

4 Calculation of the kinetic rate constants

Protein folding reactions can proceed according to a variety of different mechanisms. This chapter presents analytical solutions for kinetic rate constants and amplitudes for common reaction mechanisms.

The simplest case is that of a two-state transition, i.e., a reaction that proceeds without the occurrence of intermediates directly from the unfolded state, U, to the folded state, F (Sect. 4.2). In the transition region of the reaction $U \rightleftharpoons F$, both forward and backward reaction contribute significantly to the observed rate constant (relaxation constant, decay constant). Under conditions that strongly favor folding (or unfolding), i.e., far outside the midpoint of equilibrium between folded and unfolded state, the transition can be treated as an irreversible reaction with the observed rate constant being dominated by the folding (or unfolding) rate constant.

For reversible three-state transitions, three cases have to be distinguished: 1. The intermediate, I, is on-pathway ($U \rightleftharpoons I \rightleftharpoons F$), i.e., is always passed through in the reaction from U to F (Sect. 4.3.1.1). 2. All species may interconvert, i.e., the transition from U to F may be passed through directly and also through the intermediate, I (Sect. 4.3.1.2). 3. I is off-pathway ($I \rightleftharpoons U \rightleftharpoons F$ or $U \rightleftharpoons F \rightleftharpoons I$), i.e., the reaction from U to F cannot proceed through I (Sect. 4.3.1.3).

Derivations of solutions for four-state transitions involve the treatment of cubic equations (Sect. 4.4).

Occasionally, folding reactions are linked with monomer–multimer transitions (Sect. 4.5). Examples are, (a) the protein is monomeric in the unfolded state but dimeric in the folded state, or (b) the protein aggregates in the unfolded, folded, or an intermediate state. Since these transitions affect the observed rate constants for folding events, solutions for a few simple cases are also presented.

Many important kinetic experiments (see Chaps. 5 and 10) involve the application of perturbation methods, such as small-amplitude temperature-jumping, repetitive pressure perturbation, ultrasonic velocimetry, and dielectric relaxation. These methods utilize a small perturbation of the chemical or physical equilibrium: A small change of physical or chemical conditions initiates a relaxation process to a new equilibrium. Since the amplitude of the perturbation is small, the mathematical treatment is tremendously simplified (Sect. 4.6).

The mathematical methods and analytical solutions presented for kinetic rate constants and amplitudes are not limited to protein folding reactions, but may be applied to a large variety of other chemical or physical reactions, for example, (a) in case of unimolecular mechanisms to conformational changes of other macro-

molecules (peptides, carbohydrates, lipids, DNA), and (b) in case of bimolecular mechanisms to aggregation-, enzyme–substrate binding-, and enzyme–inhibitor binding reactions.

Kinetic rate constants and amplitudes of unimolecular and bimolecular reactions are solutions of differential equations. Since no general mathematical formalism for the analytical solution of all differential equations has been found, the finding of a particular solution is often based on a mere guess that is confirmed by inserting it into the equation. For the confirmation of a solution as the general solution it is important to check whether it fulfills every possible initial condition.

Fortunately, the rate equations of unimolecular reactions are ordinary linear differential equations which generally have solutions that are linear combinations of exponential functions.

4.1 Transition state theory

The rate constant of the formation of a product, $k_{i \rightarrow f}$, in a step of the folding reaction (Fig. 4.1; Fersht, 1985; Matouschek et al., 1989) is, in good approximation,

$$k_{i \rightarrow f} = (k_B T / h) \exp(-\Delta G_{\#-i} / (RT)), \quad (4.1)$$

where $k_B = 1.3807 \times 10^{-23} \text{ J K}^{-1}$ is the Boltzmann constant, $h = 6.6261 \times 10^{-34} \text{ J s}$ is the Planck constant, T is the absolute temperature, $R = 8.3145 \text{ J mol}^{-1} \text{ K}^{-1}$ is the molar gas constant, and $\Delta G_{\#-i}$ is the Gibbs free energy of activation.

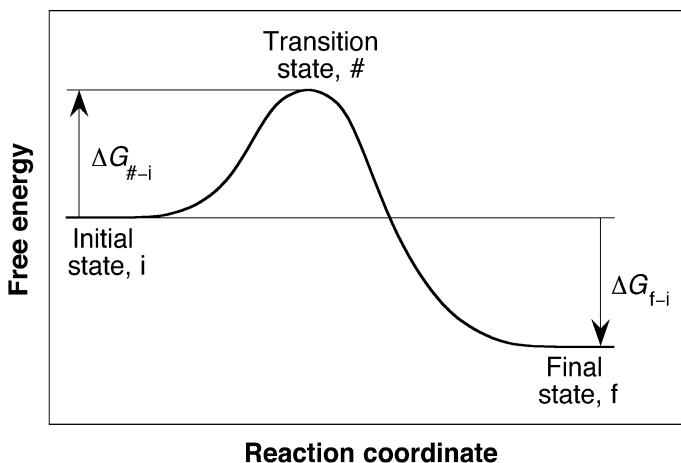


Fig. 4.1. Transition state theory. The transition state is the state of highest energy along the reaction pathway that leads from the initial state (ground state) to the final state (product). The height of the transition state barrier determines the magnitude of the rate constant of transition ($\Delta G_{\#-i}$ and $\Delta G_{\#-f} = \Delta G_{\#-i} - \Delta G_{f-i}$ determine the rate constants of $i \rightarrow f$ and $f \rightarrow i$, respectively).

The Gibbs free energy change of the reaction, ΔG_{f-i} , is connected with the equilibrium constant of the reaction, K_{f-i} , i.e., the ratio of product to reactant in equilibrium, by the well-known relation

$$\Delta G_{f-i} = \Delta H_{f-i} - T\Delta S_{f-i} = -RT \ln(K_{f-i}), \quad (4.2)$$

where ΔH_{f-i} is the enthalpy change and ΔS_{f-i} is the entropy change of the reaction.

4.2 Two-state transitions

4.2.1 Reversible two-state transition

To derive the rate equations for a reversible two-state transition between the states U and F



we have to consider that the quantity of the decay of reactant, U, per time unit is proportional to the quantity of reactant itself and the quantity of the decay of product, F, per time unit is proportional to the quantity of product:

$$\begin{aligned} \frac{d[F]}{dt} &= k_1[U] - k_{-1}[F] \\ \frac{d[U]}{dt} &= k_{-1}[F] - k_1[U], \end{aligned} \quad (4.4)$$

where $[U]$, $[F]$, k_1 , k_{-1} , and t are the concentrations of U and F, the forward rate constant, the backward rate constant, and the time, respectively. Taking into account that the total concentration of species, $[UF] \equiv [U] + [F]$, is conserved, the rate equation for the change of the folded state may be written as

$$\begin{aligned} \frac{d[F]}{dt} &= -(k_1 + k_{-1})[F] + k_1[UF] \\ [F](0) &= [F_0], \end{aligned} \quad (4.5)$$

where $[F_0]$ is the concentration of F at the start of the reaction, i.e., at $t = 0$. The solution of Eq. 4.5 is easily found by using the guess that the solution is a single-exponential function:

$$\begin{aligned} [F](t) &= C_1 \exp(-k_1 t - k_{-1} t) + C_2 \\ C_1 &= [F_0] - [UF]k_1/(k_1 + k_{-1}) \\ C_2 &= [UF]k_1/(k_1 + k_{-1}) \\ [U](t) &= [UF] - [F](t). \end{aligned} \quad (4.6)$$

It can be shown that Eq. 4.6 fulfills every initial condition $[F_0] \in [0, [UF]]$ and represents the general solution.

Consequently, $[U](t)$ and $[F](t)$ follow single-exponential functions with an observed rate constant, $k_{\text{obs}} = k_1 + k_{-1}$ (Fig. 4.2, Table 4.1 in Sect. 4.7).

