Theory of Free Energy and Entropy in Noncovalent Binding

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1. Statistical Thermodynamical Background

This section provides background information in a form that supports the present formulation of the theory of noncovalent binding.

The free energy, F, provides a measure of the stability of a system at thermal equilibrium: the lower the free energy, the higher the stability. Microscopically, low stability corresponds to a high probability of occupancy, or, equivalently, a large statistical weight, Q, which is also known as the partition function. The free energy is related to the partition function by the following expression

$$F = -k_{\mathrm{B}} T \ln Q \qquad \text{(I.1)}$$

where k_{B} is Boltzmann's constant and T is absolute temperature.

The partition function can be easily introduced for a quantized system, where the total energy takes discrete values corresponding to specific microstates of the system. One such system is a harmonic oscillator when treated quantum mechanically. Let the energies of the microstates j be E_j, $j = 1, 2, \ldots$ Then

$$Q = \sum_j e^{-\beta E_j} \qquad \text{(I.2)}$$

where $\beta = (k_{\mathrm{B}}T)^{-1}$. If the microstates are partitioned into two nonoverlapping macrostates A and B, then each macrostate is associated with a partition function, Q_{A} or Q_{B}, each given by a sum of the form of eq I.2 over its respective microstates, such that $Q=Q_A+Q_B$. The probability, p_{A} or p_{B}, of each macrostate is then proportional to the corresponding partition function, so that $p_{\mathrm{A}} = Q_{\mathrm{A}} / Q$ and $p_{\mathrm{B}} = Q_{\mathrm{B}} / Q$. This is why the partition function is referred to as a statistical weight. In the special case where a macrostate consists of a single microstate, say, j, its probability is $\exp(-\beta E_j)/Q$.

Two remarks on eq I.2 are in order. First, multiplying the expression for Q by an arbitrary constant does not alter the relative probabilities of macrostates subsumed by Q, such as A and B above. Put differently, multiplying Q by a constant shifts the free energy, F, but has no physical consequence because only differences in free energy between states are significant.

Second, the form of eq I.2 corresponds to the canonical ensemble, and the resulting free energy is therefore the Helmholtz free energy. Under the condition of constant volume, the Helmholtz free energy is the thermodynamic potential and is minimized at thermal equilibrium. The counterpart for constant pressure is the Gibbs free energy, G. The two are related by $G = F + PV$, where P is pressure and V is volume. Throughout this section, we assume that the volume of the system under consideration is constant and work with the Helmholtz free energy.

The present formulation of binding thermodynamics relies primarily on classical statistical thermodynamics. Classical statistical thermodynamics treats the energy as varying continuously, rather than in a quantized manner, and is an excellent approximation for many biomolecular applications, where the spacing of the relevant energy levels is small relative to thermal energy. Consider a system with energy, $E(x)$, that depends on a single, continuous coordinate x. At the risk of somewhat oversimplifying the relationship between quantum and classical statistical mechanics, one may identify a microstate of energy $E(x_j)$ with a narrow range of x_j values, say between x_j and $x_j + dx$.[1] Applying eq I.2, we find the partition function of the system to be

$$Q = \sum_j e^{-\beta E(x_j)} \tag{I.3a}$$

As noted in the previous paragraph, Q may be multiplied by a constant at will, and we now multiply Q by a quantity $\mathcal{N} dx$ and thereby convert the sum to an integral:

$$Q = \mathcal{N} \int dx e^{-\beta E(x)} \tag{I.3b}$$

Here the constant $\mathcal{N}$ has units reciprocal to those of x so that this integral form of Q remains unitless, but the value of $\mathcal{N}$ has no significance for the problems of interest here. When x is a spatial coordinate, then the integral of the Boltzmann factor over x is known as a configurational integral.

Generalizing to a system with more than one coordinate is straightforward. If the coordinates are collectively denoted as $\mathbf{x}$ and the energy function is $E(\mathbf{x})$, then

$$Q = \mathcal{N} \int d\mathbf{x} e^{-\beta E(\mathbf{x})} \tag{I.3c}$$

Similarly, although some of the derivations that follow are shown for systems of low dimensionality, they generalize readily to systems of arbitrary dimensionality.

1.1 Average Energy and Entropy

We now derive and elaborate expressions for the mean energy and entropy, <*E*> and *S*, for a one-dimensional system. As discussed above, Eq I.3b can be viewed as specifying the statistical weight of the microstate located between *x* and *x* + *dx* as $\mathcal{N}\exp[-\beta E(x)]dx$. The average energy is then

$$\langle E \rangle = \frac{\mathcal{N} \int dx E(x) e^{-\beta E(x)}}{Q} = \int dx E(x) \rho(x) \tag{I.4}$$

in which we have defined the equilibrium probability density in *x* as

$$\rho(x) = \frac{\mathcal{N} e^{-\beta E(x)}}{Q} = \frac{e^{-\beta E(x)}}{\int dx e^{-\beta E(x)}} \tag{I.5}$$

The entropy can be obtained from

$$S = -\left(\frac{\partial F}{\partial T} \right)_V \tag{I.6}$$

where the subscript "*V*" indicates that the derivative with respect to temperature otained at constant volume. Using eq I.1 for *F*, we find

$$\begin{aligned} S &= k_{\mathrm{B}} \ln Q + \frac{k_{\mathrm{B}} T}{Q} \left(\frac{\partial Q}{\partial T} \right)_V \\ &= k_{\mathrm{B}} \ln Q + \frac{\mathcal{N}}{TQ} \int dx E(x) e^{-\beta E(x)} \\ &= \frac{-F + \langle E \rangle}{T} \end{aligned} \tag{I.7a}$$

