

the rate at which they transit a bottleneck, such as a toll booth. The concept of a rate-determining step finds many applications in biology. The overall throughput in metabolic pathways, for example, is often controlled by regulating the key rate-limiting steps in the pathway.

B. REVERSIBLE REACTIONS, STEADY STATES, AND EQUILIBRIUM

In the first part of this chapter we have ignored reverse reactions, in which products convert back to reactants. This is a reasonable thing to do when the forward reaction is very favorable in free energy (that is, when the value of ΔG for the reaction is large and negative). In such cases, as we shall see, once products are formed, it is very unlikely that they will convert back to form the reactants. For many important biological processes, however, the magnitude of the free-energy change is not large, and both forward and reverse reactions need to be considered in the kinetic equations.

15.11 The forward and reverse rates must both be considered for a reversible reaction

A particularly simple example of a reversible reaction is a conformational rearrangement, such as the isomerization of a peptide bond where one of the residues is a proline. Recall from Section 4.8 that both the *cis* and *trans* conformers can be populated significantly if one of the residues forming a peptide bond is proline.

A reversible unimolecular reaction in which molecules A and B interconvert can be written in the following way:



where k_f and k_r are the rate constants for the forward and reverse reactions. The forward and reverse rate constants are also commonly denoted k_1 and k_{-1} , respectively.

We can work out the rate equations for concentrations as we did before, except that there will be a term that accounts for the formation of A from B in addition to the term that describes the disappearance of A as it is converted to B. The basic rate equations are then:

$$\frac{d[A]}{dt} = -k_f[A] + k_r[B] \quad (15.29)$$

and

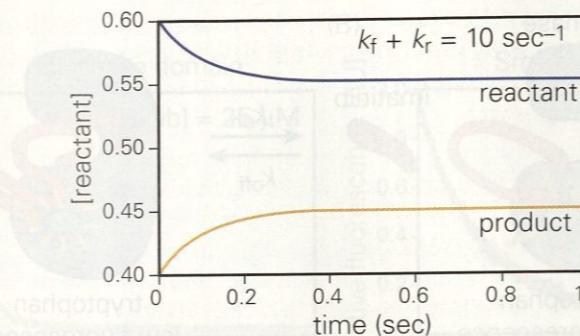
$$\frac{d[B]}{dt} = +k_f[A] - k_r[B] \quad (15.30)$$

Since A and B are the only molecules involved in the reaction, the sum of their concentrations must be constant. We can write this condition as $[A] + [B] = X$, where X is a constant. Using this substitution in the differential rate equation for [A] (Equation 15.29) and, integrating it as explained in Box 15.1, gives the following result:

$$[A] = ([A]_0 - [A]_{eq})e^{-(k_f+k_r)t} + [A]_{eq} \quad (15.31)$$

The term $[A]_{eq}$ in Equation 15.31 is the equilibrium value of the concentration of A.

There are two reactions occurring, one in the forward direction and one in reverse, and each of these reactions has a different rate constant associated with it. Nevertheless, the change in concentrations is described by a simple exponential function, as for a first-order reaction that proceeds in only one direction. The exponential relaxation has an effective rate constant that is the *sum* of the forward



and reverse rate constants, as shown in Figure 15.15. From an experiment that measures only the time-dependence of the concentrations of reactants and products for such a reaction, it is not possible to determine the values of k_f and k_r individually.

It is important to note that, for reversible reactions, the concentration of reactant does not go to zero, but rather stops changing when the equilibrium concentration ($[A]_{eq}$) is reached. This leads to the extra terms in Equation 15.31 relative to Equation 15.14, which describes the concentration dependence for a first-order reaction that proceeds in only one direction.

15.12 The on and off rates of ligand binding can be measured by monitoring the approach to equilibrium

The analysis of forward and reverse rates for a reversible reaction finds an important practical application in determining how fast a ligand (for example, a drug molecule) binds to a protein. Such a binding interaction is a bimolecular reaction, while Equation 15.31 applies to unimolecular reactions. But the analysis is simplified by the fact that we can carry out the binding reaction under conditions where the concentration of the ligand is much greater than the concentration of the protein. In that case, the concentration of the ligand does not change much during the reaction. Under such circumstances, the reaction becomes pseudo-first order (see Section 15.8) because the ligand concentration is a constant. Recall that this is an assumption that we also made in the analysis of the equilibrium constants for ligand binding (see Section 12.6).

We write the binding reaction for protein, P, and ligand, L, as:



In Equation 15.32, k_{on} and k_{off} are the forward and reverse rate constants, respectively. The subscripts "on" and "off" are commonly used for rate constants involved in ligand binding.

The basic rate equations are:

$$\frac{d[P]}{dt} = -k_{on}[P][L] + k_{off}[P \bullet L] \quad (15.33)$$

and

$$\frac{d[P \bullet L]}{dt} = +k_{on}[P][L] - k_{off}[P \bullet L] \quad (15.34)$$

Because $[L]$ is essentially constant (that is, we are considering conditions where $[L] \gg [P]$), these equations can be rewritten as:

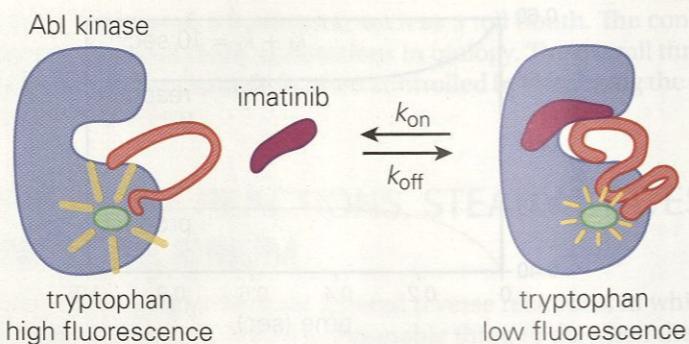
$$\frac{d[P]}{dt} = -k'_{on}[P] + k_{off}[P \bullet L] \quad (15.35)$$

and

$$\frac{d[P \bullet L]}{dt} = +k'_{on}[P] - k_{off}[P \bullet L] \quad (15.36)$$

Figure 15.15 Time dependence of reactants and products for a reversible unimolecular reaction. In this case, the concentrations of reactant and product are 0.60 and 0.40 initially and then 0.45 and 0.55 at equilibrium. The sum of the forward and reverse rate constants is 10 sec^{-1} .

Figure 15.16 Fluorescence changes can be used to detect the binding of a ligand. The schematic diagram shows the binding of the cancer drug imatinib (purple) to the protein kinase Abl. A tryptophan sidechain in the protein (illustrated in Figure 15.23) has high fluorescence when no drug is bound and low fluorescence when imatinib is bound. This change in fluorescence can be used to detect the binding of imatinib to the protein kinase.



where $k'_\text{on} = k_\text{on}[L]$ is a pseudo-first-order rate constant that has a value that depends on the total ligand concentration, $[L]_0$ (that is, we assume that $[L] \approx [L]_0$). Equations 15.35 and 15.36 are the same as Equations 15.29 and 15.30, with P corresponding to A and P•L corresponding to B. Hence, we can use Equation 15.31 to write down an expression for the concentration of the product as a function of time:

$$[P] = ([P]_0 - [P]_{\text{eq}}) e^{-(k'_\text{on} + k_\text{off})t} + [P]_{\text{eq}} \quad (15.37)$$

Here $[P]$ is the time-dependent concentration of the free protein, $[P]_0$ is the initial concentration of the free protein, and $[P]_{\text{eq}}$ is the equilibrium concentration of the free protein in the presence of ligand.

Imagine that the binding of the ligand to the protein can be monitored instantaneously as it changes. This can be readily done if the fluorescence properties of the protein change upon ligand binding, as is often the case (Figure 15.16). Equation 15.37 then tells us that, if we mix together solutions containing the protein and ligand (the drug), then we should see an exponential relaxation of the concentration of the free protein from an initial value (when it is entirely unbound) to an equilibrium value (when some of it is bound to the ligand). The apparent rate constant for the process of ligand binding can be used to derive the actual on- and off-rates, with rate constants k_on and k_off , respectively, by varying the ligand concentration as explained in the following example.

In Chapter 12, we discussed the binding of the cancer drug imatinib to the tyrosine kinase Abl. When imatinib binds to Abl, the intensity of fluorescence emission at 350 nm (upon excitation at 290 nm) is reduced (Figure 15.17). The change in fluorescence is due to changes in the environment of tryptophan residues in the protein upon drug binding. Thus, when imatinib and Abl are mixed together in a fluorescence spectrometer, the fluorescence signal is observed to decrease, as shown in Figure 15.17A. The change in the fluorescence signal is well described by an exponential curve, yielding a first-order rate constant, k_obs . According to Equation 15.37:

$$k_\text{obs} = k'_\text{on} + k_\text{off} \quad (15.38)$$

The apparent first-order forward rate constant, k'_on , is the product of the actual second-order forward rate constant and the ligand concentration ($k'_\text{on} = k_\text{on}[L]$; see the discussion following Equation 15.36). The value of k_obs depends, therefore, on the ligand concentration as follows:

$$k_\text{obs} = k_\text{on}[L] + k_\text{off} \quad (15.39)$$

According to Equation 15.39, we should get a straight line if we measure the apparent rate constant for imatinib binding to Abl as a function of total imatinib concentration ($[L]$). This is indeed the case, as shown in Figure 15.17C. The slope of the line is the second-order forward rate constant for imatinib binding to Abl (k_on). If the line is extrapolated back to zero ligand concentration, the intercept of the line is the reverse rate constant (k_off , the off-rate).

