21.1: Thermodynamics and Biomolecular Reactions

To begin, we recognize that binding and association processes are bimolecular reactions. Let's describe the basics of this process. The simplest kinetic scheme for bimolecular association is

$$A + B \rightleftharpoons C \tag{21.1.1}$$

A and *B* could be any two molecules that interact chemically or physically to result in a final bound state; for instance, an enzyme and its substrate, a ligand and receptor, or two specifically interacting proteins. From a mechanistic point of view, it is helpful to add an intermediate step:

$$A + B \rightleftharpoons AB \rightleftharpoons C$$

Here *AB* refers to transient encounter complex, which may be a metastable kinetic intermediate or a transition state. Then the initial step in this scheme reflects the rates of two molecules diffusing into proximity of their mutual target sites (including proper alignments). The second step is recognition and binding. It reflects the detailed chemical process needed to form specific contacts, execute conformational rearrangements, or perform activated chemical reactions. We separate these steps here to build a conceptual perspective, but in practice these processes may be intimately intertwined.

Equilibrium Constant

Let's start by reviewing the basic thermodynamics of bimolecular reactions, such as reaction scheme (21.1.1). The thermodynamics is described in terms of the chemical potential for the molecular species in the system (i = A,B,C)

$$\mu_i = \left(\frac{\partial G}{\partial N_i} \right)_{p,T,\{N_j, j \neq\}}$$

where N_i is the number of molecules of species *i*. The dependence of the chemical potential on the concentration can be expressed as

$$\mu_i = \mu_i^0 + RT \ln \frac{c_i}{c^0} \tag{21.1.2}$$

c_i is the concentration of reactant *i* in mol L^{-1}, and the standard state concentration is c^0 = 1 mol L^{-1}. So the molar reaction free energy for scheme (1) is

$$\begin{aligned} \Delta \overline{G} &= \sum_i v_i \mu_i \\ &= \mu_C - \mu_A \mu_B, \\ &= \Delta \overline{G}^0 + RT \ln K \end{aligned}$$

v_i is the stoichiometric coefficient for component *i*. *K* is the reaction quotient

$$K = \frac{(c_C / c^0)}{(c_A / c^0)(c_B / c^0)} \tag{21.1.3}$$

At equilibrium, $\Delta \overline{G} = 0$, so

$$\Delta \overline{G}^0 = -RT \ln K_a \tag{21.1.4}$$

where the association constant K_a is the value of the reaction quotient under equilibrium conditions. Dropping c^0, with the understanding that we must express concentration in M units:

$$K_a = \frac{c_C}{c_A c_B} \tag{21.1.5}$$

Since it is defined as a standard state quantity, K_a is a fundamental constant independent of concentration and pressure or volume, and is only dependent on temperature. The inverse of K_a is K_d the equilibrium constant for the *C* dissociation reaction $C \rightleftharpoons A + B$.

Concentration and Fraction Bound

Experimentally one controls the total mass $m_{TOT} = m_C + m_A + m_B$, or concentration

$$c_{TOT} = c_C + c_A + c_B \tag{21.1.6}$$

The composition of system can be described by the fraction of concentration due to species *i* as

$$\theta_i = \frac{c_i}{c_{TOT}}$$
$$\theta_A + \theta_B + \theta_C = 1$$

We can readily relate K_a to θ_i, but it is practical to set some specific constraint on the composition here. If we constrain the *A:B* composition to be 1:1, which is enforced either by initially mixing equal mole fractions of *A* and *B*, or by preparing the system initially with pure *C*, then

$$K_a = \frac{4\theta_C}{(1-\theta_C)^2 c_{TOT}} \qquad (\theta_A = \theta_B)$$
$$= \frac{(1-2\theta_A)}{\theta_A^2 c_{TOT}}$$

This expression might be used for mixing equimolar solutions of binding partners, such as complementary DNA oligonucleotides. Using eq. (21.1.6) (with c_A=c_B) and (21.1.7) here, we can obtain the composition as a function of total concentration fraction as a function of the total concentration

$$\theta_C = \left(1 + \tfrac{2}{K_a c_{TOT}}\right) - \sqrt{\left(1 + \tfrac{2}{K_a c_{TOT}}\right)^2 - 1} \tag{21.1.7}$$
$$\theta_A = \tfrac{1}{2}(1 - \theta_C)$$