In this derivation it is assumed that the energy function is temperature-independent; temperature-dependent energy functions are analyzed in Subsection I.3. Another useful form of the entropy is given by the Gibbs/Shannon expression:

$$S = -k_{\mathrm{B}} \int dx \rho(x) \ln \rho(x) + k_{\mathrm{B}} \ln \mathcal{N} \tag{I.7b}$$

To prove the equivalence of eqs I.7a and I.7b, we use the expression of $\rho(x)$ given by the first identity of eq I.5, which implies that $\ln\rho(x) = \ln\mathcal{N} - \ln Q - \beta E(x)$. Inserting this result in eq I.7b, we have

$$S = -k_{\mathrm{B}} \int dx \rho(x)[-\ln Q - \beta E(x) + \ln \mathcal{N}] + k_{\mathrm{B}} \ln \mathcal{N}$$

Because the integrands $-\ln Q$ and $\ln\mathcal{N}$ are independent of x and $\int dx \rho(x) = 1$, these two quantities emerge from the integral as $-k_{\mathrm{B}}\ln Q$ and $-k_{\mathrm{B}}\ln\mathcal{N}$. The former is equivalent to $-F/T$ and the latter cancels the final term in the expression for S. The integrand $-\beta E(x)$ integrates to $<E>/T$ via the second identity of eq I.4. Thus, this expression for S reduces to the result of eq I.7a.

For a multidimensional system with coordinates denoted as $\mathbf{x}$, the average energy is

$$\langle E \rangle = \int d\mathbf{x} E(\mathbf{x}) \rho(\mathbf{x}) \tag{I.8}$$

with the equilibrium probability density in $\mathbf{x}$ given by

$$\rho(\mathbf{x}) = \frac{e^{-\beta E(\mathbf{x})}}{\int d\mathbf{x} e^{-\beta E(\mathbf{x})}} \tag{I.9}$$

For the entropy eq I.7a continues to be valid, but eq I.7b now reads

$$S = -k_{\mathrm{B}} \int d\mathbf{x} \rho(\mathbf{x}) \ln \rho(\mathbf{x}) + k_{\mathrm{B}} \ln \mathcal{N} \tag{I.10}$$

1.2 Correlation and Entropy

When the coordinates of a system are uncorrelated, the entropy is the sum of contributions from individual coordinates:

$$S = \sum_i S_i \tag{I.11a}$$

where

$$S_i = -k_{\mathrm{B}} \int dx_i \; \rho(x_i) \ln \rho(x_i) + k_{\mathrm{B}} \ln \mathcal{N}_i \tag{I.12b}$$

We now show that in the general case where coordinates may be correlated,

$$S \le \sum_i S_i \tag{I.12c}$$

For simplicity, consider a system with two coordinates, x_1 and x_2. The entropy is

$$S = -k_{\mathrm{B}} \int dx_1 dx_2 \rho(x_1, x_2) \ln \rho(x_1, x_2) + k_{\mathrm{B}} \ln \mathcal{N} \tag{I.13}$$

The joint probability density, $\rho(x_1, x_2)$, is related to the marginal probability densities, $\rho(x_1)$, $\rho(x_2)$, through conditional probability densities, $\rho(x_1|x_2)$ and $\rho(x_2|x_1)$ via the product law:

$$\rho(x_1, x_2) = \rho(x_2 \mid x_1)\rho(x_1) = \rho(x_1 \mid x_2)\rho(x_2) \tag{I.14}$$

Using the first identity of eq I.14 in eq I.13, and recognizing that, for any value of x_1, $\int dx_2\, \rho(x_2 \mid x_1) = 1$, we rewrite the entropy as

$$S = -k_{\mathrm{B}} \int dx_1 \rho(x_1) \ln \rho(x_1) - k_{\mathrm{B}} \int dx_1 \rho(x_1) \int dx_2 \rho(x_2 \mid x_1) \ln \rho(x_2 \mid x_1) + k_{\mathrm{B}} \ln \mathcal{N} \tag{I.15}$$

The first term is S_1. The inequality of eq I.12c can be obtained as a result of Gibbs' inequality, which reads

$$-\int dx \rho(x) \ln \rho(x) \leq -\int dx \rho(x) \ln \rho'(x) \tag{I.16}$$

with the equality holding only if $\rho(x) = \rho'(x)$. Here, we identify x, $\rho(x)$, and $\rho'(x)$ of eq I.16 with x_2, $\rho(x_2|x_1)$, and $\rho(x_2)$, respectively, resulting in

$$-\int dx_2 \rho(x_2 \mid x_1) \ln \rho(x_2 \mid x_1) \leq -\int dx_2 \rho(x_2 \mid x_1) \ln \rho(x_2) \tag{I.17}$$

The condition for equation is $\rho(x_2|x_1) = \rho(x_2)$, i.e., x_1 and x_2 are uncorrelated. Applying eq I.17 to the second term in eq I.15, we find

$$S \leq -k_{\mathrm{B}} \int dx_1 \rho(x_1) \ln \rho(x_1) - k_{\mathrm{B}} \int dx_1 \rho(x_1) \int dx_2 \rho(x_2 \mid x_1) \ln \rho(x_2) + k_{\mathrm{B}} \ln \mathcal{N} \tag{I.18}$$

To better connect the second term and S_2, we rearrange as follows:

$$-k_{\mathrm{B}} \int dx_1 \rho(x_1) \int dx_2 \rho(x_2 \mid x_1) \ln \rho(x_2) = -k_{\mathrm{B}} \int dx_1 dx_2\, \rho(x_1, x_2) \ln \rho(x_2) = -k_{\mathrm{B}} \int dx_2\, \rho(x_2) \ln \rho(x_2)$$

Finally eq I.18 becomes

$$S \leq -k_{\mathrm{B}} \int dx_1 \rho(x_1) \ln \rho(x_1) - k_{\mathrm{B}} \int dx_2 \rho(x) \ln \rho(x_2) + k_{\mathrm{B}} \ln \mathcal{N} = S_1 + S_2$$

where the equality holds when the two coordinates are uncorrelated.