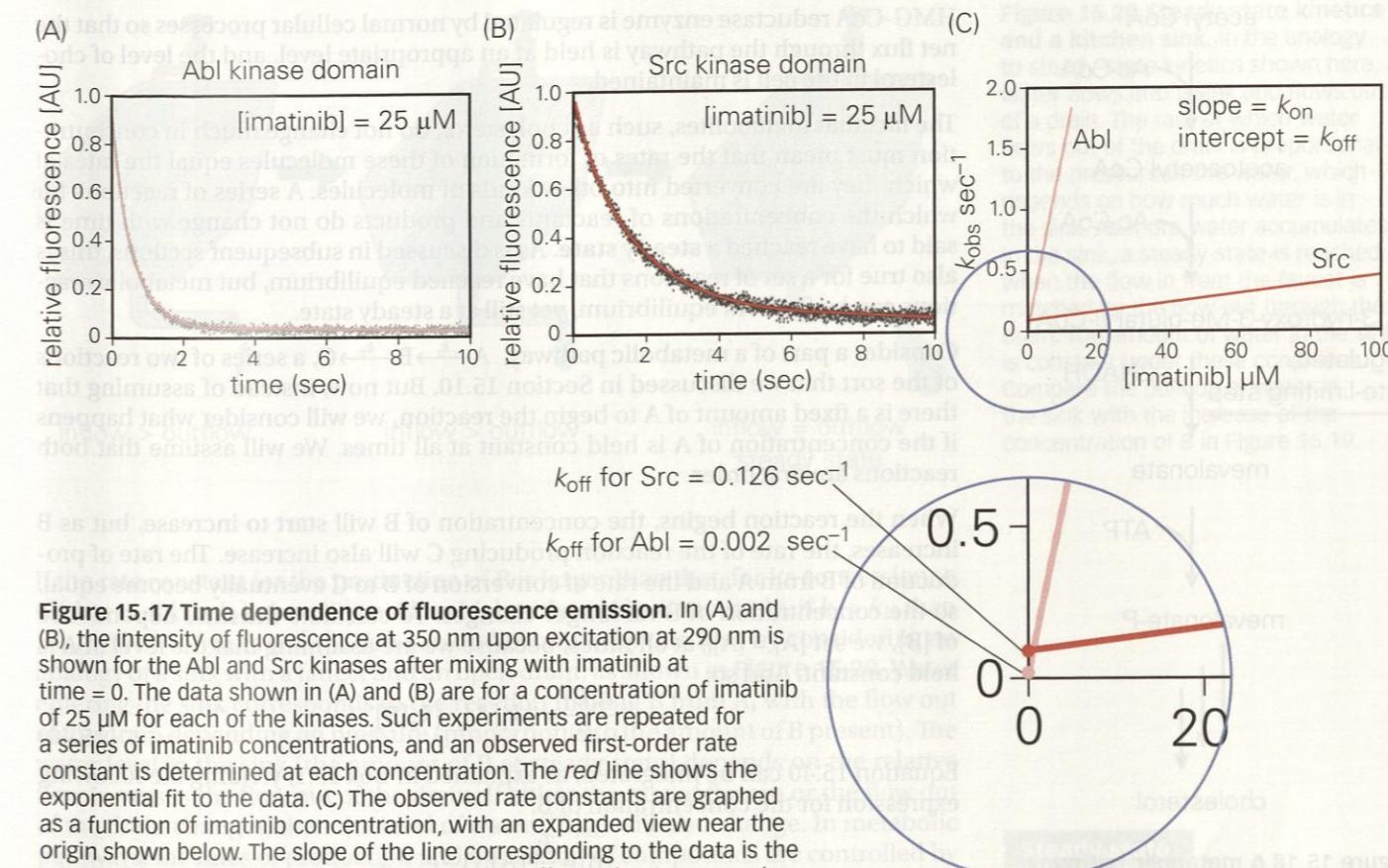


Figure 15.17 Time dependence of fluorescence emission. In (A) and (B), the intensity of fluorescence at 350 nm upon excitation at 290 nm is shown for the Abl and Src kinases after mixing with imatinib at time = 0. The data shown in (A) and (B) are for a concentration of imatinib of 25 μM for each of the kinases. Such experiments are repeated for a series of imatinib concentrations, and an observed first-order rate constant is determined at each concentration. The red line shows the exponential fit to the data. (C) The observed rate constants are graphed as a function of imatinib concentration, with an expanded view near the origin shown below. The slope of the line corresponding to the data is the on-rate, k_on , and the intercept of the line is the off-rate, k_off (see Equation 15.39). (Adapted from M.A. Seeliger et al., and J. Kuriyan, *Structure* 15: 299–311, 2007.)

Analysis of the data shown in Figure 15.17 reveals that the on-rate for imatinib binding to Abl is $0.146 \times 10^6 \text{ M}^{-1}\cdot\text{sec}^{-1}$. Recall from Section 12.17 that imatinib is ineffective as an inhibitor of the tyrosine kinase Src. Data for imatinib binding to Src are shown in Figure 15.17B, for one concentration of the drug. The line corresponding to data measured over a range of imatinib concentrations has a much smaller slope than that for the Abl data because the on-rate for imatinib binding to Src ($k_\text{on} = 0.003 \times 10^6 \text{ M}^{-1}\cdot\text{sec}^{-1}$) is much slower than for Abl (Figure 15.17C). This makes sense, because the stable conformations of Src are incompatible with imatinib binding (see Section 12.17). Presumably, imatinib is slow to bind to Src because the protein is slow to convert to a conformation that can accommodate the drug.

The off-rate of imatinib from Abl is quite low, 0.00219 sec^{-1} . This corresponds to a half-life of $t_{1/2} = 0.693/k_\text{off} = 316 \text{ sec}$. The dissociation of drug from Src is much faster, with $k_\text{off} = 0.126 \text{ sec}^{-1}$, and so $t_{1/2} = 5.5 \text{ sec}$.

15.13 Steady-state reactions are important in metabolism

As noted earlier, living organisms are never at equilibrium. It is usually the case, however, that the concentrations of many important metabolites in cells do not change much over significant periods of time, despite the fact that many different metabolic reactions are going on continuously. An example of a metabolic pathway that we have discussed earlier (Section 12.20), the production of cholesterol from acetyl coenzyme A (acetyl CoA), is shown in Figure 15.18. The rate-limiting step of this pathway is the conversion of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate by the enzyme HMG-CoA reductase. Recall from Section 12.20 that HMG-CoA reductase is the target of cholesterol-lowering drugs. The

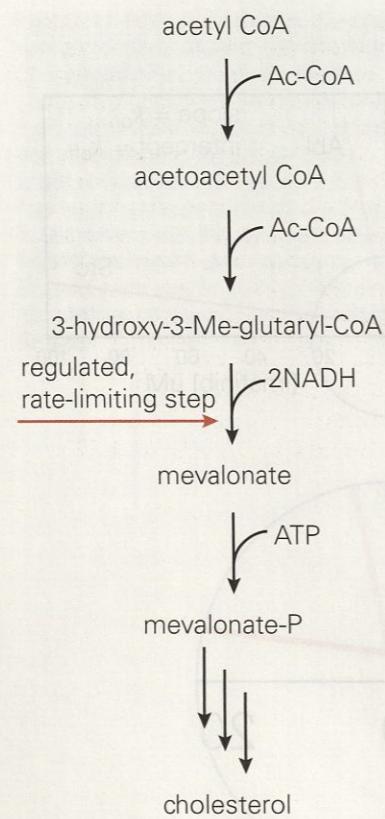


Figure 15.18 A metabolic pathway. Almost all metabolic pathways are series of reactions in which intermediates pass from one enzyme to another to either build up or degrade a product. A part of the cholesterol biosynthetic pathway is shown, with the side arrow showing a dominant, regulated rate-limiting step in this pathway (also discussed in Section 12.20 in the context of inhibitors).

HMG-CoA reductase enzyme is regulated by normal cellular processes so that the net flux through the pathway is held at an appropriate level, and the level of cholesterol in the cell is maintained.

The fact that metabolites, such as cholesterol, do not change much in concentration must mean that the rates of formation of these molecules equal the rates at which they are converted into other kinds of molecules. A series of reactions for which the concentrations of reactants and products do not change with time is said to have reached a **steady state**. As is discussed in subsequent sections, this is also true for a set of reactions that have reached equilibrium, but metabolic reactions can be far from equilibrium, yet still at a steady state.

Consider a part of a metabolic pathway, $A \xrightarrow{k_1} B \xrightarrow{k_2} C$, a series of two reactions of the sort that we discussed in Section 15.10. But now, instead of assuming that there is a fixed amount of A to begin the reaction, we will consider what happens if the concentration of A is held constant at all times. We will assume that both reactions are first order.

When the reaction begins, the concentration of B will start to increase, but as B increases, the rate of the reaction producing C will also increase. The rate of production of B from A and the rate of conversion of B to C eventually become equal, so the concentration of B no longer changes. To calculate the time dependence of [B], we set $[A] = [A]_0$ at all times, because we are assuming that the level of A is held constant. And so:

$$\frac{d[B]}{dt} = +k_1[A]_0 - k_2[B] \quad (15.40)$$

Equation 15.40 can be integrated, as explained in Box 15.1, yielding the following expression for the concentration of B:

$$[B] = \frac{k_1}{k_2}[A]_0(1 - e^{-k_2 t}) \quad (15.41)$$

Equation 15.41 shows that the concentration of [B] asymptotically approaches a final value with a time constant of $1/k_2$ (Figure 15.19; see Section 15.9 for an explanation of the time constant).

The asymptotic value of [B] is called the steady-state concentration, $[B]_{ss}$, which is obtained by setting t to infinity in Equation 15.41 and noting that $e^{-\infty}$ is zero:

$$[B]_{ss} = \frac{k_1}{k_2}[A]_0 \quad (15.42)$$

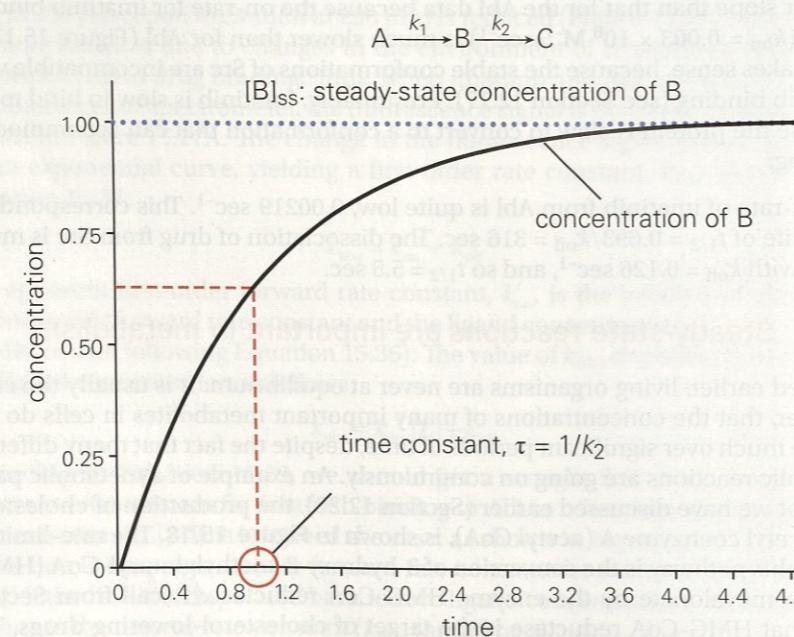


Figure 15.19 Steady-state concentration. Reactant A generates an intermediate, B, which is converted to a product, C. If the concentration of A is held fixed (that is, if it is constantly replenished), then the concentration of B will reach a steady-state value, $[B]_{ss}$. The graph shows the increase in the concentration of B with time, with a time constant equal to $1/k_2$, where k_2 is the rate constant for the conversion of B to C.

