimental measurement of V and s . (Recall that typically only the initial substrate concentration and initial velocity are used.) Then a plot of the straight line of V_{\max} against K_m can be made. Repeating this for a number of different initial substrate concentrations and velocities gives a family of straight lines, which, in an ideal world free from experimental error, intersect at the single point V_{\max} and K_m for that reaction. In reality, experimental error precludes an exact intersection, but V_{\max} and K_m can be estimated from the median of the pairwise intersections.

1.4.3 Enzyme Inhibition

An enzyme inhibitor is a substance that inhibits the catalytic action of the enzyme. Enzyme inhibition is a common feature of enzyme reactions, and is an important means by which the activity of enzymes is controlled. Inhibitors come in many different types. For example, *irreversible inhibitors*, or *catalytic poisons*, decrease the activity of the enzyme to zero. This is the method of action of cyanide and many nerve gases. For this discussion, we restrict our attention to *competitive* inhibitors and *allosteric* inhibitors.

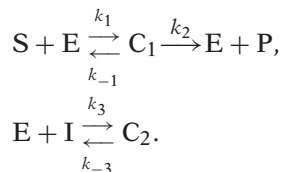
To understand the distinction between competitive and allosteric inhibition, it is useful to keep in mind that an enzyme molecule is usually a large protein, considerably larger than the substrate molecule whose reaction is catalyzed. Embedded in the large enzyme protein are one or more *active sites*, to which the substrate can bind to form the complex. In general, an enzyme catalyzes a single reaction of substrates with similar structures. This is believed to be a steric property of the enzyme that results from the three-dimensional shape of the enzyme allowing it to fit in a “lock-and-key” fashion with a corresponding substrate molecule.

If another molecule has a shape similar enough to that of the substrate molecule, it may also bind to the active site, preventing the binding of a substrate molecule, thus inhibiting the reaction. Because the inhibitor competes with the substrate molecule for the active site, it is called a competitive inhibitor.

However, because the enzyme molecule is large, it often has other binding sites, distinct from the active site, the binding of which affects the activity of the enzyme at the active site. These binding sites are called *allosteric* sites (from the Greek for “another solid”) to emphasize that they are structurally different from the catalytic active sites. They are also called *regulatory sites* to emphasize that the catalytic activity of the protein is regulated by binding at this allosteric site. The ligand (any molecule that binds to a specific site on a protein, from Latin *ligare*, to bind) that binds at the allosteric site is called an *effector* or *modifier*, which, if it increases the activity of the enzyme, is called an allosteric activator; while if it decreases the activity of the enzyme, is called an allosteric inhibitor. The allosteric effect is presumed to arise because of a conformational change of the enzyme, that is, a change in the folding of the polypeptide chain, called an *allosteric transition*.

Competitive Inhibition

In the simplest example of a competitive inhibitor, the reaction is stopped when the inhibitor is bound to the active site of the enzyme. Thus,



Using the law of mass action we find

$$\frac{ds}{dt} = -k_1se + k_{-1}c_1, \quad (1.47)$$

$$\frac{di}{dt} = -k_3ie + k_{-3}c_2, \quad (1.48)$$

$$\frac{dc_1}{dt} = k_1se - (k_{-1} + k_2)c_1, \quad (1.49)$$

$$\frac{dc_2}{dt} = k_3ie - k_{-3}c_2. \quad (1.50)$$

where $s = [S]$, $c_1 = [C_1]$, and $c_2 = [C_2]$. We know that $e + c_1 + c_2 = e_0$, so an equation for the dynamics of e is superfluous. As before, it is not necessary to write an equation for the accumulation of the product. To be systematic, the next step is to introduce dimensionless variables, and identify those reactions that are rapid and equilibrate rapidly to their quasi-steady states. However, from our previous experience (or from a calculation on a piece of scratch paper), we know, assuming the enzyme-to-substrate ratios are small, that the fast equations are those for c_1 and c_2 . Hence, the quasi-steady states are found by (formally) setting $dc_1/dt = dc_2/dt = 0$ and solving for c_1 and c_2 . Recall that this does *not* mean that c_1 and c_2 are unchanging, rather that they are changing in quasi-steady-state fashion, keeping the right-hand sides of these equations nearly zero. This gives

$$c_1 = \frac{K_i e_0 s}{K_m i + K_i s + K_m K_i}, \quad (1.51)$$

$$c_2 = \frac{K_m e_0 i}{K_m i + K_i s + K_m K_i}, \quad (1.52)$$

where $K_m = \frac{k_{-1} + k_2}{k_1}$, $K_i = k_{-3}/k_3$. Thus, the velocity of the reaction is

$$V = k_2 c_1 = \frac{k_2 e_0 s K_i}{K_m i + K_i s + K_m K_i} = \frac{V_{\max} s}{s + K_m (1 + i/K_i)}. \quad (1.53)$$

Notice that the effect of the inhibitor is to increase the effective equilibrium constant of the enzyme by the factor $1 + i/K_i$, from K_m to $K_m(1 + i/K_i)$, thus decreasing the velocity of reaction, while leaving the maximum velocity unchanged.

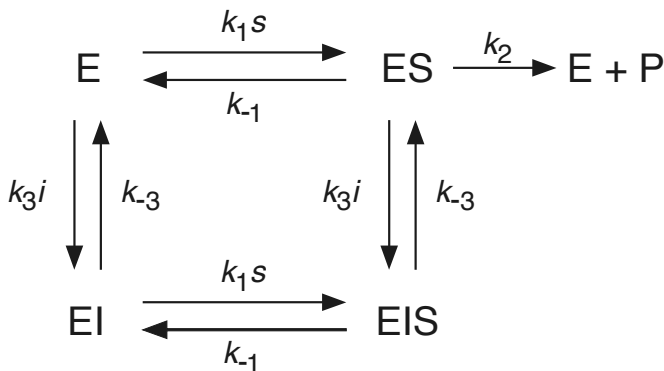


Figure 1.2 Diagram of the possible states of an enzyme with one allosteric and one catalytic binding site.

Allosteric Inhibitors

If the inhibitor can bind at an allosteric site, we have the possibility that the enzyme could bind both the inhibitor and the substrate simultaneously. In this case, there are four possible binding states for the enzyme, and transitions between them, as demonstrated graphically in Fig. 1.2.

The simplest analysis of this reaction scheme is the equilibrium analysis. (The more complicated quasi-steady-state analysis is left for Exercise 6.) We define $K_s = k_{-1}/k_1$, $K_i = k_{-3}/k_3$, and let x, y , and z denote, respectively, the concentrations of ES, EI and EIS. Then, it follows from the law of mass action that at equilibrium (take each of the 4 transitions to be at equilibrium),

$$(e_0 - x - y - z)s - K_s x = 0, \quad (1.54)$$

$$(e_0 - x - y - z)i - K_i y = 0, \quad (1.55)$$

$$ys - K_s z = 0, \quad (1.56)$$

$$xi - K_i z = 0, \quad (1.57)$$

where $e_0 = e + x + y + z$ is the total amount of enzyme. Notice that this is a linear system of equations for x, y , and z . Although there are four equations, one is a linear combination of the other three (the system is of rank three), so that we can determine x, y , and z as functions of i and s , finding

$$x = \frac{e_0 K_i}{K_i + i} \frac{s}{K_s + s}. \quad (1.58)$$

It follows that the reaction rate, $V = k_2 x$, is given by

$$V = \frac{V_{\max}}{1 + i/K_i} \frac{s}{K_s + s}, \quad (1.59)$$

where $V_{\max} = k_2 e_0$. Thus, in contrast to the competitive inhibitor, the allosteric inhibitor decreases the maximum velocity of the reaction, while leaving K_s unchanged.

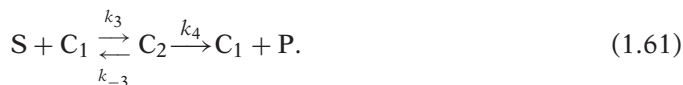
(The situation is more complicated if the quasi-steady-state approximation is used, and no such simple conclusion follows.)

1.4.4 Cooperativity

For many enzymes, the reaction velocity is not a simple hyperbolic curve, as predicted by the Michaelis–Menten model, but often has a sigmoidal character. This can result from cooperative effects, in which the enzyme can bind more than one substrate molecule but the binding of one substrate molecule affects the binding of subsequent ones.

Much of the original theoretical work on cooperative behavior was stimulated by the properties of hemoglobin, and this is often the context in which cooperativity is discussed. A detailed discussion of hemoglobin and oxygen binding is given in Chapter 13, while here cooperativity is discussed in more general terms.

Suppose that an enzyme can bind two substrate molecules, so it can exist in one of three states, namely as a free molecule E , as a complex with one occupied binding site, C_1 , and as a complex with two occupied binding sites, C_2 . The reaction mechanism is then



Using the law of mass action, one can write the rate equations for the 5 concentrations $[S]$, $[E]$, $[C_1]$, $[C_2]$, and $[P]$. However, because the amount of product $[P]$ can be determined by quadrature, and because the total amount of enzyme molecule is conserved, we only need three equations for the three quantities $[S]$, $[C_1]$, and $[C_2]$. These are

$$\frac{ds}{dt} = -k_1se + k_{-1}c_1 - k_3sc_1 + k_{-3}c_2, \quad (1.62)$$

$$\frac{dc_1}{dt} = k_1se - (k_{-1} + k_2)c_1 - k_3sc_1 + (k_4 + k_{-3})c_2, \quad (1.63)$$

$$\frac{dc_2}{dt} = k_3sc_1 - (k_4 + k_{-3})c_2, \quad (1.64)$$

where $s = [S]$, $c_1 = [C_1]$, $c_2 = [C_2]$, and $e + c_1 + c_2 = e_0$.

Proceeding as before, we invoke the quasi-steady-state assumption that $dc_1/dt = dc_2/dt = 0$, and solve for c_1 and c_2 to get

$$c_1 = \frac{K_2e_0s}{K_1K_2 + K_2s + s^2}, \quad (1.65)$$

$$c_2 = \frac{e_0s^2}{K_1K_2 + K_2s + s^2}, \quad (1.66)$$

where $K_1 = \frac{k_{-1}+k_2}{k_1}$ and $K_2 = \frac{k_4+k_{-3}}{k_3}$. The reaction velocity is thus given by

$$V = k_2c_1 + k_4c_2 = \frac{(k_2K_2 + k_4s)e_0s}{K_1K_2 + K_2s + s^2}. \quad (1.67)$$

Use of the equilibrium approximation to simplify this reaction scheme gives, as expected, similar results, in which the formula looks the same, but with different definitions of K_1 and K_2 (Exercise 10).

It is instructive to examine two extreme cases. First, if the binding sites act independently and identically, then $k_1 = 2k_3 = 2k_+$, $2k_{-1} = k_{-3} = 2k_-$ and $2k_2 = k_4$, where k_+ and k_- are the forward and backward reaction rates for the individual binding sites. The factors of 2 occur because two identical binding sites are involved in the reaction, doubling the amount of the reactant. In this case,

$$V = \frac{2k_2e_0(K+s)s}{K^2 + 2Ks + s^2} = 2\frac{k_2e_0s}{K+s}, \quad (1.68)$$

where $K = \frac{k_-+k_2}{k_+}$ is the K_m of the individual binding site. As expected, the rate of reaction is exactly twice that for the individual binding site.

In the opposite extreme, suppose that the binding of the first substrate molecule is slow, but that with one site bound, binding of the second is fast (this is large positive cooperativity). This can be modeled by letting $k_3 \rightarrow \infty$ and $k_1 \rightarrow 0$, while keeping k_1k_3 constant, in which case $K_2 \rightarrow 0$ and $K_1 \rightarrow \infty$ while K_1K_2 is constant. In this limit, the velocity of the reaction is

$$V = \frac{k_4e_0s^2}{K_m^2 + s^2} = \frac{V_{\max}s^2}{K_m^2 + s^2}, \quad (1.69)$$

where $K_m^2 = K_1K_2$, and $V_{\max} = k_4e_0$.