1.3 Temperature-Dependent Energy Function

When the energy function is a potential of mean force, arising from averaging out a subset of the coordinates of the system, it becomes temperature-dependent. Let us illustrate the situation on a system with two coordinates, x_1 and x_2. The partition function is

$$Q = \mathcal{N}\int dx_1 dx_2 \; e^{-\beta E(x_1, x_2)} \tag{I.19}$$

Integrating over x_2 defines a potential of mean force, $W_1(x_1)$:

$$e^{-\beta W_1(x_1)} = \mathcal{N}_2 \int dx_2 e^{-\beta E(x_1, x_2)} \tag{I.20}$$

Here $\mathcal{N}_2$, the part of $\mathcal{N}$ associated with variable x_2, makes the right-hand side as a whole unitless. It is clear that the potential of mean force depends on temperature. The partition function can then be written as

$$Q = \mathcal{N}_1 \int dx_1 \; e^{-\beta W_1(x_1)} \tag{I.21}$$

where $\mathcal{N}_1 = \mathcal{N}/\mathcal{N}_2$. This result is formally identical to eq I.3b, the partition function for a single coordinate, except that the energy function is now replaced by the potential of mean force.

When the energy function is a temperature-dependent potential of mean force, the entropy is

$$\begin{aligned} S &= k_{\mathrm{B}} \ln Q + \frac{k_{\mathrm{B}} T}{Q} \left(\frac{\partial Q}{\partial T} \right)_V \\ &= k_{\mathrm{B}} \ln Q + \frac{\mathcal{N}_1}{TQ} \int dx_1 W_1(x_1) e^{-\beta W_1(x_1)} - \frac{\mathcal{N}_1}{Q} \int dx_1 \left(\frac{\partial W_1(x_1)}{\partial T} \right)_V e^{-\beta W_1(x_1)} \\ &= \frac{-F + \langle W_1 \rangle}{T} - \left\langle \left(\frac{\partial W_1}{\partial T} \right)_V \right\rangle \end{aligned} \tag{I.22}$$

which generalizes eq I.7a by adding the second term for the temperature-dependence of the potential of mean force. When x_1 represents the solute degrees of freedom and x_2 the solvent degrees of freedom, the two terms of eq I.22 are referred to as the configurational entropy and the solvation entropy, respectively. We now show that these two terms correspond, respectively, to the first two terms of eq I.15.

The equivalence of the first terms of eqs I.22 and I.15 is demonstrated by the same steps used to demonstrate the equivalence of eqs I.7a and I.7b; we find that

$$\frac{-F+\langle W_1\rangle}{T} = -k_\mathrm{B}\int dx_1 \rho(x_1)\ln\rho(x_1) + k_\mathrm{B}\ln\mathcal{N}_1 \tag{I.23}$$

To prove the equivalence of the second terms, we take the temperature derivative of both sides of eq I.20:

$$\left[\frac{W_1(x_1)}{k_\mathrm{B}T^2} - \frac{1}{k_\mathrm{B}T}\left(\frac{\partial W_1(x_1)}{\partial T}\right)_V\right]e^{-\beta W_1(x_1)} = \frac{\mathcal{N}_2}{k_\mathrm{B}T^2}\int dx_2 E(x_1,x_2)e^{-\beta E(x_1,x_2)} \tag{I.24}$$

Multiplying both sides by $\beta\mathcal{N}_1/Q$ and integrating over x_1, we find

$$\frac{\langle W_1\rangle}{T} - \left\langle\left(\frac{\partial W_1}{\partial T}\right)_V\right\rangle = \frac{\langle E\rangle}{T} \tag{I.25}$$

Rearranging leads to

$$\left\langle\left(\frac{\partial W_1}{\partial T}\right)_V\right\rangle = \frac{-\langle E\rangle + \langle W_1\rangle}{T} \tag{I.26}$$

Note that by substituting this result in eq I.22, one recovers the general result of eq I.7a for the entropy.

We now work with the second term of eq I.15. The marginal probability density $\rho(x_1)$ can be expressed as (cf. eq I.5)

$$\rho(x_1) \equiv \int dx_2 \rho(x_1,x_2) = \frac{\mathcal{N}_1 e^{-\beta W_1(x_1)}}{Q} \tag{I.27}$$

and the conditional probability density $\rho(x_2|x_1)$ can be expressed as

$$\rho(x_2 \mid x_1) \equiv \frac{\rho(x_1,x_2)}{\rho(x_1)} = \frac{\mathcal{N}e^{-\beta E(x_1,x_2)}/Q}{\mathcal{N}_1 e^{-\beta W_1(x_1)}/Q} = \mathcal{N}_2 e^{-\beta[E(x_1,x_2)-W_1(x_1)]} \tag{I.28}$$

The second term of eq I.15 is now

$$\begin{aligned} &-k_\mathrm{B}\int dx_1\rho(x_1)\int dx_2\rho(x_2 \mid x_1)\ln\rho(x_2 \mid x_1) \\ &\qquad = -k_\mathrm{B}\int dx_1 dx_2 \rho(x_1,x_2)[-\beta E(x_1,x_2) + \beta W_1(x_1) + \ln\mathcal{N}_2] && \text{(I.29)} \\ &\qquad = \frac{\langle E\rangle - \langle W_1\rangle}{T} - k_\mathrm{B}\ln\mathcal{N}_2 && \text{(I.30)} \end{aligned}$$

$$= \left\langle \left(\frac{\partial W_1}{\partial T} \right)_V \right\rangle - k_\mathrm{B} \ln \mathcal{N}_2 \tag{I.31}$$

Or, equivalently,

$$\left\langle \left(\frac{\partial W_1}{\partial T} \right)_V \right\rangle = - k_\mathrm{B} \int dx_1 \rho(x_1) \int dx_2 \rho(x_2 \mid x_1) \ln \rho(x_2 \mid x_1) + k_\mathrm{B} \ln \mathcal{N}_2 \tag{I.32}$$

Eqs I.23 and I.32 complete the proof that the two terms of eq I.22, respectively, correspond with the first two terms of eq I.15.