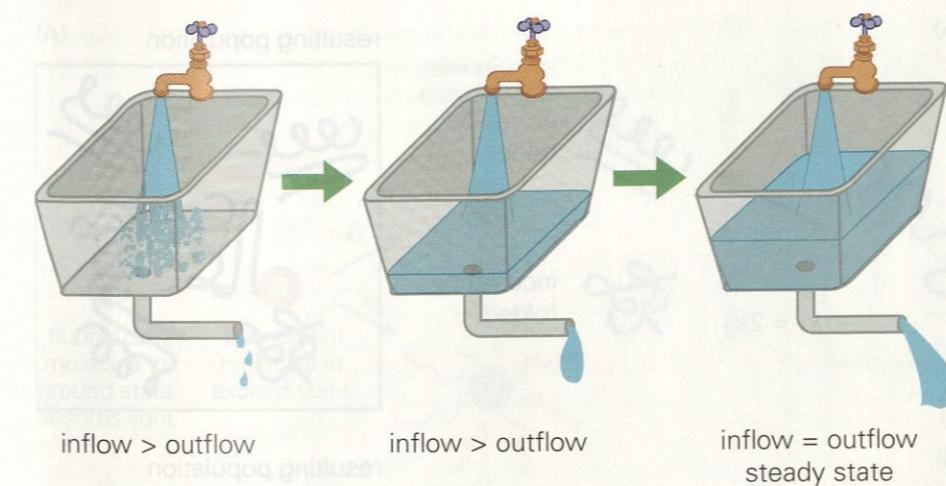


Figure 15.20 Steady-state kinetics and a kitchen sink. In the analogy to steady-state kinetics shown here, water flows into a sink and flows out of a drain. The rate at which water flows out of the drain is proportional to the pressure of the water, which depends on how much water is in the sink. As more water accumulates in the sink, a steady state is reached when the flow in from the faucet is matched by the flow out through the drain. The amount of water in the sink is constant under these conditions. Compare the buildup of water in the sink with the increase of the concentration of B in Figure 15.19.

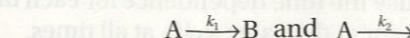
If the rate constant for the production of B is larger than that for its conversion to C (that is, if $k_1 > k_2$), then $[B]_{ss}$ will be higher than $[A]_0$. Conversely, if $k_2 > k_1$, then $[B]_{ss}$ will be lower than $[A]_0$. You can understand why this is so by considering an analogy of a sink with a faucet and an open drain, as shown in Figure 15.20. Water entering the sink corresponds to the reaction making B from A, with the flow out of the drain depending on pressure (proportional to the amount of B present). The water level in the sink (the amount of B at steady state) depends on the relative flow in versus the flow out of the drain. If either the rate of flow in or the flow out of the drain changes, then the level of water ($[B]_{ss}$) will also change. In metabolic pathways, the rates of production and utilization of compounds are controlled by enzymes whose activities are modulated by interactions with regulatory factors, including the metabolites themselves (see Section 16.17).

Note that a whole pathway, ... $A \rightarrow B \rightarrow C \rightarrow D \rightarrow E \dots$, can come to steady state with each metabolite (A, B, C...) at a different concentration. The only requirement is that the rate at each step beyond the first must depend on the concentration of the metabolites, so that as the concentrations increase, the rate also increases. It is not necessary that the processes be first order, as was used in the example above (this case was chosen because it is easy to analyze). As noted in the previous section, the rate of product formation at the end of the pathway will be determined by the rate of the slowest step. At steady state, the concentrations of metabolites before the slowest step will be higher than those after the slowest step.

15.14 For reactions with alternative products, the relative values of rate constants determine the distribution of products

We have, so far, only considered linear reaction schemes in which a reactant goes to only one kind of product. It is not uncommon, however, for reactions to be branched. Branched reactions have two or more pathways, with alternative intermediates and products generated from a reactant. One example of a branched reaction is the closure of a linear form of a sugar, which can go to either the α or the β anomer ring form (see Figure 3.4). Another branched reaction occurs when proteins go from their unfolded state to the correct, native conformation, or to one or more incorrectly folded conformations (Figure 15.21). An example that we shall discuss further in the next section is provided by fluorescence. Energy absorbed by a molecule from a photon of light can either be re-emitted as light or converted to heat.

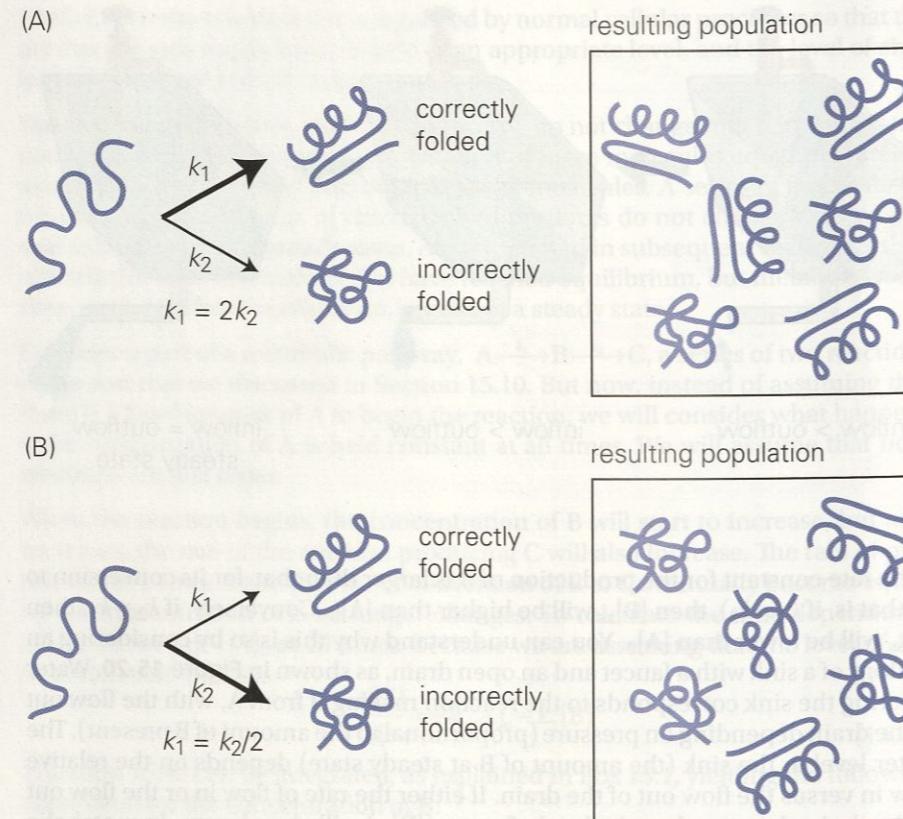
A kinetic model for a reaction with two branches is given by:



Steady state

A set of reactions for which the concentrations of reactants and products do not change with time is said to be in a steady state. The reactants and products do not need to be at equilibrium for a steady state to occur. All that is required for a steady state is that the rates of production of molecules be balanced by the rates at which they are converted to other molecules.

Figure 15.21 Protein folding as an example of a branching reaction. In this example, an unfolded protein molecule is assumed to be able to fold either into a correctly folded conformation, or an incorrectly folded one. (A) The rate constant for the formation of the correct structure, k_1 , is assumed to be twice as large as the rate constant, k_2 , for the formation of the incorrect structure. There will be twice as many correctly folded proteins formed as incorrectly folded ones. (B) If k_1 is half the value of k_2 , then the opposite happens and there will be twice as many incorrectly folded proteins as correctly folded ones.



The rate equation for the reactant, A, must account for the two parallel reactions:

$$\frac{d[A]}{dt} = -k_1[A] - k_2[A] = -(k_1 + k_2)[A] \quad (15.43)$$

There are two rate equations for the products:

$$\frac{d[B]}{dt} = +k_1[A] \text{ and } \frac{d[C]}{dt} = +k_2[A]. \quad (15.44)$$

For the reactant, A, the result is just a simple first-order behavior, but with effective rate constant, $k_{\text{eff}} = k_1 + k_2$:

$$[A] = [A]_0 e^{-k_{\text{eff}}t} \quad (15.45)$$

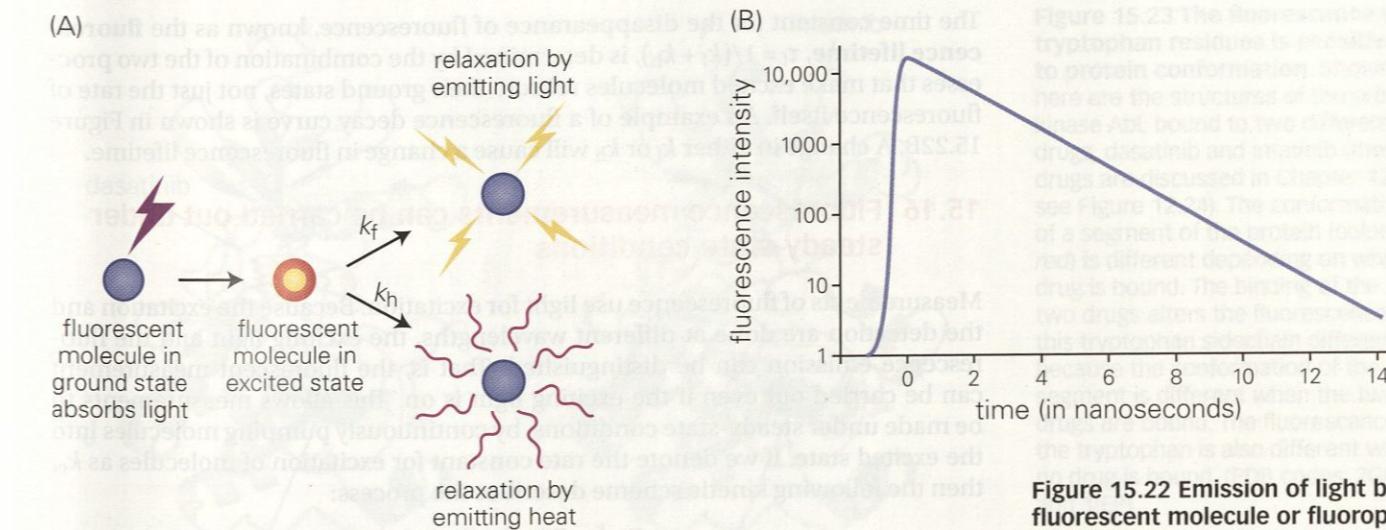
To solve for the concentration of the products ([B] and [C]), the expression for [A] is substituted into the differential equations for [B] and [C] (Equation 15.44) and integrated to give:

$$[B] = \frac{k_1[A]_0}{k_1 + k_2} \left[1 - e^{-(k_1 + k_2)t} \right] = \frac{k_1[A]_0}{k_{\text{eff}}} \left(1 - e^{-k_{\text{eff}}t} \right) \quad (15.46)$$

and

$$[C] = \frac{k_2[A]_0}{k_1 + k_2} \left[1 - e^{-(k_1 + k_2)t} \right] = \frac{k_2[A]_0}{k_{\text{eff}}} \left(1 - e^{-k_{\text{eff}}t} \right) \quad (15.47)$$

Each of these contains a ratio of rate constants, $[k_1 / (k_1 + k_2)]$ for B, the fraction of molecules that ultimately become B, and $[k_2 / (k_1 + k_2)]$ for those that become C. Equations 15.46 and 15.47 make it easy to calculate how much B is produced relative to C. The ratio of the concentrations of the two products is known as the **branching ratio**, $[B]/[C]$. Because the time dependence for each branch is identical, the branching ratio is constant at the value k_1/k_2 at all times.



15.15 Measuring fluorescence provides an easy way to monitor kinetics

Fluorescence spectroscopy and imaging are important tools in the study of biological molecules and illustrate well a number of features of kinetic processes. Fluorescence results after a molecule, known as a **fluorophore**, is put into an electronically excited state by absorbing light (usually ultraviolet light). After excitation, the fluorophore can emit a photon of light (the process of fluorescence, in which the light emitted is at a longer wavelength than that absorbed), or convert the energy to vibrations (that is, releasing it as heat, as shown in Figure 15.22). The relative rates of these two processes depend on the characteristics of the fluorophore and the environment. The sensitivity to the environment makes fluorescence useful for following processes such as ligand binding (see Section 15.12) and protein folding (Section 18.5). We have also encountered the use of fluorescence to monitor the mobility of lipids (see Section 3.18).

