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Local Fluctuations in Solution: Theory and Applications

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1 Introduction

Liquids are complicated. Liquid mixtures are even more complicated.¹ While our knowledge of gases and solids is quite extensive, and in many cases can be considered essentially complete, a deep understanding of the properties of solutions remains elusive. The major problems involve our simplistic understanding of how molecules interact, especially at high densities, and how these interactions are affected by changes in the conditions – usually temperature, pressure and composition. Even in the absence of strong intermolecular interactions, our ability to rationalize the packing of molecules in solution remains rather rudimentary. One possible solution to this problem involves the use of molecular simulation.² Computer simulations have now advanced to a stage where they have provided a wealth of information using reasonably accurate models of liquid solutions. The ability to investigate the properties of solution mixtures is clearly aided by computer simulation and research in this direction will continually evolve. However, consistent comparison with experiment is still required in order to validate many of the results. Furthermore, our ability to relate the properties of solution mixtures to the underlying interactions between the molecules is still in its infancy. For instance, when a particular model (or force field) provides an incorrect description of a given thermodynamic property, for example the isothermal compressibility or the enthalpy of mixing, it is often extremely difficult to trace this to a specific incorrectly modeled interaction between molecules. Conversely, it is also difficult to predict the thermodynamic consequences arising from variations in the intermolecular interactions. Both issues limit the effectiveness of computer driven research.

Another, more subtle, problem arises when attempting to analyze experimental data concerning solution mixtures. Experimental data is typically obtained under isothermal isobaric conditions where the system is closed to matter exchange. This is clearly the most convenient and relevant set of conditions for most experiments and real systems. However, it is well known that the thermodynamic constraints placed on a system affect the behavior of the system.³ The statistical thermodynamics of large systems ensures that ensemble averages between systems under the same average conditions are equal.⁴ In fact, many theories of solutions have traditionally been developed starting from the grand canonical ensemble where the required manipulations are often much easier to perform. However, this equivalence does not hold for fluctuating quantities. Fluctuations depend on the ensemble. We will see here that fluctuations characteristic of open systems can be used to help understand solution behavior in closed systems. In this type of approach the fluctuations then represent the fluctuations associated with a relatively small local volume of solution. We will argue that these local fluctuations should be much easier to understand and probe compared to fluctuations of the system as a whole. One then simply needs to relate these local fluctuations to thermodynamic properties of the solution under the appropriate thermodynamic constraints. This is the role that the Fluctuation Theory (FT) of solutions seeks to play.

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Fluctuations play an important role in the properties of systems. This was well established by the work of Einstein and others.⁵ However, this work has primarily focused on the fluctuations in bulk systems under different thermodynamic constraints. A major step forward was provided by Kirkwood and Buff when they related particle number fluctuations and integrals over molecular distribution functions to the properties of closed systems - specifically the changes in chemical potentials, partial molar volumes and isothermal compressibility - and to changes in the osmotic pressure for semi-open systems.⁶ This approach is generally known as the Kirkwood-Buff (KB) theory of solutions. However, the theory lay relatively unused for over 25 years. Presumably, this initial lack of interest was because the theory appeared to be unsuited for the study of salt solutions,^{7,8} and/or the theory required radial distribution functions, which are generally unknown, as input data.

More recently, KB theory has developed into a powerful tool for probing the microstructure of solution mixtures.⁹⁻¹² This can be attributed to two main factors. First, the development of the KB inversion procedure by Ben-Naim,¹³ whereby the experimental data can be used to obtain the integrals over the rdfs, the so called KB integrals (KBIs). Second, the ability of modern computer simulations to provide realistic rdfs as input for the theory has improved significantly. These two factors have prompted the development of approaches to investigate the local microstructure in solutions, i.e. deviations in the local composition from the bulk distribution, which has in turn led to a rigorous description of preferential solvation.¹⁰ Fluctuation Theory has now been widely used to understand the basic properties of solutions,⁹ to understand the effects of additives on the solubility of solutes (from small hydrocarbons to proteins) and biomolecular equilibria,¹⁴⁻²⁰ to investigate the local composition of solutions in the context of preferential solvation,²¹ to study the effects of additives on the surface tension of liquids,²² to study critical behavior,²³⁻²⁶ to interpret computer simulation data,^{19,27-29} to develop models for many of the above effects,³⁰ and to provide expressions for the volume, enthalpy, compressibility and heat capacity corresponding to simple association equilibria.^{31,32} The general formulation and application of KB theory has been outlined in detail by several workers.⁹⁻¹²