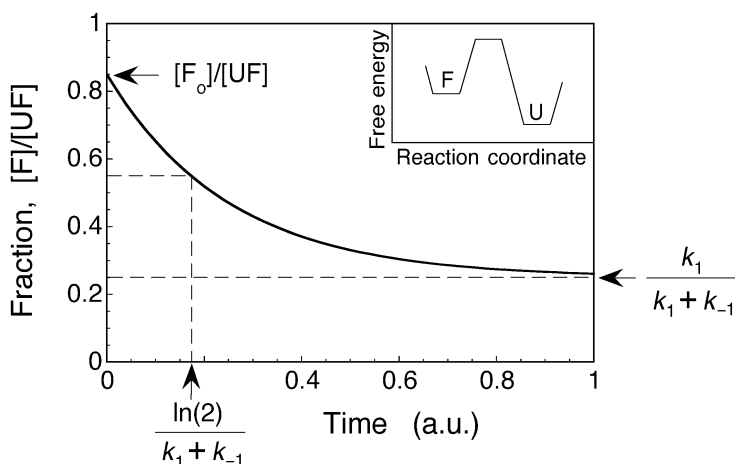


Fig. 4.2. Single-exponential change of the population of the folded state in a reversible two-state transition ($U \rightleftharpoons F$) under conditions that favor unfolding. The parameters chosen for this example are: $k_1 = 1$, $k_{-1} = 3$, $[F_0]/[UF] = 0.85$. The observed rate constant (relaxation constant) is $k_{\text{obs}} = k_1 + k_{-1} = 4$. Inset: Energy landscape.

4.2.2

Irreversible two-state transition

Under conditions which strongly favor folding, the unfolding rate constant may be neglected:



The rate equation for F in this consecutive two-state transition is

$$\frac{d[F]}{dt} = k_1[U] \quad (4.8)$$

$$[U] + [F] = [UF]$$

$$[F](0) = [F_0],$$

where $[U]$, $[F]$, k_1 , and t are the concentrations of U and F, the forward rate constant, and the time, respectively. Here the solution is (see also Table 4.1)

$$[F](t) = ([F_0] - [UF]) \exp(-k_1 t) + [UF] \quad (4.9)$$

$$[U](t) = ([UF] - [F_0]) \exp(-k_1 t).$$

$[U](t)$ and $[F](t)$ follow single-exponential functions with an observed rate constant, $k_{\text{obs}} = k_1$. In contrast to reversible reactions, here the population of U vanishes with time (Fig. 4.3).

Analogously, for conditions that strongly favor unfolding, for example, at high concentrations of denaturant, we find $k_{\text{obs}} = k_{-1}$.

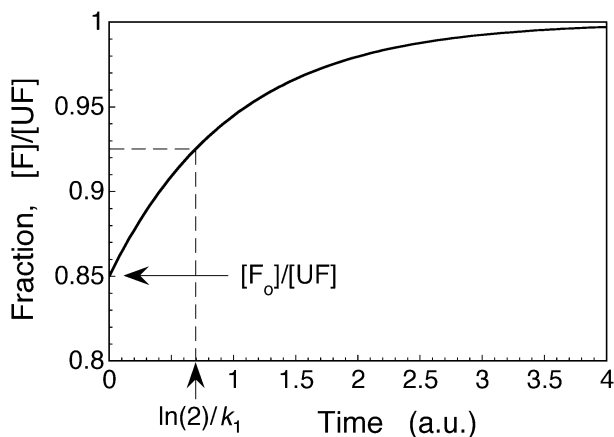


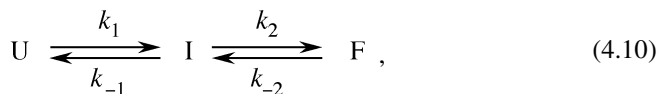
Fig. 4.3. Single exponential change of the fraction of the folded state, $[F]/[UF]$, in an irreversible two-state transition ($U \rightarrow F$). The parameters chosen for this example are: $k_1=1$; $[F_0]/[UF] = 0.85$.

4.3 Three-state transitions

4.3.1 Reversible three-state transitions

4.3.1.1 Reversible sequential three-state transition

For a reversible sequential three-state transition,



between the states U, I, and F, with the positive rate constants, k_1 , k_{-1} , k_2 , and k_{-2} , the rate equations are

$$\frac{d[U]}{dt} = k_{-1}[I] - k_1[U] \tag{4.11}$$

$$\frac{d[F]}{dt} = k_2[I] - k_{-2}[F]$$

$$[I] = [UIF] - [U] - [F],$$

where [UIF] is the total concentration of the species U, I, and F, which is conserved in the reaction. Considering for simplicity first only the changes of [U], [I], and [F],

$$\frac{d\Delta[U]}{dt} = k_{-1}\Delta[I] - k_1\Delta[U] \quad (4.12)$$

$$\frac{d\Delta[F]}{dt} = k_2\Delta[I] - k_{-2}\Delta[F]$$

$$\Delta[I] = -\Delta[U] - \Delta[F],$$

and assuming that the solution of Eq. 4.12 is of the form

$$\Delta[U](t) = C_1 \exp(-\lambda t) \quad (4.13)$$

$$\Delta[F](t) = C_2 \exp(-\lambda t),$$

we obtain,

$$-\lambda \Delta[U] = -k_{-1} (\Delta[U] + \Delta[F]) - k_1 \Delta[U] \quad (4.14)$$

$$-\lambda \Delta[F] = -k_2 (\Delta[U] + \Delta[F]) - k_{-2} \Delta[F].$$

By substituting $\Delta[U]$ or $\Delta[F]$ we find an equation for λ ,

$$0 = \lambda^2 - \lambda (k_1 + k_{-1} + k_2 + k_{-2}) + k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2}, \quad (4.15)$$

which has two solutions,

$$\lambda_{1,2} = 0.5 (k_1 + k_{-1} + k_2 + k_{-2} \pm ((k_1 + k_{-1} + k_2 + k_{-2})^2 - 4 (k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2}))^{1/2}). \quad (4.16)$$

For physically permissible, i.e., positive rate constants, the term under the root cannot be negative. Thus, both solutions are real. Both $\lambda = \lambda_1$ and $\lambda = \lambda_2$ inserted into Eq. 4.13 fulfill Eq. 4.12, and thus, represent particular solutions. The general solution of Eq. 4.11 is a superposition of the two particular solutions:

$$[U](t) = C_1 \exp(-\lambda_1 t) + C_3 \exp(-\lambda_2 t) + C_5 \quad (4.17)$$

$$[F](t) = C_2 \exp(-\lambda_1 t) + C_4 \exp(-\lambda_2 t) + C_6.$$

The constants, C_i , may be determined by inserting Eq. 4.17 into Eq. 4.11, and using the conservation relationship, $[UIF] = [U] + [I] + [F]$, and the initial conditions, $[U](0) = [U_0]$, $[F](0) = [F_0]$:

$$C_1 = ([F_0] - \xi_2[U_0] - C_6 + \xi_2 C_5) / (\xi_1 - \xi_2) \quad (4.18)$$

$$C_2 = \xi_1 C_1$$

$$C_3 = ([F_0] - \xi_1[U_0] - C_6 + \xi_1 C_5) / (\xi_2 - \xi_1)$$

$$C_4 = \xi_2 C_3$$

$$C_5 = [UIF] k_{-1} k_{-2} / (k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2})$$

$$C_6 = [UIF] k_1 k_2 / (k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2})$$

$$\xi_1 = (\lambda_1 - k_1 - k_{-1}) / k_{-1}$$

$$\xi_2 = (\lambda_2 - k_1 - k_{-1}) / k_{-1}.$$

Alternatively, we can use the relations

$$C_1 = \xi_3 C_2 \quad C_3 = \xi_4 C_4 \quad (4.19)$$

$$\xi_3 = (\lambda_1 - k_2 - k_{-2}) / k_2 \quad \xi_4 = (\lambda_2 - k_2 - k_{-2}) / k_2.$$

From $1 = \xi_1 \xi_3$ follows $\xi_1 \neq 0$ and $\xi_3 \neq 0$, and thus, $C_2 = 0$ only if $C_1 = 0$, and $C_4 = 0$ only if $C_3 = 0$.

