

Chapter 2

General Theory

2.1 Introduction

This chapter provides the necessary background for the theoretical methods and procedures to be developed in this thesis. In Section 2.2, a statistical mechanical model of molecular association is presented, wherein most of the approximations and assumptions commonly made are explicitly demonstrated and discussed. In Section 2.2.1 we demonstrate the interchangeability of the constant pressure and constant volume ensembles for systems with large enough volumes, justifying the use of the computationally simpler Helmholtz free energy for subsequent calculations. In Section 2.2.2 we introduce the fully quantum mechanical partition function for our solute–solvent system and apply simplifications afforded by the standard approximations. Section 2.2.3 derives the full free energy change associated with molecular association (the binding free energy), taking into account the conformational freedom of the reacting molecules. Section 2.2.4 discusses the properties of the infinite dilution chemical potential of the solute species needed in computing the binding free energy.

The continuum electrostatic approximation of using the linearized Poisson–Boltzmann equation to obtain electrostatic contributions to the binding free energy is derived and discussed in Section 2.3. This approximation and its special properties comprise the basis for much of the work in this thesis. Finally, general conclusions are presented in Section 2.4.

2.2 Molecular Association

In a molecular binding process, two molecules in solution associate into some configuration forming a new molecule, the complex. The reacting molecules are commonly denoted the ligand and the receptor; however technically it does not matter which is which. In general, the reactants and complexes have conformational freedom and individual pairs of reactants may bind together in multiple configurations forming multiple species of complex molecules, or multiple conformations of the same complex species. To analyze the process of molecular binding, consider a solution in which there are N distinct species of solute molecules, consisting of the reactants and their complexes (additional mobile ions may be considered part of the solvent). Denote a molecule of species (i) by

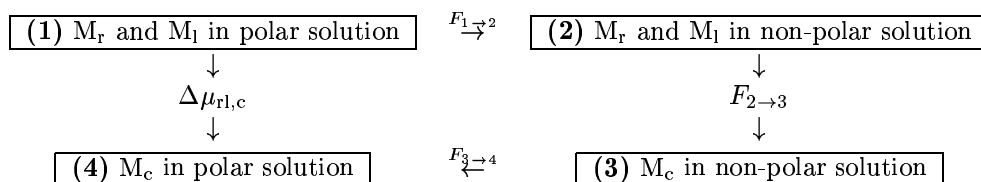


Figure 2.1: Thermodynamic Cycle for Ligand-Receptor Binding

M_i and let there be n_i molecules of each species (i) present in the solution at equilibrium. The association reaction for a ligand molecule M_l and a receptor molecule M_r , which forms a complex molecule M_c , has the form



where $r \neq c$, $l \neq c$, and $\Delta\mu_{r,l,c}$ is the free energy change of the solution due to the binding event. The solution shall be treated in a canonical ensemble with constant volume V , temperature T , and a fixed numbers of solvent molecules.^a The numbers of solute molecules in solution are subject to change due to the reversible process of complex formation.

The free energy of binding can be calculated from the thermodynamic cycle shown in Figure 2.1. Each of the four thermodynamic states in the Figure represent two separate canonical ensembles of volume V and temperature T . One ensemble is the solution ensemble just described. The other reference ensemble is a solution in which solvent consists of effectively non-polar molecules such as benzene. In thermodynamic state **1**, the non-polar solution is devoid of solute molecules and the polar solution contains n_i molecules of each solute species (i). In going from state **1** to state **2**, a ligand and a receptor molecule, M_r and M_l , are transferred into the non-polar solution (without bringing along any molecules designated as part of the solvent). In going from state **2** to state **3**, the ligand and receptor molecules in the non-polar solution have bound together forming a complex molecule M_c . Finally, the complex molecule is transferred back from the non-polar solution in states **3** to **4**. From this description, it is clear that the number of solvent molecules and the numbers of solute molecules of species other than M_r , M_l and M_c in the two solutions remain constant throughout the thermodynamic cycle.

Several common approximations are made to facilitate derivation of the solution free energies. The first approximation neglects direct solute–solute interactions, except for those within chemical complexes. The second approximation assumes that the standard part of the chemical potential of each solute species is independent of their respective concentrations. This assumption effectively ignores solvent-mediated solute–solute interactions. Both of these approximations are appropriate for low solute concentrations, and together they are known as the dilute solution limit.¹⁸ The beneficial result of these assumptions is that the solute species behave like a mixture of ideal gases.

^aThe solvent may be a vacuum (gas-phase molecular association), water with or without ions (aqueous molecular association), or a whole range of other types of solvents from methanol to DMSO.

Also, they imply that solute molecules do not affect the bulk solvent structure; therefore, the only effect of the solvent is the addition of a constant bulk solvent free energy and the introduction of a solvation chemical potential $\Delta\mu_i^{\text{solv}}$ due to solute–solvent interactions for each solute species M_i . The assumption that the solvation chemical potential of the solute is independent of the solute concentration means that $\Delta\mu_i^{\text{solv}}$ is really the free energy of transfer of one molecule M_i from an otherwise empty system of volume V into the solution. This is also infinite dilution chemical potential for transferring a molecule M_i in from ideal gas to solution.^b Note that $\Delta\mu_i^{\text{solv}}$ does not contain any direct intramolecular energy terms.

Additionally, we shall also assume that the solute molecules satisfy Boltzmann statistics.^{19,20} Also known as the classical limit,²⁰ this implies that the number of translational states available to the solute molecules is much larger than the number of solute molecules of each species present. This approximation is very good unless the temperature is close to absolute zero or the pressure is incredibly large. In both of these extreme situations, the effective volume per molecule approaches the cube of the molecule’s thermal wavelength.¹⁸ In this limit, the solute–solute intermolecular interactions can be treated classically,²⁰ however, due the dilute liquid approximation, we are already neglecting these interactions altogether. The solute–solvent and solvent–solvent interactions may still be treated fully quantum mechanically because the solvent may not be dilute.

2.2.1 Constant Pressure vs. Constant Volume Canonical Ensembles

The chemical potentials and free energies of transfer of the molecular species shall be obtained in (N, V, T) canonical ensembles, where volume, temperature and number of particles N are constant.¹⁹ The (N, P, T) ensemble, where pressure P instead of volume is held constant, is particularly important to biological applications, where pressure is generally the constant quantity;²¹ however, here we shall show that at constant pressure and sufficiently large volume, the fluctuation in the volume from equilibrium, V_0 , is negligible, and so the (N, P, T) and (N, V, T) ensembles yield equivalent descriptions of the binding phenomena. We thus may employ the computationally simpler (N, V, T) ensemble.