KB theory is restricted to the use of particle number fluctuations. It was recognized quite early that one could extend this type of approach to include particle-energy and energy-energy fluctuations.³³ Further work by DeBenedetti has also highlighted the additional insight this provides for computer simulations.³⁴⁻³⁶ However, a direct link to experimental data was absent until very recently when Ploetz and Smith provided a framework for the analysis of additional solution properties - specifically the excess partial molar enthalpies, the thermal expansion coefficient and the constant pressure heat capacity - all as a function of solution composition.³⁷ Furthermore, all the fluctuating properties can also be obtained directly from available experimental data. The approach has since been extended to treat molecular association and conformational equilibria.³²

Here, we outline a practical general theory of solution fluctuations, representing local regions within a solution, which relates the local fluctuations to thermodynamic properties of bulk closed isothermal isobaric systems. We argue that this type of approach leads to a much simpler, though certainly not trivial, description of the behavior of solution mixtures. Furthermore, this appears to be the most appropriate and convenient approach for analyzing a variety of computer simulation data.

2 Outline, Notation and General Remarks

The outline of this review is as follows. Some basic background material in the areas of thermodynamics (Section 3) and statistical thermodynamics (Section 4) is presented with an emphasis on the Gibbs (NpT) and grand canonical (μ VT) ensembles of the latter. This is

followed by the derivation of a general fluctuation theory of solutions (Sections 5–7) involving any number of components, which relates fluctuating quantities in the grand canonical ensemble to thermodynamic properties of solutions under isothermal isobaric conditions. The theory is essentially complete at the end of Section 7, but the specific forms relating to various applications are not always obvious. Thus, this leads to several sections outlining the application to a variety of binary (Section 8) and ternary (Section 9) systems. This is illustrated by an analysis of experimental data corresponding to representative binary mixtures (Section 8). This is followed by an analysis and discussion of the local fluctuations in terms of a multivariate Gaussian distribution with examples from computer simulation (Section 10). The application of FT to describe molecular association and conformational equilibria is then provided (Section 11). The role of higher moments of the distributions in understanding the effects of temperature, pressure, and composition on the fluctuating quantities provided by FT is then presented (Section 12). Finally, we discuss some technical issues surrounding the implementation of the theory for the analysis of computer simulation data, the relationship to ideal solution behavior, and the application to salt solutions (Sections 13, 15 & 16). We conclude with a summary and future outlook.

In presenting the expressions below it quickly becomes apparent that a general matrix formulation is possible in many cases, but this often suffers from a lack of transparency. Hence, in many places we provide expressions involving a combination of local fluctuating and bulk thermodynamic quantities. We believe this provides clarity to the situation and often helps during subsequent manipulations that might be of interest, e.g. infinitely dilute conditions.

Throughout this work we refer to a collection of species (1, 2, 3, ...) in a system of interest. We consider this to represent a primary solvent (1), a solute of interest (2), and a series of additional cosolutes or cosolvents (3, 4, ...) which may also be present in the solution. It should be noted that when we refer to solutions we are primarily concerned with liquid mixtures. FT can, however, be applied to mixtures of gases. The summations appearing here involve sums over all thermodynamically independent components (n_c) in the mixture unless stated otherwise. The composition of the solution is denoted by either mole fractions ($x_i = N_i/N$), dimensionless molalities ($m_i = N_i/N_1$), or number densities ($\rho_i = N_i/V$), where N_i and N are the number of molecules of i and the total number of molecules, respectively. The classic definitions of molalities and molarities can be obtained by a simple scaling of the dimensionless molalities and number densities, respectively, but this does not affect the final relationships provided here. Our formulation will focus on the use of $\beta = (k_B T)^{-1}$ rather than the absolute temperature as an independent variable, where k_B is the Boltzmann constant. The former is much more convenient for statistical thermodynamic derivatives involving temperature. The temperature derivatives can be obtained by simple substitution, $-k_B T^2 d\beta = dT$, although second derivatives are more involved.