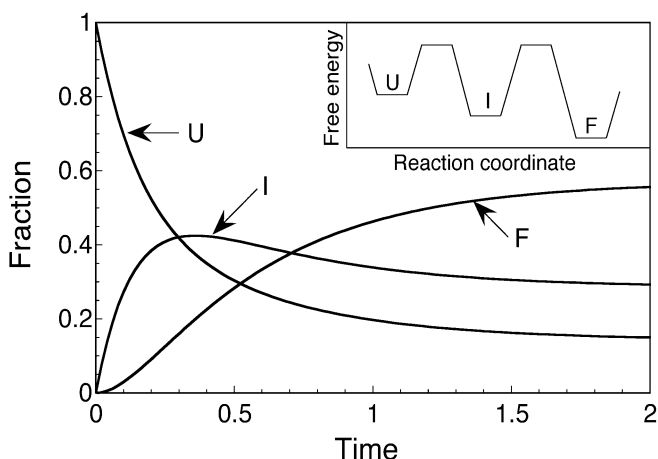


Fig. 4.4. Double-exponential change of the populations of folded, F, intermediate, I, and unfolded, U, state in a reversible sequential three-state transition ($U \rightleftharpoons I \rightleftharpoons F$) under conditions that favor folding. The parameters chosen for this example are: $k_1 = 4$, $k_{-1} = 2$, $k_2 = 2$, $k_{-2} = 1$, $[F_0]/[UIF] = 0$, $[U_0]/[UIF] = 1$. Observed rate constants ($\lambda \equiv k_{\text{obs}}$) are $\lambda_1 = 7$ and $\lambda_2 = 2$. Inset: Energy landscape.

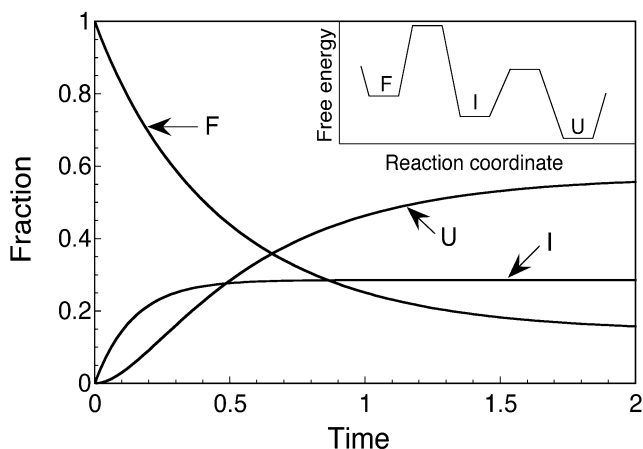


Fig. 4.5. Double-exponential change of the populations of folded, F, intermediate, I, and unfolded, U, state in a reversible sequential three-state transition ($U \rightleftharpoons I \rightleftharpoons F$) under conditions that favor unfolding. The parameters chosen for this example are: $k_1 = 2$, $k_{-1} = 4$, $k_2 = 1$, $k_{-2} = 2$, $[F_0]/[UIF] = 1$, $[U_0]/[UIF] = 0$. Observed rate constants ($\lambda \equiv k_{\text{obs}}$) are $\lambda_1 = 7$ and $\lambda_2 = 2$. Inset: Energy landscape.

Summarizing, the general solutions for the reversible sequential three-state transition (Eq. 4.10) usually are double-exponential functions, given by Eqs. 4.17 and 4.18 (see Table 4.1). Two special cases, where both [F] and [U] follow only single-exponential functions are: $C_1 = C_2 = 0$, and $C_3 = C_4 = 0$. In case $C_1 = C_2 = C_3 = C_4 = 0$, no kinetic event is macroscopically observed because the reaction is already in equilibrium.

In the example for Fig. 4.4, one can see that the intermediate accumulates kinetically. This is because the intermediate first becomes rapidly populated by the fast transition from U to I, but then becomes partially trapped by the high barrier of transition from I to F. In contrast, under conditions that favor unfolding, the early refolding intermediate is not accumulated, i.e., it is never populated above the level of population in equilibrium (Fig. 4.5).

After infinite time the system approaches an equilibrium,

$$\begin{aligned} [U](\infty) / [I](\infty) &= K_{UI} = k_{-1} / k_1 \\ [I](\infty) / [F](\infty) &= K_{IF} = k_{-2} / k_2, \end{aligned} \quad (4.20)$$

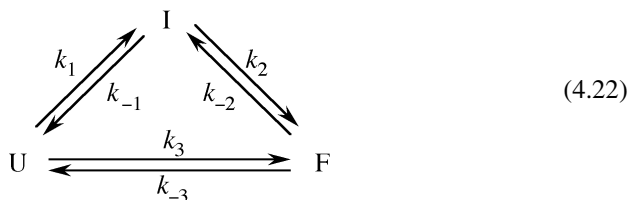
where K_{UI} , K_{IF} are the equilibrium constants for unfolding. Using the conservation relation, $[I] = [UIF] - [U] - [F]$, we obtain the equilibrium concentrations:

$$\begin{aligned} [U](\infty) &= [UIF] k_{-1}k_{-2} / (k_1k_2 + k_1k_{-2} + k_{-1}k_{-2}) \\ [I](\infty) &= [UIF] k_1k_{-2} / (k_1k_2 + k_1k_{-2} + k_{-1}k_{-2}) \\ [F](\infty) &= [UIF] k_1k_2 / (k_1k_2 + k_1k_{-2} + k_{-1}k_{-2}). \end{aligned} \quad (4.21)$$

Rate constants for the special case of a much faster transition $U \rightleftharpoons I$ than $I \rightleftharpoons F$ are given in Sect. 8.3.3.2.

4.3.1.2

Reversible two-pathway three-state transition



The rate equations for the two-pathway three-state reaction (Eq. 4.22) are:

$$\begin{aligned} \frac{d[U]}{dt} &= k_{-1}[I] - k_1[U] + k_{-3}[F] - k_3[U] \\ \frac{d[F]}{dt} &= k_2[I] - k_{-2}[F] + k_3[U] - k_{-3}[F] \\ [I] &= [UIF] - [U] - [F]. \end{aligned} \quad (4.23)$$

The free energy difference between U and F must be independent of the pathway:

$$k_3/k_{-3} = k_1k_2/(k_{-1}k_{-2}) \quad (4.24)$$

The method of solving Eq. 4.23 is analogous to the method for the reversible sequential three-state transition (Sect. 4.3.1.1): Consider first for simplicity only the changes of $[U]$, $[I]$, and $[F]$,

$$\frac{d\Delta[U]}{dt} = k_{-1}\Delta[I] - k_1\Delta[U] + k_{-3}\Delta[F] - k_3\Delta[U] \quad (4.25)$$

$$\frac{d\Delta[F]}{dt} = k_2\Delta[I] - k_{-2}\Delta[F] + k_3\Delta[U] - k_{-3}\Delta[F]$$

$$\Delta[I] = -\Delta[U] - \Delta[F],$$

and assume that a particular solution has the form:

$$\Delta[U](t) = C_1 \exp(-\lambda t) \quad (4.26)$$

$$\Delta[F](t) = C_2 \exp(-\lambda t).$$

This assumption leads to:

$$-\lambda \Delta[U] = -k_{-1}(\Delta[U] + \Delta[F]) - k_1\Delta[U] + k_{-3}\Delta[F] - k_3\Delta[U] \quad (4.27)$$

$$-\lambda \Delta[F] = -k_2(\Delta[U] + \Delta[F]) - k_{-2}\Delta[F] + k_3\Delta[U] - k_{-3}\Delta[F].$$