In general, if n substrate molecules can bind to the enzyme, there are n equilibrium constants, K_1 through K_n . In the limit as $K_n \rightarrow 0$ and $K_1 \rightarrow \infty$ while keeping K_1K_n fixed, the rate of reaction is

$$V = \frac{V_{\max}s^n}{K_m^n + s^n}, \quad (1.70)$$

where $K_m^n = \prod_{i=1}^n K_i$. This rate equation is known as the *Hill equation*. Typically, the Hill equation is used for reactions whose detailed intermediate steps are not known but for which cooperative behavior is suspected. The exponent n and the parameters V_{\max} and K_m are usually determined from experimental data. Observe that

$$n \ln s = n \ln K_m + \ln \left(\frac{V}{V_{\max} - V} \right), \quad (1.71)$$

so that a plot of $\ln(\frac{V}{V_{\max}-V})$ against $\ln s$ (called a *Hill plot*) should be a straight line of slope n . Although the exponent n suggests an n -step process (with n binding sites), in practice it is not unusual for the best fit for n to be noninteger.

An enzyme can also exhibit negative cooperativity (Koshland and Hamadani, 2002), in which the binding of the first substrate molecule *decreases* the rate of subsequent

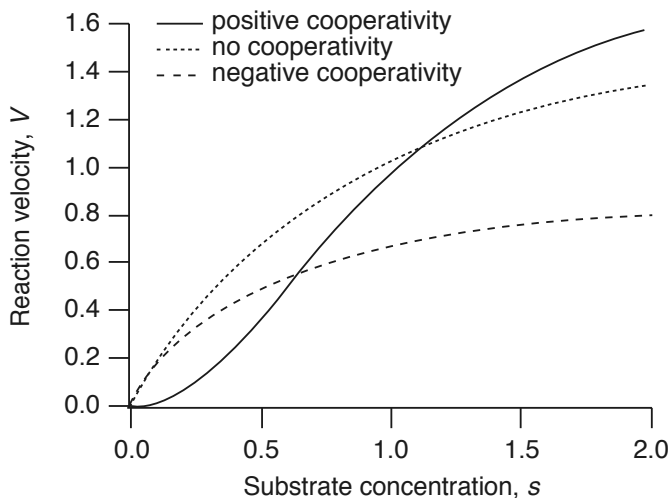


Figure 1.3 Reaction velocity plotted against substrate concentration, for three different cases. Positive cooperativity, $K_1 = 1000$, $K_2 = 0.001$; independent binding sites, $K_1 = 0.5$, $K_2 = 2$; and negative cooperativity, $K_1 = 0.5$, $K_2 = 100$. The other parameters are $e_0 = 1$, $k_2 = 1$, $k_4 = 2$. Concentration and time units are arbitrary.

binding. This can be modeled by decreasing k_3 . In Fig. 1.3 we plot the reaction velocity against the substrate concentration for the cases of independent binding sites (no cooperativity), extreme positive cooperativity (the Hill equation), and negative cooperativity. From this figure it can be seen that with positive cooperativity, the reaction velocity is a sigmoidal function of the substrate concentration, while negative cooperativity primarily decreases the overall reaction velocity.

The Monod–Wyman–Changeux Model

Cooperative effects occur when the binding of one substrate molecule alters the rate of binding of subsequent ones. However, the above models give no explanation of how such alterations in the binding rate occur. The earliest model proposed to account for cooperative effects in terms of the enzyme's conformation was that of Monod, Wyman, and Changeux (1965). Their model is based on the following assumptions about the structure and behavior of enzymes.

1. Cooperative proteins are composed of several identical reacting units, called *protomers*, or subunits, each containing one binding site, that occupy equivalent positions within the protein.
2. The protein has two conformational states, usually denoted by R and T, which differ in their ability to bind ligands.

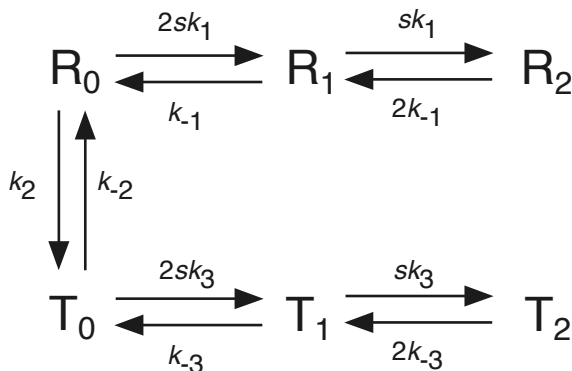


Figure 1.4 Diagram of the states of the protein, and the possible transitions, in a six-state Monod–Wyman–Changeux model.

3. If the binding of a ligand to one protomer induces a conformational change in that protomer, an identical conformational change is induced in all protomers. Because of this assumption, Monod–Wyman–Changeux (MWC) models are often called *concerted* models, as each subunit acts in concert with the others.

To illustrate how these assumptions can be quantified, we consider a protein with two binding sites. Thus, the protein can exist in one of six states: $R_i, i = 0, 1, 2$, or $T_i, i = 0, 1, 2$, where the subscript i is the number of bound ligands. (In the original model of Monod, Wyman and Changeux, R denoted a *relaxed* state, while T denoted a *tense* state.) For simplicity, we also assume that R_1 cannot convert directly to T_1 , or vice versa, and similarly for R_2 and T_2 . The general case is left for Exercise 7. The states of the protein and the allowable transitions are illustrated in Fig. 1.4. As with other enzyme models, we assume that the production rate of product is proportional to the amount of substrate that is bound to the enzyme.

We now assume that all the reactions are in equilibrium. We let a lowercase letter denote a concentration, and thus r_i and t_i denote the concentrations of chemical species R_i and T_i respectively. Also, as before, we let s denote the concentration of the substrate. Then, the fraction Y of occupied sites (also called the *saturation function*) is

$$Y = \frac{r_1 + 2r_2 + t_1 + 2t_2}{2(r_0 + r_1 + r_2 + t_0 + t_1 + t_2)}. \quad (1.72)$$

(This is also proportional to the production rate of product.) Furthermore, with $K_i = k_{-i}/k_i$, for $i = 1, 2, 3$, we find that

$$r_1 = 2sK_1^{-1}r_0, \quad r_2 = s^2K_1^{-2}r_0, \quad (1.73)$$

$$t_1 = 2sK_3^{-1}t_0, \quad t_2 = s^2K_3^{-2}t_0. \quad (1.74)$$

Substituting these into (1.72) gives

$$Y = \frac{sK_1^{-1}(1 + sK_1^{-1}) + K_2^{-1}[sK_3^{-1}(1 + sK_3^{-1})]}{(1 + sK_1^{-1})^2 + K_2^{-1}(1 + sK_3^{-1})^2}, \quad (1.75)$$

where we have used that $r_0/t_0 = K_2$. More generally, if there are n binding sites, then

$$Y = \frac{sK_1^{-1}(1 + sK_1^{-1})^{n-1} + K_2^{-1}[sK_3^{-1}(1 + sK_3^{-1})^{n-1}]}{(1 + sK_1^{-1})^n + K_2^{-1}(1 + sK_3^{-1})^n}. \quad (1.76)$$

In general, Y is a sigmoidal function of s .

It is not immediately apparent how cooperative binding kinetics arises from this model. After all, each binding site in the R conformation is identical, as is each binding site in the T conformation. In order to get cooperativity it is necessary that the binding affinity of the R conformation be different from that of the T conformation. In the special case that the binding affinities of the R and T conformations are equal (i.e., $K_1 = K_3 = K$, say) the binding curve (1.76) reduces to

$$Y = \frac{s}{K + s}, \quad (1.77)$$

which is simply noncooperative Michaelis–Menten kinetics.

Suppose that one conformation, T say, binds the substrate with a higher affinity than does R. Then, when the substrate concentration increases, T_0 is pushed through to T_1 faster than R_0 is pushed to R_1 , resulting in an increase in the amount of substrate bound to the T state, and thus increased overall binding of substrate. Hence the cooperative behavior of the model.

If $K_2 = \infty$, so that only one conformation exists, then once again the saturation curve reduces to the Michaelis–Menten equation, $Y = s/(s + K_1)$. Hence each conformation, by itself, has noncooperative Michaelis–Menten binding kinetics. It is only when the overall substrate binding can be biased to one conformation or the other that cooperativity appears.

Interestingly, MWC models cannot exhibit negative cooperativity. No matter whether $K_1 > K_3$ or *vice versa*, the binding curve always exhibits positive cooperativity.

The Koshland–Nemethy–Filmer model

One alternative to the MWC model is that proposed by Koshland, Nemethy and Filmer in 1966 (the KNF model). Instead of requiring that all subunit transitions occur in concert, as in the MWC model, the KNF model assumes that substrate binding to one subunit causes a conformational change in that subunit only, and that this conformational change causes a change in the binding affinity of the neighboring subunits. Thus, in the KNF model, each subunit can be in a different conformational state, and transitions from one state to the other occur sequentially as more substrate is bound. For this reason KNF models are often called *sequential* models. The increased generality of the KNF model allows for the possibility of negative cooperativity, as the binding to one subunit can *decrease* the binding affinity of its neighbors.

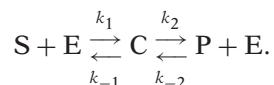
When binding shows positive cooperativity, it has proven difficult to distinguish between the MWC and KNF models on the basis of experimental data. In one of the most intensely studied cooperative mechanisms, that of oxygen binding to hemoglobin,

there is experimental evidence for both models, and the actual mechanism is probably a combination of both.

There are many other models of enzyme cooperativity, and the interested reader is referred to Dixon and Webb (1979) for a comprehensive discussion and comparison of other models in the literature.

1.4.5 Reversible Enzyme Reactions

Since all enzyme reactions are reversible, a general understanding of enzyme kinetics must take this reversibility into account. In this case, the reaction scheme is



Proceeding as usual, we let $e + c = e_0$ and make the quasi-steady-state assumption

$$0 = \frac{dc}{dt} = k_1s(e_0 - c) - (k_{-1} + k_2)c + k_{-2}p(e_0 - c), \quad (1.78)$$

from which it follows that

$$c = \frac{e_0(k_1s + k_{-2}p)}{k_1s + k_{-2}p + k_{-1} + k_2}. \quad (1.79)$$

The reaction velocity, $V = \frac{dP}{dt} = k_2c - k_{-2}pe$, can then be calculated to be

$$V = e_0 \frac{k_1k_2s - k_{-1}k_{-2}p}{k_1s + k_{-2}p + k_{-1} + k_2}. \quad (1.80)$$

When p is small (e.g., if product is continually removed), the reverse reaction is negligible and we get the previous answer (1.43).

In contrast to the irreversible case, the equilibrium and quasi-steady-state assumptions for reversible enzyme kinetics give qualitatively different answers. If we assume that S, E, and C are in fast equilibrium (instead of assuming that C is at quasi-steady state) we get

$$k_1s(e_0 - c) = k_{-1}c, \quad (1.81)$$

from which it follows that

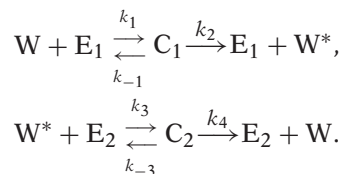
$$V = k_2c - k_{-2}p(e_0 - c) = e_0 \frac{k_1k_2s - k_{-1}k_{-2}p}{k_1s + k_{-1}}. \quad (1.82)$$

Comparing this to (1.80), we see that the quasi-steady-state assumption gives additional terms in the denominator involving the product p . These differences result from the assumption underlying the fast-equilibrium assumption, that k_{-1} and k_1 are both substantially larger than k_{-2} and k_2 , respectively. Which of these approximations is best depends, of course, on the details of the reaction.