To describe the kinetics of the fluorescence process, we start with some population of fluorophores, F, that have been excited, F^* , and consider the two alternative reactions by which they return to the ground state:



with rate constants k_f for fluorescence and k_h for producing heat. Using Equation 15.45, we can obtain the following equation for the concentration of the excited fluorophores, F^* :

$$[F^*] = [F^*]_0 e^{-(k_f + k_h)t} \quad (15.49)$$

In a fluorescence experiment, what is actually measured is the intensity of light emitted, I_{light} , as a function of time. I_{light} is the fluorescence intensity, and has units of photons per second. The fluorescence intensity corresponds directly to the rate of production of light as a product—that is:

$$I_{\text{light}} = \frac{d[F]}{dt} = k_f [F^*] \quad (15.50)$$

In this case, the direct measurement is the *rate* of reaction, rather than the concentration of product.

Fluorophores can be excited using a very short pulse of light (from a flash lamp or a pulsed laser), creating the initial concentration of excited fluorophores, $[F^*]_0$. Using the last two equations together, the time dependence of fluorescence intensity, I_{light} , is:

$$I_{\text{light}} = k_f [F^*]_0 e^{-(k_f + k_h)t} \quad (15.51)$$

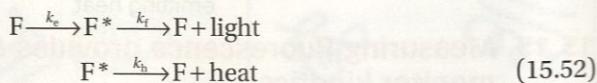
Figure 15.23 The fluorescence of tryptophan residues is sensitive to protein conformation. Some segments of a protein chain are bound to tryptophan residues. The tryptophan side chains are different colors depending on the environment. When a tryptophan residue is bound to a protein, its fluorescence is quenched. The tryptophan residue is bound to a protein chain in the presence of a drug. The tryptophan residue is bound to a protein chain in the absence of a drug.

Figure 15.22 Emission of light by a fluorescent molecule or fluorophore. (A) Fluorophores can absorb light of an appropriate wavelength and convert to an excited electronic state (red). The fluorophore can then relax back to the ground state by two processes. In one process, known as fluorescence, the molecule emits light of longer wavelength than that absorbed. In an alternative process, the molecule gives up its extra energy in the form of heat. The rate constants for the two processes, k_f and k_h , vary depending on the environment of the fluorescent molecule. (B) A fluorescence decay curve. A logarithmic plot of fluorescence intensity versus time, t , is shown. The rise near $t = 0$ comes from the pulse of exciting light, and thereafter the intensity decays exponentially, giving a straight line in the log plot. Note that the fluorescence decays in a few nanoseconds, which is typical and reflects both the emission of light and the conversion to heat.

The time constant for the disappearance of fluorescence, known as the **fluorescence lifetime**, $\tau_f = 1/(k_f + k_h)$, is determined by the combination of the two processes that make excited molecules relax to their ground states, not just the rate of fluorescence itself. An example of a fluorescence decay curve is shown in Figure 15.22B. A change in either k_f or k_h will cause a change in fluorescence lifetime.

15.16 Fluorescence measurements can be carried out under steady-state conditions

Measurements of fluorescence use light for excitation. Because the excitation and the detection are done at different wavelengths, the exciting light and the fluorescence emission can be distinguished. That is, the fluorescent measurement can be carried out even if the exciting light is on. This allows measurements to be made under steady-state conditions, by continuously pumping molecules into the excited state. If we denote the rate constant for excitation of molecules as k_e , then the following kinetic scheme describes the process:



Some short time after turning on the exciting light, the system will reach a steady state:

$$\frac{d[F^*]_{ss}}{dt} = 0 = k_e[F] - k_f[F^*] - k_h[F^*] \quad (15.53)$$

By rearranging Equation 15.53, we can see that the steady-state concentration of the excited fluorophore, $[F^*]_{ss}$ is given by:

$$[F^*]_{ss} = \frac{k_e}{k_f + k_h}[F] \quad (15.54)$$

Recall from Equation 15.50 that the intensity of fluorescence emission is given by the product of the rate constant for fluorescence and the concentration of excited molecules. Combining this information with Equation 15.42, we get:

$$I_{\text{light}} = k_f[F^*]_{ss} = \frac{k_e k_f}{k_f + k_h}[F]_0 \quad (15.55)$$

To obtain Equation 15.55, we assume that the excitation is fairly weak, so that almost all fluorophores are in their ground state at any given time (that is, the concentration of unexcited fluorophores, $[F]$, is essentially the same as the total fluorophore concentration, $[F]_0$). Note that this assumption is similar to the assumption we made when analyzing ligand binding—namely, that the free ligand concentration is the same as the total ligand concentration (see Section 12.6). For the analysis of fluorescence, as for ligand binding, the advantage of working under such conditions is that the total concentration of fluorophore (or ligand, in the case of binding), a parameter that is known to the experimenter, is sufficient to calculate the rates or extent of binding.

Note that, according to Equation 15.55, the fluorescence intensity will change if any of the rate constants, k_e , k_f , or k_h , changes. These rate constants can change when the fluorescent group changes its environment. If the fluorescent group is attached to a protein, then conformational changes in the protein, or the binding of the ligand, can change the fluorescence intensity.

Tryptophan sidechains in proteins are fluorescent, and monitoring fluorescence from tryptophan residues is often a good way to detect the binding of a ligand, such as a drug molecule, to a protein. This is illustrated in Figure 15.17 for two drugs that inhibit the protein kinase Abl, which was discussed earlier, in Section 15.12. The conformation of Abl is different when bound to these two drugs, dasatinib and imatinib (Section 12.17). A tryptophan sidechain in Abl is in quite different environments when these two drugs are bound, as shown in Figure 15.23. As a consequence, the fluorescence intensity is different in the two cases and is also different when no drug is bound.

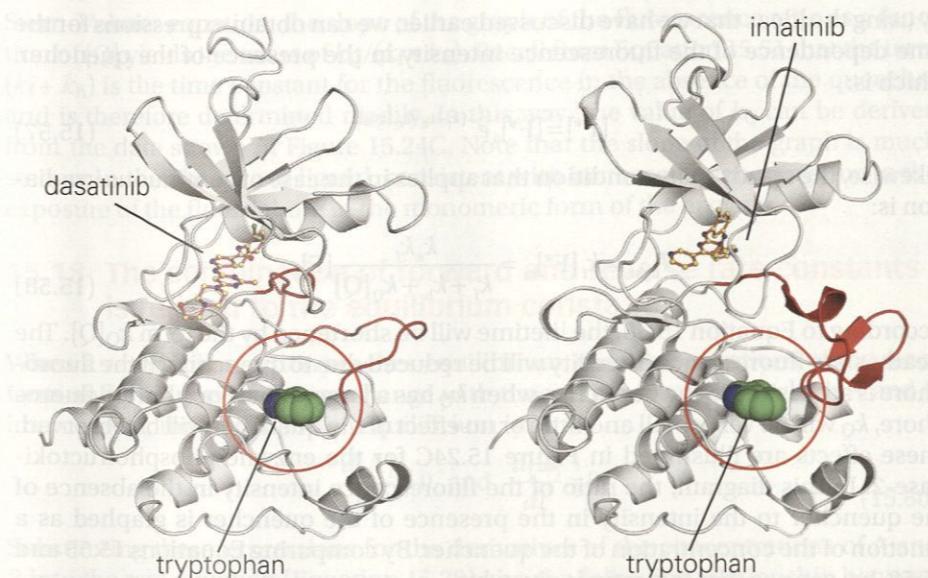


Figure 15.23 The fluorescence of tryptophan residues is sensitive to protein conformation. Shown here are the structures of the protein kinase Abl, bound to two different drugs, dasatinib and imatinib (these drugs are discussed in Chapter 12; see Figure 12.24). The conformation of a segment of the protein (colored red) is different depending on which drug is bound. The binding of the two drugs alters the fluorescence of this tryptophan sidechain differently because the conformation of the red segment is different when the two drugs are bound. The fluorescence of the tryptophan is also different when no drug is bound. (PDB codes: 2GQ and 1IEP)

Fluorescence from tryptophan sidechains is also commonly used both to follow the kinetics of unfolding of proteins and to determine the amount of unfolded protein at equilibrium, as discussed in Section 18.5.

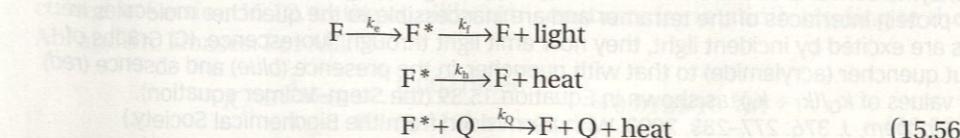
15.17 Fluorescence quenchers provide a way to detect whether a fluorophore on a protein is accessible to the solvent

Another useful application of fluorescence comes about because it is easy to probe whether or not a fluorophore that is attached to a protein or RNA molecule is buried. This can be done by the addition of a **fluorescence quencher** to the solution. The quencher is a compound, such as acrylamide, to which the excitation energy is readily transferred from the fluorophore, and the quencher converts this energy rapidly to heat. In this way, fluorescence quenchers reduce the amount of light emitted by fluorescent molecules. The quencher must come into close contact with the fluorophore for energy to be transferred to it and, hence, the quencher will be ineffective if the fluorophore is buried.

For example, a tryptophan residue in the hydrophobic core of a protein would not have its fluorescence reduced efficiently by a quencher. But if the protein were to unfold, the tryptophan would be exposed to the solvent, and addition of a quencher would then drastically reduce its fluorescence.

Fluorescence quenching also provides a way to monitor the oligomerization of proteins. In the example shown in Figure 15.24, an enzyme, phosphofructokinase-2, switches between a monomeric and a tetrameric form depending on whether ligand is bound to it. In order to detect which state the enzyme is in, scientists attached a fluorophore to a cysteine residue that is exposed in the monomer but buried in the tetramer. In the absence of the ligand, fluorescence from the fluorophore is readily quenched by a quencher, Q. But, when the ligand is added to the enzyme, the amount of quenching is reduced substantially.