By simplifying Eq. 4.27 and substituting $[U]$ or $[F]$, we obtain an equation for λ ,

$$0 = \lambda^2 - \lambda(k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_{-3}) + k_1k_2 + k_1k_{-2} + k_1k_{-3} + k_{-1}k_{-2} + k_{-1}k_3 + k_{-1}k_{-3} + k_2k_3 + k_2k_{-3} + k_{-2}k_3, \quad (4.28)$$

which has two real solutions (λ_1 corresponds to the positive sign before the root; the “-” corresponds to λ_2):

$$\lambda_{1,2} = 0.5 (k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_{-3}) \quad (4.29)$$

$$\pm ((k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_{-3})^2 - 4 (k_1k_2 + k_1k_{-2} + k_1k_{-3} + k_{-1}k_{-2} + k_{-1}k_3 + k_{-1}k_{-3} + k_2k_3 + k_2k_{-3} + k_{-2}k_3))^{1/2}$$

$$[U](t) = C_1 \exp(-\lambda_1 t) + C_3 \exp(-\lambda_2 t) + C_5 \quad (4.30)$$

$$[F](t) = C_2 \exp(-\lambda_1 t) + C_4 \exp(-\lambda_2 t) + C_6$$

$$C_1 = \begin{cases} ([F_0] - \xi_2[U_0] - C_6 + \xi_2 C_5) / (\xi_1 - \xi_2) & \text{for } k_{-1} \neq k_{-3} \\ [U_0] - [UIF]k_{-1} / (k_1 + k_{-1} + k_3) & \text{for } k_{-1} = k_{-3} \end{cases}$$

$$C_2 = \begin{cases} C_1(\lambda_1 - k_1 - k_{-1} - k_3) / (k_{-1} - k_{-3}) & \text{for } k_{-1} \neq k_{-3} \\ C_1(k_3 - k_2) / ((k_2 + k_{-2})(k_1 - k_{-2})) & \text{for } k_{-1} = k_{-3}, k_1 \neq k_{-2} \\ 0 & \text{for } k_{-1} = k_{-3}, k_1 = k_{-2} \end{cases}$$

$$C_3 = \begin{cases} ([F_0] - \xi_1[U_0] - C_6 + \xi_1 C_5) / (\xi_2 - \xi_1) & \text{for } k_{-1} \neq k_{-3} \\ 0 & \text{for } k_{-1} = k_{-3} \end{cases}$$

$$C_4 = \begin{cases} C_3(\lambda_2 - k_1 - k_{-1} - k_3)/(k_{-1} - k_{-3}) & \text{for } k_{-1} \neq k_{-3} \\ [F_0] - C_2 - C_6 & \text{for } k_{-1} = k_{-3} \end{cases}$$

$$C_5 = [\text{UIF}](k_{-1}k_{-2} + k_{-1}k_{-3} + k_2k_3) / (k_1k_2 + k_1k_{-2} + k_1k_{-3} + k_{-1}k_{-2} + k_{-1}k_3 + k_{-1}k_{-3} + k_2k_3 + k_2k_{-3} + k_{-2}k_3)$$

$$C_6 = [\text{UIF}](k_1k_2 + k_{-1}k_3 + k_2k_3) / (k_1k_2 + k_1k_{-2} + k_1k_{-3} + k_{-1}k_{-2} + k_{-1}k_3 + k_{-1}k_{-3} + k_2k_3 + k_2k_{-3} + k_{-2}k_3)$$

$$\xi_1 = (\lambda_1 - k_1 - k_{-1} - k_3)/(k_{-1} - k_{-3}) \quad \text{for } k_{-1} \neq k_{-3}$$

$$\xi_2 = (\lambda_2 - k_1 - k_{-1} - k_3)/(k_{-1} - k_{-3}) \quad \text{for } k_{-1} \neq k_{-3}.$$

Similar to the sequential three-state transition ($U \rightleftharpoons I \rightleftharpoons F$), $[U](t)$, $[I](t)$, and $[F](t)$ usually display a double-exponential behavior (Eq. 4.30) with two observed rate constants given by Eq. 4.29 (Figs. 4.6, 4.7, Table 4.1). An important implication is that the information of kinetic rate constants alone is generally not sufficient to distinguish between the two different mechanisms, two-pathway or sequential reaction. There are a few special cases in which $[F](t)$ or $[U](t)$ or both follow single-exponential functions. At the limits $k_3 \rightarrow 0$ and $k_{-3} \rightarrow 0$, Eqs. 4.29 and 4.30 transform into the solution for the reversible sequential three-state transition (Sect. 4.3.1.1).

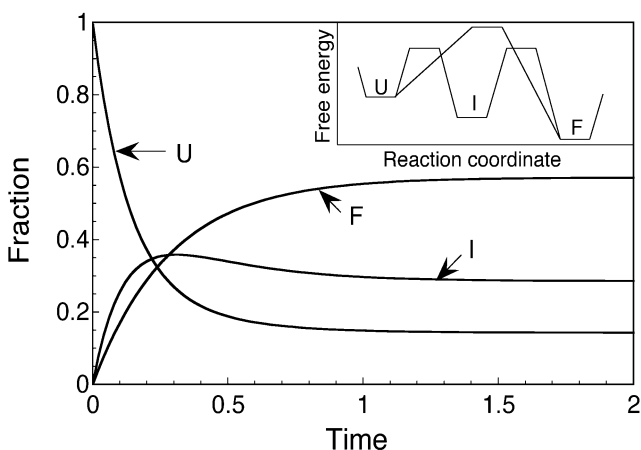


Fig. 4.6. Change of the populations of folded, F, intermediate, I, and unfolded state, U, in a reversible three-state transition with two parallel pathways ($U \rightleftharpoons I \rightleftharpoons F$ and $U \rightleftharpoons F$) under conditions that favor folding. The parameters chosen for this example are: $k_1 = 4$, $k_{-1} = 2$, $k_2 = 2$, $k_{-2} = 1$, $k_3 = 2$, $k_{-3} = 0.5$, $[F_0]/[\text{UIF}] = 0$, $[U_0]/[\text{UIF}] = 1$. Observed rate constants ($\lambda \equiv k_{\text{obs}}$) are $\lambda_1 = 8$ and $\lambda_2 = 3.5$. Inset: Energy landscape.

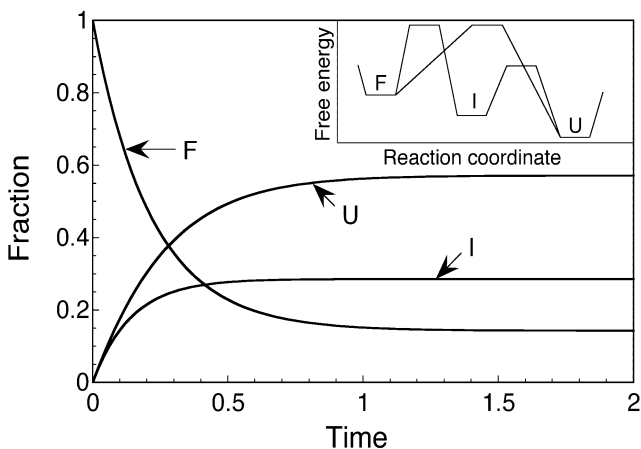
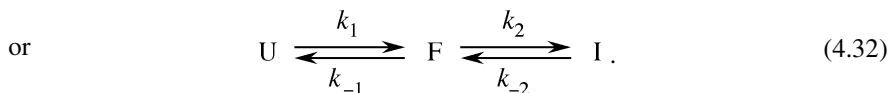
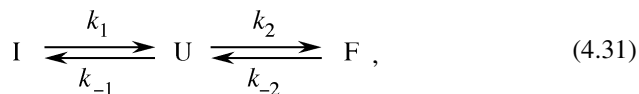


Fig. 4.7. Change of the populations of folded, F, intermediate, I, and unfolded state, U, in a reversible three-state transition with two parallel pathways ($U \rightleftharpoons I \rightleftharpoons F$ and $U \rightleftharpoons F$) under conditions that favor unfolding. The parameters chosen for this example are: $k_1 = 2$, $k_{-1} = 4$, $k_2 = 1$, $k_{-2} = 2$, $k_3 = 0.5$, $k_{-3} = 2$, $[F_0]/[UIF] = 1$, $[U_0]/[UIF] = 0$. Inset: Energy landscape.