In the case where *A*=*B*, applicable to homodimerization or hybridization of self-complementary oligonucleotides, we rewrite scheme (21.1.1) as the association of monomers to form a dimer

$$2M \rightleftharpoons D$$

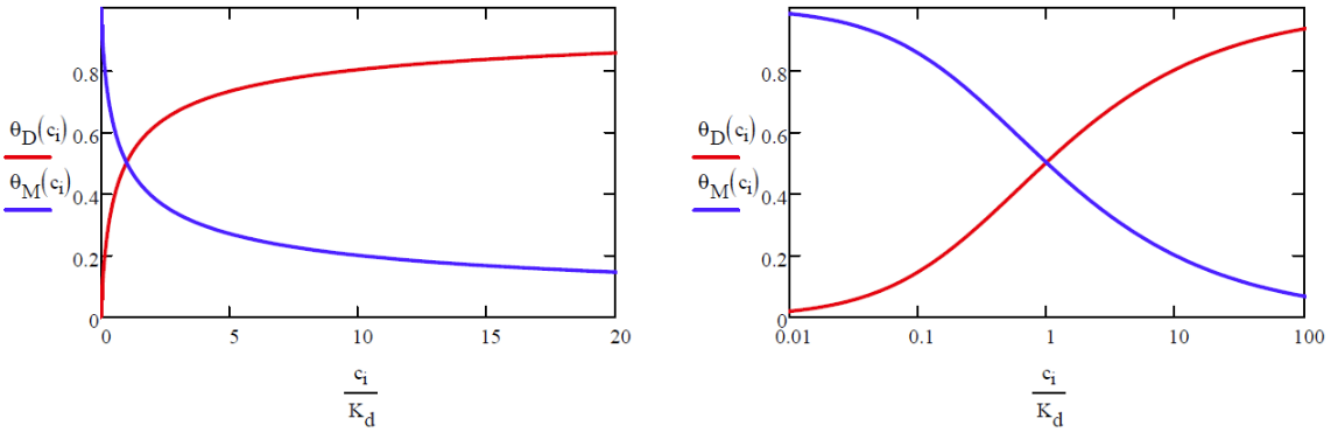
and find:

$$K_a = \theta_D / 2(1-\theta_D)^2 c_{TOT}$$
$$K_a = (1-\theta_M)/2\theta_M^2 c_{TOT}$$

$$\theta_D = 1 + \frac{1}{4 c_{TOT} K_a}\left(1 - \sqrt{1 + 8 c_{TOT} K_a}\right) \tag{21.1.8}$$

$$\theta_M = 1 - \theta_D \tag{21.1.9}$$

These expressions for the fraction of monomer and dimer, and the corresponding concentrations of monomer and dimer are shown below. An increase in the total concentration results in a shift of the equilibrium toward the dimer state. Note that c_{TOT}= $(9K_a)^{-1}$ = K_d/9 at θ_M = θ_D = 0.5,



For ligand receptor binding, ligand concentration will typically be much greater than that of the receptor, and we are commonly interested in fraction of receptors that have a ligand bound, θ_{bound}. Re-writing our association reaction as

$$L + R \rightleftharpoons LR \qquad K_a = \frac{c_{LR}}{c_L c_R} \tag{21.1.10}$$

we write the fraction bound as

$$\begin{aligned} \theta_{bound} &= \frac{c_{LR}}{c_R + c_{LR}} \\ &= \frac{c_L K_a}{1 + c_L K_a} \end{aligned}$$

This is equivalent to a **Langmuir absorption isotherm**.

Temperature Dependence

The temperature dependence of K_a is governed by eq. (21.1.4) and the fundamental relation