1.4 System of Non-Interacting, Identical Molecules

We wish to analyze the statistical thermodynamics of an ideal solution containing receptors, ligands and their complexes. McMillan-Mayer solution theory[2] shows that such a system has a close analogy to an ideal gas of these solutes, where their interaction potentials are replaced by solvent-modified potentials of mean force. We therefore consider a system N_α non-interacting and identical molecules of type α, each with a classical molecular partition function Q_α (Subsection 2.3.2 of the main text). The ideality of the solution (or gas) means that the molecules are non-interacting, so the total energy is additive, the motions of the various molecules are not correlated with each other, and the partition function of the system appears to be $Q_\alpha^{N_\alpha}$. However, the indistinguishability of the molecules means that swapping of their coordinates does not make a new contribution to the statistical weight of the system. The overcounting is determined by recognizing that each unique set of coordinates can be assigned to the molecules in $N_\alpha!$ ways. The partition function of the system, after accounting for the indistinguishability of the molecules, is thus

$$Q = \frac{Q_\alpha^{N_\alpha}}{N_\alpha!} \tag{I.33}$$

Correspondingly the free energy is

$$F = -k_\mathrm{B} T \ln Q \approx -N_\alpha k_\mathrm{B} T \ln \frac{Q_\alpha e}{N_\alpha} \tag{I.34}$$

where we have used Stirling's approximation $\ln N! \approx N\ln N - N$, and e is the base of the natural logarithm. Applying eq I.6, the entropy is found to be

$$S = N_\alpha k_\mathrm{B} \ln \frac{Q_\alpha e}{N_\alpha} + N_\alpha k_\mathrm{B} T \left(\frac{\partial \ln Q_\alpha}{\partial T} \right)_V \tag{I.35}$$

If we now consider adding more types of molecule to the system, the assumption of non-interaction means that each additional type makes its own additive contribution to F and S.

2. Binding at Constant Pressure

2.1 General Expressions

We now address the binding of two molecules, A and B, to form a complex C under the typical experimental condition of constant pressure. In the reactant state, our system has N_α α molecules, α = A, B, and C, at temperature T and pressure P, and the volume of the system is V. After binding, the numbers of A and B molecules are each decreased by 1 and the number of C molecules is increased by 1; the temperature remains at T. The pressure remains at P, but the volume will likely be slightly different from the initial value V, due to the difference between the partial molar volumes of the reactants and product molecules.

Under constant pressure, the Gibbs free energy is the thermodynamic potential. Here we are interested in the change in Gibbs free energy upon binding. In general a change in Gibbs free energy is given by

$$dG = -SdT + VdP + \sum_\alpha \mu_\alpha dN_\alpha \tag{II.1}$$

where μ_α is the chemical potential of molecule α. Upon binding under constant pressure, $dP = 0$, $dT = 0$, $dN_\mathrm{C} = -dN_\mathrm{A} = -dN_\mathrm{B} = 1$. Therefore the change in Gibbs free energy upon binding under constant pressure is

$$\Delta G_\mathrm{b} = \mu_\mathrm{C} - \mu_\mathrm{A} - \mu_\mathrm{B} \tag{II.2}$$

We now present explicit results for the binding free energy and its entropy and enthalpy components. Our starting point is the expression for the Helmholtz free energy given by eq I.34, here generalized to three species of solute molecules:

$$F = -\sum_{\alpha} N_\alpha k_{\mathrm{B}} T \ln \frac{Q_\alpha e}{N_\alpha} \tag{II.3}$$

The chemical potential of molecule α is

$$\mu_\alpha = \left(\frac{\partial F}{\partial N_\alpha} \right)_{T,V,N_{\alpha' \neq \alpha}} = -k_{\mathrm{B}} T \ln \frac{Q_\alpha}{N_\alpha} \tag{II.4a}$$

The partition function of each molecule is proportional to the volume (e.g., see eq 2.23 of the main text) so we may write

$$\mu_\alpha = -k_{\mathrm{B}} T \ln \left(\frac{Q_\alpha / V}{N_\alpha / V} \right) = -k_{\mathrm{B}} T \ln \left(\frac{Q_\alpha / V}{C_\alpha} \right) \tag{II.4b}$$

to remove the volume dependence from the partition function; C_α is the concentration of molecule α. Using eq II.4b in eq II.2, we find

$$\begin{aligned} \Delta G_{\mathrm{b}} &= -k_{\mathrm{B}} T \ln \left[\frac{C_{\mathrm{A}} C_{\mathrm{B}}}{C_{\mathrm{C}}} \frac{Q_{\mathrm{C}} / V}{(Q_{\mathrm{A}} / V)(Q_{\mathrm{B}} / V)} \right] \\ &= -k_{\mathrm{B}} T \ln \left(\frac{C_{\mathrm{A}} C_{\mathrm{B}}}{C_{\mathrm{C}}} K_{\mathrm{a}} \right) \end{aligned} \tag{II.5}$$

where the binding constant K_{a} is given by eq 3.2 of the main text.