Derivatives of the chemical potentials with respect to composition form a central component of the theory. Unfortunately, these derivatives can take many forms depending on the concentration scale of interest. This is further complicated if one switches to a particular activity coefficient. Hence, we choose to use chemical potentials and define,

$$\mu_{ij} = \left(\frac{\partial \beta \mu_i}{\partial \ln N_j} \right)_{p, T, \{N\}'} = \left(\frac{\partial \beta \mu_i}{\partial \ln m_j} \right)_{p, T, \{m\}'} \quad (1)$$

as our primary derivative of interest. Using this definition we also have $N_i \mu_{ij} = N_j \mu_{ji}$. Our focus in this work will be on fluctuating properties of solutions. A general fluctuation in a property X is written as $\delta X = X - \langle X \rangle$, where the angular brackets denote an ensemble or

time average. All ensemble averages will refer to the grand canonical ensemble unless stated otherwise. We use the symbol E to represent the instantaneous internal energy for each member of the ensemble, or each configuration of a simulation trajectory, whose average value is the thermodynamic internal energy (U). In many applications we are interested in the expressions for an infinitely dilute solute. These are denoted with an infinity superscript, while the pure solvent properties are indicated by a zero superscript. Finally, the Kronecker delta function (δ_{ij}) is used consistently during the theoretical development and is equal to unity when $i = j$, and zero otherwise.

3 Thermodynamic Background

In this section we define many of the thermodynamic properties of interest to FT, and also present several useful relationships which aid in the development and application of the theory. The experienced reader may skip this section. Our focus will be on two types of systems. In the first type of system the independent variables are the number of particles ($\{N\}$), the absolute temperature (T), and the pressure (p), while in the second type of system the independent variables are the chemical potentials ($\{\beta\mu\}$), the volume (V), and temperature. Starting with the entropy formulation of Gibbs' fundamental equation and then transforming to the above sets of variables provides,³

$$\begin{aligned} d\beta G &= H d\beta + \beta V dp + \sum_i \beta \mu_i dN_i \\ d\beta p V &= -U d\beta + \beta p dV + \sum_i N_i d\beta \mu_i \end{aligned} \quad (2)$$

The first set of variables corresponds to the most common situation of experimental interest, while the second set refer to a system that is open to matter exchange with its surroundings. The above equations dictate the response of the system at equilibrium to changes in the independent variables. The enthalpy ($H = U + pV$), volume, and chemical potentials are therefore provided by first derivatives of the Gibbs free energy ($G = H - TS$) for our closed system, while the internal energy, the pressure, and the average number of particles are provided by derivatives of the thermodynamic function (pV) characterizing our open system.

Several global properties of solution mixtures will be relevant to FT. These include the isothermal compressibility (κ_T), the isobaric thermal expansion coefficient (α_p), and the constant pressure molar heat capacity ($C_{p,m}$) given by the following derivatives,

$$\begin{aligned} \kappa_T &= -\frac{1}{V} \left(\frac{\partial V}{\partial p} \right)_{T, \{N\}} = -\frac{1}{V_m} \left(\frac{\partial V_m}{\partial p} \right)_T = \frac{1}{\rho} \left(\frac{\partial \rho}{\partial p} \right)_T \\ \alpha_p &= \frac{1}{V} \left(\frac{\partial V}{\partial T} \right)_{p, \{N\}} = \frac{1}{V_m} \left(\frac{\partial V_m}{\partial T} \right)_p = -\frac{1}{\rho} \left(\frac{\partial \rho}{\partial T} \right)_p \\ C_{p,m} &= \left(\frac{\partial H_m}{\partial T} \right)_p \end{aligned} \quad (3)$$

where ρ is the total particle number or mass density and X_m is the corresponding molar quantity, $X_m = \langle X \rangle / N$. All three properties can be obtained directly from experiment. They involve second derivatives of the Gibbs free energy. It is the second derivatives of the Gibbs free energy shown above that one can express in terms of second derivatives of the thermodynamic function (pV) for open systems. Second derivatives are typically related to fluctuations in the system as we shall see below.

Chemical potentials are central to our understanding of material and phase equilibrium and phase stability. However, the vast majority of the studies using the FT of solutions involve a

single stable phase with multiple components. Here, we are concerned with the relationships between the chemical potentials (and their derivatives) and the solution properties. Thermodynamically, we have from Equation 2 that,

$$\mu_i = \bar{G}_i = \left(\frac{\partial G}{\partial N_i} \right)_{p, T, \{N\}'} \quad (4)$$

Hence, the chemical potential quantifies the change in the Gibbs free energy on addition of a single particle of species i while keeping p , T , and the number of all other species constant (indicated by the prime superscript). There are additional useful relationships between the partial molar quantities which can be obtained from interchanging the second derivatives of the Gibbs free energy. These are provided in Table 1.