A kinetic model for this process just requires adding one more term to the scheme shown in Equation 15.52:



By using the ideas that we have discussed earlier, we can obtain expressions for the time dependence of the fluorescence intensity in the presence of the quencher, which is:

$$[F^*] = [F^*]_0 e^{-(k_f + k_h + k_Q[Q])t} \quad (15.57)$$

Likewise, the steady-state condition that applies in the case of continuous irradiation is:

$$I_{\text{light}} = k_f [F^*]_{\text{ss}} = \frac{k_e k_f}{k_f + k_h + k_Q[Q]} [F]_0 \quad (15.58)$$

According to Equation 15.58, the lifetime will be shortened by the term $k_Q[Q]$. The steady-state fluorescence intensity will be reduced due to quenching if the fluorophore is accessible to the quencher, when k_Q has a large value. For a buried fluorophore, k_Q will be very small and little or no effect of the quencher will be observed. These effects are illustrated in Figure 15.24C for the enzyme phosphofructokinase-2. In this diagram, the ratio of the fluorescence intensity in the absence of the quencher to the intensity in the presence of the quencher is graphed as a function of the concentration of the quencher. By comparing Equations 15.55 and 15.58, we can see that this ratio is given by:

$$\frac{\text{fluorescence intensity in the absence of quencher}}{\text{fluorescence intensity in the presence of quencher}} = 1 + \left(\frac{k_Q}{k_f + k_h} \right) [Q] \quad (15.59)$$

Equation 15.59 is known as the **Stern–Volmer equation**, and it allows us to determine the rate constant for fluorescence quenching, k_Q . According to the

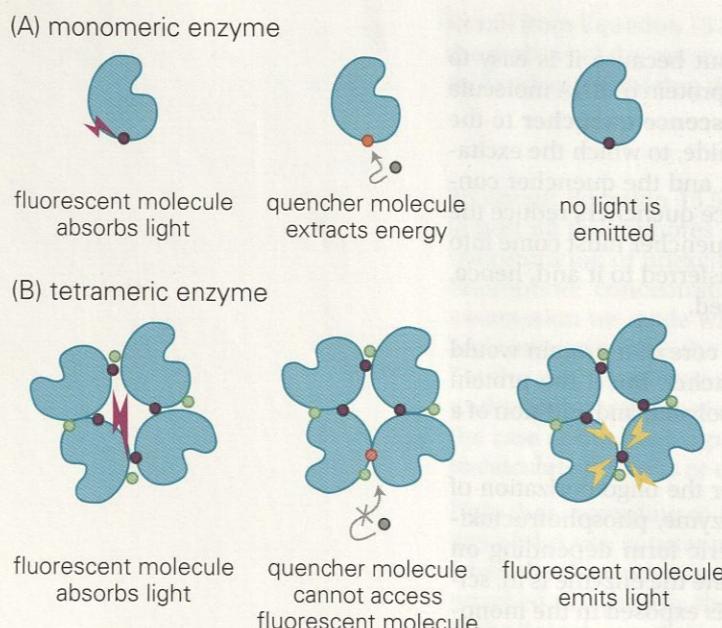
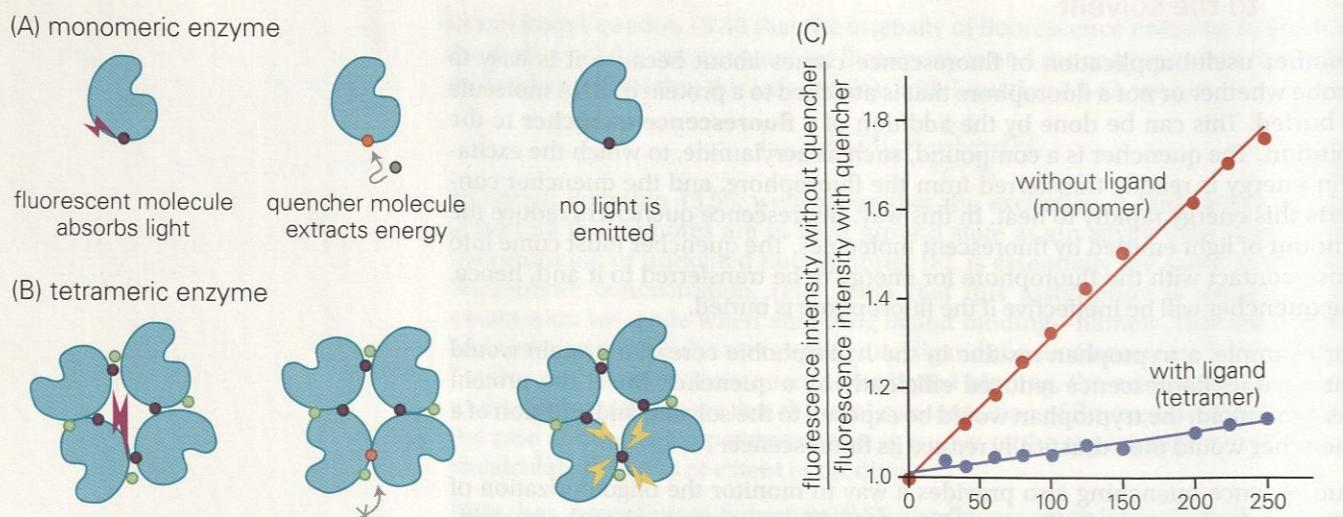


Figure 15.24 Fluorescence quenching. An enzyme that can exist in either a monomeric or a tetrameric form is shown schematically in (A) and (B). The surface of the enzyme is labeled with a fluorophore. (A) In the monomeric form, the fluorescent molecule is exposed. The fluorescent molecule absorbs light and converts to an excited electronic state (red). The presence of fluorescence quenchers in the solution results in the transfer of the excitation energy to the quenchers, and the amount of light emitted as fluorescence is reduced. (B) The enzyme converts to a tetrameric form upon binding to a ligand molecule (green). The fluorescent molecules are buried at the inter-protein interfaces of the tetramer and are inaccessible to the quencher molecules in the solution. When the fluorescent molecules are excited by incident light, they now emit light through fluorescence. (C) Graphs of the ratio of the fluorescence intensity without quencher (acrylamide) to that with quencher, in the presence (blue) and absence (red) of the ligand. The slopes of the lines give the values of $k_Q/(k_f + k_h)$, as shown in Equation 15.59 (the Stern–Volmer equation). (C, adapted from M. Baez et al., and V. Guixe, *Biochem. J.* 376: 277–283, 2003. With permission from the Biochemical Society.)



Stern–Volmer equation, the slope of the graph of the fluorescence ratio as a function of $[Q]$ yields the value of $k_Q/(k_f + k_h)$. Recall from Equation 15.51 that the term $(k_f + k_h)$ is the time constant for the fluorescence in the absence of the quencher and is therefore determined readily. In this way, the value of k_Q can be derived from the data shown in Figure 15.24C. Note that the slope of the graph is much steeper in the absence of ligand, consistent with increased quenching due to the exposure of the fluorophore in the monomeric form of the enzyme.

15.18 The combination of forward and reverse rate constants is related to the equilibrium constant

When a reversible reaction reaches equilibrium, the concentrations of the reactants and products no longer change with time. For a reaction in which A and B interconvert, the condition for equilibrium is:

$$\frac{d[A]}{dt} = 0 \quad \text{and} \quad \frac{d[B]}{dt} = 0 \quad (15.60)$$

Substituting these expressions for the derivatives of the concentrations of A and B into the rate equation (Equation 15.29) give the following relationship between the rate constants and the equilibrium concentrations of A and B:

$$k_f [A]_{\text{eq}} = k_r [B]_{\text{eq}} \quad (15.61)$$

Equation 15.61 can be rearranged to give:

$$\frac{k_f}{k_r} = \frac{[B]_{\text{eq}}}{[A]_{\text{eq}}} = K_{\text{eq}} \quad (15.62)$$

where K_{eq} is the thermodynamic equilibrium constant. This shows that, although the equilibrium constant for a reaction does not define the values of k_f or k_r , it does constrain their ratio. The actual values of k_f and k_r can be very large or very small, but the ratio of the two rate constants is always equal to the equilibrium constant.

The relationship between rate constants and equilibrium constant (Equation 15.62) holds for any order of reaction. For example, consider a second-order binding reaction between a protein, P, and a ligand, L. Recall that the dissociation constant (K_D) is related to the protein and ligand concentrations as follows:

$$K_D = \frac{[P][L]}{[P \cdot L]} \quad (15.63)$$

At equilibrium,

$$\frac{d[P]}{dt} = 0 \quad (15.64)$$

Combining Equation 15.63 with Equation 15.64, we get:

$$\frac{d[P]}{dt} = -k_{\text{on}} [P][L] + k_{\text{off}} [P \cdot L] = 0 \quad (15.65)$$

Thus,

$$-k_{\text{on}} [P][L] = -k_{\text{off}} [P \cdot L] \quad (15.66)$$

and so:

$$\frac{k_{\text{off}}}{k_{\text{on}}} = \frac{[P][L]}{[P \cdot L]} = K_D = \frac{1}{K_A} \quad (15.67)$$

where K_A is the association constant. In Equations 15.63–15.67, the concentrations $[P]$, $[L]$ and $[P \cdot L]$ are all equilibrium concentrations.

Equation 15.67 allows us to link the on- and off-rates for imatinib binding, discussed in Section 15.12, to the equilibrium constants for imatinib binding to the Abl and Src kinases. For Abl:

$$K_D = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{0.00219 \text{ sec}^{-1}}{0.146 \times 10^6 \text{ M}^{-1} \cdot \text{sec}^{-1}} = 0.015 \times 10^{-6} \text{ M} \quad (15.68)$$

For Src:

$$K_D = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{0.126 \text{ sec}^{-1}}{0.003 \times 10^6 \text{ M}^{-1} \cdot \text{sec}^{-1}} = 42 \times 10^{-6} \text{ M} \quad (15.69)$$

Knowing the values of the dissociation constants from the kinetic measurements we can apply the ideas of specificity of binding that were developed in Chapter 13. The maximum specificity of imatinib, which will be at very low ligand concentrations, is given by the ratio of the dissociation constants:

$$\frac{(K_D)_{\text{Src}}}{(K_D)_{\text{Abl}}} = \frac{42 \times 10^{-6}}{0.015 \times 10^{-6}} = 2800 \quad (15.70)$$

While this specificity is very high, the relevant concentration range for therapeutic applications requires that most Abl molecules have imatinib bound while few Src molecules do. This requirement corresponds to:

$$(K_D)_{\text{Abl}} < [\text{imatinib}] < (K_D)_{\text{Src}}$$

With the actual K_D values we can see that the typical drug concentration range in the body of 100 nM to 1 μM corresponds well to the range in which high specificity is maintained.

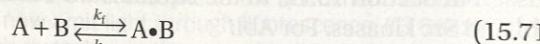
15.19 Relaxation methods provide a way to obtain rate constants for reversible reactions

The obvious way to determine the kinetic parameters governing a reaction is to mix the reactants together and monitor how quickly the products are formed. This is the approach underlying the analysis of drug binding to the Abl kinase, discussed in Section 15.12. There are many situations where it is inconvenient, or perhaps even impossible, to start with pure reactants and watch how fast the products are generated. Consider, for example, the dimerization of a protein. The monomeric and dimeric forms of the protein are always interconverting, and so it is not straightforward to separate monomers from dimers and then recombine them. How, then, might we learn about the rates of interconversion between monomers and dimers?

A different way of obtaining kinetic information is to start with a system at equilibrium, with both reactants and products present, and to perturb it so that it is no longer at equilibrium. By monitoring how the system returns to equilibrium, we can learn about the rates of interconversion between the molecules that define the system. Such approaches are known as **relaxation methods**. Relaxation methods provide a very powerful way to study kinetics, but they require that both the reactants and the products be present at measurable concentrations (that is, the reaction must have an equilibrium constant that is neither very large nor very small).

One way of perturbing the system is to change the temperature in a **temperature jump experiment** (Figure 15.25). This can be done within microseconds by using voltage pulses, or even within nanoseconds by using pulses of laser light. In this way, the kinetics of very fast processes can be measured.