4.3.1.3

Reversible off-pathway intermediate

The third important type of reversible three-state transitions is illustrated in Eqs. 4.31 and 4.32. Strictly speaking, I is a side-product, but because it is often spectroscopically in-between U and F, it is commonly referred to as an off-pathway intermediate:



The solution for Eq. 4.31 is found by transforming it into Eq. 4.10 (Sect. 4.3.1.1) by exchanging I and U:

$$[I](t) = C_1 \exp(-\lambda_1 t) + C_3 \exp(-\lambda_2 t) + C_5 \quad (4.33)$$

$$[F](t) = C_1 \xi_1 \exp(-\lambda_1 t) + C_3 \xi_2 \exp(-\lambda_2 t) + C_6$$

$$[U](t) = [UIF] - [I](t) - [F](t)$$

$$\lambda_{1,2} = 0.5 (k_1 + k_{-1} + k_2 + k_{-2}) \pm ((k_1 + k_{-1} + k_2 + k_{-2})^2 - 4(k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2}))^{1/2} \quad (4.34)$$

$$\xi_1 = (\lambda_1 - k_1 - k_{-1}) / k_{-1}$$

$$\begin{aligned}\xi_2 &= (\lambda_2 - k_1 - k_{-1}) / k_{-1} \\ C_1 &= ([F_0] - \xi_2[I_0] - C_6 + \xi_2 C_5) / (\xi_1 - \xi_2) \\ C_3 &= ([F_0] - \xi_1[I_0] - C_6 + \xi_1 C_5) / (\xi_2 - \xi_1) \\ C_5 &= [UIF]k_{-1}k_{-2} / (k_1k_2 + k_1k_{-2} + k_{-1}k_{-2}) \\ C_6 &= [UIF]k_1k_2 / (k_1k_2 + k_1k_{-2} + k_{-1}k_{-2}),\end{aligned}$$

where $[I_0] = [I](t=0)$, $[F_0] = [F](t=0)$, and $[UIF] = [U] + [I] + [F]$. Analogously, the solution for Eq. 4.32 is obtained by cyclically exchanging U, I, and F in Eq. 4.31. λ_1 and λ_2 are the same as for the mechanism $U \rightleftharpoons I \rightleftharpoons F$ (Eq. 4.16).

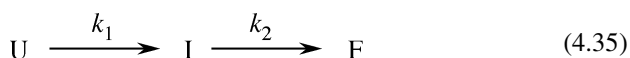
An important implication is that the information of kinetic rate constants alone is generally not sufficient to distinguish between on-pathway (Sect. 4.3.1.1) or off-pathway intermediates. A method for distinguishing these two cases is the Φ -value analysis (Sect. 8.3). Originally this method was designed for the structural resolution of transition states and intermediates (Goldenberg et al., 1989; Matouschek et al., 1989, 1990; Fersht et al., 1991, 1992; Matouschek and Fersht, 1991; Fersht, 1992, 1993, 1995a, b; Clarke and Fersht, 1993; Otzen et al., 1994; Itzhaki et al., 1995b; Nölting et al., 1995, 1997a; Nölting, 1998a, 1999), but it also provides information about the kinetic mechanism: In the Φ -value analysis mutants are used as reporters of structural consolidation along the folding pathway. In the case of an off-pathway intermediate, the calculated total Φ for the whole reaction from U to F is not 1 for all mutants when erroneously assuming an on-pathway mechanism (Sect. 8.3.4.2).

4.3.2

Irreversible three-state transitions

4.3.2.1

Irreversible consecutive three-state transition



The solution (Fig. 4.8, Table 4.1) is derived by using the methods presented in the previous sections:

$$\begin{aligned}[U](t) &= [U_0] \exp(-k_1 t) & (4.36) \\ [F](t) &= C_2 \exp(-k_1 t) + C_4 \exp(-k_2 t) + [UIF] \\ C_2 &= \begin{cases} k_2[U_0]/(k_1 - k_2) & \text{for } k_1 \neq k_2 \\ -k_1[U_0]t & \text{for } k_1 = k_2 \end{cases} \\ C_4 &= \begin{cases} [F_0] - k_2[U_0]/(k_1 - k_2) - [UIF] & \text{for } k_1 \neq k_2 \\ [F_0] - [UIF] & \text{for } k_1 = k_2 \end{cases}\end{aligned}$$

$$\begin{aligned}
 [U_0] &= [U](0) \\
 [F_0] &= [F](0) \\
 [UIF] &= [U] + [I] + [F] .
 \end{aligned}$$

The definitions are as in Sect. 4.3.1. One should mention, that in case $k_1 = k_2$, C_2 is a function of time, and thus, $[F](t)$ and $[I](t)$ are not pure superpositions of two exponential functions anymore.

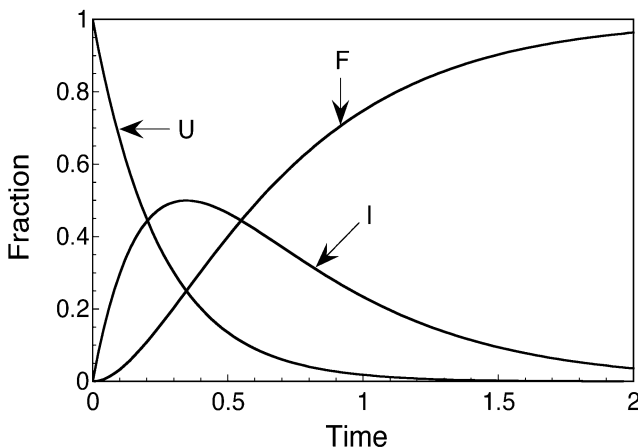
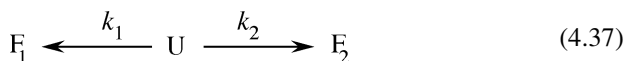


Fig. 4.8. Change of the populations of folded, F, intermediate, I, and unfolded, U, state in an irreversible three-state transition ($U \rightarrow I \rightarrow F$). The parameters chosen for this example are: $k_1 = 4, k_2 = 2, [F_0]/[UIF] = 0, [U_0]/[UIF] = 1$.

4.3.2.2 Irreversible parallel decay



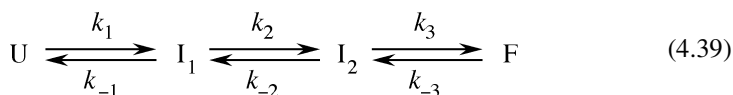
This type of reaction may occur under conditions that strongly favor folding when two conformations (e.g., a correctly folded and a misfolded, see Sect. 9.3) are produced. Here the populations of the species are

$$\begin{aligned}
 [U](t) &= [U_0] \exp(-(k_1 + k_2)t) \\
 [F_1](t) &= -[U_0] \exp(-(k_1 + k_2)t) \frac{k_1}{(k_1 + k_2)} + C_1 \\
 [F_2](t) &= -[U_0] \exp(-(k_1 + k_2)t) \frac{k_2}{(k_1 + k_2)} + C_2 ,
 \end{aligned}
 \tag{4.38}$$

where the definitions are analogous to those in Sect. 4.3.1, and the constants C_1 and C_2 depend on the initial conditions.

4.4

Reversible sequential four-state transition



The solution of this case involves the treatment of a cubic equation (Eq. 4.40) (Beyer, 1991). With the exception of a few special cases, a superposition of three exponential functions is observed:

$$0 = \lambda^3 + p\lambda^2 + q\lambda + r \quad (4.40)$$

$$p = -(k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_{-3})$$

$$q = k_1k_2 + k_1k_{-2} + k_1k_3 + k_1k_{-3} + k_{-1}k_{-2} + k_{-1}k_3 + k_{-1}k_{-3} + k_2k_3 + k_2k_{-3} + k_{-2}k_{-3}$$

$$r = -(k_1k_2k_3 + k_1k_2k_{-3} + k_1k_{-2}k_{-3} + k_{-1}k_{-2}k_{-3})$$

$$\lambda_1 = A + B - p/3$$

$$\lambda_{2,3} = -0.5(A + B) \pm 0.5(A - B)\sqrt{-3} - p/3$$

$$A = \sqrt[3]{-0.5b + c}$$

$$B = \sqrt[3]{-0.5b - c}$$

$$c = \sqrt{\frac{b^2}{4} + \frac{a^3}{27}}$$

$$a = (3q - p^2)/3$$

$$b = (2p^3 - 9pq + 27r)/27$$

$$[U](t) = C_1 \exp(-\lambda_1 t) + C_3 \exp(-\lambda_2 t) + C_5 \exp(-\lambda_3 t) + C_7$$

$$[F](t) = C_2 \exp(-\lambda_1 t) + C_4 \exp(-\lambda_2 t) + C_6 \exp(-\lambda_3 t) + C_8 .$$

For example, for $k_1 = 4$, $k_{-1} = 2$, $k_2 = 2$, $k_{-2} = 1$, $k_3 = 2$, $k_{-3} = 0.5$, one obtains $c = -5.449i$, $A = 1.630 - 0.733i$, $B = 1.630 + 0.733i$, where the imaginary number i is defined as $i \equiv \sqrt{-1}$, and $\lambda_1 = 7.093$, $\lambda_2 = 3.473$, $\lambda_3 = 0.934$. Compared with the example for three-state transitions with two parallel pathways (Fig. 4.6), the observed rate constants, λ_1 and λ_2 , are slower, and there is an additional, even slower, phase, λ_3 .