Calculation of the equations for a reversible enzyme reaction in which the enzyme has multiple binding sites is left for the exercises (Exercise 11).

1.4.6 The Goldbeter–Koshland Function

As is seen in many ways in this book, cooperativity is an important ingredient in the construction of biochemical switches. However, highly sensitive switching behavior requires large Hill coefficients, which would seem to require multiple interacting enzymes or binding sites, making these unlikely to occur. An alternative mechanism by which highly sensitive switching behavior is possible, suggested by Goldbeter and Koshland (1981), uses only two enzymatic transitions. In this model reaction, a substrate can be in one of two forms, say W and W^* , and transferred from state W to W^* by one enzyme, say E_1 , and transferred from state W^* to W by another enzyme, say E_2 . For example, W^* could be a phosphorylated state of some enzyme, E_1 could be the kinase that phosphorylates W , and E_2 could be the phosphatase that dephosphorylates W^* . Numerous reactions of this type are described in Chapter 10, where W is itself an enzyme whose activity is determined by its phosphorylation state. Thus, the reaction scheme is



Although the full analysis of this reaction scheme is not particularly difficult, a simplified analysis quickly shows the salient features. If we suppose that the enzyme reactions take place at Michaelis–Menten rates, the reaction simplifies to



where

$$r_1 = \frac{V_1 E_1}{K_1 + W}, \quad r_{-1} = \frac{V_2 E_2}{K_2 + W^*}, \quad (1.84)$$

and the concentration of W is governed by the differential equation

$$\frac{dW}{dt} = r_{-1}(W_t - W) - r_1 W, \quad (1.85)$$

where $W + W^* = W_t$. In steady state, the forward and backward reaction rates are the same, leading to the equation

$$\frac{V_1 E_1}{V_2 E_2} = \frac{W^*(K_1 + W)}{W(K_2 + W^*)}. \quad (1.86)$$

This can be rewritten as

$$\frac{v_1}{v_2} = \frac{(1-y)(\hat{K}_1 + y)}{y(\hat{K}_2 + 1 - y)}, \quad (1.87)$$

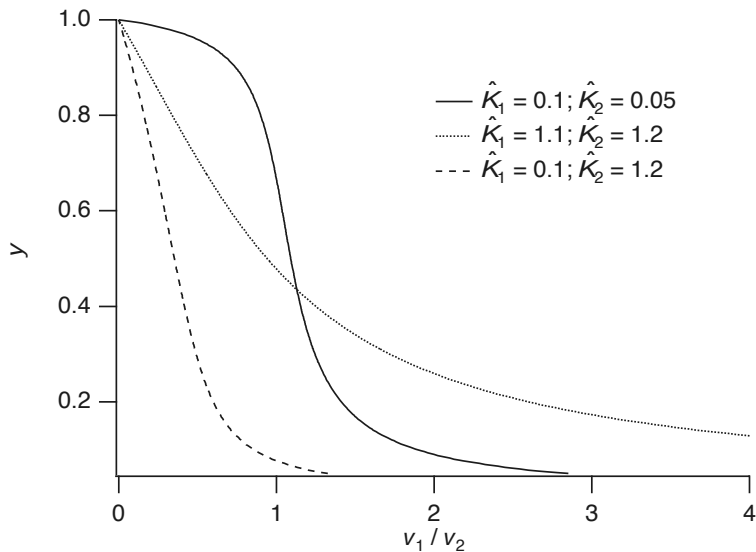


Figure 1.5 Plots of y as a function of the ratio $\frac{v_1}{v_2}$.

where $y = \frac{W}{W_t}$, $\hat{K}_i = K_i/W_t$, $v_i = V_i E_i$, for $i = 1, 2$. Plots of y as a function of the ratio $\frac{v_1}{v_2}$ are easy to draw. One simply plots $\frac{v_1}{v_2}$ as a function of y and then reverses the axes. Examples of these are shown in Fig. 1.5. As is seen in this figure, the ratio $\frac{v_1}{v_2}$ controls the relative abundance of y in a switch-like fashion. In particular, the switch becomes quite sharp when the equilibrium constants \hat{K}_1 and \hat{K}_2 are small compared to 1. In other words, if the enzyme reactions are running at highly saturated levels, then there is sensitive switch-like dependence on the enzyme velocity ratio $\frac{v_1}{v_2}$.

Equation (1.87) is a quadratic polynomial in y , with explicit solution

$$y = \frac{\beta - \sqrt{\beta^2 - 4\alpha\gamma}}{2\alpha}, \quad (1.88)$$

where

$$\alpha = \frac{v_1}{v_2} - 1, \quad (1.89)$$

$$\beta = (1 - \hat{K}_1) - \frac{v_1}{v_2}(\hat{K}_2 + 1), \quad (1.90)$$

$$\gamma = \hat{K}_1. \quad (1.91)$$

The function

$$G(v_1, v_2, \hat{K}_1, \hat{K}_2) = \frac{\beta - \sqrt{\beta^2 - 4\alpha\gamma}}{2\alpha} \quad (1.92)$$

is called the *Goldbeter-Koshland function*. The Goldbeter-Koshland function is often used in descriptions of biochemical networks (Chapter 10). For example, V_1 and V_2

could depend on the concentration of another enzyme, \tilde{E} say, leading to switch-like regulation of the concentration of W as a function of the concentration of \tilde{E} . In this way, networks of biochemical reactions can be constructed in which some of the components are switched on or switched off, relatively abruptly, by other components.

1.5 Glycolysis and Glycolytic Oscillations

Metabolism is the process of extracting useful energy from chemical bonds. A metabolic pathway is the sequence of enzymatic reactions that take place in order to transfer chemical energy from one form to another. The common carrier of energy in the cell is the chemical adenosine triphosphate (ATP). ATP is formed by the addition of an inorganic phosphate group (HPO_4^{2-}) to adenosine diphosphate (ADP), or by the addition of two inorganic phosphate groups to adenosine monophosphate (AMP). The process of adding an inorganic phosphate group to a molecule is called *phosphorylation*. Since the three phosphate groups on ATP carry negative charges, considerable energy is required to overcome the natural repulsion of like-charged phosphates as additional groups are added to AMP. Thus, the hydrolysis (the cleavage of a bond by water) of ATP to ADP releases large amounts of energy.

Energy to perform chemical work is made available to the cell by the oxidation of glucose to carbon dioxide and water, with a net release of energy. The overall chemical reaction for the oxidation of glucose can be written as



but of course, this is not an elementary reaction. Instead, this reaction takes place in a series of enzymatic reactions, with three major reaction stages, *glycolysis*, the *Krebs cycle*, and the *electron transport* (or *cytochrome*) *system*.

The oxidation of glucose is associated with a large negative free energy, $\Delta G^0 = -2878.41$ kJ/mol, some of which is dissipated as heat. However, in living cells much of this free energy is stored in ATP, with one molecule of glucose resulting in 38 molecules of ATP.

Glycolysis involves 11 elementary reaction steps, each of which is an enzymatic reaction. Here we consider a simplified model of the initial steps. (To understand more of the labyrinthine complexity of glycolysis, interested readers are encouraged to consult a specialized book on biochemistry, such as Stryer, 1988.) The first three steps of glycolysis are (Fig. 1.6)

1. the phosphorylation of glucose to glucose 6-phosphate;
2. the isomerization of glucose 6-phosphate to fructose 6-phosphate; and
3. the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate.

The direct reaction of glucose with phosphate to form glucose 6-phosphate has a relatively large positive standard free energy change ($\Delta G^0 = 14.3$ kJ/mol) and so

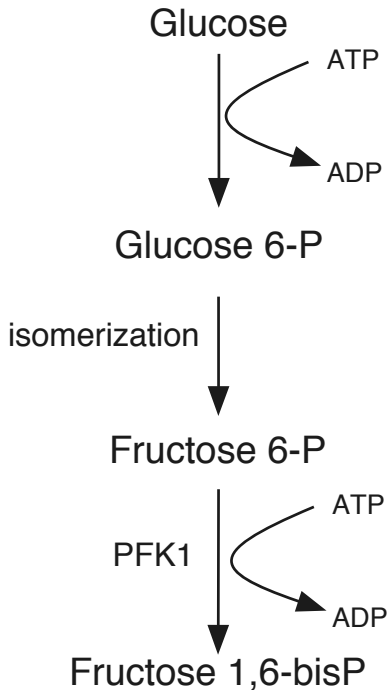


Figure 1.6 The first three reactions in the glycolytic pathway.

does not occur significantly under physiological conditions. However, the first step of metabolism is coupled with the hydrolysis of ATP to ADP (catalyzed by the enzyme hexokinase), giving this step a net negative standard free energy change and making the reaction strongly spontaneous. This feature turns out to be important for the efficient operation of glucose membrane transporters, which are described in the next chapter.

The second step of glycolysis has a relatively small positive standard free energy change ($\Delta G^0 = 1.7 \text{ kJ/mol}$), with an equilibrium constant of 0.5. This means that significant amounts of product are formed under normal conditions.

The third step is, like the first step, energetically unfavorable, were it not coupled with the hydrolysis of ATP. However, the net standard free energy change ($\Delta G^0 = -14.2 \text{ kJ/mol}$) means that not only is this reaction strongly favored, but also that it augments the reaction in the second step by depleting the product of the second step.

This third reaction is catalyzed by the enzyme phosphofructokinase (PFK1). PFK1 is an example of an allosteric enzyme as it is allosterically inhibited by ATP. Note that ATP is both a substrate of PFK1, binding at a catalytic site, and an allosteric inhibitor, binding at a regulatory site. The inhibition due to ATP is removed by AMP, and thus the activity of PFK1 increases as the ratio of ATP to AMP decreases. This feedback enables PFK1 to regulate the rate of glycolysis based on the availability of ATP. If ATP levels fall, PFK1 activity increases thereby increasing the rate of production of ATP, whereas, if ATP levels become high, PFK1 activity drops shutting down the production of ATP.

As PFK1 phosphorylates fructose 6-P, ATP is converted to ADP. ADP, in turn, is converted back to ATP and AMP by the reaction



which is catalyzed by the enzyme adenylate kinase. Since there is normally little AMP in cells, the conversion of ADP to ATP and AMP serves to significantly decrease the ATP/AMP ratio, thus activating PFK1. This is an example of a positive feedback loop; the greater the activity of PFK1, the lower the ATP/AMP ratio, thus further increasing PFK1 activity.

It was discovered in 1980 that in some cell types, another important allosteric activator of PFK1 is fructose 2,6-bisphosphate (Stryer, 1988), which is formed from fructose 6-phosphate in a reaction catalyzed by phosphofructokinase 2 (PFK2), a different enzyme from phosphofructokinase (PFK1) (you were given fair warning about the labyrinthine nature of this process!). Of particular significance is that an abundance of fructose 6-phosphate leads to a corresponding abundance of fructose 2,6-bisphosphate, and thus a corresponding increase in the activity of PFK1. This is an example of a negative feedback loop, where an increase in the substrate concentration leads to a greater rate of substrate reaction and consumption. Clearly, PFK1 activity is controlled by an intricate system of reactions, the collective behavior of which is not obvious a priori.

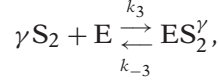
Under certain conditions the rate of glycolysis is known to be oscillatory, or even chaotic (Nielsen et al., 1997). This biochemical oscillator has been known and studied experimentally for some time. For example, Hess and Boiteux (1973) devised a flow reactor containing yeast cells into which a controlled amount of substrate (either glucose or fructose) was continuously added. They measured the pH and fluorescence of the reactants, thereby monitoring the glycolytic activity, and they found ranges of continuous input under which glycolysis was periodic.