Let $Q(NPT)$ be the full quantum mechanical partition function for a solute-solvent system in the (N, P, T) ensemble. Similarly, let $Q(NVT)$ be the full quantum mechanical partition function for a solute-solvent system in the (N, V, T) canonical ensemble. Then, $F(NVT) = -kT \ln(Q(NVT))$ is the Helmholtz free energy of the entire system in the (NVT) ensemble.¹⁹ One can use a Laplace transform to relate the two partition functions²²

$$Q(NPT) = \int_0^\infty e^{-\beta PV} Q(NVT) dV = \int_0^\infty e^{-\beta PV - \beta F(NVT)} dV, \quad (2.2)$$

^bSince the binding free energy $\Delta\mu_{r,c}$ involves mainly differences in free energies between the polar and non-polar solutions together with changes in internal molecular free energies (as we shall see more clearly below), the ideal gas reference state will cancel in the differences of $\Delta\mu_i^{\text{solv}}$ between the non-polar and polar solutions. Therefore, one may computationally use a different reference state (such as the non-polar solution) to obtain $\Delta\mu_i^{\text{solv}}$ and obtain equivalent transfer free energies and binding free energies. However, for a rigorous description of this quantity, we must refer to a solution-free ideal gas reference state.

where $\beta = 1/(kT)$. This transformation makes intuitive sense, because it allows the volume to vary weighted by the pressure–volume Boltzmann factor. Expanding the Helmholtz free energy about the average thermodynamic system volume, V_0 ,

$$F(NVT) \approx F(NV_0T) + (V - V_0) \left. \frac{\partial F(NVT)}{\partial V} \right|_{V_0} + \frac{(V - V_0)^2}{2} \left. \frac{\partial^2 F(NVT)}{\partial V^2} \right|_{V_0}, \quad (2.3)$$

and using the thermodynamic relations^{19, 22}

$$\left. \frac{\partial F(NVT)}{\partial V} \right|_{V_0} = -P \quad (2.4)$$

$$\left. \frac{\partial P}{\partial V} \right|_{V_0} = \frac{-1}{V_0 \kappa_T}, \quad (2.5)$$

where κ_T is the isothermal compressibility of the system, we can re-write Eq. 2.2 as

$$Q(NPT) \approx \int_0^\infty dV e^{-\beta PV_0 - \beta F(NV_0T) - \beta(V - V_0)^2 / (2V_0 \kappa_T)} \quad (2.6)$$

$$= Q(NV_0T) e^{-\beta PV_0} \int_0^\infty dV e^{-\beta(V - V_0)^2 / (2V_0 \kappa_T)} \quad (2.7)$$

$$\approx Q(NV_0T) e^{-\beta PV_0} \sqrt{2\pi V_0 \kappa_T kT}. \quad (2.8)$$

Therefore, the $Q(NPT)$ partition function is proportional to the $Q(NVT)$ partition function to second order in the volume fluctuation.

From the expansion of the Helmholtz free energy, Eq. 2.3, the integrand in Eq. 2.7, as in any Gaussian distribution,²³ has a quadratic exponent of the form $\frac{(V - V_0)^2}{2(\overline{\Delta V})^2}$, where $\overline{\Delta V}$ is the standard deviation in the volume fluctuation from V_0 . Therefore,

$$\frac{\overline{\Delta V}}{V_0} = \sqrt{\frac{kT \kappa_T}{V_0}}. \quad (2.9)$$

For sufficiently large volumes V_0 , the fluctuation in volume will be negligible.^c In these cases, the Gibbs free energy, $G = -kT \ln Q(NPT)$, and Helmholtz free energy, F , are related by a constant,

$$G \approx F + PV_0 - \frac{kT}{2} \ln [2\pi V_0 \kappa_T kT]. \quad (2.10)$$

The last term becomes negligible for large volumes, and the thermodynamic relation $G = F + PV_0$ is recovered. Because P and V are both effectively constant in the limit of large volume, their values are independent of the binding processes and thus the Gibbs (N, P, T) and the Helmholtz (N, V, T) free energies will yield equivalent statistical mechanical descriptions of binding.

2.2.2 The Partition Function

In this section we present the fully quantum mechanical solution partition function and show how, based on the dilute liquid and Boltzmann statistics approximations, it may be expressed as the product of a bulk solvent partition function Q^v and a series of ideal gas and solute-solvent factors

^cExcept for certain exceptional situations such as near a phase transition.¹⁸

for the solute molecules. The exact quantum mechanical partition function Q of the solution can be written

$$Q = \text{Tr} e^{-\beta \mathcal{H}(\{x^\nu\}, \{x^\mu\})}, \quad (2.11)$$

where \mathcal{H} is the exact quantum mechanical Hamiltonian for the system, $\{x^\nu\}$ and $\{x^\mu\}$ are the solvent and solute degrees of freedom, respectively, and Tr , the ‘‘trace,’’ is a sum over these degrees of freedom. In this ensemble, the Helmholtz free energy is given by $F = -kT \ln(Q)$ and the chemical potential of solute species (i) is given by¹⁹

$$\mu_i = \frac{\partial F(n_i)}{\partial n_i}, \quad (2.12)$$

where $F(n_i)$ is the Helmholtz free energy when there are n_i molecules of solute species (i) in the system.

In order to compute Q , we first apply the dilute liquid approximation, which allows us to write the Hamiltonian in a factorized form

$$\mathcal{H}(\{x^\nu\}, \{x^\mu\}) = \mathcal{H}^\nu(\{x^\nu\}) + \mathcal{H}^{\mu-\nu}(\{x^\mu\}, \{x^\nu\}) + \mathcal{H}^\mu(\{x^\mu\}). \quad (2.13)$$

\mathcal{H}^ν is the exact quantum mechanical solvent–solvent Hamiltonian, which is free of solute–solvent and solute–solute terms. $\mathcal{H}^{\mu-\nu}$ is the exact quantum mechanical solute–solvent Hamiltonian,

$$\mathcal{H}^{\mu-\nu} = \sum_i^s \sum_j^{n_i} \mathcal{H}_i^{\mu-\nu}(x_{ij}^\mu, \{x^\nu\}) \quad (2.14)$$

which is independent of solute–solute interactions. In this equation, s represents the number of chemically distinct solute species, and x_{ij}^μ are the variables describing the state of the j^{th} solute molecule of species (i). \mathcal{H}^μ is the quantum mechanical intramolecular solute Hamiltonian describing the internal free energy of the solute molecules; it has the form

$$\mathcal{H}^\mu = \sum_i^s \sum_j^{n_i} \mathcal{H}_i^\mu(x_{ij}^\mu), \quad (2.15)$$

independent of the solvent and with no solute–solute intermolecular energy terms.