Concerning the calculation of the roots in Eq. 4.40, it should be mentioned that a complex number $z = z_r + iz_i$ can be transformed to $z = r \times (\cos \phi + i \sin \phi)$, where $r = (z_r^2 + z_i^2)^{0.5}$ and $\phi = \arctan(z_i/z_r)$. From *de Moivre's* identity, $(e^{i\phi})^n = e^{in\phi}$, and *Euler's* formula, $e^{i\phi} = \cos \phi + i \sin \phi$, follows $(\cos \phi + i \sin \phi)^n = \cos(n\phi) + i \sin(n\phi)$. Thus,

$$z^{1/n} = (r \times (\cos \phi + i \sin \phi))^{1/n} \quad (4.41)$$

$$= r^{1/n} \times (\cos((\phi + 2\pi k)/n) + i \sin((\phi + 2\pi k)/n))$$

$$n = 2, 3, \dots ; \quad k = 0, 1, \dots, n-1$$

$$z = z_r + iz_i \quad r = (z_r^2 + z_i^2)^{0.5} \quad \phi = \arctan(z_i/z_r) .$$

For example, the three third roots of 1 are 1 and $-1/2 \pm \sqrt{-3}/2$.

4.5 Reactions with monomer–dimer transitions

4.5.1

Monomer–dimer transition



In contrast to the cases treated in the previous sections, here we are encountering a bimolecular reaction. The rate equation for $[A]$ is a first-order non-linear differential equation:

$$\frac{d[A]}{dt} = -2k_1[A]^2 - k_{-1}[A] + k_{-1}[A_{\text{tot}}] \quad (4.43)$$

$$[A](0) = [A_0]$$

$$[A_2] = 0.5 ([A_{\text{tot}}] - [A]),$$

where $[A_{\text{tot}}]$ is the total protein concentration in equivalents of monomers, and the other definitions are analogous to those in Sect. 4.2. We start with the guess that particular solutions are of the types:

$$[A] = B + C \tanh[D(t + E)] \quad (4.44)$$

$$[A] = B + C \coth[D(t + E)]$$

$$\tanh(u) \equiv \frac{\exp(u) - \exp(-u)}{\exp(u) + \exp(-u)}$$

$$\coth(u) \equiv \frac{\exp(u) + \exp(-u)}{\exp(u) - \exp(-u)}.$$

By inserting Eq. 4.44 into Eq. 4.43 and using the relations:

$$d[\tanh(u)]/dt = (1 - \tanh^2(u)) \times du/dt \quad (4.45)$$

$$d[\coth(u)]/dt = (1 - \coth^2(u)) \times du/dt,$$

the guess is verified and the constants are calculated (Figs. 4.9, 4.10):

$$B = -0.25 [A_{\text{tot}}] K \quad (4.46)$$

$$C = 0.25 [A_{\text{tot}}] (K^2 + 8K)^{0.5}$$

$$D = 2 k_1 C$$

$$K = k_{-1}/([A_{\text{tot}}]k_1)$$

$$E = \begin{cases} \operatorname{arctanh}([(A_0] - B)/C]/D & \text{for } |([A_0] - B)/C| < 1 \\ \operatorname{arccoth}([(A_0] - B)/C]/D & \text{for } |([A_0] - B)/C| > 1 \end{cases}$$

$$[A] = \begin{cases} B + C \tanh[D(t + E)] & \text{for } |([A_0] - B)/C| < 1 \\ B + C \coth[D(t + E)] & \text{for } |([A_0] - B)/C| > 1. \end{cases}$$

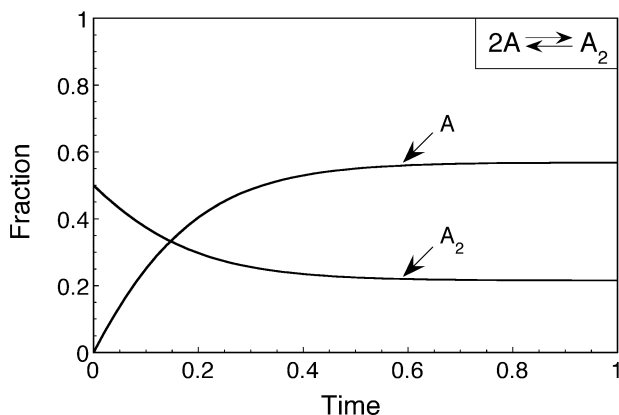


Fig. 4.9. Change of the populations of monomers and dimers in a monomer–dimer transition ($2A \rightleftharpoons A_2$). The parameters chosen for this example are: $k_1 = 2/[A_{\text{tot}}]$, $k_{-1} = 3$, $[A_0]/[A_{\text{tot}}] = 0$.

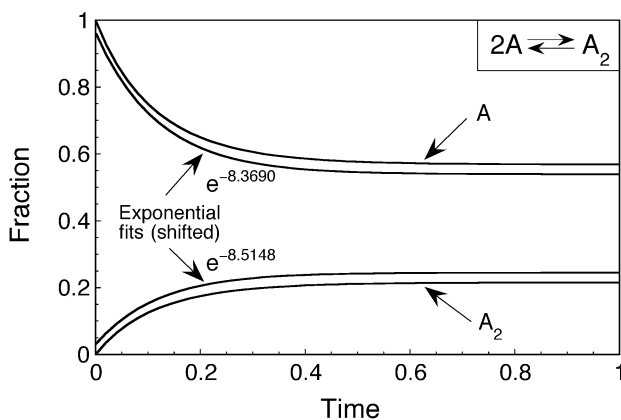


Fig. 4.10. Change of the populations of monomers and dimers in a monomer–dimer transition ($2A \rightleftharpoons A_2$), compared with single-exponential fits which are shifted by 0.03 units for better visibility. Only little differences are observed between the kinetic traces and the wrong curve fits. Thus, the information about the shape of the kinetic traces is often not sufficient to distinguish between bimolecular and unimolecular reactions. The parameters chosen for this example are: $k_1 = 2/[A_{\text{tot}}]$, $k_{-1} = 3$, $[A_0]/[A_{\text{tot}}] = 1$.

Alternatively, the solution of Eq. 4.43 may be represented as a linear combination of both particular solutions (Eq. 4.44). The equilibrium concentrations are:

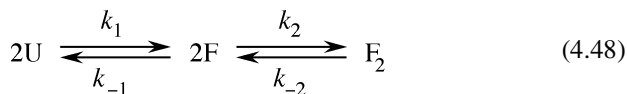
$$\begin{aligned}
 [A](\infty) &= 0.25 [A_{\text{tot}}] ((K^2 + 8K)^{0.5} - K) \\
 [A_2](\infty) &= 0.5 ([A_{\text{tot}}] - [A](\infty)) .
 \end{aligned}
 \tag{4.47}$$

In contrast to the first-order reactions in Sects. 4.2–4.4, the rate constants for reactions that involve a monomer–dimer transition are always dependent on the concentration.

4.5.2

Reversible two-state folding transition linked with a monomer–dimer transition

Another common case of a kinetic reaction is a reversible two-state folding transition of monomeric molecules followed by the dimerization in the folded state (Fig. 4.11):



The rate equations are:

$$\begin{aligned}
 \frac{d[U]}{dt} &= k_{-1}[F] - k_1[U] \\
 \frac{d[F_2]}{dt} &= k_2[F]^2 - k_{-2}[F_2] \\
 [F] &= [UF] - [U] - 2[F_2] ,
 \end{aligned}
 \tag{4.49}$$

where $[UF]$ is the total concentration in equivalents of monomers. The rate equation for $[U]$ is quite complicated:

$$\begin{aligned}
 k_{-1} \frac{d^2[U]}{dt^2} + 2k_2 \left(\frac{d[U]}{dt} \right)^2 + 4k_1k_2[U] \frac{d[U]}{dt} + k_{-1}(k_1 + k_{-1} + k_{-2}) \frac{d[U]}{dt} \\
 + 2k_1^2k_2[U]^2 + k_{-1}k_{-2}(k_1 + k_{-1})[U] - k_{-1}^2k_2[UF] = 0
 \end{aligned}
 \tag{4.50}$$

Exponential functions are solutions only for a few special cases. A very crude approximation for $[U]$ which is valid for several non-trivial cases is given by:

$$[U] = C + \frac{\sum_{i=1}^n A_i e^{-i\lambda t}}{\sum_{i=1}^n B_i e^{-i\lambda t}} ,
 \tag{4.51}$$

where $n \geq 4$, A_i , B_i , and C are constants. By inserting Eq. 4.51 into Eq. 4.50 and using the boundary condition for $[U](0)$, one obtains several algebraic equations for A_i , B_i , and C . These can be solved for the time-independent terms and the terms which contain only low-order exponential functions. Then the error due to the non-equality of the terms which contain high-order exponential functions is occasionally found to be relatively small.