Recall from Section 10.14 that the free energy, and hence the equilibrium constant, is temperature dependent. If the temperature is changed suddenly, then the reactants and products will no longer be at their equilibrium values at the new temperature. It turns out that, if the perturbation is small, the concentrations then relax to their new equilibrium values with simple exponential behavior, regardless of the order of the reaction (Box 15.3). For example, consider the following bimolecular reaction:



Here k_f and k_r are the rate constants for association and dissociation, respectively. If this reaction is perturbed slightly from equilibrium, then the concentrations will

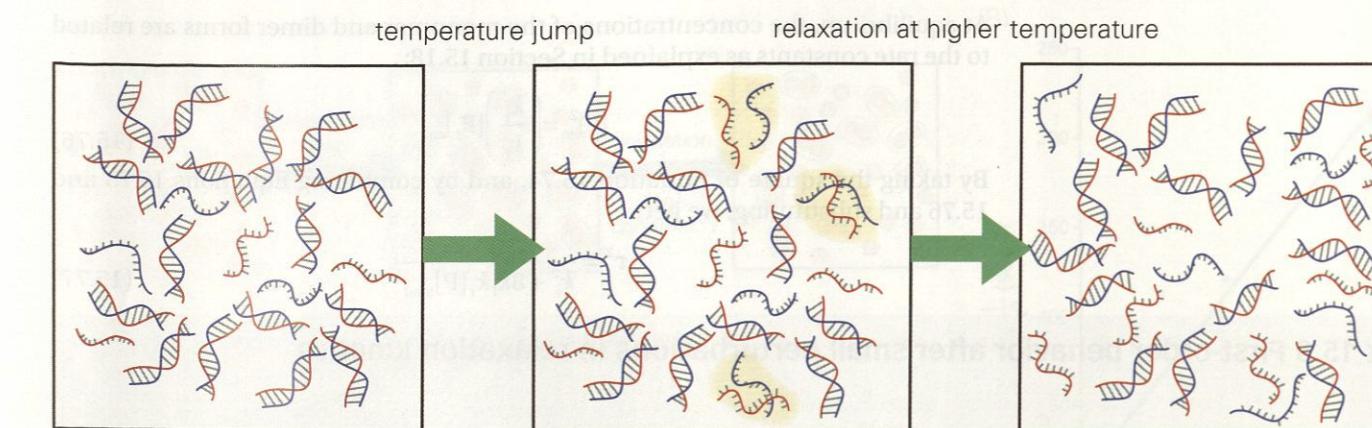


Figure 15.25 An example of a temperature jump experiment. A test tube containing two DNA molecules with complementary sequences (red and blue) is shown schematically. At equilibrium, most of the DNA molecules are in a double-helical form. The temperature is increased suddenly and some of the double helices begin to separate into individual strands of DNA (middle panel). The system relaxes to a new equilibrium at the higher temperature.

relax back to equilibrium with exponential kinetics, with a time constant, τ , given by:

$$\tau = \frac{1}{k_{\text{apparent}}} = \frac{1}{k_r + k_f([A]_{\text{eq}} + [B]_{\text{eq}})} \quad (15.72)$$

The time constant, τ , is known as the **relaxation time**. The rate constant, k_{apparent} , is the apparent first-order rate constant (“apparent” because the reaction is not really first order). The subscript “eq” indicates that the concentrations in Equation 15.72 are the equilibrium concentrations after relaxation. An explanation of how Equation 15.72 comes about is given in Box 15.3.

15.20 Temperature jump experiments can be used to determine the association and dissociation rate constants for dimerization

Temperature jump experiments are particularly useful for studying the rates of dimerization of a protein (Figure 15.26). Consider a protein, P, that undergoes a monomer-dimer equilibrium:



If the monomer and dimer forms are at equilibrium and the system is subject to a temperature jump, then the system will relax to a new equilibrium with the following time constant, τ :

$$\tau = \frac{1}{k_{\text{apparent}}} = \frac{1}{4k_f[P]_{\text{eq}} + k_r} \quad (15.74)$$

Equation 15.74 can be derived by following the same set of steps, explained in Box 15.3, that led to Equation 15.72 for the more general bimolecular reaction.

The term $[P]_{\text{eq}}$ in Equation 15.74 is the equilibrium concentration of the monomeric form of the protein. It is more convenient to express the relaxation time in terms of the total concentration of protein subunits, $[P]_{\text{total}}$, because that value is set by the experimenter when making up the protein solution. The total subunit concentration, $[P]_{\text{total}}$, is related to $[P]_{\text{eq}}$ in the following way:

$$[P]_{\text{total}} = [P] + 2[P_2] \quad (15.75)$$

Relaxation method

A relaxation method follows the rate of return to equilibrium after a sudden perturbation that disturbs the equilibrium.

At equilibrium, the concentrations of the monomer and dimer forms are related to the rate constants as explained in Section 15.18:

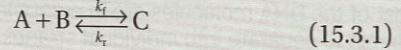
$$[P]_{\text{eq}}^2 = \left(\frac{k_f}{k_r} \right) [P_2]_{\text{eq}} \quad (15.76)$$

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

$$\tau^2 = \frac{1}{k_r^2 + 8k_f k_r [P]_{\text{total}}} \quad (15.77)$$

Box 15.3 First-order behavior after small perturbations in relaxation kinetics

Consider a second-order reaction in which two reactants form a single product, as discussed in Section 15.19 of the main text:



A, B and C are initially at equilibrium. The system is then subjected to a temperature jump and allowed to relax to a new equilibrium. We define the equilibrium concentrations *after* the perturbation to be $[A]_{\text{eq}}$, $[B]_{\text{eq}}$, and $[C]_{\text{eq}}$. The concentrations at any time during the change following the perturbation can be written as:

$$[A] = [A]_{\text{eq}} + \Delta[A] \quad (15.3.2)$$

$$[B] = [B]_{\text{eq}} + \Delta[B] \quad (15.3.3)$$

$$[C] = [C]_{\text{eq}} + \Delta[C] \quad (15.3.4)$$

The terms $\Delta[A]$, $\Delta[B]$, and $\Delta[C]$ are the small deviations from equilibrium in the concentrations of A, B, and C, respectively, that are brought about by the temperature jump. Because C can only be produced from A and B, it follows that:

$$\Delta[A] = \Delta[B] = -\Delta[C] \quad (15.3.5)$$

The equilibrium concentrations of the reactants and products are fixed. Hence, the expression for the rate of change in the concentration of the product, C, depends only on the perturbation in the concentration:

$$\frac{d[C]}{dt} = \frac{d([C]_{\text{eq}} + \Delta[C])}{dt} = \frac{d\Delta[C]}{dt} \quad (15.3.6)$$

Combining Equation 15.3.6 with the rate equation for the second-order reaction depicted in Equation 15.3.1, we get:

$$\begin{aligned} \frac{d[C]}{dt} &= \frac{d\Delta[C]}{dt} = k_f[A][B] - k_r[C] = k_f([A]_{\text{eq}} + \\ &\Delta[A])([B]_{\text{eq}} + \Delta[B]) - k_r([C]_{\text{eq}} + \Delta[C]) \end{aligned} \quad (15.3.7)$$

Thus,

$$\begin{aligned} \frac{d\Delta[C]}{dt} &= k_f[A]_{\text{eq}}[B]_{\text{eq}} + k_f[A]_{\text{eq}}\Delta[B] + k_f[B]_{\text{eq}}\Delta[A] + \\ &k_f\Delta[A]\Delta[B] - k_r[C]_{\text{eq}} - k_r\Delta[C] \end{aligned} \quad (15.3.8)$$

Recall from Section 15.18 that the ratio of the forward

and reverse rates is equal to the equilibrium constant:

$$\frac{k_f}{k_r} = \frac{[A]_{\text{eq}}[B]_{\text{eq}}}{[C]_{\text{eq}}} \quad (15.3.9)$$

Rearranging Equation 15.3.9, we get:

$$k_f[A]_{\text{eq}}[B]_{\text{eq}} = k_r[C]_{\text{eq}} \quad (15.3.10)$$

Substituting Equation 15.3.10 in Equation 15.3.8, and by using Equation 15.3.5, we get:

$$\frac{d\Delta[C]}{dt} = -k_f[A]_{\text{eq}}\Delta[C] - k_f[B]_{\text{eq}}\Delta[C] - k_r\Delta[C] + k_r\Delta[C]^2 \quad (15.3.11)$$

Now we use the fact that, if the perturbation is small, then the term $\Delta[C]^2$ is small. We can therefore neglect $\Delta[C]^2$, which is very small and can be ignored relative to the other terms. In this case, Equation 15.3.11 simplifies to:

$$\frac{d\Delta[C]}{dt} = -[k_f([A]_{\text{eq}} + [B]_{\text{eq}}) + k_r]\Delta[C] = -k_{\text{eff}}\Delta[C] \quad (15.3.12)$$

This is a simple first-order kinetic equation that gives rise to exponential time dependence, where the effective (or apparent) rate constant, k_{eff} , is:

$$k_{\text{eff}} = k_f([A]_{\text{eq}} + [B]_{\text{eq}}) + k_r \quad (15.3.13)$$

The associated time constant, or relaxation time, is:

$$\tau_{\text{eff}} = \frac{1}{k_{\text{eff}}} = \frac{1}{k_f([A]_{\text{eq}} + [B]_{\text{eq}}) + k_r} \quad (15.3.14)$$

The central point in this derivation, leading to a simple exponential dependence on time, is that only terms that are first order in $\Delta[X]$ (where X is any of the chemical species) are kept. All higher-order terms (regardless of what they are in the actual kinetic mechanism) are dropped. This yields a first-order equation and thus gives an exponential approach to equilibrium. The relationship between the observed effective rate constant and the rate constants for specific steps in the reaction mechanism depends on the details of the mechanism. This relationship can be worked out through a process analogous to that presented above for the reaction of A and B forming C.

(A) At equilibrium, the concentrations of the monomer and dimer forms are related to the rate constants as explained in Section 15.18:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

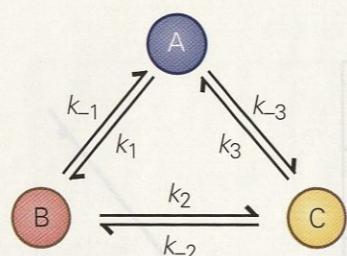
By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the square of Equation 15.74, and by combining Equations 15.75 and 15.76 and substituting, we get:

By taking the



15.21 The rate constants for a cyclic set of reactions are coupled

In thinking about chemical reactions, an interesting case to consider is a cyclic set of reactions. An example of such a cyclic set would be three sequential reactions that lead back to the initial reactant. Schematically we can draw this as shown in **Figure 15.27**, in which all of the individual rate constants are identified. When such a system reaches equilibrium, there will be no further change in concentrations with time.