The application of Euler's Theorem to the extensive properties ($X = U, S, V, H, G$ and C_p) of a system at constant T and P indicates that,³⁸

$$X = \sum_i N_i \left(\frac{\partial X}{\partial N_i} \right)_{p, T, \{N\}'} = \sum_i N_i \bar{X}_i = \sum_i x_i \bar{X}_i \quad (5)$$

where $\bar{X}_i$ is known as a partial molar quantity. All the extensive quantities depend on T , p , and composition and hence one can write differentials of each X quantity which, when subtracting the differential obtained from Equation 5, provides a series of relationships between partial molar quantities. The most useful being when $X = G$,

$$-\beta V dp - H d\beta + \sum_i N_i d\beta \mu_i = 0 \quad (6)$$

which is known as the Gibbs-Duhem (GD) equation. At constant T and p the above expressions indicate that changes in the partial molar quantities are not independent.

In practice, many of the extensive functions (U, S, H, G), and their corresponding partial molar quantities, can only be determined up to an additive constant. Hence, their values are expressed with respect to a set of well-defined reference states. These are typically taken as the pure solutions of each component at the same T and p . One can then define a series of mixing quantities such that,

$$\Delta_{mix} X_m = X_m - \sum_i x_i X_m^o = \sum_i x_i (\bar{X}_i - X_m^o) \quad (7)$$

This concept is also often applied to the volume of the solution even though absolute molar volumes are clearly available. A series of excess quantities may then be defined using the properties of ideal solutions such that,

$$X_m^E = \Delta_{mix} X_m - \Delta_{mix} X_m^{id} = \sum_i x_i \bar{X}_i^E \quad (8)$$

where the excess mixing quantities are expressed in terms of the corresponding excess partial molar quantities. It is these excess quantities ($X = G, V, H, S$) that are normally available experimentally and are typically represented as a function of composition by a suitable fitting function. First derivatives of the fitting function with respect to composition then provide the corresponding excess partial molar volumes, enthalpies, and chemical potentials, while second derivatives of the excess Gibbs energy with respect to composition then provide derivatives of the chemical potentials.

In many cases it is necessary to transform the derivatives of the chemical potentials between different concentration units. This can be achieved by use of the following relationships,

$$\begin{aligned} \left(\frac{\partial \ln \rho_i}{\partial \ln m_j} \right)_{p,T} &= \delta_{ij} - \phi_j \left(\frac{\partial \ln x_i}{\partial \ln m_j} \right)_{p,T} = \delta_{ij} - x_j \left(\frac{\partial \ln \rho_i}{\partial \ln x_j} \right)_{p,T} = \frac{\delta_{ij} - \phi_j}{1 - x_j} \\ \left(\frac{\partial \ln \rho_i}{\partial \ln \rho_j} \right)_{p,T} &= \frac{\delta_{ij} - \phi_j}{1 - \phi_j} \left(\frac{\partial \ln x_i}{\partial \ln x_j} \right)_{p,T} = \frac{\delta_{ij} - x_j}{1 - x_j} \left(\frac{\partial \ln m_i}{\partial \ln m_j} \right)_{p,T} = \delta_{ij} \end{aligned} \quad (9)$$

which apply to solutions with any number of components and where $\phi_i = \rho_i \bar{V}_i$ are the volume fractions.

The above expressions are all that we require for the manipulation of the available experimental data. Most of the relationships correspond to closed systems which are of primary interest to the vast majority of researchers. FT will be used to provide expressions for many of these closed system quantities in terms of fluctuations in an equivalent open system. This open system can then be considered as a local region of fixed volume within the bulk system that possesses the same average pressure and temperature, but allows matter exchange in order to maintain the same chemical potentials. This is illustrated in Figure 1. The spatial extent of the local region will depend on the nature of the components, the solution composition, and the particular values of temperature and pressure concerned.

4 Statistical Thermodynamics Background

Statistical thermodynamics is used to provide the characteristic functions in terms of the appropriate partition functions, which will formally involve a sum over the microscopic states available to the system. It is also used to provide relevant expressions for the fluctuations under one set of variables, which can then be used to rationalize the thermodynamic properties of a system characterized by a different set of variables.⁴ Again, the experienced reader may skip this section.