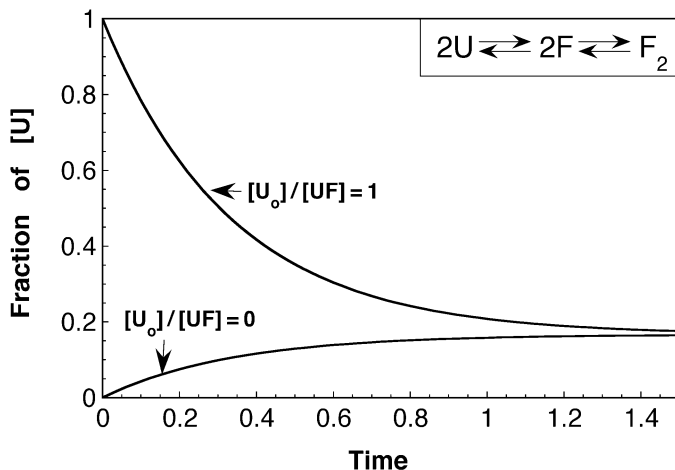
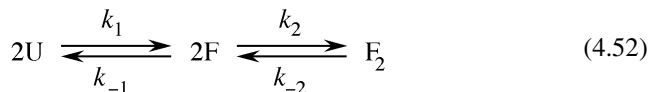


Fig. 4.11. Change of the population of the unfolded state in a two-state folding transition that is in sequence with a monomer–dimer transition ($2U \rightleftharpoons 2F \rightleftharpoons F_2$). The parameters chosen for this example are: $k_1 = 3$, $k_{-1} = 1$, $k_2 = 2/[UF]$, $k_{-2} = 3$. Two cases are shown: $[U_0]/[UF] = 1$, and $[U_0]/[UF] = 0$.

4.6

Kinetic rate constants for perturbation methods

The mathematical treatment of bimolecular reaction kinetics is tremendously simplified for small-amplitude perturbation methods, such as small-amplitude temperature-jumping, pressure perturbation, ultrasonic velocimetry, and dielectric relaxation (Table 4.2). In these methods, the changes of the populations of the states are small because the equilibrium of the species involved is only slightly perturbed by a small change in the physical or chemical conditions. Consider, for example, Eq. 4.52:



In equilibrium, per definition, the macroscopic changes in concentration are zero:

$$\frac{d[U]}{dt} = k_{-1}[F] - k_1[U] = 0 \quad (4.53)$$

$$\frac{d[F_2]}{dt} = k_2[F]^2 - k_{-2}[F_2] = 0$$

$$[F] = [UF] - [U] - 2[F_2],$$

where $[UF]$ is the total concentration in equivalents of monomers. Thus, the equilibrium concentrations are:

$$[U]_{\text{eq}} = \frac{k_{-1}}{4k_1^2 k_2} (\sqrt{(k_1 + k_{-1})^2 k_2^2 + 8k_1^2 k_2 k_{-2} [UF]} - (k_1 + k_{-1}) k_{-2}) \quad (4.54)$$

$$[F]_{\text{eq}} = \frac{1}{4k_1 k_2} (\sqrt{(k_1 + k_{-1})^2 k_2^2 + 8k_1^2 k_2 k_{-2} [UF]} - (k_1 + k_{-1}) k_{-2})$$

$$[F_2]_{\text{eq}} = 0.5([UF] - [U]_{\text{eq}} - [F]_{\text{eq}}).$$

Upon perturbation, the equilibrium moves to a new position:

$$[U] = [U]_{\text{eq}} + [u] \quad (4.55)$$

$$[F] = [F]_{\text{eq}} + [f] \quad [F_2] = [F_2]_{\text{eq}} + [f_2],$$

where $[u]$, $[f]$, and $[f_2]$ are the small changes of $[U]_{\text{eq}}$, $[F]_{\text{eq}}$, and $[F_2]_{\text{eq}}$, respectively. Thus, the relaxation of $[F_2]$ is described by

$$\frac{d[F_2]_{\text{eq}}}{dt} + \frac{d[f_2]}{dt} = k_2([F]_{\text{eq}} + [f])^2 - k_{-2}([F_2]_{\text{eq}} + [f_2]), \quad (4.56)$$

and so,

$$\frac{d[f_2]}{dt} = k_2(2[f][F]_{\text{eq}} + [f]^2) - k_{-2}[f_2]. \quad (4.57)$$

Neglecting the second-order term, $[f]^2$, leads to

$$\frac{d[f_2]}{dt} = 2k_2[f][F]_{\text{eq}} - k_{-2}[f_2]. \quad (4.58)$$

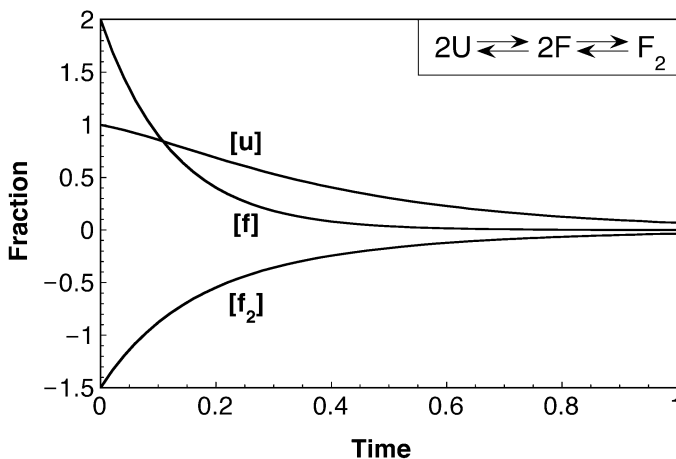


Fig. 4.12. Change in the populations of the monomeric unfolded state, monomeric folded state, and dimeric folded state upon a small perturbation of their equilibrium ($2U \rightleftharpoons 2F \rightleftharpoons F_2$). $[u]$, $[f]$, and $[f_2]$ are the small changes in the equilibrium concentrations, $[U]_{\text{eq}}$, $[F]_{\text{eq}}$, and $[F_2]_{\text{eq}}$, for the states U, F, and F_2 , respectively. Parameters chosen for this example are: $k_1 = 3$, $k_{-1} = 1$, $k_2 = 2/[UF]$, $k_{-2} = 3$, $2[u_0] = [f_0]$. Observed rate constants ($\lambda \equiv k_{\text{obs}}$) are $\lambda_1 = 8$ and $\lambda_2 = 3$.

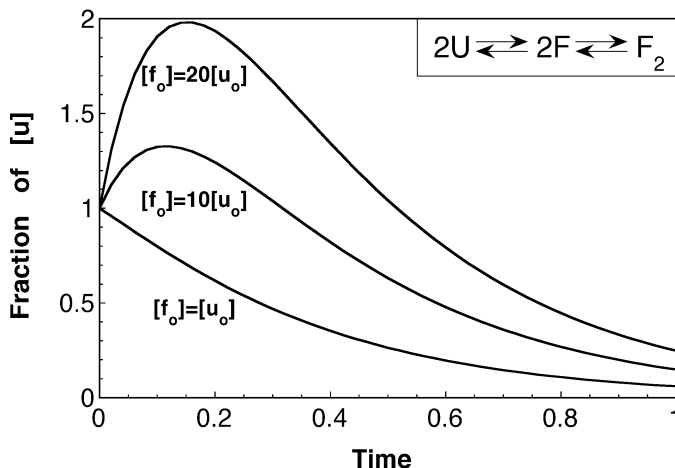


Fig. 4.13. Change in the population of the unfolded state upon a small perturbation of the equilibrium between monomeric unfolded, monomeric folded, and dimeric folded state ($2U \rightleftharpoons 2F \rightleftharpoons F_2$). The parameters chosen for this example are: $k_1 = 3$, $k_{-1} = 1$, $k_2 = 2/[UF]$, $k_{-2} = 3$, and $[f_0] = [u_0]$, $[f_0] = 10 [u_0]$, $[f_0] = 20 [u_0]$, respectively, as indicated. $[u_0]$ and $[f_0]$ depend on the magnitude and type of the perturbation and on the physical and chemical properties of the involved species. Observed rate constants ($\lambda \equiv k_{\text{obs}}$) are $\lambda_1 = 8$ and $\lambda_2 = 3$.