Interestingly, the oscillatory behavior is different in intact yeast cells and in yeast extracts. In intact cells the oscillations are sinusoidal in shape, and there is strong evidence that they occur close to a Hopf bifurcation (Danø et al., 1999). In yeast extract the oscillations are of relaxation type, with widely differing time scales (Madsen et al., 2005).

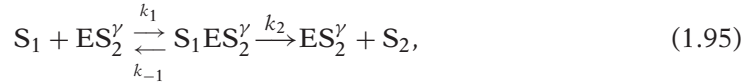
Feedback on PFK is one, but not the only, mechanism that has been proposed as causing glycolytic oscillations. For example, hexose transport kinetics and autocatalysis of ATP have both been proposed as possible mechanisms (Madsen et al., 2005), while some authors have claimed that the oscillations arise as part of the entire network of reactions, with no single feedback being of paramount importance (Bier et al., 1996; Reijenga et al., 2002). Here we focus only on PFK regulation as the oscillatory mechanism.

A mathematical model describing glycolytic oscillations was proposed by Sel'kov (1968) and later modified by Goldbeter and Lefever (1972). It is designed to capture only the positive feedback of ADP on PFK1 activity. In the Sel'kov model, PFK1 is inactive

in its unbound state but is activated by binding with several ADP molecules. Note that, for simplicity, the model does not take into account the conversion of ADP to AMP and ATP, but assumes that ADP activates PFK1 directly, since the overall effect is similar. In the active state, the enzyme catalyzes the production of ADP from ATP as fructose-6-P is phosphorylated. Sel'kov's reaction scheme for this process is as follows: PFK1 (denoted by E) is activated or deactivated by binding or unbinding with γ molecules of ADP (denoted by S_2)



and ATP (denoted S_1) can bind with the activated form of enzyme to produce a product molecule of ADP. In addition, there is assumed to be a steady supply rate of S_1 , while product S_2 is irreversibly removed. Thus,



Note that (1.95) is an enzymatic reaction of exactly Michaelis–Menten form so we should expect a similar reduction of the governing equations.

Applying the law of mass action to the Sel'kov kinetic scheme, we find five differential equations for the production of the five species $s_1 = [S_1]$, $s_2 = [S_2]$, $e = [E]$, $x_1 = [ES_2^\gamma]$, $x_2 = [S_1 ES_2^\gamma]$:

$$\frac{ds_1}{dt} = v_1 - k_1 s_1 x_1 + k_{-1} x_2, \quad (1.97)$$

$$\frac{ds_2}{dt} = k_2 x_2 - \gamma k_3 s_2^\gamma e + \gamma k_{-3} x_1 - v_2 s_2, \quad (1.98)$$

$$\frac{dx_1}{dt} = -k_1 s_1 x_1 + (k_{-1} + k_2) x_2 + k_3 s_2^\gamma e - k_{-3} x_1, \quad (1.99)$$

$$\frac{dx_2}{dt} = k_1 s_1 x_1 - (k_{-1} + k_2) x_2. \quad (1.100)$$

The fifth differential equation is not necessary, because the total available enzyme is conserved, $e + x_1 + x_2 = e_0$. Now we introduce dimensionless variables $\sigma_1 = \frac{k_1 s_1}{k_2 + k_{-1}}$, $\sigma_2 = (\frac{k_3}{k_{-3}})^{1/\gamma} s_2$, $u_1 = x_1/e_0$, $u_2 = x_2/e_0$, $t = \frac{k_2 + k_{-1}}{e_0 k_1 k_2} \tau$ and find

$$\frac{d\sigma_1}{d\tau} = v - \frac{k_2 + k_{-1}}{k_2} u_1 \sigma_1 + \frac{k_{-1}}{k_2} u_2, \quad (1.101)$$

$$\frac{d\sigma_2}{d\tau} = \alpha \left[u_2 - \frac{\gamma k_{-3}}{k_2} \sigma_2^\gamma (1 - u_1 - u_2) + \frac{\gamma k_{-3}}{k_2} u_1 \right] - \eta \sigma_2, \quad (1.102)$$

$$\epsilon \frac{du_1}{d\tau} = u_2 - \sigma_1 u_1 + \frac{k_{-3}}{k_2 + k_{-1}} [\sigma_2^\gamma (1 - u_1 - u_2) - u_1], \quad (1.103)$$

$$\epsilon \frac{du_2}{d\tau} = \sigma_1 u_1 - u_2, \quad (1.104)$$

where $\epsilon = \frac{e_0 k_1 k_2}{(k_2 + k_{-1})^2}$, $\nu = \frac{\nu_1}{k_2 e_0}$, $\eta = \frac{\nu_2 (k_2 + k_{-1})}{k_1 k_2 e_0}$, $\alpha = \frac{k_2 + k_{-1}}{k_1} \left(\frac{k_3}{k_{-3}}\right)^{1/\gamma}$. If we assume that ϵ is a small number, then both u_1 and u_2 are fast variables and can be set to their quasi-steady values,

$$u_1 = \frac{\sigma_2^\gamma}{\sigma_2^\gamma \sigma_1 + \sigma_2^\gamma + 1}, \quad (1.105)$$

$$u_2 = \frac{\sigma_1 \sigma_2^\gamma}{\sigma_2^\gamma \sigma_1 + \sigma_2^\gamma + 1} = f(\sigma_1, \sigma_2), \quad (1.106)$$

and with these quasi-steady values, the evolution of σ_1 and σ_2 is governed by

$$\frac{d\sigma_1}{d\tau} = \nu - f(\sigma_1, \sigma_2), \quad (1.107)$$

$$\frac{d\sigma_2}{d\tau} = \alpha f(\sigma_1, \sigma_2) - \eta \sigma_2. \quad (1.108)$$

The goal of the following analysis is to demonstrate that this system of equations has oscillatory solutions for some range of the supply rate ν . First observe that because of saturation, the function $f(\sigma_1, \sigma_2)$ is bounded by 1. Thus, if $\nu > 1$, the solutions of the differential equations are not bounded. For this reason we consider only $0 < \nu < 1$. The nullclines of the flow are given by the equations

$$\sigma_1 = \frac{\nu}{1 - \nu} \frac{1 + \sigma_2^\gamma}{\sigma_2^\gamma} \quad \left(\frac{d\sigma_1}{d\tau} = 0 \right), \quad (1.109)$$

$$\sigma_1 = \frac{1 + \sigma_2^\gamma}{\sigma_2^{\gamma-1} (p - \sigma_2)} \quad \left(\frac{d\sigma_2}{d\tau} = 0 \right), \quad (1.110)$$

where $p = \alpha/\eta$. These two nullclines are shown plotted as dotted and dashed curves respectively in Fig. 1.7.

The steady-state solution is unique and satisfies

$$\sigma_2 = \nu, \quad (1.111)$$

$$\sigma_1 = \frac{\nu(1 + \sigma_2^\gamma)}{(1 - \nu)\sigma_2^\gamma}. \quad (1.112)$$

The stability of the steady solution is found by linearizing the differential equations about the steady-state solution and examining the eigenvalues of the linearized system. The linearized system has the form

$$\frac{d\tilde{\sigma}_1}{d\tau} = -f_1 \tilde{\sigma}_1 - f_2 \tilde{\sigma}_2, \quad (1.113)$$

$$\frac{d\tilde{\sigma}_2}{d\tau} = \alpha f_1 \tilde{\sigma}_1 + (\alpha f_2 - \eta) \tilde{\sigma}_2, \quad (1.114)$$

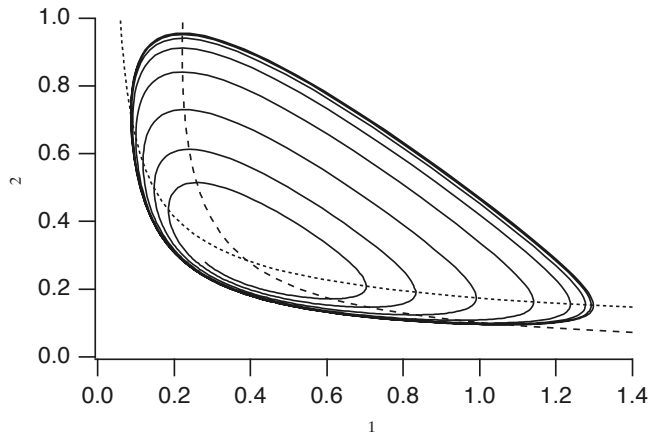


Figure 1.7 Phase portrait of the Sel'kov glycolysis model with $\nu = 0.0285$, $\eta = 0.1$, $\alpha = 1.0$, and $\gamma = 2$. Dotted curve: $\frac{d\sigma_1}{d\tau} = 0$. Dashed curve: $\frac{d\sigma_2}{d\tau} = 0$.

where $f_j = \frac{\partial f}{\partial \sigma_j}$, $j = 1, 2$, evaluated at the steady-state solution, and where $\tilde{\sigma}_i$ denotes the deviation from the steady-state value of σ_i . The characteristic equation for the eigenvalues λ of the linear system (1.113)–(1.114) is

$$\lambda^2 - (\alpha f_2 - \eta - f_1)\lambda + f_1\eta = 0. \quad (1.115)$$

Since f_1 is always positive, the stability of the linear system is determined by the sign of $H = \alpha f_2 - \eta - f_1$, being stable if $H < 0$ and unstable if $H > 0$. Changes of stability, if they exist, occur at $H = 0$, and are Hopf bifurcations to periodic solutions with approximate frequency $\omega = \sqrt{f_1\eta}$.

The function $H(\nu)$ is given by

$$H(\nu) = \frac{(1-\nu)}{(1+\nu)}(\eta\gamma + (\nu-1)y) - \eta, \quad (1.116)$$

$$y = (p\nu)^\gamma. \quad (1.117)$$

Clearly, $H(0) = \eta(\gamma - 1)$, $H(1) = -\eta$, so for $\gamma > 1$, there must be at least one Hopf bifurcation point, below which the steady solution is unstable. Additional computations show that this Hopf bifurcation is supercritical, so that for ν slightly below the bifurcation point, there is a stable periodic orbit.

An example of this periodic orbit is shown in Fig. 1.7 with coefficients $\nu = 0.0285$, $\eta = 0.1$, $\alpha = 1.0$, and $\gamma = 2$. The evolution of σ_1 and σ_2 are shown plotted as functions of time in Fig. 1.8.

A periodic orbit exists only in a very small region of parameter space, rapidly expanding until it becomes infinitely large in amplitude as ν decreases. For still smaller values of ν , there are no stable trajectories. This information is summarized in a bifurcation diagram (Fig. 1.9), where we plot the steady state, σ_1 , against one of the

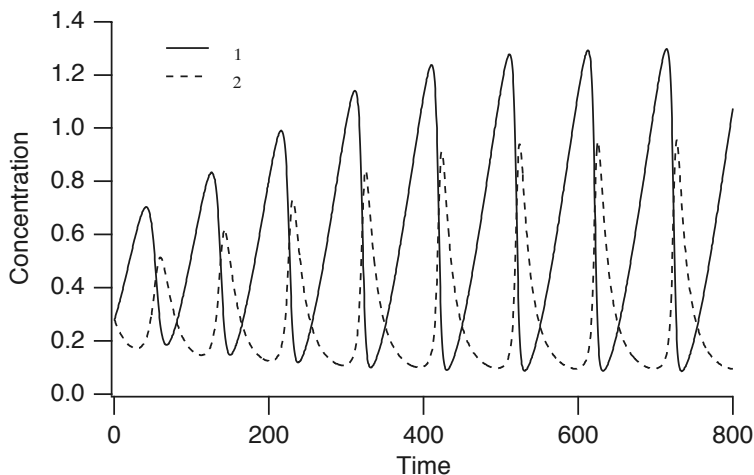


Figure 1.8 Evolution of σ_1 and σ_2 for the Sel'kov glycolysis model toward a periodic solution. Parameters are the same as in Fig. 1.7.