Our application of statistical thermodynamics will focus on the grand canonical ensemble, where we shall develop expressions for the local fluctuations, and the isothermal isobaric (or Gibbs) ensemble, which is representative of most experimental conditions. The characteristic functions provided in Equation 2 can be expressed in terms of the corresponding classical multicomponent partition functions such that,⁴

$$\begin{aligned} \beta G &= -\ln \Delta(\{N\}, p, \beta) \beta p V = \ln \Xi(\{\beta \mu\}, V, \beta) \\ \Delta(\{N\}, p, \beta) &= V_o^{-1} \int_0^\infty Q(\{N\}, V, \beta) e^{-\beta p V} dV \\ \Xi(\{\beta \mu\}, V, \beta) &= \sum_{\{N\}=0}^\infty Q(\{N\}, V, \beta) e^{\beta \mu \cdot N} \\ Q(\{N\}, V, \beta) &= \prod_i \frac{1}{N_i!} \left(\frac{q_i^{\text{int}}}{h^3} \right)^{N_i} \iint_{-\infty}^\infty e^{-\beta E} \{dr\} \{dp\} \end{aligned} \quad (10)$$

where E is the internal energy for a particular set of molecular positions $\{r\}$ and momenta $\{p\}$, q_i^{int} is the internal molecular partition function, h is the Planck constant, and we have used the shorthand notation $\mu \cdot N = \mu_1 N_1 + \mu_2 N_2 \cdots$ for simplicity. It should be noted that the focus on classical systems does not imply that FT is invalid for quantum systems, but is merely an acknowledgement that these are the most common systems of interest.

Using these expressions in Equation 3 and evaluating the derivatives leads to expressions for the compressibility, thermal expansion, and heat capacity in the Gibbs ensemble. The results are,

$$\begin{aligned}k_B T \kappa_T &= \frac{\langle \delta V \delta V \rangle}{\langle V \rangle} \\k_B T^2 \alpha_p &= \frac{\langle \delta H \delta V \rangle}{\langle V \rangle} \\k_B T^2 C_{p,m} &= \frac{\langle \delta H \delta H \rangle}{N}\end{aligned}\quad (11)$$

where all the ensemble averages correspond to the NpT ensemble. It is important to note that the above formulas represent fluctuations in the properties of the whole system, i.e. bulk fluctuations. Expressions for the chemical potentials, partial molar enthalpies and volumes, and also derivatives of the chemical potentials can (in principle) also be provided in terms of properties of the whole system. They are valuable expressions, but they provide little or no information concerning fluctuations in the local vicinity of atoms or molecules. It is these local quantities that will prove more useful.

If we turn to the grand canonical ensemble, one finds the expressions provided in Table 2. Several of the second derivatives involve particle-particle, particle-energy, or energy-energy fluctuations, and will play a central role in the FT of solutions. We emphasize that the expressions in Table 2 can be used to describe an ensemble of systems in the grand canonical ensemble, in which case they correspond to bulk properties, or to small regions of any bulk system in any ensemble at constant T, in which case they represent local fluctuations in a much larger bulk system.

Finally, before leaving this section we briefly discuss the use of the pseudo chemical potential and associated quantities developed by Ben-Naim.¹⁰ The pseudo chemical potential (pcp) formulation offers certain advantages over other traditional approaches to understanding the properties of solutions. The reader is referred to the detailed discussions of Ben-Naim for further information.¹⁰ The pseudo chemical potential μ_i^* of species i is defined by,

$$\beta \mu_i = \beta \mu_i^* + \ln \Lambda_i^3 \rho_i \quad (12)$$

where Λ is the thermal de Broglie wavelength. The major advantage of this definition is that one separates the total chemical potential into contributions from the translational degrees of freedom (the $\ln$ term) and contributions from specific interactions with the other molecules in the system (the pseudo chemical potential term). Using the above definition and taking the appropriate derivatives one also finds,

$$\begin{aligned}\bar{V}_i &= V_i^* + k_B T \kappa_T \\ \bar{H}_i &= H_i^* + k_B T^2 \alpha_p + \frac{3}{2} k_B T \\ \mu_{ij} &= \mu_{ij}^* + \delta_{ij} - \phi_j\end{aligned}\quad (13)$$

which provide the pseudo volume and enthalpy, and derivatives of the pseudo chemical potential. Derivatives of the pcp are most closely related to changes in the molar activity coefficient. Standard thermodynamic transformations then provide relationships to Lewis-Randall (mole fraction) activity coefficients and the rational (molal) activity coefficients. The pcp is also closely related to the excess chemical potential often determined from

computer simulation using, for example, the Widom particle insertion approach.² Here, we find,

$$\beta\mu_i^* = \beta\mu_i^{ex} - \ln q_i^{\text{int}} \quad (14)$$

In many applications the changes to the internal partition function are neglected, in which case changes to the pc_p are equal to the changes in the excess chemical potential.