The entropy component of the binding free energy is given by

$$\Delta S_{\mathrm{b}} = -\left(\frac{\partial \Delta G_{\mathrm{b}}}{\partial T} \right)_{P,N_\alpha} \tag{II.6}$$

where the subscripted quantities are held constant when calculating the derivative with respect to temperature. Note that, under the condition of constant P and N_α, the concentrations can vary with temperature due to thermal expansion, so C_α should, in general, be viewed as a function of not only pressure but also temperature. With this in mind, and using eq II.5, we find

$$\Delta S_{\mathrm{b}} = k_{\mathrm{B}} \ln \frac{C_{\mathrm{A}} C_{\mathrm{B}} K_{\mathrm{a}}}{C_{\mathrm{C}}} + k_{\mathrm{B}} T \left(\frac{\partial \ln K_{\mathrm{a}}}{\partial T} \right)_P + k_{\mathrm{B}} T \left(\frac{\partial}{\partial T} \ln \frac{C_{\mathrm{A}} C_{\mathrm{B}}}{C_{\mathrm{C}}} \right)_{P,N_\alpha} \tag{II.7}$$

where the last term results from the temperature dependence of the concentrations. The combination of the three concentrations in this term contributes a single volume factor, so it can be rewritten as

$$k_{\mathrm{B}}T\left(\frac{\partial}{\partial T}\ln\frac{C_{\mathrm{A}}C_{\mathrm{B}}}{C_{\mathrm{C}}}\right)_{P,N_{\alpha}} = k_{\mathrm{B}}T\left(\frac{\partial}{\partial T}\ln\frac{1}{V}\right)_{P} = -\frac{k_{\mathrm{B}}T}{V}\left(\frac{\partial V}{\partial T}\right)_{P} \equiv -k_{\mathrm{B}}T\kappa \qquad \text{(II.8)}$$

where κ is the thermal expansion if the solution. The thermal expansion of aqueous solutions is very small around room temperature. Pure water has $\kappa \sim 2.6 \times 10^{-4}\ \mathrm{K}^{-1}$ at 25 °C, which would lead to a value of $-0.08k_{\mathrm{B}}$ for the last term of eq II.7, and the result for a dilute solution will not be very different. Therefore, for binding in solution, the last term of eq II.7 may be neglected. Substituting the standard concentration $C°$ for C_{A}, C_{B} and C_{C}, then, yields eq 4.1 in the main text for the standard entropy change on binding. On the other hand, if the solute molecules had been treated as ideal gases, as done in the rigid rotor, harmonic oscillator (RRHO) approximation, rather than as solutes in a nearly incompressible solvent, then $\kappa = 1/T$ and the last term would equal $-k_{\mathrm{B}}$. This neglected term will surface again in an explicit calculation of binding entropy given below (eq II.13).

The binding enthalpy can be obtained as

$$\Delta H_{\mathrm{b}} = \Delta G_{\mathrm{b}} - T\Delta S_{\mathrm{b}} \qquad \text{(II.9a)}$$

Upon neglecting the last term of eq II.7, we find

$$\Delta H_{\mathrm{b}} = -k_{\mathrm{B}}T^{2}\left(\frac{\partial \ln K_{\mathrm{a}}}{\partial T}\right)_{P} \qquad \text{(II.9b)}$$

2.2 Example: Formation of a Diatomic Complex with a Harmonic Bond

We now calculate the binding entropy for the model in Subsection 3.1 of the main text, in which two atoms bind to form a diatomic complex held together by a harmonic potential $E(r)$ (eq 3.5) with equilibrium distance r_0 and force constant k.

First we present the result from the RRHO approximateion, $\Delta S^{\circ}_{\mathrm{RRHO}}$. The general treatment in Subsection 5.3.2 of the main text (eqs 5.3 and 5.4) is specialized to the binding of a monatomic receptor with a monatomic ligand, which is also discussed briefly in Subsection 3.1.1 of the main text. $\Delta S^{\circ}_{\mathrm{RRHO}}$ consists of three contributions: $\Delta S^{\circ}_{\mathrm{RRHO,\,t}}$, $\Delta S_{\mathrm{RRHO,\,r}}$, and $\Delta S_{\mathrm{RRHO,\,vib}}$ for the changes in translational, rotational, and vibrational entropies upon binding. The translational contribution is

$$\Delta S^{\mathrm{o}}_{\mathrm{RRHO,\,t}} = -k_{\mathrm{B}} \ln\left[\left(\frac{2\pi m}{\beta h^2}\right)^{\frac{3}{2}} \frac{e^{\frac{5}{2}}}{C^{\circ}}\right] \tag{II.10a}$$

where *m* is the reduced mass of the diatomic complex. The unbound atoms have no rotational degrees of freedom and the diatomic complex has two rotational degrees of freedom. The change in rotational entropy is

$$\Delta S_{\mathrm{RRHO,\,r}} = k_{\mathrm{B}} \ln\left(\frac{8\pi^2 Ie}{\beta h^2}\right) \tag{II.10b}$$

where $I = mr_0^2$ is the moment of inertia of the diatomic complex. Only the diatomic complex has a single vibrational degree of freedom. The change in rotational entropy is

$$\Delta S_{\mathrm{RRHO,\,vib}}(\mathrm{quant}) = k_{\mathrm{B}}\left[\frac{\beta h\omega}{2\pi}\left(e^{\frac{\beta h\omega}{2\pi}} - 1\right)^{-1} - \ln\left(1 - e^{-\frac{\beta h\omega}{2\pi}}\right)\right] \tag{II.10c}$$

where $\omega = (k/m)^{1/2}$ is the angular frequency of vibration, and "(quant)" indicates that this is a quantum statistical mechanical result. The classical limit of this result can be obtained by taking the $\beta \rightarrow 0$ limit, leading to

$$\Delta S_{\mathrm{RRHO,\,vib}}(\mathrm{class}) = k_{\mathrm{B}}\left[1 - \ln\left(\frac{\beta h\omega}{2\pi}\right)\right] \tag{II.10d}$$

Adding up the results of eqs II.10a, II.10b, and II.10b, we find the classical limit of the RRHO binding entropy is

$$\Delta S^{\mathrm{o}}_{\mathrm{RRHO}}(\mathrm{class}) = k_{\mathrm{B}} \ln\left[4\pi r_0^{\,2}\left(\frac{2\pi}{\beta k}\right)^{\frac{1}{2}} C^{\circ}\right] - \frac{k_{\mathrm{B}}}{2} \tag{II.11}$$

The final result is independent of both mass and Planck's constant.