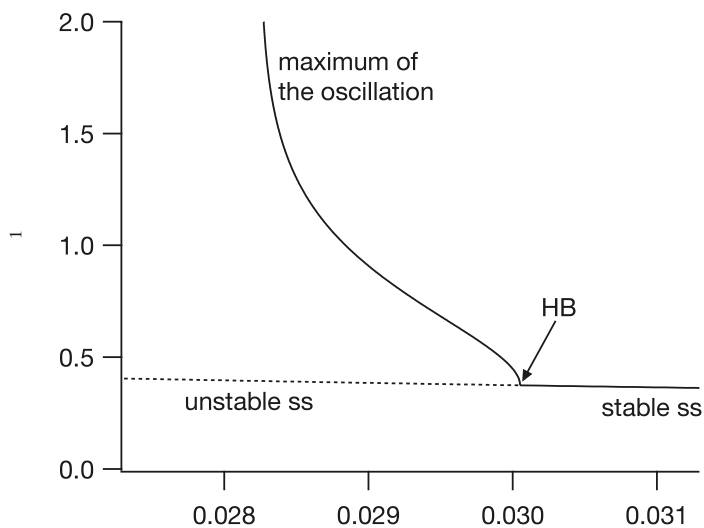


Figure 1.9 Bifurcation diagram for the Sel'kov glycolysis model.

parameters, in this case v . Thus, v is called the *bifurcation parameter*. The dashed line labeled “unstable ss” is the curve of unstable steady states as a function of v , while the solid line labeled “stable ss” is the curve of stable steady states as a function of v . As is typical in such bifurcation diagrams, we also include the maximum of the oscillation (when it exists) as a function of v . We could equally have chosen to plot the minimum

of the oscillation (or both the maximum and the minimum). Since the oscillation is stable, the maximum of the oscillation is plotted with a solid line.

From the bifurcation diagram we see that the stable branch of oscillations originates at a supercritical Hopf bifurcation (labeled HB), and that the periodic orbits only exist for a narrow range of values of ν . The question of how this branch of periodic orbits terminates is not important for the discussion here, so we ignore this important point for now.

We use bifurcation diagrams throughout this book, and many are considerably more complicated than that shown in Fig. 1.9. Readers who are unfamiliar with the basic theory of nonlinear bifurcations, and their representation in bifurcation diagrams, are urged to consult an elementary book such as Strogatz (1994).

While the Sel'kov model has certain features that are qualitatively correct, it fails to agree with the experimental results at a number of points. Hess and Boiteux (1973) report that for high and low substrate injection rates, there is a stable steady-state solution. There are two Hopf bifurcation points, one at the flow rate of 20 mM/hr and another at 160 mM/hr. The period of oscillation at the low flow rate is about 8 minutes and decreases as a function of flow rate to about 3 minutes at the upper Hopf bifurcation point. In contrast, the Sel'kov model has but one Hopf bifurcation point.

To reproduce these additional experimental features we consider a more detailed model of the reaction. In 1972, Goldbeter and Lefever proposed a model of Monod–Wyman–Changeux type that provided a more accurate description of the oscillations. More recently, by fitting a simpler model to experimental data on PFK1 kinetics in skeletal muscle, Smolen (1995) has shown that this level of complexity is not necessary; his model assumes that PFK1 consists of four independent, identical subunits, and reproduces the observed oscillations well. Despite this, we describe only the Goldbeter–Lefever model in detail, as it provides an excellent example of the use of Monod–Wyman–Changeux models.

In the Goldbeter–Lefever model of the phosphorylation of fructose-6-P, the enzyme PFK1 is assumed to be a dimer that exists in two states, an active state R and an inactive state T. The substrate, S_1 , can bind to both forms, but the product, S_2 , which is an activator, or positive effector, of the enzyme, binds only to the active form. The enzymatic forms of R carrying substrate decompose irreversibly to yield the product ADP. In addition, substrate is supplied to the system at a constant rate, while product is removed at a rate proportional to its concentration. The reaction scheme for this is as follows: let T_j represent the inactive T form of the enzyme bound to j molecules of substrate and let R_{ij} represent the active form R of the enzyme bound to i substrate molecules and j product molecules. This gives the reaction diagram shown in Fig. 1.10. In this system, the substrate S_1 holds the enzyme in the inactive state by binding with T_0 to produce T_1 and T_2 , while product S_2 holds the enzyme in the active state by binding with R_{00} to produce R_{01} and binding with R_{01} to produce R_{02} . There is a factor of two in the rates of reaction because a dimer with two available binding sites reacts like twice the same amount of monomer.

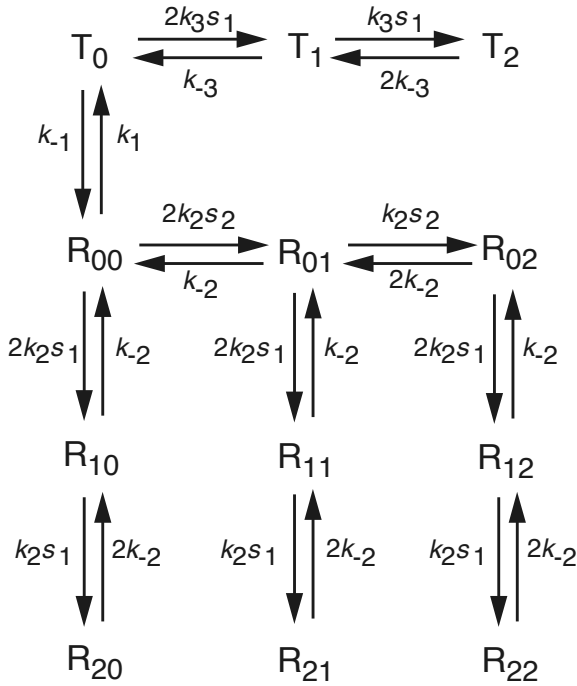


Figure 1.10 Possible states of the enzyme PFK1 in the Goldbeter-Lefever model of glycolytic oscillations.

In addition to the reactions shown in Fig. 1.10, the enzyme complex can disassociate to produce product via the reaction



provided $i \geq 1$.

The analysis of this reaction scheme is substantially more complicated than that of the Sel'kov scheme, although the idea is the same. We use the law of mass action to write differential equations for the fourteen chemical species. For example, the equation for $s_1 = [S_1]$ is

$$\frac{ds_1}{dt} = v_1 - F, \quad (1.119)$$

where

$$\begin{aligned} F = & k_{-2}(r_{10} + r_{11} + r_{12}) + 2k_{-2}(r_{20} + r_{21} + r_{22}) \\ & - 2k_2s_1(r_{00} + r_{01} + r_{02}) - k_2s_1(r_{10} + r_{11} + r_{12}) \\ & - 2k_3s_1t_0 - k_3s_1t_1 + k_{-3}t_1 + 2k_{-3}t_2, \end{aligned} \quad (1.120)$$

and the equation for $r_{00} = [R_{00}]$ is

$$\frac{dr_{00}}{dt} = -(k_1 + 2k_2s_1 + 2k_2s_2)r_{00} + (k_{-2} + k)r_{10} + k_{-2}r_{01} + k_{-1}t_0. \quad (1.121)$$

We then assume that all twelve of the intermediates are in quasi-steady state. This leads to a 12 by 12 linear system of equations, which, if we take the total amount of enzyme to be e_0 , can be solved. We substitute this solution into the differential equations for s_1 and s_2 with the result that

$$\frac{ds_1}{dt} = v_1 - F(s_1, s_2), \quad (1.122)$$

$$\frac{ds_2}{dt} = F(s_1, s_2) - v_2 s_2, \quad (1.123)$$

where

$$F(s_1, s_2) = \left(\frac{2k_2 k_{-1} k e_0}{k + k_{-2}} \right) \left(\frac{s_1 \left(1 + \frac{k_2}{k + k_{-2}} s_1 \right) (s_2 + K_2)^2}{K_2^2 k_1 \left(\frac{k_3}{k_{-3}} s_1 + 1 \right)^2 + k_{-1} \left(1 + \frac{k_2}{k + k_{-2}} s_1 \right)^2 (K_2 + s_2)^2} \right), \quad (1.124)$$

where $K_2 = \frac{k_{-2}}{k_2}$. Now we introduce dimensionless variables $\sigma_1 = \frac{s_1}{K_2}$, $\sigma_2 = \frac{s_2}{K_2}$, $t = \frac{\tau}{\tau_c}$ and parameters $\nu = \frac{k_2 v_1}{k_{-2} \tau_c}$, $\eta = \frac{v_2}{\tau_c}$, where $\tau_c = \frac{2k_2 k_{-1} k e_0}{k_1 (k + k_{-2})}$, and arrive at the system (1.107)–(1.108), but with a different function $f(\sigma_1, \sigma_2)$, and with $\alpha = 1$. If, in addition, we assume that

1. the substrate does not bind to the T form ($k_3 = 0$, T is completely inactive),
2. T_0 is preferred over R_{00} ($k_1 \gg k_{-1}$), and
3. if the substrate S_1 binds to the R form, then formation of product S_2 is preferred to dissociation ($k \gg k_{-2}$),

then we can simplify the equations substantially to obtain

$$f(\sigma_1, \sigma_2) = \sigma_1 (1 + \sigma_2)^2. \quad (1.125)$$

The nullclines for this system of equations are somewhat different from the Sel'kov system, being

$$\sigma_1 = \frac{\nu}{(1 + \sigma_2)^2} \quad \left(\frac{d\sigma_1}{d\tau} = 0 \right), \quad (1.126)$$

$$\sigma_1 = \frac{\eta \sigma_2}{(1 + \sigma_2)^2} \quad \left(\frac{d\sigma_2}{d\tau} = 0 \right), \quad (1.127)$$

and the unique steady-state solution is given by

$$\sigma_1 = \frac{\nu}{(1 + \sigma_2)^2}, \quad (1.128)$$

$$\sigma_2 = \frac{\nu}{\eta}. \quad (1.129)$$

The stability of the steady-state solution is again determined by the characteristic equation (1.115), and the sign of the real part of the eigenvalues is the same as the sign of

$$H = f_2 - f_1 - \eta = 2\sigma_1 (1 + \sigma_2) - (1 + \sigma_2)^2 - \eta, \quad (1.130)$$

evaluated at the steady state (1.126)–(1.127). Equation (1.130) can be written as the cubic polynomial

$$\frac{1}{\eta}y^3 - y + 2 = 0, \quad y = 1 + \frac{\nu}{\eta}. \quad (1.131)$$

For η sufficiently large, the polynomial (1.131) has two roots greater than 2, say, y_1 and y_2 . Recall that ν is the nondimensional flow rate of substrate ATP. To make some correspondence with the experimental data, we assume that the flow rate ν is proportional to the experimental supply rate of glucose. This is not strictly correct, although ATP is produced at about the same rate that glucose is supplied. Accepting this caveat, we see that to match experimental data, we require

$$\frac{y_2 - 1}{y_1 - 1} = \frac{\nu_2}{\nu_1} = \frac{160}{20} = 8. \quad (1.132)$$

Requiring (1.131) to hold at y_1 and y_2 and requiring (1.132) to hold as well, we find numerical values

$$y_1 = 2.08, \quad y_2 = 9.61, \quad \eta = 116.7, \quad (1.133)$$

corresponding to $\nu_1 = 126$ and $\nu_2 = 1005$.