Next, we re-write Eq. 2.11 in terms of traces over the solute and solvent coordinates

$$Q = \text{Tr}_\nu \left[e^{-\beta \mathcal{H}^\nu} \text{Tr}_\mu e^{-\beta \mathcal{H}^{\mu-\nu}} e^{-\beta \mathcal{H}^\mu} \right], \quad (2.16)$$

where Tr_ν and Tr_μ are traces over all solvent and solute degrees of freedom, respectively. Multiplying and dividing by the bulk solvent partition function $Q^\nu = \text{Tr}_\nu e^{-\beta \mathcal{H}^\nu}$, one obtains

$$Q = Q^\nu \frac{\text{Tr}_\nu \left[e^{-\beta \mathcal{H}^\nu} \text{Tr}_\mu e^{-\beta \mathcal{H}^{\mu-\nu}} e^{-\beta \mathcal{H}^\mu} \right]}{\text{Tr}_\nu e^{-\beta \mathcal{H}^\nu}}. \quad (2.17)$$

the expectation value of the solute–solvent partition function in the bulk solvent, which we shall denote by the subscript ν on the angle brackets

$$Q = Q^\nu \left\langle \text{Tr}_\mu e^{-\beta \mathcal{H}^{\mu-\nu}} e^{-\beta \mathcal{H}^\mu} \right\rangle_\nu. \quad (2.18)$$

Substituting Eqs. 2.14 and 2.15 and taking advantage of the fact that the average of a sum is the sum of the averages, we obtain

$$Q = Q^\nu \text{Tr}_\mu \left\langle \prod_i^s \prod_j^{n_i} e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_{ij}^\mu, \{x^\nu\})} e^{-\beta \mathcal{H}_i^\mu(x_{ij}^\mu)} \right\rangle_\nu. \quad (2.19)$$

Because there are no solute–solute interaction energy terms in the Hamiltonians \mathcal{H}_i^μ , the products may also be brought outside of the average

$$Q = Q^\nu \text{Tr}_\mu \prod_i^s \prod_j^{n_i} \left\langle e^{-\beta \mathcal{H}_i^\mu(x_{ij}^\mu)} e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_{ij}^\mu, \{x^\nu\})} \right\rangle_\nu. \quad (2.20)$$

Another way to see the validity of this step is to realize that the neglect of solute–solute interactions eliminates any correlation of solute properties. Therefore, the solutes are all independent and the average of the products will be the product of the averages. Also, because we are in the dilute liquid approximation, each solute molecule is effectively surrounded by its own independent region of solvent, so one can imagine the expectation value in Eq. 2.19 factoring into expectation values over solvent states in each region.

The next step involves bringing the trace over solute coordinates through the products. Here we use our assumption that the solute molecules satisfy Boltzmann statistics, so that

$$\text{Tr}_\mu = \prod_i^s \text{Tr}_{\{x_i^\mu\}} = \prod_i^s \frac{1}{n_i!} \prod_j^{n_i} \text{Tr}_{x_{ij}}, \quad (2.21)$$

where $\{x_i^\mu\}$ are the variables describing all molecules of species (i) and the second equality is a result of Boltzmann statistics and the indistinguishability of molecules of the same species.^d

Substituting this back into Eq. 2.20, and noting that every solute molecule of the same species must have the same average Boltzmann factor in the solvent,

$$Q = Q^\nu \prod_i^s \frac{1}{n_i!} \prod_j^{n_i} \text{Tr}_{x_{ij}} \left\langle e^{-\beta \mathcal{H}_i^\mu(x_{ij}^\mu)} e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_{ij}^\mu, \{x^\nu\})} \right\rangle_\nu \quad (2.22)$$

$$= Q^\nu \prod_i^s \frac{1}{n_i!} \left[\text{Tr}_{x_i} \left\langle e^{-\beta \mathcal{H}_i^\mu(x_i^\mu)} e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_i^\mu, \{x^\nu\})} \right\rangle_\nu \right]^{n_i} \quad (2.23)$$

$$= Q^\nu \prod_i^s \frac{1}{n_i!} [Q_i^0]^{n_i}, \quad (2.24)$$

where Q_i^0 is an effective infinite-dilution partition function for a single solute molecule of species (i) in bulk solvent. We have dropped the molecule index ‘ j ’ because all solute molecules of the same species are equivalent. So x_i^μ are the degrees of freedom describing some particular solute molecule of species (i), whereas $\{x_i^\mu\}$ represent the set of degrees of freedom representing all molecules of species (i).

^dProtein molecules are large enough that in solution there may be many isotopically distinguishable variants. In practice, these variations are usually ignored and they are treated as indistinguishable. However, a correct treatment would consider each isotopic variant as a different molecular species present in solution with different internal energetics.

The single-solute infinite-dilution partition functions

$$Q_i^0 = \text{Tr}_{x_i} \left\langle e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_i^{\mu}, \{x^{\nu}\})} e^{-\beta \mathcal{H}_i^{\mu}(x_i^{\mu})} \right\rangle_{\nu} \quad (2.25)$$

must be computed to gain additional insight into the binding processes. Up to now, all solute conformational freedom has been implicit in the Hamiltonian \mathcal{H}^{ν} which, among other things, imposes all connectivity and steric constraints and restraints on the components of each molecule. Without loss of generality, we shall now make the conformational freedom of each solute explicit by factoring out a trace over all allowed molecular conformations $\{c_i\}$; this may be a discrete set or a continuous distribution,

$$Q_i^0 = \text{Tr}_j^{\{c_i\}} \text{Tr}_{x_{i,j}} \left\langle e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_{i,j}^{\mu}, \{x^{\nu}\})} e^{-\beta \mathcal{H}_i^{\mu}(x_{i,j}^{\mu})} \right\rangle_{\nu}, \quad (2.26)$$

where $x_{i,j}$ are the degrees of freedom describing a molecule of species (i) in the ‘‘rigid’’ conformation j . This is not to be confused with the notation x_{ij} representing the degrees of freedom of molecule j of species (i), which will no longer be used in this chapter.

By ‘‘rigid’’ we mean a fixed arrangement of the molecule’s atoms produced by a certain choice of torsional angles. Degrees of freedom such as nuclear, electronic, and vibrational motion are much faster than rotations about torsions and molecular association reactions. We assume that these fast degrees of freedom are separable in the Hamiltonian so that their contribution to the free energy may be independently determined. This approximation may be systematically corrected by adding small interaction terms to the Hamiltonian, but this is often not done. In fact, these small corrections and, indeed many of the internal partition functions, are often unaffected by the binding process or conformational change, and thus do not contribute to the overall free energy of binding anyway. The equilibrium positions of the atoms, with respect these fast degrees of freedom, are used as the coordinates for the conformation based on the specified set of torsional angles.