Analogously,

$$\frac{d[u]}{dt} = k_{-1}[f] - k_1[u] \quad (4.59)$$

$$[f] + [u] + 2[f_2] = 0.$$

By combination of Eqs. 4.58 and 4.59 we obtain:

$$\begin{aligned} \frac{d^2[u]}{dt^2} + \frac{d[u]}{dt}(k_1 + k_{-1} + k_{-2} + 4k_2[F]_{\text{eq}}) \\ + [u](k_1k_{-2} + k_{-1}k_{-2} + 4k_1k_2[F]_{\text{eq}}) = 0 \end{aligned} \quad (4.60)$$

$$[u](t) = [u_0]C \exp(-\lambda_1 t) + [u_0](1 - C) \exp(-\lambda_2 t) \quad (4.61)$$

$$C = (k_{-1}[f_0]/[u_0] + \lambda_2 - k_1)/(\lambda_2 - \lambda_1)$$

$$\lambda_{1,2} = 0.5p \pm (0.25p^2 - q)^{0.5}$$

$$p = k_1 + k_{-1} + k_{-2} + 4k_2[F]_{\text{eq}}$$

$$q = k_1k_{-2} + k_{-1}k_{-2} + 4k_1k_2[F]_{\text{eq}}$$

$$[f] = \left(\frac{d[u]}{dt} + k_1[u] \right) / k_{-1}$$

$$= ([u_0]/k_{-1}) \{ C(k_1 - \lambda_1) \exp(-\lambda_1 t) + (1 - C)(k_1 - \lambda_2) \exp(-\lambda_2 t) \}$$

$$[f_2] = -0.5([f] + [u]) ,$$

where $[u_0]$ and $[f_0]$ change with magnitude and type of the perturbation, and depend on the physical and chemical properties of the species involved. Depending on these parameters, convex or concave curve shapes, with or without transient accumulation, are observed (Figs. 4.12, 4.13).

Analogously, the simple case,



is easily derived:

$$\frac{d[f_2]}{dt} = -4k_1[f_2][F]_{\text{eq}} - k_{-1}[f_2] \quad (4.63)$$

$$[F]_{\text{eq}} = 0.25(\sqrt{K^2 + 8K[F_{\text{tot}}]} - K)$$

$$[F_{\text{tot}}] = [F] + 2[F_2]$$

$$K = k_{-1} / k_1 .$$

Here the observed rate constant, λ , is

$$\lambda = k_{-1} + 4k_1[F]_{\text{eq}} . \quad (4.64)$$

The rate constants of unimolecular reactions are the same as those derived in Sects. 4.2–4.4 (see Table 4.1).

4.7 Summary

In reactions which involve solely unimolecular transitions, so-called first-order reactions, the fractions of the species involved usually change with time according to superpositions of exponential functions with concentration-independent rate constants (Table 4.1).

As soon as bimolecular reactions are at least partly involved, the speed of reaction becomes concentration-dependent (Table 4.2). However, under pseudo-first-order experimental conditions this concentration-dependence may be undetectably small, for example, under conditions where the process is dominated by a dissociation event.

The kinetic traces of bimolecular reactions often have non-exponential shapes. However, in small perturbation methods, generally, exponential shapes of the kinetic traces are observed also for bimolecular reactions.

Table 4.1. Rate constants for first-order reaction mechanisms. For special cases and for magnitudes of changes of reactants and products see Sects. 4.2–4.4.

Reaction	Observed rate constants, $\lambda \equiv k_{\text{obs}}^a$ (relaxation constants)
$U \xrightarrow{k_1} F$	$\lambda = k_1$
$U \xrightleftharpoons[k_{-1}]{k_1} F$	$\lambda = k_1 + k_{-1}$
$U \xrightarrow{k_1} I \xrightarrow{k_2} F$	$\lambda_{1,2} = k_1, k_2$
$U \xrightleftharpoons[k_{-1}]{k_1} I \xrightleftharpoons[k_{-2}]{k_2} F$	$\lambda_{1,2} = 0.5 (k_1 + k_{-1} + k_2 + k_{-2} \pm ((k_1 + k_{-1} + k_2 + k_{-2})^2 - 4(k_1k_2 + k_1k_{-2} + k_{-1}k_{-2}))^{1/2})$
$\begin{array}{ccc} & & I \\ & \nearrow^{k_1} & \nwarrow_{k_2} \\ U & & F \\ & \xleftarrow[k_{-3}]{k_3} & \end{array}$	$\lambda_{1,2} = 0.5 (k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_{-3} \pm ((k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_{-3})^2 - 4(k_1k_2 + k_1k_{-2} + k_1k_{-3} + k_{-1}k_{-2} + k_{-1}k_{-3} + k_{-2}k_3 + k_2k_{-3} + k_{-2}k_3))^{1/2})$
	$\lambda_1 = A + B - p/3$
	$\lambda_{2,3} = -0.5(A + B) \pm 0.5(A - B)\sqrt{-3} - p/3$
	$A = \sqrt[3]{-0.5b + c} \quad B = \sqrt[3]{-0.5b - c}$
	$c = \sqrt{\frac{b^2}{4} + \frac{a^3}{27}}$
	$a = (3q - p^2)/3$
	$b = (2p^3 - 9pq + 27r)/27$
	$p = -(k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_{-3})$
	$q = k_1k_2 + k_1k_{-2} + k_1k_3 + k_1k_{-3} + k_{-1}k_{-2} + k_{-1}k_{-3} + k_{-1}k_3 + k_{-1}k_{-3} + k_2k_3 + k_2k_{-3} + k_{-2}k_{-3}$
	$r = -(k_1k_2k_3 + k_1k_2k_{-3} + k_1k_{-2}k_{-3} + k_{-1}k_{-2}k_{-3})$
$U \xrightleftharpoons[k_{-1}]{k_1} I_1 \xrightleftharpoons[k_{-2}]{k_2} I_2 \xrightleftharpoons[k_{-3}]{k_3} F$	

^a In special cases, some of the given rate constants do not apply.

Table 4.2. Rate constants for second-order reaction mechanisms in small-amplitude perturbation methods (see Sect. 4.6).

Reaction	Observed rate constants, $\lambda \equiv k_{\text{obs}}^a$ (relaxation constants)
$2\text{F} \xrightleftharpoons[k_{-1}]{k_1} \text{F}_2$	$\lambda = k_{-1} + 4k_1[\text{F}]_{\text{eq}}$
$\text{A} + \text{B} \xrightleftharpoons[k_{-1}]{k_1} \text{AB}$	$\lambda = k_{-1} + k_1([\text{A}]_{\text{eq}} + [\text{B}]_{\text{eq}})$
$2\text{U} \xrightleftharpoons[k_{-1}]{k_1} 2\text{F} \xrightleftharpoons[k_{-2}]{k_2} \text{F}_2$	$\lambda_{1,2} = 0.5p \pm (0.25p^2 - q)^{0.5}$ $p = k_1 + k_{-1} + k_{-2} + 4k_2[\text{F}]_{\text{eq}}$ $q = k_1k_{-2} + k_{-1}k_{-2} + 4k_1k_2[\text{F}]_{\text{eq}}$

^a The rate constants are calculated for experimental conditions under which a chemical equilibrium is approached prior to application of a small perturbation. $[\text{F}]_{\text{eq}}$, $[\text{A}]_{\text{eq}}$, and $[\text{B}]_{\text{eq}}$ are the equilibrium concentrations of F, A, and B, respectively. In special cases, some of the given rate constants do not apply.