$$\Delta G^0(T) = \Delta H^0(T) - T\Delta S^0(T) \tag{21.1.11}$$

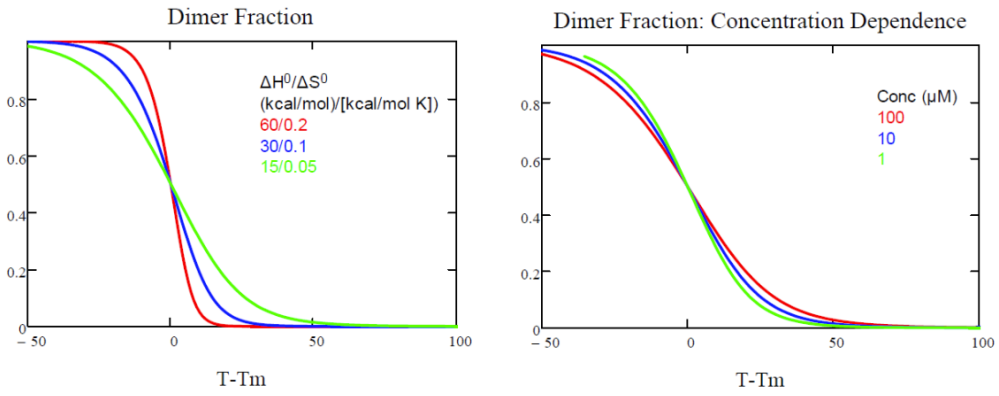
Under the assumption that ΔH^0 and ΔS^0 are temperature independent, we find

$$K_a(T) = exp\left[-\frac{\Delta H_a^0}{RT} + \frac{\Delta S_a^0}{R}\right] \tag{21.1.12}$$

This allows us to describe the temperature-dependent composition of a system using the expressions above for θ_i. While eq. (12) allows you to predict a melting curve for a given set of thermodynamic parameters, it is more difficult to use it to extract those parameters from experiments because it only relates the value of K_d at one temperature to another.

Temperature is often used to thermally dissociate or melt dsDNA or proteins, and the analysis of these experiments requires that we define a reference temperature. In the case of DNA melting, the most common and readily accessible reference temperature is the melting temperature Tm defined as the point where the mole fractions of ssDNA (monomer) and dsDNA (dimer) are equal, $\theta_M = \theta_D = 0.5$. This definition is practically motivated, since DNA melting curves typically have high and low temperature limits that correspond to pure dimer or pure monomer. Then Tm is commonly associated with the inflection point of the melting curve or the peak of the first derivative of the melting curve. From eq. (21.1.9), we see that the equilibrium constants for the association and dissociation reaction are given by the total concentration of DNA: $K_a(T_m) = K_d(T_m)^{-1} = c_{tot}^{-1}$ and $\Delta G_d^0(T_m) = -RT_m \ln c_{tot}$. Furthermore, eq. (21.1.12) implies $T_m = \Delta H^0/\Delta S^0$.

The examples below show the dependence of melting curves on thermodynamic parameters, T_m, and concentration. These examples set a constant value of T_m ($\Delta H^0/\Delta S^0$). The concentration dependence is plotted for ΔH^0 = 15 kcal mol^{-1} and ΔS^0 = 50 cal mol^{-1} K^{-1}.



For conformational changes in macromolecules, it is expected that the enthalpy and entropy will be temperature dependent. Drawing from the definition of the heat capacity,

$$C_p = \left(\frac{\partial H}{\partial T}\right)_{N,P} = T\left(\frac{\partial S}{\partial T}\right)_{N,P}$$

we can describe the temperature dependence of ΔH^0 and ΔS^0 by integrating from a reference temperature T^0 to T. If ΔC_p is independent of temperature over a small enough temperature range, then we obtain a linear temperature dependence to the enthalpy and entropy of the form

$$\Delta H^0(T) = \Delta H^0(T_0) + \Delta C_p[T - T_0] \tag{21.1.13}$$

$$\Delta S^0(T) = \Delta S^0(T_0) + \Delta C_p\left(\frac{T}{T_0}\right) \tag{21.1.14}$$

These expressions allow us to relate values of ΔH^0, ΔS^0, and ΔG^0 at temperature T to its value at the reference temperature T^0. From these expressions, we obtain a more accurate description of the temperature dependence of the equilibrium constant is

$$K_d(T) = exp\left[-\frac{\Delta H_m^0}{RT} + \frac{\Delta S_m^0}{R} - \frac{C_p}{R}\left[1 - \frac{T_m}{T} - \ln\left(\frac{T}{T_m}\right)\right]\right] \tag{21.1.15}$$

where $\Delta H_m^0 = \Delta H^0(T_m)$ and $\Delta S_m^0 = \Delta S^0(T_m)$ are the enthalpy and entropy for the dissociation reaction evaluated at T_m.