If the reaction was just the interconversion of A and B, then equilibrium has to occur through a balance of the forward and reverse reactions. In the cyclic case, it seems possible that the amount of A converted to B can be balanced by B converting to C and then C to A without requiring B to undergo the back reaction to A at all. But, in fact, each of these individual steps must be in equilibrium, and so the rate constants have to satisfy the following relationships:

$$\left. \begin{array}{l} k_1[A] = k_{-1}[B] \\ k_2[B] = k_{-2}[C] \\ k_3[C] = k_{-3}[A] \end{array} \right\} \quad (15.79)$$

This rule is known as the **principle of microscopic reversibility**. This idea, which is also known as the principle of **detailed balance**, is a consequence of a principle in physics, which is that the equations of motion hold true when time is reversed. For any trajectory of atoms that obeys the laws of motion, a trajectory that follows the same path, but has the direction reversed, also obeys the laws of motion. An extension of this idea is that, for any molecular reaction at equilibrium, the forward rate must equal the reverse rate—that is, the forward and backward flux must be equal at every step. If this were not true, then it would be possible to create a kind of perpetual motion in which A converts to B, which converts to C, which then converts to A, always in a forward direction.

The principle of microscopic reversibility applies whether or not the reactions are unimolecular. An example is provided by the phosphorylation of glucose, which is depicted in **Figure 15.28**. Here, A is glucose + P_i (phosphate ion); B is glucose-6-P (glucose phosphorylated at position 6); and C is glucose-1-P (glucose phosphorylated at the 1 position). Glucose-1-P and glucose-6-P are interconverted by the enzyme glucophosphomutase. Microscopic reversibility says that the amount of glucose + P_i reacting to form glucose-1-P must equal the amount of glucose-1-P hydrolyzed in the same time, regardless of the rates of the other reactions.

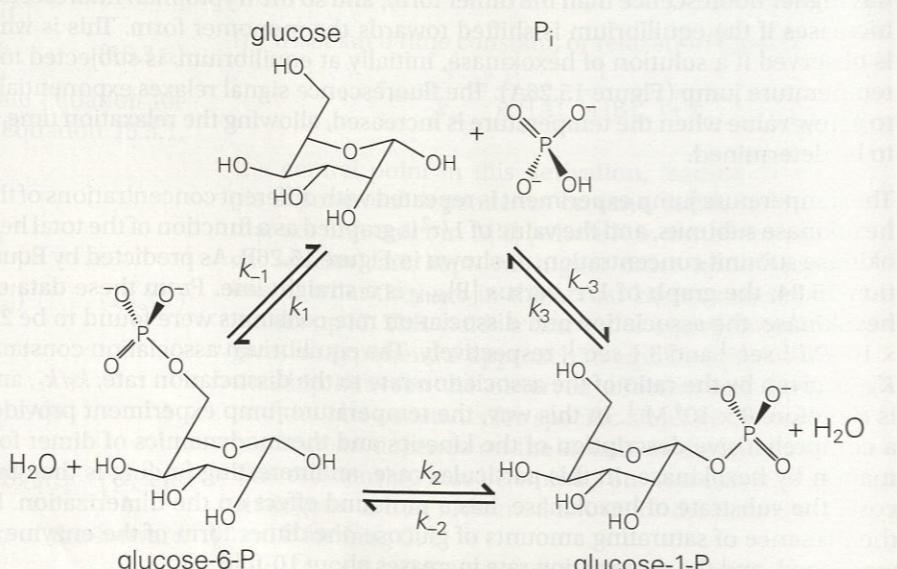


Figure 15.28 The phosphorylation of glucose is an example of a cyclic set of reactions. Glucose can be phosphorylated on either the 1 or 6 position, and these two isomers are interconverted by the enzyme glucophosphomutase.

For this set of cyclic reactions, the equilibrium constants are related to the rate constants as follows:

$$K_1 = \frac{[\text{glucose-6-P}]}{[\text{glucose}][\text{P}_i]} = \frac{k_{-1}}{k_1} \quad (15.80)$$

$$K_2 = \frac{[\text{glucose-1-P}]}{[\text{glucose-6-P}]} = \frac{k_{-2}}{k_2} \quad (15.81)$$

$$K_3 = \frac{[\text{glucose}][\text{P}_i]}{[\text{glucose-1-P}]} = \frac{k_{-3}}{k_3} \quad (15.82)$$

The concentrations in Equations 15.80–15.82 are all equilibrium concentrations. If we multiply the three equilibrium constants together, we get the following result:

$$K_1 K_2 K_3 = \left(\frac{[\text{glucose-6-P}]}{[\text{glucose}][\text{P}_i]} \right) \left(\frac{[\text{glucose-1-P}]}{[\text{glucose-6-P}]} \right) \left(\frac{[\text{glucose}][\text{P}_i]}{[\text{glucose-1-P}]} \right) \\ = 1 = \frac{k_{-1} k_{-2} k_{-3}}{k_1 k_2 k_3} \quad (15.83)$$

This means that:

$$k_1 k_2 k_3 = k_{-1} k_{-2} k_{-3} \quad (15.84)$$

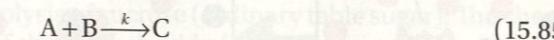
Thus, the cyclic nature of the set of coupled reactions places an additional constraint on the values of the rate constants, which is that the product of the forward rate constants must equal the product of the reverse rate constants. This constraint is in addition to the requirements for them to agree with the individual equilibrium constants.

C. FACTORS THAT AFFECT THE RATE CONSTANT

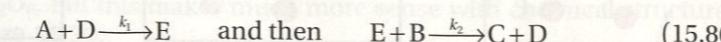
The rate constants specified in elementary reactions are “constant” in the sense that their values do not change with changes in concentrations of any reactants or products under a defined set of reaction conditions. They do change, however, when conditions change. For example, they are almost always strongly temperature dependent and often also depend on the solvent used. It is important to understand why the rate constants vary with such parameters and to learn how structural information about changes in the molecules during the reaction can be deduced from the behavior of the rate constants. In this part of the chapter, we discuss some of the factors that affect the observed rates of reactions.

15.22 Catalysts accelerate the rates of chemical reactions without being consumed in the process

A thermodynamic analysis of a chemical reaction depends only on the nature of the reactants and products and their concentrations. If there is another substance present that participates in terms of the reaction mechanism, but is neither consumed nor produced in the *overall* reaction, it does not enter into the thermodynamic analysis. For example, given the following overall reaction:



a substance D could participate in the actual mechanism without appearing in the overall reaction:



D is unchanged in the *overall net* reaction and E is created in the first step, but used in the second step. For these two reactions, the net equilibrium constant is:

$$K_{\text{eq}} = K_1 K_2 = \frac{[C]}{[A][B]} \quad (15.87)$$

with

$$K_1 = \frac{[E]}{[A][D]}$$

and

$$K_2 = \frac{[C][D]}{[E][B]}$$

Although D and E do not enter into the thermodynamic equilibrium constant, K_{eq} , the kinetics of the overall reaction can be affected enormously by the presence of D. For example, it is quite often the case that the process by which A collides with B to immediately form C can occur, but is very slow (that is, the value of the rate constant, k , is small). But, the overall rate of the reaction can be much faster if A collides rapidly with D to form E, which then collides rapidly with B to form C and release D (that is, the rate constants, k_1 and k_2 , are much larger than k).

Substances such as D, which accelerate reactions but do not appear in the overall balanced reaction, are called **catalysts** (Figure 15.29). Proteins (and some key RNAs) act as catalysts that accelerate the rates of many biochemical transformations so that these reactions can occur fast enough to sustain life. Protein and RNA catalysts are called **enzymes**, and are discussed in detail in Chapter 16.

Catalyst

A catalyst is a substance that accelerates the rate of a reaction, but does not appear in the overall balanced reaction. Such substances participate in the reaction mechanism, but are regenerated in their original form in the course of the reaction.

15.23 Rate laws for reactions must be determined experimentally

Given the role of catalysts in affecting reaction rates, and the possibility of complex multistep mechanisms, it should now be apparent that it is often impossible to simply look at an overall reaction and write down the kinetic rate law that applies. If we know something about the chemistry of the reactions, a rational mechanism and rate law can be developed, but these must be tested experimentally to verify that the experimental concentration dependence is indeed in agreement with the proposed mechanism.

Even for complicated mechanisms, rate laws can usually be written as the products of concentrations of participating molecules:

$$\text{rate} = k[A]^a[B]^b[C]^c \quad (15.88)$$

For a complex reaction mechanism, some of the chemical species that appear in the rate law may not appear in the overall chemical reaction. This is the case for catalysts, for example. The exponents a , b , and c need not have integer values. If one just picks initial concentrations, $[A]_0$, $[B]_0$, $[C]_0$, etc., with no idea of the overall

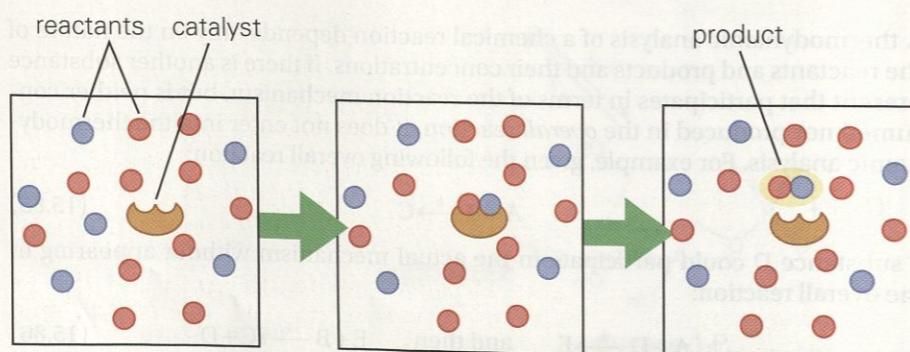


Figure 15.29 A catalyst participates in a chemical reaction but is left unaltered by the reaction. Shown here are two kinds of molecules (red and blue) that can react to form a covalent bond. A catalyst (brown) binds to both kinds of molecules and promotes their reaction by bringing them together. Once the reaction is completed the catalyst releases the product and can participate in more reactions.

mechanism, then it is not generally possible to fit measured concentrations as a function of time to an appropriate time-dependent concentration equation.

Fortunately, there are simple approaches that allow the exponents (and hence the rate law) to be determined experimentally. The most generally used approach is to measure the initial reaction rates (that is, the reaction rates are measured at times short enough that concentrations have not changed by a large fraction of their total value). Such measurements are made for different initial concentrations of one reactant. To determine the value of the exponent of [A], for example, measurements could be done using starting concentrations $[A]_0$ and then $2[A]_0$, and so on.

In terms of unknown exponents a , b , c , the rate is then written as:

$$\text{rate}_1 = k[A]_0^a[B]_0^b[C]_0^c \quad (15.89)$$

where $[A]_0$, $[B]_0$, and $[C]_0$ are the initial concentrations of A, B, and C. Equation 15.89 is valid if the measurement is done over a period of time that is short enough that the concentrations of the reactants remain close to their initial values (for example, $[A] \approx [A]_0$).

If we now double the initial concentration of A, from $[A]_0$ to $2[A]_0$, then the rate is given by:

$$\text{rate}_2 = k(2[A]_0)^a[B]_0^b[C]_0^c \quad (15.90)$$

The ratio of the two rates is:

$$\frac{\text{rate}_1}{\text{rate}_2} = \frac{k[A]_0^a[B]_0^b[C]_0^c}{k(2[A]_0)^a[B]_0^b[C]_0^c} = \left(\frac{1}{2}\right)^a \quad (15.91)$$

Taking the logarithm of both sides of Equation 15.91, we can see that the value of the exponent, a , is given by:

$$a = \frac{1}{\log 2} \log \left(\frac{\text{rate}_2}{\text{rate}_1} \right) \quad (15.92)$$

To determine the other exponents, b and c , the same procedure would need to be repeated, varying the concentrations of the other reactants, one at a time.