At the Hopf bifurcation point, the period of oscillation is

$$T_i = \frac{2\pi}{\omega_i} = \frac{2\pi}{\sqrt{\eta}(1 + \sigma_2)} = \frac{2\pi}{\sqrt{\eta}y_i}. \quad (1.134)$$

For the numbers (1.133), we obtain a ratio of periods $T_1/T_2 = 4.6$, which is acceptably close to the experimentally observed ratio $T_1/T_2 = 2.7$.

The behavior of the solution as a function of the parameter ν is summarized in the bifurcation diagram, Fig. 1.11, shown here for $\eta = 120$. The steady-state solution is stable below $\eta = 129$ and above $\eta = 1052$. Between these values of η the steady-state solution is unstable, but there is a branch of stable periodic solutions which terminates and collapses into the steady-state solution at the two points where the stability changes, the Hopf bifurcation points.

A typical phase portrait for the periodic solution that exists between the Hopf bifurcation points is shown in Fig. 1.12, and the concentrations of the two species are shown as functions of time in Fig. 1.13.

1.6 Appendix: Math Background

It is certain that some of the mathematical concepts and tools that we routinely invoke here are not familiar to all of our readers. In this first chapter alone, we have used nondimensionalization, phase-plane analysis, linear stability analysis, bifurcation theory, and asymptotic analysis, all the while assuming that these are familiar to the reader.

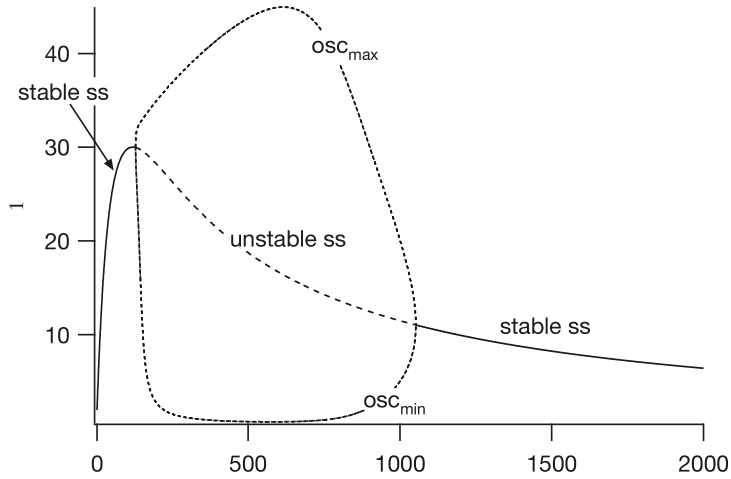


Figure 1.11 Bifurcation diagram for the reduced Goldbeter–Lefever glycolysis model, with $\eta = 120$.

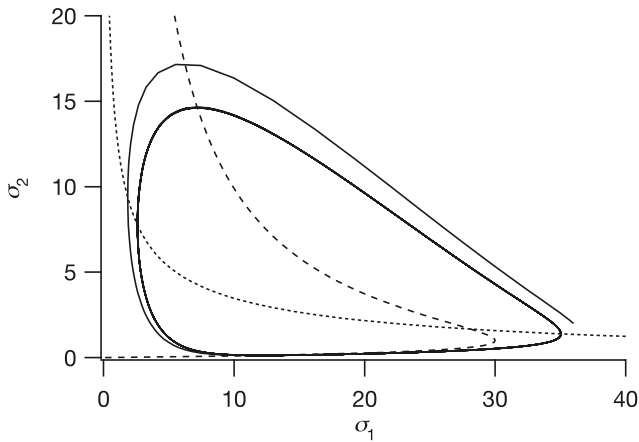


Figure 1.12 Phase portrait of the Goldbeter–Lefever model with $\nu = 200, \eta = 120$. Dotted curve: $\frac{d\sigma_1}{d\tau} = 0$. Dashed curve: $\frac{d\sigma_2}{d\tau} = 0$.

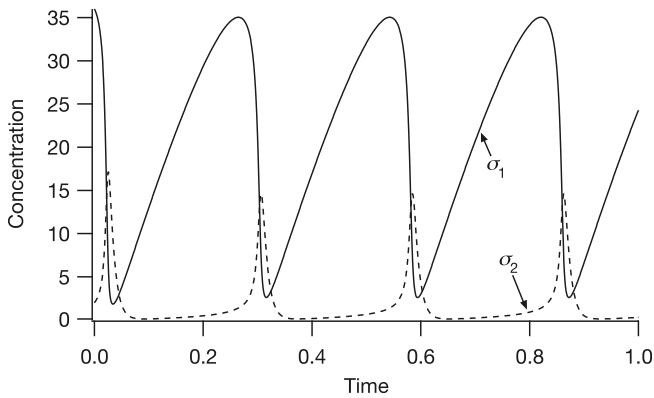


Figure 1.13 Solution of the Goldbeter–Lefever model with $\nu = 200, \eta = 120$.

The purpose of this appendix is to give a brief guide to those techniques that are a basic part of the applied mathematician's toolbox.

1.6.1 Basic Techniques

In any problem, there are a number of parameters that are dictated by the problem. However, it often happens that not all parameter variations are *independent*; that is, different variations in different parameters may lead to identical changes in the behavior of the model. Second, there may be parameters whose influence on a behavior is negligible and can be safely ignored for a given context.

The way to identify independent parameters and to determine their relative magnitudes is to nondimensionalize the problem. Unfortunately, there is not a unique algorithm for nondimensionalization; nondimensionalization is as much art as it is science.

There are, however, rules of thumb to apply. In any system of equations, there are a number of independent variables (time, space, etc.), dependent variables (concentrations, etc.) and parameters (rates of reaction, sizes of containers, etc.). Nondimensionalization begins by rescaling the independent and dependent variables by "typical" units, rendering them thereby dimensionless. One goal may be to ensure that the dimensionless variables remain of a fixed order of magnitude, not becoming too large or negligibly small. This usually requires some a priori knowledge about the solution, as it can be difficult to choose typical scales unless something is already known about typical solutions. Time and space scales can be vastly different depending on the context.

Once this selection of scales has been made, the governing equations are written in terms of the rescaled variables and dimensionless combinations of the remaining parameters are identified. The number of remaining free dimensionless parameters is usually less than the original number of physical parameters. The primary difficulty (at least to understand and apply the process) is that there is not necessarily a single way to scale and nondimensionalize the equations. Some scalings may highlight certain features of the solution, while other scalings may emphasize others. Nonetheless, nondimensionalization often (but not always) provides a good starting point for the analysis of a model system.

An excellent discussion of scaling and nondimensionalization can be found in Lin and Segel (1988, Chapter 6). A great deal of more advanced work has also been done on this subject, particularly its application to the quasi-steady-state approximation, by Segel and his collaborators (Segel, 1988; Segel and Slemrod, 1989; Segel and Perelson, 1992; Segel and Goldbeter, 1994; Borghans et al., 1996; see also Frenzen and Maini, 1988).

Phase-plane analysis and linear stability analysis are standard fare in introductory courses on differential equations. A nice introduction to these topics for the biologically inclined can be found in Edelstein-Keshet (1988, Chapter 5) or Braun (1993, Chapter 4). A large number of books discuss the qualitative theory of differential equations,

for example, Boyce and Dippima (1997), or at a more advanced level, Hale and Koçak (1991), or Hirsch and Smale (1974).

Bifurcation Theory

Bifurcation theory is a topic that is gradually finding its way into introductory literature. The most important terms to understand are those of *steady-state* bifurcations, *Hopf* bifurcations, *homoclinic* bifurcations, and *saddle-node* bifurcations, all of which appear in this book. An excellent introduction to these concepts is found in Strogatz (1994, Chapters 3, 6, 7, 8), and an elementary treatment, with particular application to biological systems, is given by Beuter et al. (2003, Chapters 2, 3). More advanced treatments include those in Guckenheimer and Holmes (1983), Arnold (1983) or Wiggins (2003).

One way to summarize the behavior of the model is with a bifurcation diagram (examples of which are shown in Figs. 1.9 and 1.11), which shows how certain features of the model, such as steady states or limit cycles, vary as a parameter is varied. When models have many parameters there is a wide choice for which parameter to vary. Often, however, there are compelling physiological or experimental reasons for the choice of parameter. Bifurcation diagrams are important in a number of chapters of this book, and are widely used in the analysis of nonlinear systems. Thus, it is worth the time to become familiar with their properties and how they are constructed. Nowadays, most bifurcation diagrams of realistic models are constructed numerically, the most popular choice of software being AUTO (Doedel, 1986; Doedel et al., 1997, 2001). The bifurcation diagrams in this book were all prepared with XPPAUT (Ermentrout, 2002), a convenient implementation of AUTO.

In this text, the bifurcation that is seen most often is the Hopf bifurcation. The Hopf bifurcation theorem describes conditions for the appearance of small periodic solutions of a differential equation, say

$$\frac{du}{dt} = f(u, \lambda), \quad (1.135)$$

as a function of the parameter λ . Suppose that there is a steady-state solution, $u = u_0(\lambda)$, and that the system linearized about u_0 ,

$$\frac{dU}{dt} = \frac{\partial f(u_0(\lambda), \lambda)}{\partial u} U, \quad (1.136)$$

has a pair of complex eigenvalues $\mu(\lambda) = \alpha(\lambda) \pm i\beta(\lambda)$. Suppose further that $\alpha(\lambda_0) = 0$, $\alpha'(\lambda_0) \neq 0$, and $\beta(\lambda_0) \neq 0$, and that at $\lambda = \lambda_0$ no other eigenvalues of the system have zero real part. Then λ_0 is a Hopf bifurcation point, and there is a branch of periodic solutions emanating from the point $\lambda = \lambda_0$. The periodic solutions could exist (locally) for $\lambda > \lambda_0$, for $\lambda < \lambda_0$, or in the degenerate (nongeneric) case, for $\lambda = \lambda_0$. If the periodic solutions occur in the region of λ for which $\alpha(\lambda) > 0$, then the periodic solutions are stable (provided all other eigenvalues of the system have negative real part), and this branch of solutions is said to be supercritical. On the other hand, if the periodic

solutions occur in the region of λ for which $\alpha(\lambda) < 0$, then the periodic solutions are unstable, and this branch of solutions is said to be subcritical.

The Hopf bifurcation theorem applies to ordinary differential equations and delay differential equations. For partial differential equations, there are some technical issues having to do with the nature of the spectrum of the linearized operator that complicate matters, but we do not concern ourselves with these here. Instead, rather than checking all the conditions of the theorem, we find periodic solutions by looking only for a change of the sign of the real part of an eigenvalue, using numerical computations to verify the existence of periodic solutions, and calling it good.

1.6.2 Asymptotic Analysis

Applied mathematicians love small parameters, because of the hope that the solution of a problem with a small parameter might be approximated by an *asymptotic representation*. A commonplace notation has emerged in which ϵ is often the small parameter. An asymptotic representation has a precise mathematical meaning. Suppose that $G(\epsilon)$ is claimed to be an asymptotic representation of $g(\epsilon)$, expressed as

$$g(\epsilon) = G(\epsilon) + O(\phi(\epsilon)). \quad (1.137)$$

The precise meaning of this statement is that there is a constant A such that

$$\left| \frac{g(\epsilon) - G(\epsilon)}{\phi(\epsilon)} \right| \leq A \quad (1.138)$$

for all ϵ with $|\epsilon| \leq \epsilon_0$ and $\epsilon > 0$. The function $\phi(\epsilon)$ is called a *gauge function*, a typical example of which is a power of ϵ .

Perturbation Expansions

It is often the case that an asymptotic representation can be found as a power series in powers of the small parameter ϵ . Such representations are called *perturbation expansions*. Usually, a few terms of this power series representation suffice to give a good approximation to the solution. It should be kept in mind that under no circumstances does this power series development imply that a complete power series (with an infinite number of terms) exists or is convergent. Terminating the series at one or two terms is deliberate.