In order to proceed, we must make the form of the Hamiltonian more explicit. As discussed above, we assume that the intramolecular Hamiltonian is separable into independent nuclear, electronic, vibrational, rotational, translational, and conformational terms²⁰

$$\mathcal{H}_i^{\mu} = \mathcal{H}_i^{\mu,\text{nuc}} + \mathcal{H}_i^{\mu,\text{elec}} + \mathcal{H}_i^{\mu,\text{vib}} + \mathcal{H}_i^{\mu,\text{rot}} + \mathcal{H}_i^{\mu,\text{trans}} + \mathcal{H}_i^{\mu,\text{conf}}. \quad (2.27)$$

Because all terms are assumed to be independent of the solvent, the traces over the degrees of freedom describing them may be factored out of the expectation value in Eq. 2.26 and evaluated using the standard ideal gas partition functions for a polyatomic molecule,²⁰

$$Q_i^0 = \mathcal{Z}_i^T \text{Tr}_j^{\{c_i\}} \mathcal{Z}_{i,j}^I e^{-\beta \mathcal{H}_i^{\mu,\text{conf}}(x_{i,j}^{\mu})} \left\langle e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_{i,j}^{\mu}, \{x^{\nu}\})} \right\rangle_{\nu}, \quad (2.28)$$

where

$$\mathcal{Z}_i^T = V \left(\frac{2\pi m_i kT}{h^2} \right)^{3/2} \quad (2.29)$$

is the translational partition function²⁰ of solute species (i), m_i is the mass of the molecule, h is Planck’s constant, and $\mathcal{Z}_{i,j}^I$ is the internal partition function for conformation j (i.e., the traces over the nuclear, electronic, rotational and vibrational degrees of freedom).

Let us define $E_{i,j}^0 = H_i^{\mu,\text{conf}}(x_{i,j}^\mu)$ as the internal conformational energy of the molecule in conformation j . This term includes torsion and angle strain, electrostatic and van der Waals energies associated with the intramolecular interactions that change with conformation. We shall also identify the solvation chemical potential

$$\Delta\mu_{i,j}^{\text{solv}} = -kT \ln \left\langle e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_{i,j}^\mu, \{x^\nu\})} \right\rangle_\nu \quad (2.30)$$

with the Boltzmann-averaged solute-solvent interaction energy of the rigid molecule in the specified conformation with the solvent. This chemical potential is equivalent to the free energy of transfer of a molecule of species (i) in conformation j from infinite dilution in an ideal gas to infinite dilution in the solution. The partition function Eq. 2.26 can now be completely re-written as

$$Q_i^0 = \mathcal{Z}_i^T \text{Tr}_j^{\{c_i\}} \mathcal{Z}_{i,j}^I e^{-\beta(E_{i,j}^0 + \Delta\mu_{i,j}^{\text{solv}})}. \quad (2.31)$$

From Eq. 2.24 the Helmholtz free energy of the entire solution is

$$F = F^\nu + kT \sum_i^s \left[\ln n_i! - n_i \ln \mathcal{Z}_i^T - n_i \ln \left(\text{Tr}_j^{\{c_i\}} \mathcal{Z}_{i,j}^I e^{-\beta(\Delta\mu_{i,j}^{\text{solv}} + E_{i,j}^0)} \right) \right]. \quad (2.32)$$

2.2.3 Binding Free Energy

Traversing each leg of the thermodynamic cycle in Figure 2.1 results in a change in free energy as denoted in the Figure as particles move to and from non-polar solvent and are allowed to interact. The leg of interest, $1 \rightarrow 4$, yields the overall free energy of binding, $\Delta\mu_{\text{r1,c}}$. The free energy changes along the other legs, when summed, must equal $\Delta\mu_{\text{r1,c}}$. The free energy change along each of these other legs is given by the difference in total free energy of the states involved.

The total free energy of any state is given by the sum of the Helmholtz free energy of the polar solution, Eq. 2.32, and the non-polar solution for that state. Equating paths in the thermodynamic cycle and using Eq. 2.32, one has

$$\begin{aligned} F_{1 \rightarrow 4} &= F_{1 \rightarrow 2} + F_{2 \rightarrow 3} + F_{3 \rightarrow 4} \\ \Delta\mu_{\text{r1,c}} &= (F_2 - F_1) + (F_3 - F_2) + (F_4 - F_3) \\ &= F_4 - F_1 \\ &= kT \ln \left[V \frac{(n_c + 1)}{n_r n_l} \left(\frac{2\pi m_r m_l kT}{(m_r + m_l) \hbar^2} \right)^{3/2} \right] \\ &\quad + kT \ln \left[\text{Tr}_{j,k}^{\{c_r\}, \{c_l\}} \mathcal{Z}_{r,j}^I \mathcal{Z}_{l,k}^I e^{-\beta(E_{r,j}^0 + \Delta\mu_{r,j}^{\text{solv}} + E_{l,k}^0 + \Delta\mu_{l,k}^{\text{solv}})} \right] \\ &\quad - kT \ln \left[\text{Tr}_j^{\{c_c\}} \mathcal{Z}_{c,j}^I e^{-\beta(E_{c,j}^0 + \Delta\mu_{c,j}^{\text{solv}})} \right], \end{aligned} \quad (2.33)$$

where it has been assumed that that mass is conserved in complex formation, $m_c = m_r + m_l$, and that there are initially n_r molecules of species M_r , n_l molecules of type M_l and n_c molecules of complex species M_c . Also, it has been assumed that the receptor and ligand molecules are of different species.^e The free energy of binding, in the current approximation, does not depend on the

^eIf they are molecules of the same species, then the term $n_r n_l$ becomes $n_r(n_r - 1)$ in this and all future equations.

presence of other solute species and the dependence on the solvent enters only through the solvation chemical potentials.