Another approach to determine the value of the exponents is simply to begin with a large excess of all reagents except for one. For example, we keep $[A]_0$ small, but make $[B]_0$ and $[C]_0$ large. In this case, the concentrations of B and C essentially remain constant at $[B]_0$ and $[C]_0$ during the reaction, because only a small amount of B and C can react with the small amount of A that is present. This is the approach used to simplify the analysis of ligand binding to a protein in Section 15.12. In this case, the rate is given by:

$$\text{rate} = k'[A]^a \quad (15.93)$$

where

$$k' = k[B]_0^b[C]_0^c \quad (15.94)$$

Since there is only one reactant that is time dependent, the resulting rate (determined by measuring $[A]$ as a function of time) can be fitted to give the value of the exponent, a .

15.24 The hydrolysis of sucrose provides an example of how a reaction mechanism is analyzed

An example of a reaction that demonstrates some aspects of the kinetic models that have been discussed is the hydrolysis of sucrose (ordinary table sugar). The chemical reaction is the hydrolysis of the disaccharide [sucrose = α -D-glucopyranosyl (1 → 2) β -D-fructofuranoside; see Figure 3.1] to give two monosaccharides, glucose and fructose. Written as a chemical equation, this is $C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$, but this makes much more sense with chemical structures, as shown in Figure 15.30.

FIGURE 15.21 Structures of sucrose and its hydrolytic products glucose and fructose. The disaccharide sucrose is formed by the linkage of a glucose molecule (a six-membered ring) and a fructose molecule (a five-membered ring) via their anomeric carbons. Hydrolysis cleaves this linkage to regenerate the two monosaccharides.

Pre-equilibrium approximation

A pre-equilibrium approximation is often used to analyze reaction mechanisms that involve multiple steps. In the first step, the forward reaction is much faster than the reverse reaction, so the reactants and products for the first step are treated as if they were fully at equilibrium.

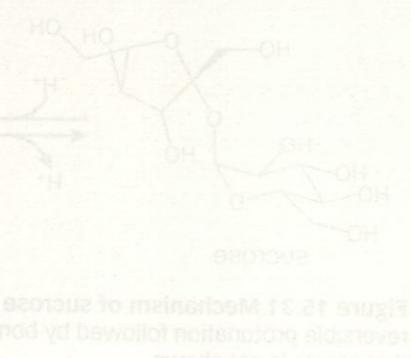
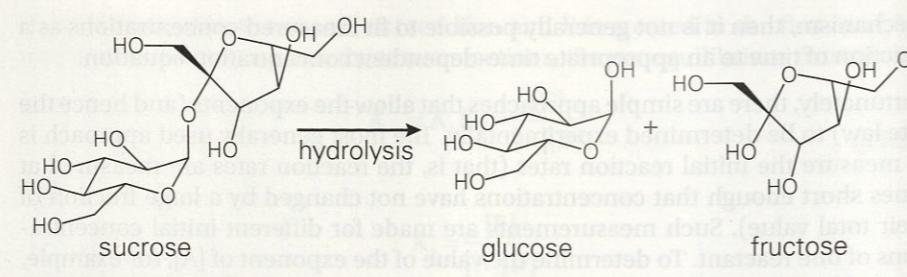


Figure 15.30 Structures of sucrose and its hydrolysis products, glucose and fructose. The process of sucrose hydrolysis is known as inversion because it was first followed by measuring how much the plane of polarization of light was rotated by passing through the solution. As the reaction proceeds in this case, the sense of rotation inverts for products relative to reactants.



The kinetics of sucrose hydrolysis was studied many years ago by following the reaction through observation of the rotation of polarized light by solutions containing sucrose. A solution of sucrose is dextrorotatory (that is, it rotates light in a counterclockwise fashion, as explained in Section 3.4). Glucose is dextrorotatory and fructose is levorotatory, but because fructose rotates the polarization of light more strongly than glucose, the net polarization of light by the products is levorotatory. Thus, as the reaction proceeds, the polarization of light by the solution switches from counterclockwise to clockwise (that is, the polarization inverts). This phenomenon led to the hydrolysis of sucrose being referred to as an **inversion reaction**.

The mechanism of sucrose hydrolysis can be deduced from three important observations concerning the kinetics of the reaction. The first observation is that although the hydrolysis reaction is very slow in pure water, the rate increases dramatically as the pH is lowered (that is, as the proton concentration increases). The nature of the acid used to lower the pH of the solution does not matter.

The second observation is that during the course of the reaction the pH of the solution does not change. Thus, although protons are clearly participating in the reaction (because the rate depends on the pH), the protons must be acting as catalysts.

The third observation is that the reaction is first order in proton concentration and also first order in sucrose. Thus, the rate law seems to be:

$$\frac{d[\text{sucrose}]}{dt} = k[\text{sucrose}][\text{H}^+] \quad (15.95)$$

We now combine these observations with some chemical knowledge to deduce the chemical mechanism. First, in reactions that break bonds, the stability of the leaving group is very important. From a study of other organic reactions, it is known that protonation of an oxygen can create a better leaving group (HOCH is a better leaving group than the unstable -OCH group). In this case, it is the bridging oxygen in sucrose that must be protonated (Figure 15.31). But this is unfavorable, because ether oxygens ($\text{C}-\text{O}-\text{C}$) have a very low pK_a value. Nevertheless,

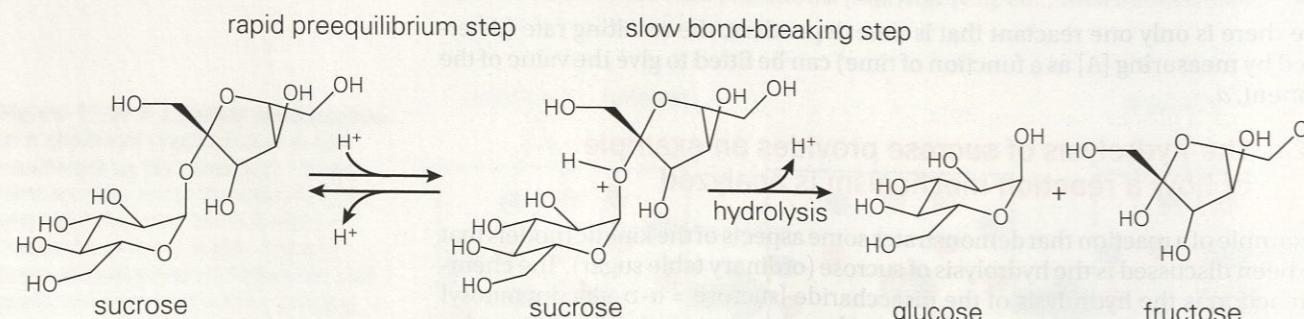
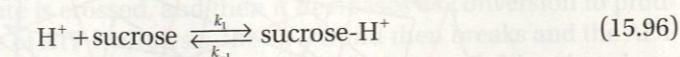


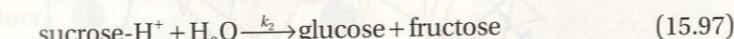
Figure 15.31 Mechanism of sucrose hydrolysis. A mechanism that is consistent with the observed rate law is shown. It involves reversible protonation followed by bond cleavage in the protonated form of sucrose to give glucose + fructose. The water molecule that reacts is not shown.

in solution we know that there will be a small amount of the protonated sugar present. This reaction is:



and the rates for protonation/deprotonation reactions are generally rapid.

If we assume that the protonated sucrose reacts with water, then we can write:



The back reaction is not included because the forward reaction is thermodynamically favorable and the reaction goes essentially to completion (that is, all of the sucrose is converted to glucose and fructose). We can now develop a kinetic model from these two reactions.

First, note that product formation is an elementary reaction of sucrose- H^+ with H_2O , so the rate law is:

$$\frac{d[\text{glucose}]}{dt} = \frac{d[\text{fructose}]}{dt} = k_2[\text{sucrose-H}^+][\text{H}_2\text{O}] \quad (15.98)$$

To obtain an expression for [sucrose- H^+], we consider the reaction depicted in Equation 15.96. Protonation/deprotonation is much faster than the bond-breaking step that occurs in the reaction depicted in Equation 15.97. This means that the rate constants for the protonation/deprotonation step, k_1 and k_{-1} , are much greater than the rate constant for the bond-breaking step, k_2 . This fact allows us to make a “**preequilibrium approximation**”—that is, we assume that the following is true:

$$[\text{sucrose-H}^+] \approx \frac{k_1}{k_{-1}}[\text{sucrose}][\text{H}^+] \quad (15.99)$$

Equation 15.99 is equivalent to stating that the protonated and deprotonated sucrose essentially remains at equilibrium throughout the reaction—that is, we are equating the ratio of the two rate constants to the equilibrium constant for the reaction (compare Equation 15.67). Then, by inserting Equation 15.99 into Equation 15.98, we find that the rate of product production is given by:

$$\begin{aligned} \frac{d[\text{glucose}]}{dt} &= k_2 \frac{k_1}{k_{-1}} [\text{sucrose}][\text{H}^+][\text{H}_2\text{O}] \\ &= k'_2 \frac{k_1}{k_{-1}} [\text{sucrose}][\text{H}^+] \end{aligned} \quad (15.100)$$

In the last step we have taken $k'_2 = k_2[\text{H}_2\text{O}]$ because the reaction is in water (where $[\text{H}_2\text{O}] = 55 \text{ mol L}^{-1}$), and the concentration of water does not change significantly during the reaction.

This kinetic model, which is shown in Figure 15.31 and consists of a reversible protonation step followed by an essentially irreversible hydrolysis step, is consistent with experimental observation. The rate depends linearly on sucrose concentration and also on hydrogen ion concentration. It is important to note, however, that this agreement does not prove that this kinetic model is correct. It is often the case that there are other kinetic models that can give the same concentration dependence and, hence, would also be consistent with the kinetic observations. To distinguish among such possibilities we would need to carry out more complicated experiments, such as those that probe whether other intermediates are present.

15.25 The fastest possible bimolecular reaction rate is determined by the diffusion-limited rate of collision

In Section 15.2, we noted that the rate constant, k , for a bimolecular reaction must reflect the frequency of collisions per unit concentration. The rate constant also depends on the fraction of collisions that lead to formation of product. Both of these factors depend on reaction conditions, including temperature.

emit noisutib ent TCS.25 eno
Hilme gti tsi oto
molecules in solution
benzene even though zan
re propanone maste the ethyl
methyl ester maste the
benzyl bromide could be ent
yonepen enT (S boham shilao
yd benimite el analloko rbia to
erb bns noitulos ent to ylazoev ent
shutneomet

Preequilibrium approximation

A preequilibrium approximation refers to a case in which there are forward and backward reactions that are both much faster than subsequent reaction steps. In this case, the reactants and products for this fast step always remain at relative concentrations determined as if they were fully at equilibrium.