However, there are times when a full power series could be found and would be convergent in some nontrivial ϵ domain. Such problems are called *regular perturbation problems* because their solutions are regular, or analytic, in the parameter ϵ .

There are numerous examples of regular perturbation problems, including all of those related to bifurcation theory. These problems are regular because their solutions can be developed in a convergent power series of some parameter.

There are, however, many problems with small parameters whose solutions are not regular, called *singular perturbation problems*. Singular perturbation problems are

characterized by the fact that their dependence on the small parameter is not regular, but *singular*, and their convergence as a function of ϵ is not uniform.

Singular problems come in two basic varieties. Characteristic of the first type is a small region of width ϵ somewhere in the domain of interest (either space or time) in which the solution changes rapidly. For example, the solution of the boundary value problem

$$\epsilon u'' + u' + u = 0 \quad (1.139)$$

subject to boundary conditions $u(0) = u(1) = 1$ is approximated by the asymptotic representation

$$u(x; \epsilon) = (1 - e)e^{-x/\epsilon} + e^{1-x} + O(\epsilon). \quad (1.140)$$

Notice the nonuniform nature of this solution, as

$$e = \lim_{x \rightarrow 0^+} \left(\lim_{\epsilon \rightarrow 0^+} u(x; \epsilon) \right) \neq \lim_{\epsilon \rightarrow 0^+} \left(\lim_{x \rightarrow 0^+} u(x; \epsilon) \right) = 1.$$

Here the term $e^{-x/\epsilon}$ is a *boundary layer correction*, as it is important only in a small region near the boundary at $x = 0$.

Other terms that are typical in singular perturbation problems are *interior layers* or *transition layers*, typified by expressions of the form $\tan(\frac{x-x_0}{\epsilon})$, and *corner layers*, locations where the derivative changes rapidly but the solution itself changes little. Transition layers are of great significance in the study of excitable systems (Chapter 5). While corner layers show up in this book, we do not study or use them in any detail.

Singular problems of this type can often be identified by the fact that the order of the system decreases if ϵ is set to zero. An example that we have already seen is the quasi-steady-state analysis used to simplify reaction schemes in which some reactions are significantly faster than others. Setting ϵ to zero in these examples reduces the order of the system of equations, signaling a possible problem. Indeed, solutions of these equations typically have *initial layers* near time $t = 0$. We take a closer look at this example below.

The second class of singular perturbation problems is that in which there are two scales in operation everywhere in the domain of interest. Problems of this type show up throughout this book. For example, action potential propagation in cardiac tissue is through a cellular medium whose detailed structure varies rapidly compared to the length scale of the action potential wave front. Physical properties of the cochlear membrane in the inner ear vary slowly compared to the wavelength of waves that propagate along it. For problems of this type, one must make explicit the dependence on multiple scales, and so solutions are often expressed as functions of two variables, say x and x/ϵ , which are treated as independent variables. Solution techniques that exploit the multiple-scale nature of the solution are called *multiscale methods* or *averaging methods*.

Detailed discussions of these asymptotic methods may be found in Murray (1984), Kevorkian and Cole (1996), and Holmes (1995).

1.6.3 Enzyme Kinetics and Singular Perturbation Theory

In most of the examples of enzyme kinetics discussed in this chapter, extensive use was made of the quasi-steady-state approximation (1.44), according to which the concentration of the complex remains constant during the course of the reaction. Although this assumption gives the right answers (which, some might argue, is justification enough), mathematicians have sought for ways to justify this approximation rigorously. Bowen et al. (1963) and Heineken et al. (1967) were the first to show that the quasi-steady-state approximation can be derived as the lowest-order term in an asymptotic expansion of the solution. This has since become one of the standard examples of the application of singular perturbation theory to biological systems, and it is discussed in detail by Rubinow (1973), Lin and Segel (1988), and Murray (2002), among others.

Starting with (1.37) and (1.38),

$$\frac{d\sigma}{d\tau} = -\sigma + x(\sigma + \alpha), \quad (1.141)$$

$$\epsilon \frac{dx}{d\tau} = \sigma - x(\sigma + \kappa), \quad (1.142)$$

with initial conditions

$$\sigma(0) = 1, \quad (1.143)$$

$$x(0) = 0, \quad (1.144)$$

we begin by looking for solutions of the form

$$\sigma = \sigma_0 + \epsilon\sigma_1 + \epsilon^2\sigma_2 + \dots, \quad (1.145)$$

$$x = x_0 + \epsilon x_1 + \epsilon^2 x_2 + \dots. \quad (1.146)$$

We substitute these solutions into the differential equations and equate coefficients of powers of ϵ . To lowest order (i.e., equating all those terms with no ϵ) we get

$$\frac{d\sigma_0}{d\tau} = -\sigma_0 + x_0(\sigma_0 + \alpha), \quad (1.147)$$

$$0 = \sigma_0 - x_0(\sigma_0 + \kappa). \quad (1.148)$$

Note that, because we are matching powers of ϵ , the differential equation for x has been converted into an algebraic equation for x_0 , which can be solved to give

$$x_0 = \frac{\sigma_0}{\sigma_0 + \kappa}. \quad (1.149)$$

It follows that

$$\frac{d\sigma_0}{d\tau} = -\sigma_0 + x_0(\sigma_0 + \alpha) = -\sigma_0 \left(\frac{\kappa - \alpha}{\sigma_0 + \kappa} \right). \quad (1.150)$$

These solutions for x_0 and σ_0 (i.e., for the lowest-order terms in the power series expansion) are the quasi-steady-state approximation of Section 1.4.2. We could carry

on and solve for σ_1 and x_1 , but since the calculations rapidly become quite tedious, with little to no benefit, the lowest-order solution suffices.

However, it is important to notice that this lowest-order solution cannot be correct for all times. For, clearly, the initial conditions $\sigma(0) = 1$, $x(0) = 0$ are inconsistent with (1.149). In fact, by setting ϵ to be zero, we have decreased the order of the differential equations system, making it impossible to satisfy the initial conditions.

There must therefore be a brief period of time at the start of the reaction during which the quasi-steady-state approximation does not hold. It is not that ϵ is not small, but rather that $\epsilon \frac{dx}{dt}$ is not small during this initial period, since dx/dt is large. Indeed, it is during this initial time period that the enzyme is “filling up” with substrate, until the concentration of complexed enzyme reaches the value given by the quasi-steady-state approximation. Since there is little enzyme compared to the total amount of substrate, the concentration of substrate remains essentially constant during this period.

For most biochemical reactions this transition to the quasi-steady state happens so fast that it is not physiologically important, but for mathematical reasons, it is interesting to understand these kinetics for early times as well. To see how the reaction behaves for early times, we make a change of time scale, $\eta = \tau/\epsilon$. This change of variables expands the time scale on which we look at the reaction and allows us to study events that happen on a fast time scale. To be more precise, we also denote the solution on this fast time scale by a tilde. In the new time scale, (1.37)–(1.38) become

$$\frac{d\tilde{\sigma}}{d\eta} = \epsilon(-\tilde{\sigma} + \tilde{x}(\tilde{\sigma} + \alpha)), \quad (1.151)$$

$$\frac{d\tilde{x}}{d\eta} = \tilde{\sigma} - \tilde{x}(\tilde{\sigma} + \kappa). \quad (1.152)$$

The initial conditions are $\tilde{\sigma}(0) = 1$, $\tilde{x}(0) = 0$.

As before, we expand $\tilde{\sigma}$ and \tilde{x} in power series in ϵ , substitute into the differential equations, and equate coefficients of powers of ϵ . To lowest order in ϵ this gives

$$\frac{d\tilde{\sigma}_0}{d\eta} = 0, \quad (1.153)$$

$$\frac{d\tilde{x}_0}{d\eta} = \tilde{\sigma}_0 - \tilde{x}_0(\tilde{\sigma}_0 + \kappa). \quad (1.154)$$

Simply stated, this means that $\tilde{\sigma}_0$ does not change on this time scale, so that $\tilde{\sigma}_0 = 1$. Furthermore, we can solve for \tilde{x}_0 as

$$\tilde{x}_0 = \frac{1}{1 + \kappa} (1 - e^{-(1+\kappa)\eta}), \quad (1.155)$$

where we have used the initial condition $\tilde{x}_0(0) = 0$.

Once again, we could go on to solve for $\tilde{\sigma}_1$ and \tilde{x}_1 , but such calculations, being long and of little use, are rarely done. Thus, from now on, we omit the subscript 0, since it plays no essential role.

One important thing to notice about this solution for $\tilde{\sigma}$ and \tilde{x} is that it cannot be valid at large times. After all, σ cannot possibly be a constant for all times. Thus, $\tilde{\sigma}$ and

\tilde{x} are valid for small times (since they satisfy the initial conditions), but not for large times.

At first sight, it looks as if we are at an impasse. We have a solution, σ and x , that works for large times but not for small times, and we have another solution, $\tilde{\sigma}$ and \tilde{x} , that works for small times, but not for large ones. The goal now is to match them to obtain a single solution that is valid for all times. Fortunately, this is relatively simple to do for this example.

In terms of the original time variable τ , the solution for \tilde{x} is

$$\tilde{x}(\tau) = \frac{\tilde{\sigma}}{\tilde{\sigma} + \kappa} (1 - e^{-(1+\kappa)\frac{\tau}{\epsilon}}). \quad (1.156)$$

As τ gets larger than order ϵ , the exponential term disappears, leaving only

$$\tilde{x}(\tau) = \frac{\tilde{\sigma}}{\tilde{\sigma} + \kappa}, \quad (1.157)$$

which has the same form as (1.149). It thus follows that the solution

$$x(\tau) = \frac{\sigma}{\sigma + \kappa} (1 - e^{-(1+\kappa)\frac{\tau}{\epsilon}}) \quad (1.158)$$

is valid for all times.

The solution for σ is obtained by direct solution of (1.150), which gives

$$\sigma + \kappa \log \sigma = (\alpha - \kappa)t + 1, \quad (1.159)$$

where we have used the initial condition $\sigma(0) = 1$. Since σ does not change on the short time scale, this solution is valid for both small and large times.

This simple analysis shows that there is first a time span during which the enzyme products rapidly equilibrate, consuming little substrate, and after this initial “layer” the reaction proceeds according to Michaelis–Menten kinetics along the quasi-steady-state curve. This is shown in Fig. 1.14. In the phase plane one can see clearly how the solution moves quickly until it reaches the quasi-steady-state curve (the slow manifold) and

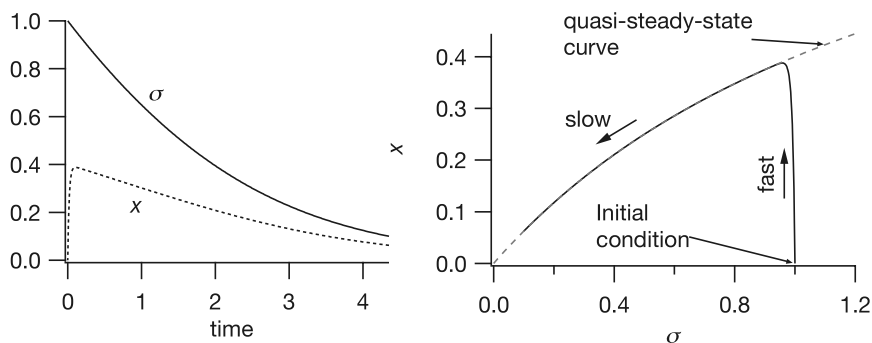


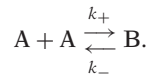
Figure 1.14 The solution to the quasi-steady-state approximation, plotted as functions of time (left panel) and in the phase plane (right panel). Calculated using $\kappa = 1.5$, $\alpha = 0.5$, $\epsilon = 0.05$.

then moves slowly along that curve toward the steady state. Note that the movement to the quasi-steady-state curve is almost vertical, since during that time σ remains approximately unchanged from its initial value. A similar procedure can be followed for the equilibrium approximation (Exercise 20). In this case, the fast movement to the slow manifold is not along lines of constant σ , but along lines of constant $\sigma + \alpha x$, where $\alpha = e_0/s_0$.