5 A General Fluctuation Theory of Solutions

5.1 Theory

Many derivations of the principle expressions involving only particle-particle fluctuations have appeared in the literature.^{6,10,39–43} They all start with expressions valid for the grand canonical ensemble, followed by a series of transformations to provide expressions for properties representative of the Gibbs ensemble. Here, we will present a relatively simple derivation that attempts to reduce many of the thermodynamic manipulations appearing in other approaches. The general approach for multicomponent systems will involve the manipulation of a few matrices. However, for binary and ternary systems the expressions can be obtained directly from a few simultaneous equations. This offers greater transparency for those less familiar with matrix operations and their properties.

The average number of particles of species *i* and the average internal energy can be considered a function of the temperature, volume and set of chemical potentials. Using the expressions provided in Table 2 the following set of differentials can be written,

$$d\langle X \rangle = -\langle \delta X \delta E \rangle d\beta + \frac{\langle X \rangle}{V} dV + \sum_j \langle \delta X \delta N_j \rangle d\beta \mu_j \quad (15)$$

where *X* = *N_i* or *E*. In the traditional formulation of KB theory, *T* remains constant.⁶ Hence, the *dβ* term disappears and one has a series of simultaneous differentials, one for each component, which can be solved after taking the appropriate derivatives. Here, we wish to include temperature related effects and therefore our expressions will involve energy fluctuations. We now define a set of fluctuating quantities and, in order to make these more compatible with experimentally available data, we choose a reference energy equal to the enthalpy of the pure components such that,

$$\delta_{ij} + N_{ij} = \frac{\langle \delta N_i \delta N_j \rangle}{\langle N_i \rangle} F_i = \frac{\langle \delta N_i \delta \varepsilon \rangle}{\langle N_i \rangle} \Delta_m = \frac{\langle \delta \varepsilon \delta \varepsilon \rangle}{\langle N \rangle} \varepsilon = E - \sum_j N_j H_j^o \quad (16)$$

where *ε* corresponds to an excess energy for each member of the ensemble. We note that the above definitions are slightly different from the ones used in the original study.³⁷ However, the new definitions lead to a significant simplification of the resulting expressions. With these steps our differentials can be written,

$$\begin{aligned} d \ln \rho_i &= -F_i d\beta + \sum_j (\delta_{ij} + N_{ij}) (d\beta \mu_j - H_j^o d\beta) \\ d\langle H \rangle &= -[\langle \delta E \delta \varepsilon \rangle + k_B T H^E] d\beta + \frac{\langle H \rangle}{V} dV + \sum_j [\langle \delta E \delta N_j \rangle + \langle N_j \rangle] (d\beta \mu_j - H_j^o d\beta) \end{aligned} \quad (17)$$

The last expression for the enthalpy was obtained by a combination of Equations 2 and 15. We note that the following relationships follow directly from the above expressions,

$$\begin{aligned}
 k_B T \kappa_T &= \sum_j (\delta_{ij} + N_{ij}) \bar{V}_j \\
 \delta_{ik} - \rho_k \bar{V}_k &= \sum_j (\delta_{ij} + N_{ij}) \mu_{jk} \\
 k_B T^2 \alpha_p &= -F_i + \sum_j (\delta_{ij} + N_{ij}) \bar{H}_j^E = \rho k_B T \kappa_T H_m^E - \sum_j \phi_i F_i \quad (18) \\
 k_B T^2 C_{p,m} &= \Delta_m + k_B T^2 \alpha_p H_m^E - \sum_j x_j F_j \bar{H}_j^E
 \end{aligned}$$

where $\rho = N/V$ is the total number density. Equations 17 and 18, coupled with the Gibbs-Duhem equation, are all that is required to derive the basic theory and provide a series of relationships between fluctuating quantities and thermodynamic properties. All the properties on the right hand side of the above expressions involve fluctuations or properties that can also be expressed in terms of fluctuations.