Next we calculate the binding entropy, $\Delta S_{\mathrm{b}}^{\circ}$, by the flexible molecule (FM) approach. The expression for $\Delta S_{\mathrm{b}}^{\circ}$ is given by eq 4.4b of the main text, in which the potential of mean force $W(\mathbf{r})$ is the harmonic energy function $E(r)$. Because of the temperature independence of $E(r)$, $\Delta S_{\mathrm{b}}^{\circ}$ is identical to $\Delta S_{\mathrm{t}}^{\circ}$ of eq 4.5, which is our definition of the change in translational entropy upon binding. Specializing that equation to the present case, we find

$$\Delta S_{\mathrm{t}}^{\circ} = k_{\mathrm{B}} \ln(C^{\circ} K_{\mathrm{a}}) + \frac{1}{T} \frac{\int dr\ 4\pi r^2\ E(r) e^{-\beta E(r)}}{\int dr\ 4\pi r^2\ e^{-\beta E(r)}} \tag{II.12a}$$

in which we have written, $\langle E(r) \rangle$, the average of $E(r)$ in the bound state, in its explicit form in the second term. As noted in Subsection 3.1.2, when the force constant k is great enough that r deviates minimally from r_0, the exact result for K_{a} in eq 3.9 is well approximated by the expression of eq 3.7. A similar approximation allows $\langle E(r) \rangle$, appearing in the second term of eq II.12a, to be written as $E_0 + k_{\mathrm{B}}T/2$. Consequently we have

$$\Delta S_{\mathrm{t}}^{\circ} = k_{\mathrm{B}} \ln\left[4\pi r_0^{\,2} \left(\frac{2\pi}{\beta k}\right)^{\frac{1}{2}} C^{\circ}\right] + \frac{k_{\mathrm{B}}}{2} \tag{II.12b}$$

These expressions for $\langle E(r) \rangle$ and $\Delta S_{\mathrm{t}}^{\circ}$ closely resemble those for a one-dimensional harmonic potential (eq 2.9 of the main text).

Comparing eqs II.11 and II.12b, we find that the results for the binding entropy obtained from the two approaches differ by k_{B}:

$$\Delta S_{\mathrm{RRHO}}^{\circ} = \Delta S_{\mathrm{t}}^{\circ} - k_{\mathrm{B}} \tag{II.13}$$

This discrepancy can be traced to the last term of eq II.7, which, as noted above, has been neglected in deriving the expression for $\Delta S_{\mathrm{b}}^{\circ}$, and hence eq II.12a for $\Delta S_{\mathrm{t}}^{\circ}$.

The calculation for $\Delta S_{\mathrm{t}}^{\circ}$ presented here also provides an opportunity to illustrate V_{b}, the effective volume accessible to the ligand in the bound state for the case of a harmonic, rather than a square-well, potential. V_{b} is obtained by identifying $\Delta S_{\mathrm{t}}^{\circ}$ with $k_{\mathrm{B}}\ln(V_{\mathrm{b}}C^{\circ})$. Neglecting the second, small term of eq II.12b for $\Delta S_{\mathrm{t}}^{\circ}$, we find

$$V_\mathrm{b} = 4\pi r_0^{\,2}\left(\frac{2\pi}{\beta k}\right)^{\frac{1}{2}} \tag{II.14a}$$

This expression appears as the pre-exponential factor of eq 3.7 of the main text. To gain some physical insight into this result, we note that the $(\beta k)^{-1/2}$ gives the root-mean-square deviation $<(r - r_0)^2>^{1/2}$. Therefore

$$V_\mathrm{b} = 4\pi r_0^{\,2}\left\langle 2\pi(r - r_0)^2\right\rangle^{\frac{1}{2}} \tag{II.14b}$$

As expected, V_b is determined by the extent of motions within the binding site, as measured by $<(r - r_0)^2>^{1/2}$.

3. General Expression for $K_\mathbf{a}$

Here we derive the expression for the binding constant given in eq 3.15 of the main text, for the case where both binding partners, A and B, are polyatomic molecules. Following eq 3.10a of the main text, the partition functions of these molecules can be written as

$$Q_\alpha = 8\pi^2 V \int d\mathbf{x}_\alpha J_\alpha(\mathbf{x}_\alpha)^{-\beta E_\alpha(\mathbf{x}_\alpha)} \tag{III.1}$$

where α = A or B. The partition function of the complex is given by a similar expression, where the internal coordinates of C consist of $\mathbf{x}_\mathrm{A}$, $\mathbf{x}_\mathrm{B}$, and six additional coordinates, $\mathbf{r}$ and $\boldsymbol{\omega}$, that specify, respectively, the relative separation and orientation of the two molecules within the complex. That is, $\mathbf{x}_\mathrm{C} = (\mathbf{x}_\mathrm{A}, \mathbf{x}_\mathrm{B}, \mathbf{r}, \boldsymbol{\omega})$. For C, the integration of the partition function is restricted to the region in configurational space where the complex is deemed formed. Inserting these partition functions into eq 3.2 of the main text, we obtain the binding constant as

$$K_\mathrm{a} = \frac{(8\pi^2)^{-1}\int_\mathrm{b} d\mathbf{x}_\mathrm{C} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) J_\mathrm{B}(\mathbf{x}_\mathrm{B}) J(\boldsymbol{\omega}) e^{-\beta E_\mathrm{C}(\mathbf{x}_\mathrm{C})}}{\int d\mathbf{x}_\mathrm{A} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) e^{-\beta E_\mathrm{A}(\mathbf{x}_\mathrm{A})} \int d\mathbf{x}_\mathrm{B} J_\mathrm{B}(\mathbf{x}_\mathrm{B}) e^{-\beta E_\mathrm{B}(\mathbf{x}_\mathrm{B})}} \tag{III.2}$$