In the special case when the receptor, ligand and complex molecules have only single rigid conformations j , k , and p , respectively, Eq. 2.33 reduces to the ‘‘rigid free energy of binding’’

$$\begin{aligned} \Delta\mu_{r1,c}^{jk,p} &= kT \ln \left[V \frac{(n_c + 1)}{n_r n_l} \left(\frac{2\pi m_r m_l kT}{(m_r + m_l)h^2} \right)^{3/2} \frac{Z_{r,j}^I Z_{1,k}^I}{Z_{c,p}^I} \right] \\ &\quad + \Delta\mu_{c,p}^{\text{solv}} - \Delta\mu_{r,j}^{\text{solv}} - \Delta\mu_{1,k}^{\text{solv}} + E_{c,p}^0 - E_{r,j}^0 - E_{1,k}^0. \end{aligned} \quad (2.34)$$

Now, of all pairs of unbound ligand and receptor conformations, it is possible that only a subset of these may be able to bind forming a specific complex. Therefore, the trace in the second term of Eq. 2.33 may be broken up into pairs which bind forming the specified complex (bind) and those which do not (nb)

$$\begin{aligned} \Delta\mu_{r1,c} &= kT \ln \left[V \frac{(n_c + 1)}{n_r n_l} \left(\frac{2\pi m_r m_l kT}{(m_r + m_l)h^2} \right)^{3/2} \right] \\ &\quad + kT \ln \left[\text{Tr}_{j,k}^{\text{nb}} Z_{r,j}^I Z_{1,k}^I e^{-\beta(E_{r,j}^0 + \Delta\mu_{r,j}^{\text{solv}} + E_{1,k}^0 + \Delta\mu_{1,k}^{\text{solv}})} \right] \\ &\quad + kT \ln \left[\text{Tr}_{j,k}^{\text{bind}} Z_{r,j}^I Z_{1,k}^I e^{-\beta(E_{r,j}^0 + \Delta\mu_{r,j}^{\text{solv}} + E_{1,k}^0 + \Delta\mu_{1,k}^{\text{solv}})} \right] \\ &\quad - kT \ln \left[\text{Tr}_j^{\{c_c\}} Z_{c,j}^I e^{-\beta(E_{c,j}^0 + \Delta\mu_{c,j}^{\text{solv}})} \right]. \end{aligned} \quad (2.35)$$

The last two terms can be combined to form

$$kT \ln \left[\frac{\text{Tr}_{j,k}^{\text{bind}} Z_{r,j}^I Z_{1,k}^I e^{-\beta(E_{r,j}^0 + \Delta\mu_{r,j}^{\text{solv}} + E_{1,k}^0 + \Delta\mu_{1,k}^{\text{solv}})}}{\text{Tr}_j^{\{c_c\}} Z_{c,j}^I e^{-\beta(E_{c,j}^0 + \Delta\mu_{c,j}^{\text{solv}})}} \right] \quad (2.36)$$

which, bringing the terms in the numerator into the denominator, is

$$kT \ln \left[\text{Tr}_{j,k}^{\text{bind}} \frac{1}{\text{Tr}_p^{\{c_c\}} \frac{Z_{c,p}^I}{Z_{r,j}^I Z_{1,k}^I} e^{-\beta(E_{c,p}^0 + \Delta\mu_{c,p}^{\text{solv}} - E_{r,j}^0 - \Delta\mu_{r,j}^{\text{solv}} - E_{1,k}^0 - \Delta\mu_{1,k}^{\text{solv}})}} \right] \quad (2.37)$$

This form can be fed back into Eq. 2.35 and merged with the first term there to yield

$$\begin{aligned} \Delta\mu_{r1,c} &= kT \ln \left[\text{Tr}_{j,k}^{\text{nb}} Z_{r,j}^I Z_{1,k}^I e^{-\beta(E_{r,j}^0 + \Delta\mu_{r,j}^{\text{solv}} + E_{1,k}^0 + \Delta\mu_{1,k}^{\text{solv}})} \right] \\ &\quad + kT \ln \left[V \frac{(n_c + 1)}{n_r n_l} \left(\frac{2\pi m_r m_l kT}{(m_r + m_l)h^2} \right)^{3/2} \right. \\ &\quad \left. \times \text{Tr}_{j,k}^{\text{bind}} \frac{1}{\text{Tr}_p^{\{c_c\}} \frac{Z_{c,p}^I}{Z_{r,j}^I Z_{1,k}^I} e^{-\beta(E_{c,p}^0 + \Delta\mu_{c,p}^{\text{solv}} - E_{r,j}^0 - \Delta\mu_{r,j}^{\text{solv}} - E_{1,k}^0 - \Delta\mu_{1,k}^{\text{solv}})}} \right] \end{aligned} \quad (2.38)$$

Comparing this to equation Eq. 2.34, one can see how the total free energy of binding for conformationally flexible molecules is a function of the free energies of binding of their sets of rigid conformations

$$\Delta\mu_{r1,c} = kT \ln \left[\sum_{j,k}^{\text{nb}} Z_{r,j}^I Z_{1,k}^I e^{-\beta(E_{r,j}^0 + \Delta\mu_{r,j}^{\text{solv}} + E_{1,k}^0 + \Delta\mu_{1,k}^{\text{solv}})} \right] + kT \ln \left[\sum_{j,k}^{\text{bind}} \frac{1}{\sum_p^{\{c_c\}} e^{-\beta\Delta\mu_{r1,c}^{jk,p}}} \right], \quad (2.39)$$

plus a term to account for the entropy loss upon binding due to loss of conformational freedom. In the case where an individual ligand and receptor can bind in different conformations to form different complex molecules, the second term may be modified to include a sum over complexes formed for each ligand and receptor configuration

$$\begin{aligned} \Delta\mu_{\text{rl,c}} = & kT \ln \left[\text{Tr}_{j,k}^{\text{nb}} Z_{\text{r},j}^I Z_{\text{l},k}^I e^{-\beta(E_{\text{r},j}^0 + \Delta\mu_{\text{r},j}^{\text{solv}} + E_{\text{l},k}^0 + \Delta\mu_{\text{l},k}^{\text{solv}})} \right] \\ & + kT \ln \left[\text{Tr}_{\text{c}}^{\text{allowed}} \text{Tr}_{j,k}^{\text{bindc}} \frac{1}{\sum_p^{\{\text{c}_c\}} e^{-\beta\Delta\mu_{\text{rl,c}}^{j,k,p}}} \right]. \end{aligned} \quad (2.40)$$

Now, if one can compute the free energies of binding, internal partition functions and configurational energies for a set of rigid reactants and complexes, one can explicitly compute the total free energy of binding.

For any particular rigid conformation, the conformational free energy $E_{i,j}^0$ can be obtained a number a ways, including using quantum mechanics and empirical molecular force fields such as the CHARMM PARAM19 parameter set²⁴ or the AMBER parameter set.²⁵ The translational and rotational partition functions are straight forward to evaluate, knowing the mass distribution of the molecular species. It can be seen from Eq. 2.40 that free energy loss due to restriction of translational, rotational and internal degrees of freedom is fully included. The internal partition functions can be obtained, in the gas phase, to various degrees of accuracy using standard methods.^{18,20,26,27} Obtaining the solvation chemical potentials is more difficult and is the subject of the next section.