A general formulation for the chemical potential derivatives and partial molar volumes is most easy if one first considers the constant temperature case and transforms to molalities. Using component 1 as the primary solvent and noting that $d \ln m_i = d \ln \rho_i - d \ln \rho_1$ provides,

$$d \ln m_i = - (F_i - F_1) d\beta + \sum_j [\delta_{ij} + N_{ij} - (\delta_{1j} + N_{1j})] (d\beta \mu_j - H_j^o d\beta) \quad (19)$$

Using this expression, the GD relation at constant T and p, and the expressions provided in Equation 18, it is a simple process to obtain relationships for μ_{22} , V_2 , and κ_T using a few simple steps. However, for ternary or higher solutions a series of matrices becomes more convenient. We write the number fluctuations in the above expression in matrix form where we also include the number densities (GD expression) in the first row,

$$\begin{pmatrix} \rho_1 & \rho_2 & \cdots & \rho_n \\ -(1+N_{11} - N_{21}) & 1+N_{22} - N_{12} & \cdots & N_{2n} - N_{1n} \\ \vdots & \vdots & \ddots & \vdots \\ -(1+N_{11} - N_{n1}) & N_{n2} - N_{12} & \cdots & 1+N_{nn} - N_{1n} \end{pmatrix} \quad (20)$$

This will prove to be a central matrix for the development of FT. Therefore, if we signify the above matrix as M, then take derivatives of Equation 19 with respect to molality at constant p (and T), and also take derivatives with respect to p with composition (and T) constant, the results can be expressed in the form,

$$\begin{pmatrix} \bar{V}_1 & \mu_{12} & \cdots & \mu_{1n} \\ \bar{V}_2 & \mu_{22} & \cdots & \mu_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \bar{V}_n & \mu_{n2} & \cdots & \mu_{nn} \end{pmatrix} = M^{-1} \bar{V}_i = \frac{M^{i1}}{|M|} \mu_{ij>1} = \frac{M^{ij}}{|M|} \quad (21)$$

i.e. the expressions or values for the partial molar volumes and activity derivatives are just the elements of the inverse of the M matrix, which can also be expressed in terms of cofactors of the M matrix. This also leads to an expression for the compressibility,

$$k_B T \kappa_T = \frac{|NN|}{|M|} \quad (22)$$

where NN is the matrix of $\delta_{ij} + N_{ij}$ elements. At this point we have a matrix formulation for the chemical potential derivatives, partial molar volumes, and isothermal compressibility. This is essentially the theory of Kirkwood and Buff.

To complete the general theory we require expressions for the partial molar excess enthalpies. The excess enthalpies can be obtained from the expressions provided above (Equation 18), or in matrix form as $H^E = M^{-1} F^*$, where F^* is a vector with elements $\delta_{1i} H_m^E + F_i - F_1$. A general expression for the enthalpies then becomes,

$$\bar{H}_i^E = \rho \bar{V}_i H_m^E + \sum_j F_j \mu_{ij} \quad (23)$$

after the use of Equation 21 and some rearrangement via the GD expression. The fluctuation expressions for the partial molar volumes and chemical potential derivatives could be inserted into the above equation. However, the expression becomes rather complicated. We prefer the above form as the limiting expressions at low concentrations are easily obtained. The fluctuation expressions for the thermal expansion and heat capacity then follow from Equation 18.

In this section we have related a series of properties for solution mixtures to fluctuations in local regions of the solution. The expressions are somewhat complicated, especially as the number of components increases. However, this is primarily due to closure of the bulk system to matter exchange. The corresponding fluctuations for local regions are much simpler in form and do not change with the number of components – there are simply more fluctuating quantities. This suggests that a focus on the local properties may prove more beneficial to our understanding of solution mixtures than a focus on bulk properties.

5.2 Inversion of FT

The KB inversion procedure is the process of obtaining expressions for the particle number fluctuations in terms of experimentally available (isothermal isobaric) data.¹³ Again, there are multiple approaches to the inversion procedure.^{13,44} Analysis of Equation 18 indicates that an inversion to obtain particle-energy and energy-energy fluctuations is rather trivial once one has the particle-particle formulas. Arguably, the simplest approach to obtain these involves the pseudo chemical potential and partial molar volumes. First, we note that one can add the expressions for $\delta_{ik} - \rho_k V_k^*$ and $\rho_k k_B T \kappa_T$, provided in Equation 18, and then convert to pcg derivatives using Equation 13 to give,

$$\delta_{ik} - \rho_i V_k^* = \sum_j (\delta_{ij} + N_{ji})(\delta_{kj} + \mu_{kj}^*) \quad (24)$$