As in eq 3.11 of the main text, the potential energy of the complex can be written as

$$E_\mathrm{C}(\mathbf{x}_\mathrm{C}) = E_\mathrm{A}(\mathbf{x}_\mathrm{A}) + E_\mathrm{B}(\mathbf{x}_\mathrm{B}) + w(\mathbf{x}_\mathrm{C}) \tag{III.3}$$

where the third term represents the energy arising from interactions between the binding partners. Following eq 3.13a of the main text, we define a potential of mean force $W(\mathbf{r}, \boldsymbol{\omega})$ by[3,4]

$$e^{-\beta W(\mathbf{r},\boldsymbol{\omega})} = \frac{\int d\mathbf{x}_\mathrm{A} d\mathbf{x}_\mathrm{B} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) J_\mathrm{B}(\mathbf{x}_\mathrm{B}) e^{-\beta E_\mathrm{C}(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\mathbf{r},\boldsymbol{\omega})}}{\int d\mathbf{x}_\mathrm{A} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) e^{-\beta E_\mathrm{A}(\mathbf{x}_\mathrm{A})} \int d\mathbf{x}_\mathrm{B} J_\mathrm{B}(\mathbf{x}_\mathrm{B}) e^{-\beta E_\mathrm{B}(\mathbf{x}_\mathrm{B})}} \tag{III.4}$$

The binding constant can now be written as

$$K_\mathrm{a} = (8\pi^2)^{-1} \int_\mathrm{b} d\mathbf{r} d\boldsymbol{\omega} J(\boldsymbol{\omega})\, e^{-\beta W(\mathbf{r},\boldsymbol{\omega})} \tag{III.5}$$

which is the desired result.

One can further integrate over $\boldsymbol{\omega}$ to define a potential of mean force in $\mathbf{r}$ only:

$$e^{-\beta \overline{W}(\mathbf{r})} = (8\pi^2)^{-1} \int d\boldsymbol{\omega}\ J(\boldsymbol{\omega}) e^{-\beta W(\mathbf{r},\boldsymbol{\omega})} \tag{III.6}$$

Then

$$K_\mathrm{a} = \int_\mathrm{b} d\mathbf{r}\ e^{-\beta \overline{W}(\mathbf{r})} \tag{III.7}$$

For a monatomic ligand, $\overline{W}(\mathbf{r})$ is simply the potential of mean force $W(\mathbf{r})$, and eq III.7 reduces to eq 3.14a of the main text.

4. Effective Concentration in Intramolecular Binding

Here we present a complete formulation of the intramolecular binding constant K_i and then describe the approximation required for deriving the effective concentration. As in the case of intermolecular binding, we assume that there are no new covalent bonds created upon intramolecular receptor-ligand binding. The whole molecule consists of the receptor, the ligand, and the linker joining them. The internal coordinates of the whole molecule are fully specified by $\mathbf{x}_\mathrm{A}$, $\mathbf{x}_\mathrm{B}$, $\boldsymbol{\omega}$, and $\mathbf{x}_\mathrm{L}$, where $\mathbf{x}_\mathrm{A}$ represents the internal coordinates of the receptor which is, for convenience, considered to be fixed translationally and rotationally in the lab frame of reference; $\mathbf{x}_\mathrm{B}$ represents the internal coordinates of the ligand; $\boldsymbol{\omega}$ represents the external rotation of the ligand around its own origin of coordinates; and $\mathbf{x}_\mathrm{L}$ represents the position, orientation, and conformation of the linker (Figure 4A), and therefore implicitly specifies a vector $\mathbf{r}$ from the receptor to the ligand and which specifies the location of the ligand relative to the receptor. A basic premise of this derivation is that the bound state, in both intermolecular binding and intramolecular binding, occupies the same region in the space of receptor-ligand relative translation (**r**) and rotation (**ω**). The intramolecular binding constant is given by the ratio of the partition functions in the bound and unbound states. If the potential energy of the whole molecule is $E(\mathbf{x}_\mathrm{A}, \mathbf{x}_\mathrm{B}, \boldsymbol{\omega}, \mathbf{x}_\mathrm{L})$, then

$$K_\mathrm{i} = \frac{\int_\mathrm{b} d\mathbf{x}_\mathrm{A} d\mathbf{x}_\mathrm{B} d\boldsymbol{\omega} d\mathbf{x}_\mathrm{L} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) J_\mathrm{B}(\mathbf{x}_\mathrm{B}) J(\boldsymbol{\omega}) e^{-\beta E(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\boldsymbol{\omega},\mathbf{x}_\mathrm{L})}}{\int_\mathrm{u} d\mathbf{x}_\mathrm{A} d\mathbf{x}_\mathrm{B} d\boldsymbol{\omega} d\mathbf{x}_\mathrm{L} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) J_\mathrm{B}(\mathbf{x}_\mathrm{B}) J(\boldsymbol{\omega}) e^{-\beta E(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\boldsymbol{\omega},\mathbf{x}_\mathrm{L})}} \tag{IV.1}$$

where the subscripts "b" and "u" signify that the integrations are restricted to the bound and unbound states, respectively.