2.2.4 Solvation Chemical Potential

Perhaps the most non-trivial term in the binding free energy, Eq. 2.40, is the solvation chemical potential of the rigid solute species, $\Delta\mu_{i,j}^{\text{solv}}$. This term is complicated due to the many-body nature of solvation and the long-range effects that a solute molecule can have on the solvent. There are many ways of attempting to compute this quantity including integral equation theories²⁸⁻³¹ (also see Appendix B), molecular dynamics, and Monte Carlo simulations.³²⁻³⁷ However, simulation methods are often very computationally expensive and difficult to converge,³⁸⁻⁴¹ and integral equation methods are moderately accurate only for very small solutes.

In order to compute the infinite-dilution chemical potential in an approximate but efficient and relatively fast way, it is usually broken up into independent non-polar and electrostatic terms.^{1,2} Of course, all solute–solvent interactions are inherently electrostatic in nature; i.e., van der Waals interactions are merely the mutual attractions of induced dipoles coupled with the Pauli exclusion principle. However, most molecules have what appear to be net electrostatic charge distributions: dipoles, quadrupole and even formal monopole charges. Unlike the induced dipoles of van der Waals interactions, these charge distributions fluctuate about a non-zero average. We define the “electrostatic” component of the chemical potential as the Boltzmann average interaction of this formal average electrostatic charge distribution with the solvent. The “non-polar” component of the chemical potential contains all other solute–solvent interaction terms.

Because a solute molecule M_i of species i has atomic coordinates which are conformationally fixed,^f the shape and conformation of the solute is effectively constant in the determination of the solvent average

$$\Delta\mu_{i,j}^{\text{solv}} = -kT \ln \left\langle e^{-\beta \mathcal{H}_i^{\mu-\nu}(x_{i,j}^{\mu}, \{x^{\nu}\})} \right\rangle_{\nu}.$$

Assuming that the electrostatic solute–solvent interactions due to the formal electrostatic charge distribution of the solute, $\rho_{i,j}(\mathbf{x})$, is separable from the non-electrostatic energy terms,

$$\mathcal{H}_i^{\mu-\nu}(x_{i,j}^{\mu}, \{x^{\nu}\}) = \mathcal{H}_i^{\mu-\nu, \text{non-polar}}(x_{i,j}^{\mu}, \{x^{\nu}\}) + \int d\mathbf{x} \rho_{i,j}(\mathbf{x}) \Phi^{\nu}(\mathbf{x}), \quad (2.41)$$

where $\Phi^{\nu}(\mathbf{x})$ is the net electrostatic potential felt by the solute due to a particular configuration of solvent.

The non-polar contribution to the solvation chemical potential is the hypothetical chemical potential obtained when $\rho_{i,j}^{\mu}(\mathbf{x}) = 0$,

$$\Delta\mu_{i,j}^{\text{solv, non-polar}} = -kT \ln \left\langle e^{-\beta \mathcal{H}_i^{\mu-\nu, \text{non-polar}}(x_{i,j}^{\mu}, \{x^{\nu}\})} \right\rangle_{\nu}. \quad (2.42)$$

It is in general difficult to compute this term; however, it has been found^{42,43} that it scales roughly with the solvent-accessible surface area^g (SAS) of the solute molecule. The coefficient of proportionality between SAS and the non-polar chemical potential contribution is determined by fitting to experimental free energies of transfer of hydrocarbons from a non-polar hydrocarbon environment to water.^h It has been found that the constant of proportionality varies between 5 and 75 cal/mol-Å² depending on the solvent, fitting method, and parameters used, and has some dependence on the curvature of the solute surface.⁴³ In any case, with a suitable choice of parameters, reasonable non-polar contribution to the chemical potential may be obtained with little computational cost. This leaves the evaluation of the electrostatic component, which is the subject of the next section.

2.3 Continuum Electrostatics

The electrostatic component of the solvation chemical potential is given by

$$\Delta\mu_{i,j}^{\text{solv, es}} = \Delta\mu_{i,j}^{\text{solv}} - \Delta\mu_{i,j}^{\text{solv, non-polar}}, \quad (2.43)$$

and can be re-expressed in terms of the solvent averages

$$\Delta\mu_{i,j}^{\text{solv, es}} = -kT \ln \left[\frac{\left\langle e^{-\beta (\mathcal{H}_i^{\mu-\nu, \text{non-polar}}(x_{i,j}^{\mu}, \{x^{\nu}\}) + \int d\mathbf{x} \rho_{i,j}(\mathbf{x}) \Phi^{\nu}(\mathbf{x}))} \right\rangle_{\nu}}{\left\langle e^{-\beta \mathcal{H}_i^{\mu-\nu, \text{non-polar}}(x_{i,j}^{\mu}, \{x^{\nu}\})} \right\rangle_{\nu}} \right]. \quad (2.44)$$

^fThey only move with the fast internal molecular degrees of freedom. Solute–solvent interactions are considered slow and thus we may fix the solute coordinates at their equilibrium values for conformation j in order to obtain the chemical potential $\Delta\mu_{i,j}^{\text{solv}}$.

^gThe solvent-accessible surface area is defined as the locus of points of closest approach to the solute molecule of a water molecule.^{44,45}

^hThis is exactly the situation presented here: a transfer from non-polar solution to, for example, water, a polar solution. We have already discussed that it is only this difference that actually arises in the overall binding free energies, as the ideal-gas states of the molecules cancel in the thermodynamic cycle, Figure 2.1

Looking back at Eq. 2.17 for the original definition of the solvent expectation value, the explicit form of Eq. 2.44 is

$$\Delta\mu_{i,j}^{\text{solv,es}} = -kT \ln \left[\frac{\text{Tr}_\nu e^{-\beta\mathcal{H}^\nu} e^{-\beta(\mathcal{H}_i^{\mu-\nu,\text{non-polar}}(x_{i,j}^\mu, \{x^\nu\}) + \int d\mathbf{x} \rho_{i,j}(\mathbf{x}) \Phi^\nu(\mathbf{x}))}}{\text{Tr}_\nu e^{-\beta\mathcal{H}^\nu} e^{-\beta\mathcal{H}_i^{\mu-\nu,\text{non-polar}}(x_{i,j}^\mu, \{x^\nu\})}} \right]. \quad (2.45)$$

The expectation value $\langle \cdot \rangle_\nu$ represents the Boltzmann-weighted expectation value over all bulk-solvent states, even those wherein solvent molecules overlap the solute molecule. We now define $\langle \cdot \rangle_{\nu,i,j}$ as the Boltzmann-weighted expectation value over all bulk solvent states, with the non-polar solute-solvent interaction energy of solute species (i) in conformation j factored in, so that states where the solute and solvent overlap are suppressed by this additional Boltzmann factor. Eq. 2.45 is then more simply written as

$$\Delta\mu_{i,j}^{\text{solv,es}} = -kT \ln \left\langle e^{-\beta \int d\mathbf{x} \rho_{i,j}(\mathbf{x}) \Phi^\nu(\mathbf{x})} \right\rangle_{\nu,i,j}, \quad (2.46)$$

the expectation value of the electrostatic Boltzmann-factor in the electrostatically-unperturbed ensemble.