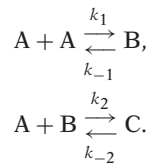
In this problem, the analysis of the initial layer is relatively easy and not particularly revealing. However, this type of analysis is of much greater importance later in this book when we discuss the behavior of excitable systems.

1.7 EXERCISES

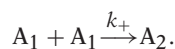
1. Consider the simple chemical reaction in which two monomers of A combine to form a dimer B, according to



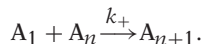
- (a) Use the law of mass action to find the differential equations governing the rates of production of A and B.
 - (b) What quantity is conserved? Use this conserved quantity to find an equation governing the rate of production of A that depends only on the concentration of A.
 - (c) Nondimensionalize this equation and show that these dynamics depend on only one dimensionless parameter.
2. In the real world trimolecular reactions are rare, although trimerizations are not. Consider the following trimerization reaction in which three monomers of A combine to form the trimer C,



- (a) Use the law of mass action to find the rate of production of the trimer C.
 - (b) Suppose $k_{-1} \gg k_{-2}, k_2 A$. Use the appropriate quasi-steady-state approximation to find the rates of production of A and C, and show that the rate of production of C is proportional to $[A]^3$. Explain in words why this is so.
3. The length of microtubules changes by a process called treadmilling, in which monomer is added to one end of the microtubule and taken off at the other end. To model this process, suppose that monomer A_1 is self-polymerizing in that it can form dimer A_2 via



Furthermore, suppose A_1 can polymerize an n -polymer A_n at one end making an $n + 1$ -polymer A_{n+1}



Finally, degradation can occur one monomer at a time from the opposite end at rate k_- . Find the steady-state distribution of polymer lengths after an initial amount of monomer A_0 has fully polymerized.

- Suppose that the reaction rates for the three reactant loop of Fig. 1.1 do not satisfy detailed balance. What is the net rate of conversion of A into B when the reaction is at steady state?
- Consider an enzymatic reaction in which an enzyme can be activated or inactivated by the same chemical substance, as follows:



Suppose further that X is supplied at a constant rate and removed at a rate proportional to its concentration. Use quasi-steady-state analysis to find the nondimensional equation describing the degradation of X,

$$\frac{dx}{dt} = \gamma - x - \frac{\beta xy}{1 + x + y + \frac{\alpha}{\delta} x^2}. \quad (1.163)$$

Identify all the parameters and variables, and the conditions under which the quasi-steady state approximation is valid.

- Using the quasi-steady-state approximation, show that the velocity of the reaction for an enzyme with an allosteric inhibitor (Section 1.4.3) is given by

$$V = \left(\frac{V_{\max} K_3}{i + K_3} \right) \left(\frac{s(k_{-1} + k_3 i + k_1 s + k_{-3})}{k_1(s + K_1)^2 + (s + K_1)(k_3 i + k_{-3} + k_2) + k_2 k_{-3} / k_1} \right). \quad (1.164)$$

Identify all parameters. Under what conditions on the rate constants is this a valid approximation? Show that this reduces to (1.59) in the case $K_1 = \kappa_1$.

- (a) Derive the expression (1.76) for the fraction of occupied sites in a Monod–Wyman–Changeux model with n binding sites.
 (b) Modify the Monod–Wyman–Changeux model shown in Fig. 1.4 to include transitions between states R_1 and T_1 , and between states R_2 and T_2 . Use the principle of detailed balance to derive an expression for the equilibrium constant of each of these transitions. Find the expression for Y , the fraction of occupied sites, and compare it to (1.72).
- An enzyme-substrate system is believed to proceed at a Michaelis–Menten rate. Data for the (initial) rate of reaction at different concentrations is shown in Table 1.1.
 (a) Plot the data V vs. s . Is there evidence that this is a Michaelis–Menten type reaction?
 (b) Plot V vs. V/s . Are these data well approximated by a straight line?

Table 1.1 Data for Problem 8.

Substrate Concentration (mM)	Reaction Velocity (mM/s)
0.1	0.04
0.2	0.08
0.5	0.17
1.0	0.24
2.0	0.32
3.5	0.39
5.0	0.42

Table 1.2 Data for Problem 9.

Substrate Concentration (mM)	Reaction Velocity (mM/s)
0.2	0.01
0.5	0.06
1.0	0.27
1.5	0.50
2.0	0.67
2.5	0.78
3.5	0.89
4.0	0.92
4.5	0.94
5.0	0.95

- (c) Use linear regression and (1.46) to estimate K_m and V_{\max} . Compare the data to the Michaelis–Menten rate function using these parameters. Does this provide a reasonable fit to the data?
9. Suppose the maximum velocity of a chemical reaction is known to be 1 mM/s, and the measured velocity V of the reaction at different concentrations s is shown in Table 1.2.
- (a) Plot the data V vs. s . Is there evidence that this is a Hill type reaction?
- (b) Plot $\ln\left(\frac{V}{V_{\max}-V}\right)$ vs. $\ln(s)$. Is this approximately a straight line, and if so, what is its slope?
- (c) Use linear regression and (1.71) to estimate K_m and the Hill exponent n . Compare the data to the Hill rate function with these parameters. Does this provide a reasonable fit to the data?
10. Use the equilibrium approximation to derive an expression for the reaction velocity of the scheme (1.60)–(1.61).

Answer:

$$V = \frac{(k_2 K_3 + k_4 s) e_0 s}{K_1 K_3 + K_3 s + s^2}, \quad (1.165)$$

where $K_1 = k_{-1}/k_1$ and $K_3 = k_{-3}/k_3$.

11. (a) Find the velocity of reaction for an enzyme with three active sites.
- (b) Under what conditions does the velocity reduce to a Hill function with exponent three? Identify all parameters.
- (c) What is the relationship between rate constants when the three sites are independent? What is the velocity when the three sites are independent?
12. The Goldbeter–Koshland function (1.92) is defined using the solution of the quadratic equation with a negative square root. Why?
13. Suppose that a substrate can be broken down by two different enzymes with different kinetics. (This happens, for example, in the case of cAMP or cGMP, which can be hydrolyzed by two different forms of phosphodiesterase—see Chapter 19).

- (a) Write the reaction scheme and differential equations, and nondimensionalize, to get the system of equations

$$\frac{d\sigma}{dt} = -\sigma + \alpha_1(\mu_1 + \sigma)x + \alpha_2(\mu_2 + \sigma)y, \quad (1.166)$$

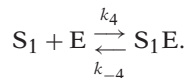
$$\epsilon_1 \frac{dx}{dt} = \frac{1}{\lambda_1} \sigma(1-x) - x, \quad (1.167)$$

$$\epsilon_2 \frac{dy}{dt} = \frac{1}{\lambda_2} \sigma(1-y) - y. \quad (1.168)$$

where x and y are the nondimensional concentrations of the two complexes. Identify all parameters.

- (b) Apply the quasi-steady-state approximation to find the equation governing the dynamics of substrate σ . Under what conditions is the quasi-steady-state approximation valid?
- (c) Solve the differential equation governing σ .
- (d) For this system of equations, show that the solution can never leave the positive octant $\sigma, x, y \geq 0$. By showing that $\sigma + \epsilon_1 x + \epsilon_2 y$ is decreasing everywhere in the positive octant, show that the solution approaches the origin for large time.
14. For some enzyme reactions (for example, the hydrolysis of cAMP by phosphodiesterase in vertebrate retinal cones) the enzyme is present in large quantities, so that e_0/s_0 is not a small number. Fortunately, there is an alternate derivation of the Michaelis–Menten rate equation that does not require that $\epsilon = \frac{e_0}{s_0}$ be small. Instead, if one or both of k_{-1} and k_2 are much larger than $k_1 e_0$, then the formation of complex c is a rapid exponential process, and can be taken to be in quasi-steady state. Make this argument systematic by introducing appropriate nondimensional variables and then find the resulting quasi-steady-state dynamics. (Segel, 1988; Frenzen and Maini, 1988; Segel and Slemrod, 1989; Sneyd and Tranchina, 1989).

15. ATP is known to inhibit its own dephosphorylation. One possible way for this to occur is if ATP binds with the enzyme, holding it in an inactive state, via



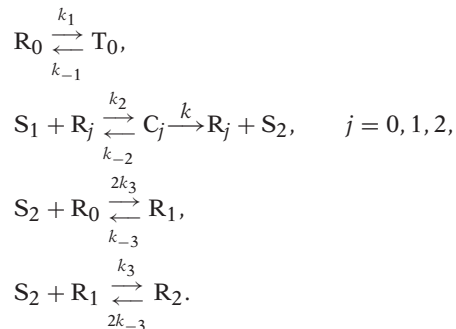
Add this reaction to the Sel'kov model of glycolysis and derive the equations governing glycolysis of the form (1.107)–(1.108). Explain from the model why this additional reaction is inhibitory.

16. In the case of noncompetitive inhibition, the inhibitor combines with the enzyme-substrate complex to give an inactive enzyme-substrate-inhibitor complex which cannot undergo further reaction, but the inhibitor does not combine directly with free enzyme or affect its reaction with substrate. Use the quasi-steady-state approximation to show that the velocity of this reaction is

$$V = V_{\max} \frac{s}{K_m + s + \frac{i}{K_i} s}. \quad (1.169)$$

Identify all parameters. Compare this velocity with the velocity for other types of inhibition discussed in the text.

17. The following reaction scheme is a simplified version of the Goldbeter–Lefever reaction scheme for glycolytic oscillations:



Show that, under appropriate assumptions about the ratios k_1/k_{-1} and $\frac{k_{-2}+k_3}{k_2}$ the equations describing this reaction are of the form (1.107)–(1.108) with $f(\sigma_1, \sigma_2)$ given by (1.125).

18. Use the law of mass action and the quasi-steady-state assumption for the enzymatic reactions to derive a system of equations of the form (1.107)–(1.108) for the Goldbeter–Lefever model of glycolytic oscillations. Verify (1.124).
19. When much of the ATP is depleted in a cell, a considerable amount of cAMP is formed as a product of ATP degradation. This cAMP activates an enzyme phosphorylase that splits glycogen, releasing glucose that is rapidly metabolized, replenishing the ATP supply. Devise a model of this control loop and determine conditions under which the production of ATP is oscillatory.
20. (a) Nondimensionalize (1.26)–(1.29) in a way appropriate for the equilibrium approximation (rather than the quasi-steady-state approximation of Section 1.4.2). Hint: Recall that for the equilibrium approximation, the assumption is that $k_1 e_0$ and k_{-1} are large

compared to k_2 . You should end up with equations that look something like

$$\epsilon \frac{d\sigma}{d\tau} = \alpha x - \beta \alpha \sigma (1 - x), \quad (1.170)$$

$$\epsilon \frac{dx}{d\tau} = \beta \sigma (1 - x) - x - \epsilon x, \quad (1.171)$$

where $\epsilon = k_2/k_{-1}$, $\alpha = e_0/s_0$ and $\beta = s_0/K_1$.

- (b) Find the behavior, to lowest order in ϵ for this system. (Notice that the slow variable is $\sigma + \alpha x$.)
 - (c) To lowest order in ϵ , what is the differential equation for σ on this time scale?
 - (d) Rescale time to find equations valid for small times.
 - (e) Show that, to lowest order in ϵ , $\sigma + \alpha x = \text{constant}$ for small times.
 - (f) Without calculating the exact solution, sketch the solution in the phase plane, showing the initial fast movement to the slow manifold, and then the movement along the slow manifold.
-