The potential energy of the whole molecule can be written as (cf. eq III.3)

$$E(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\boldsymbol{\omega},\mathbf{x}_\mathrm{L}) = E_\mathrm{C}(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\mathbf{r},\boldsymbol{\omega}) + E_\mathrm{L}(\mathbf{x}_\mathrm{L}) + w(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\boldsymbol{\omega},\mathbf{x}_\mathrm{L}) \tag{IV.2}$$

where E_L is the potential energy of the linker and w is the linker's noncovalent interaction energy with the receptor and ligand. The approximation that allows K_i to be related to K_a is the neglect of the interaction energy w. Then

$$K_\mathrm{i} = \frac{\int_\mathrm{b} d\mathbf{x}_\mathrm{A} d\mathbf{x}_\mathrm{B} d\boldsymbol{\omega} d\mathbf{x}_\mathrm{L} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) J_\mathrm{B}(\mathbf{x}_\mathrm{B}) J(\boldsymbol{\omega}) e^{-\beta E_\mathrm{C}(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\mathbf{r},\boldsymbol{\omega})} e^{-\beta E_\mathrm{L}(\mathbf{x}_\mathrm{L})}}{8\pi^2 \int d\mathbf{x}_\mathrm{A} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) e^{-\beta E_\mathrm{A}(\mathbf{x}_\mathrm{A})} \int d\mathbf{x}_\mathrm{B} J_\mathrm{B}(\mathbf{x}_\mathrm{B}) e^{-\beta E_\mathrm{B}(\mathbf{x}_\mathrm{B})} \int d\mathbf{x}_\mathrm{L} e^{-\beta E_\mathrm{L}(\mathbf{x}_\mathrm{L})}} \tag{IV.3}$$

where we have replaced E_C in the unbound state by $E_\mathrm{A} + E_\mathrm{B}$ on the consideration that the ligand and receptor have no direct interaction in this state. To proceed further, we make the approximation that the linker does not influence the rotation of the ligand, **ω**, and introduce the intrinsic probability density for the linker's end-to-end vector (**r**),

$$\rho_\mathrm{L}(\mathbf{r}) = \frac{\int d\mathbf{x}_\mathrm{L} \delta(\mathbf{r}' - \mathbf{r}) e^{-\beta E_\mathrm{L}(\mathbf{x}_\mathrm{L})}}{\int d\mathbf{x}_\mathrm{L} e^{-\beta E_\mathrm{L}(\mathbf{x}_\mathrm{L})}} \tag{IV.4}$$

Introducing $\int d\mathbf{r}' \, \delta(\mathbf{r}' - \mathbf{r})$, which equals 1, into the numerator of eq IV.3, and using the above expression to replace the integration over $\mathbf{x}_\mathrm{L}$, we find

$$K_\mathrm{i} = \frac{\int_\mathrm{b} d\mathbf{x}_\mathrm{A} d\mathbf{x}_\mathrm{B} d\mathbf{r}' d\boldsymbol{\omega} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) J_\mathrm{B}(\mathbf{x}_\mathrm{B}) J(\boldsymbol{\omega}) e^{-\beta E_\mathrm{C}(\mathbf{x}_\mathrm{A},\mathbf{x}_\mathrm{B},\mathbf{r}',\boldsymbol{\omega})} \rho_\mathrm{L}(\mathbf{r}')}{8\pi^2 \int d\mathbf{x}_\mathrm{A} J_\mathrm{A}(\mathbf{x}_\mathrm{A}) e^{-\beta E_\mathrm{A}(\mathbf{x}_\mathrm{A})} \int d\mathbf{x}_\mathrm{B} J_\mathrm{B}(\mathbf{x}_\mathrm{B}) e^{-\beta E_\mathrm{B}(\mathbf{x}_\mathrm{B})}} \tag{IV.5a}$$

$$= (8\pi^2)^{-1} \int_\mathrm{b} d\mathbf{r} d\boldsymbol{\omega} \; J(\boldsymbol{\omega}) e^{-\beta W(\mathbf{r},\boldsymbol{\omega})} \rho_\mathrm{L}(\mathbf{r}) \tag{IV.5b}$$

We now consider two extreme situations. In the first, the linker is fully flexible. The ligand is held in the binding site by the potential of mean force $W(\mathbf{r}, \boldsymbol{\omega})$. The integration of eq IV.5b is then dominated by a small region around the energy minimum at $(\mathbf{r}_0, \boldsymbol{\omega}_0)$, and $\rho_\mathrm{L}(\mathbf{r})$ can be replaced by its value at $\mathbf{r}_0$. Hence

$$K_\mathrm{i} = \left[(8\pi^2)^{-1}\int_\mathrm{b} d\mathbf{r}d\boldsymbol{\omega}\, J(\boldsymbol{\omega})e^{-\beta W(\mathbf{r},\boldsymbol{\omega})}\right]\rho_\mathrm{L}(\mathbf{r}_0) = K_\mathrm{a}\rho_\mathrm{L}(\mathbf{r}_0) \tag{IV.5c}$$

which is eq 6.2b of the main text. This probability density $\rho_\mathrm{L}(\mathbf{r})$ can be obtained by modeling the linker as a polymer chain.[5,6] In the opposite extreme, the linker is rigid. Such a linker can be modeled by a delta-function probability density:[7]

$$\rho_\mathrm{L}(\mathbf{r}) = \delta(\mathbf{r} - \mathbf{r}_0)$$

where $\mathbf{r}_0$ is the end vector of the rigid linker relative to the receptor. The unbound state in this case corresponds to a fictitious situation where the ligand is held at $\mathbf{r}_0$ but does not experience an interaction with the receptor. Then

$$K_\mathrm{i} = (8\pi^2)^{-1}\int d\boldsymbol{\omega} J(\boldsymbol{\omega})e^{-\beta W(\mathbf{r}_0,\boldsymbol{\omega})} = e^{-\beta \overline{W}(\mathbf{r}_0)} \tag{IV.5d}$$

It should be recalled that, for a monatomic ligand, $\overline{W}(\mathbf{r}_0)$ is simply the potential of mean force $W(\mathbf{r}_0)$.

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