Now, consider the functional derivative of Eq. 2.46 with respect to $\rho_{i,j}(\mathbf{x})$,

$$\frac{\partial}{\partial \rho_{i,j}(\mathbf{x})} \Delta\mu_{i,j}^{\text{solv}} = \langle \Phi^\nu(\mathbf{x}) \rangle_{\nu,i,j}. \quad (2.47)$$

Defining $d\rho_{i,j}(\mathbf{x}; \lambda) = \rho_{i,j}(\mathbf{x})d\lambda$, one can write²⁰

$$\Delta\mu_{i,j}^{\text{solv,es}} = \int d\mathbf{x} \rho_{i,j}(\mathbf{x}) \int_0^1 d\lambda \langle \Phi^\nu(\mathbf{x}; \lambda) \rangle_{\nu,i,j}. \quad (2.48)$$

In this expression, $\langle \Phi^\nu(\mathbf{x}; \lambda) \rangle_{\nu,i,j}$ is the Boltzmann-average electrostatic potential at \mathbf{x} due to the solvent, when the solute is charged to a degree λ . Without loss of generality, we may assume that the solute molecule in question is located at the center of the coordinate system.

In order to obtain an expression for $\langle \Phi^\nu(\mathbf{x}; \lambda) \rangle_{\nu,i,j}$, we examine the non-ionic solvent and mobile solvent ions separately. The non-ionic solvent is treated as a polarizable continuum with dielectric constant ϵ .⁴⁶ Space is divided into two regions — solvent and solute, see Figure 2.2. The solvent region consists of all space accessible to solvent and is assigned a dielectric constant ϵ . The boundary between these regions is typically obtained by rolling a probe solvent molecule over the surface of the solute and obtaining the locus of points of contact. The interior solute region (which actually may include some volume exterior to the solute but inaccessible to the solvent — see Figure 2.2) contains the solute charge distribution $\rho_{i,j}(\mathbf{x})$ and is assigned a dielectric constant ϵ_m . The solute dielectric constant should technically be 1; however, as molecular vibrations, polarization and other fast degrees of freedom are not usually accounted for in the sum over allowed conformations, a value of greater than 1 is typically used to account for them in a mean field sense.

The contribution to $\langle \Phi^\nu(\mathbf{x}; \lambda) \rangle_{\nu,i,j}$ due to the polarizable continuum solvent is obtained by solving the Poisson equation,⁴⁶

$$\nabla \cdot (\epsilon(\mathbf{x}) \nabla \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu,i,j}) = -4\pi \lambda \rho_{i,j}(\mathbf{x}), \quad (2.49)$$

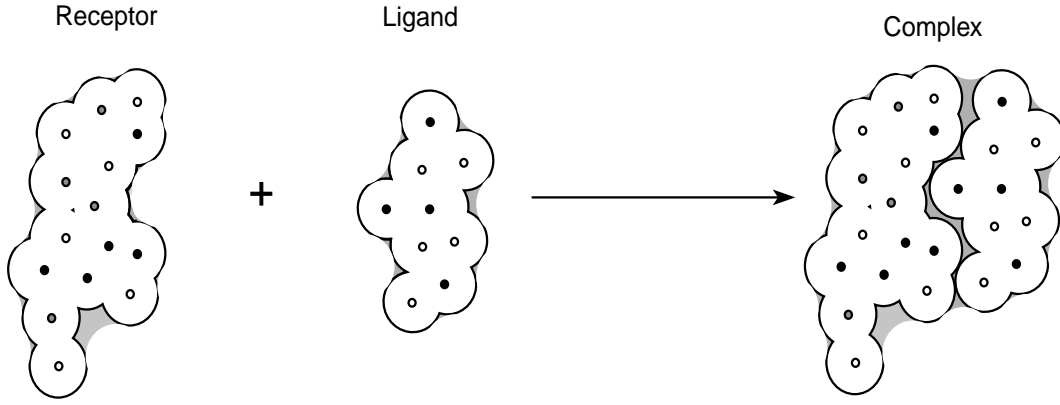


Figure 2.2: Illustration of a general rigid binding process wherein a receptor and ligand associate forming a complex. The small circles represent example locations of atomic centers shaded to indicate possible differences in charge. The solid line encloses the atomic volume of the molecules, which is essentially the union of the spherical volumes occupied by the molecule's atoms. The shaded region outside the atomic volume represents the additional volume assigned to the molecule when the molecular surface is used to designate the molecule–solvent boundary. This excess volume arises because the polarizable solvent molecules cannot fit into all of the crevices left by the atomic volume. The region exterior to this is the solvent volume.

where $\epsilon(\mathbf{x})$ is the position dependent dielectric constant of space (ϵ in the solvent and ϵ_m in the solute). However, as this gives the total electrostatic potential $\langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j}$, one must subtract out the Coulombic potential of the solute,

$$\langle \Phi^\nu(\mathbf{x}; \lambda) \rangle_{\nu, i, j} = \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j} - \lambda \int d\mathbf{x}' \frac{\rho_{i, j}(\mathbf{x}')}{\epsilon_u |\mathbf{x} - \mathbf{x}'|} \quad (2.50)$$

to obtain the polarization potential due to the solvent.

Now we shall incorporate the additional effect that mobile solvent ions may have on the solvent electrostatic potential. Denoting the equilibrium density of ionic species (k) by $c_k(\mathbf{x}, \lambda)$, the electrostatic charge distribution of the ions isⁱ

$$\rho^{\text{ion}}(\mathbf{x}, \lambda) = \sum_k q_k c_k(\mathbf{x}, \lambda), \quad (2.51)$$

where q_k is the net charge of ionic species (k). It is generally assumed that the ionic concentration is small, so that the solvent dielectric constant can be approximated to be independent of ionic concentration. To obtain $\langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j}$ for the full solvent system with ions present, one must solve

$$\nabla \cdot \left(\epsilon(\mathbf{x}) \nabla \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j} \right) = -4\pi \lambda \rho_{i, j}(\mathbf{x}) - 4\pi \rho^{\text{ion}}(\mathbf{x}, \lambda), \quad (2.52)$$

because, at equilibrium, the ionic species merely contribute an additional net charge distribution within the solvent. Of course, the distributions $c_k(\mathbf{x}, \lambda)$ must still be obtained. The centers of the

ⁱIons are treated as spheres with formal charges located at their centers. There is no provision for excluding ion-ion overlap, as they are treated in a mean-field sense.

ions are explicitly excluded from approaching any closer to the solute than would be physically possible of real ions. This is done by creating a Stern layer^{47, 48} described by the locus of points of the center of an ion-sized sphere (typically taken to be 2.0 Å in radius) as it is rolled over the solute molecule. I.e. ion centers cannot approach as closely as the continuum solvent can.

In order to explicitly determine the equilibrium density of the ions, we examine their individual chemical potentials. At equilibrium, the chemical potential of each ionic species μ_k must be uniform throughout the accessible regions of the solution⁴⁹

$$\mu_k(\mathbf{x}, \lambda) = \mu_k^0 + kT \ln a_k(\mathbf{x}, \lambda) + q_k \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, k}, \quad (2.53)$$

where μ_k^0 is the standard-state chemical potential of ionic species (k). Note that by assigning $q_k \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j}$ to be the potential of mean force on the ions, we have made the first approximation of Debye–Hückel theory.²⁰ The activity $a_k(\mathbf{x}, \lambda)$ is usually assumed to be equal to the local ionic concentration,^{18, 49} $a_k(\mathbf{x}, \lambda) = c_k(\mathbf{x}, \lambda)$. In the bulk, the average electrostatic potential is zero and the chemical potential of each ionic species is

$$\mu_k^b = \mu_k^0 + kT \ln c_k^b. \quad (2.54)$$

Equating Eqs. 2.53 and 2.54, we obtain an expression for the equilibrium ionic concentration in ion-accessible regions

$$c_k(\mathbf{x}, \lambda) = c_k^b e^{-\beta q_k \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j}}. \quad (2.55)$$

The net ionic charge density is then

$$\rho^{\text{ion}}(\mathbf{x}, \lambda) = \sum_k q_k c_k^b e^{-\beta q_k \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j}}. \quad (2.56)$$

Substitution of this expression into the Poisson equation results in the Poisson–Boltzmann equation, a complicated non-linear expression.

The second Debye–Hückel approximation²⁰ involves linearizing the charge density by expanding the exponentials to first order,

$$\rho^{\text{ion}}(\mathbf{x}, \lambda) = \sum_k q_k c_k^b - \beta \sum_k q_k^2 c_k^b \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j}. \quad (2.57)$$

Imposition of an electroneutrality constraint results a vanishing of the first term.^j Defining the inverse Debye screening length as

$$\kappa(\mathbf{x}) = \left\{ \begin{array}{ll} 0 & \text{Inside the Stern Layer} \\ \sqrt{\frac{8\pi I}{kT\epsilon}} & \text{Elsewhere} \end{array} \right\}, \quad (2.58)$$

with the bulk ionic strength $I = \frac{1}{2} \sum_i q_i^2 c_i^b$, the Poisson equation, Eq. 2.52, becomes the linearized Poisson–Boltzmann equation,

$$\nabla \cdot \left(\epsilon(\mathbf{x}) \nabla \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j} \right) - \epsilon \kappa^2(\mathbf{x}) \langle \Phi(\mathbf{x}; \lambda) \rangle_{\nu, i, j} = -4\pi \lambda \rho_{i, j}(\mathbf{x}). \quad (2.59)$$

^jIf the solute proteins are charged, overall electroneutrality may result in an imbalance in the bulk concentrations of the positive and negative ions. This would result in a non-zero first term of Eq. 2.57. However, because we are in the dilute liquid approximation, the bulk concentration of solute will be much less than the bulk concentration of the mobile ions, so this discrepancy can be neglected compared to the second term in Eq. 2.57.

The solution to this equation, under the appropriate boundary conditions,⁴⁶ gives the total electrostatic potential in space. Eq. 2.50 then yields $\langle \Phi^\nu(\mathbf{x}; \lambda) \rangle_{\nu,i,j}$, the total electrostatic potential from the solvent polarization and ionic screening resulting from the existence of the solute charge distribution. From the linearity of Eq. 2.59, it is not difficult to see that

$$\langle \Phi^\nu(\mathbf{x}; \lambda) \rangle_{\nu,i,j} = \lambda \langle \Phi^\nu(\mathbf{x}; \lambda = 1) \rangle_{\nu,i,j}, \quad (2.60)$$

so the electrostatic portion of the rigid chemical potential, Eq. 2.48, is simply

$$\Delta\mu_{i,j}^{\text{solv,es}} = \frac{1}{2} \int d\mathbf{x} \rho_{i,j}(\mathbf{x}) \langle \Phi^\nu(\mathbf{x}; \lambda = 1) \rangle_{\nu,i,j}, \quad (2.61)$$

which can be obtained by solving the linearized Poisson–Boltzmann equation. Note that in this expression $\rho_{i,j}(\mathbf{x})$ is the solute charge distribution and is independent of the ionic charge density.

The electrostatic component of the chemical potential, Eq. 2.61, is truly a *free energy* and not merely an energy. The actual solute–solvent electrostatic energy component is simply the integral of the solute charge distribution times the average solvent potential.⁴⁶ The factor of one half comes in through the charging integral, Eq. 2.48, where, in this linear approximation, an amount of energy equal to one half of the total electrostatic energy is lost to pay for re-arranging the solvent (including the mobile ions) about the solute.⁴⁹ Thus, this entropic rearrangement penalty and the total electrostatic energy combine to yield Eq. 2.61, the electrostatic free energy component.

It is interesting to note that while solutions to the linearized Poisson–Boltzmann equation satisfy the necessary reciprocity relations for an accurate theory, solutions to the nonlinear Poisson–Boltzmann equation do not, thus linearization is a necessary step in obtaining a self consistent theory describing the free energies.²⁰ The Debye–Hückel approximations for describing the ionic effects are valid for low ionic concentrations, but have been successful at physiological levels and higher.

In succeeding chapters, we shall employ a simplified notation, denoting $\langle \Phi^\nu(\mathbf{x}; \lambda = 1) \rangle_{\nu,i,j}$ by $\Phi_i(\mathbf{x})$, where the subscript j is dropped because the desired solute conformation will be implied.

2.4 Conclusions

This chapter has provided a general theoretical background for the topics discussed in this thesis. In particular, we have started from first principles and derived equations for finding the free energies of molecular reactants. In the process we have explicitly shown the approximations and assumptions made, and obtained expressions relating the rigid binding free energies of various solute conformations to the overall binding free energy. Furthermore, we have derived the linearized Poisson–Boltzmann equation and shown how its solution can be used to obtain the electrostatic component of the rigid standard state chemical potential of a solute molecule. These results are of central importance in the following chapters.