This can then be expressed in matrix form,

$$\begin{pmatrix} 1+N_{11} & \cdots & N_{n1} \\ \vdots & \ddots & \vdots \\ N_{1n} & \cdots & 1+N_{nn} \end{pmatrix} = \begin{pmatrix} 1 - \rho_1 V_1^* & \cdots & -\rho_1 V_n^* \\ \vdots & \ddots & \vdots \\ -\rho_n V_1^* & \cdots & 1 - \rho_n V_n^* \end{pmatrix} \begin{pmatrix} 1+\mu_{11}^* & \cdots & \mu_{n1}^* \\ \vdots & \ddots & \vdots \\ \mu_{1n}^* & \cdots & 1+\mu_{nn}^* \end{pmatrix}^{-1} \quad (25)$$

to provide a simple expression for the elements of the transpose of the NN matrix (and thereby the KBIs) in terms of a matrix containing just the pseudo partial molar volumes and the inverse of a matrix containing just the pseudo chemical potentials, both of which can be expressed in terms of experimental data. Other approaches are also available.⁴⁴ The particle-energy and energy-energy fluctuations are then easily obtained from Equation 18. The overall inversion process is outlined in Figure 2.

In summary, FT provides a link between fluctuating properties in the grand canonical ensemble and thermodynamic properties of systems in the Gibbs ensemble. This is illustrated schematically as,

$$\{\langle \delta N_i \delta N_j \rangle\}, \{\langle \delta N_i \delta \varepsilon \rangle\}, \langle \delta \varepsilon \delta \varepsilon \rangle \leftrightarrow \{\mu_{ij}\}, \{\bar{V}_i\}, \{\bar{H}_i^E\}, \kappa_T, \alpha_p, C_{p,m}$$

For an n_c component solution there are $n_c(n_c+1)/2$ unique particle number fluctuations, n_c unique particle-excess energy fluctuations and one excess energy-energy fluctuation. These can be used to provide, or can be extracted from, $n_c(n_c-1)/2$ independent chemical potential derivatives, n_c-1 independent partial molar volumes, n_c-1 independent excess partial molar enthalpies, together with the compressibility, thermal expansion and heat capacity. Alternatively speaking, $(n_c^2+3n_c)/2 + 1$ fluctuating quantities are related to the same number of independent thermodynamic properties. All of these properties vary with composition.

6 Fluctuations in Terms of Molecular Distribution Functions

The previous expressions involve particle number (and energy) fluctuations. It is more common, and totally equivalent, to use distribution functions to replace the number fluctuations. However, in doing so one has to remember that these distributions correspond to a system volume which is open to all species. Consequently, integration over the system volume does not lead to the trivial results observed for closed systems.¹⁰ In the grand canonical ensemble the probability that any N_1 molecules of species 1, and N_2 molecules of species 2, etc, are within $d\{r\}$ at $\{r\}$ is given by $\rho^{(n)}(\{r\})\{dr\}$ where,⁴

$$\int \rho^{(n)}(\{r\})\{dr\} = \langle \prod_i \frac{N_i!}{(N_i - n_i)!} \rangle \quad (26)$$

the product is over the different species in the mixture, and n_i is the number of i molecules in the n particle probability distribution. Hence, we can evaluate the following integrals,

$$\begin{aligned} \int \rho_i^{(1)}(r_1) dr_1 &= \langle N_i \rangle \\ \iint \rho_{ij}^{(2)}(r_1, r_2) dr_1 dr_2 &= \langle N_i N_j \rangle - \delta_{ij} \langle N_i \rangle \\ \iiint \rho_{ijk}^{(3)}(r_1, r_2, r_3) dr_1 dr_2 dr_3 &= \langle N_i N_j N_k \rangle - \delta_{ik} \langle N_i N_j \rangle - \delta_{ij} \langle N_i N_k \rangle - \delta_{jk} \langle N_i N_j \rangle + 2\delta_{ij} \delta_{jk} \langle N_i \rangle \end{aligned} \quad (27)$$

where the integrals are over all possible positions of the particles. Various combinations of the above integrals are generally encountered in statistical thermodynamic theories of solutions.^{4,38} The two most common integrals are,

$$\begin{aligned} \rho_i \rho_j V G_{ij} &= \iint [\rho_{ij}^{(2)}(r_1, r_2) - \rho_i^{(1)}(r_1) \rho_j^{(1)}(r_2)] dr_1 dr_2 \\ \rho_i \rho_j \rho_k V G_{ijk} &= \iiint [\rho_{ijk}^{(3)}(r_1, r_2, r_3) - \rho_{ij}^{(2)}(r_1, r_2) \rho_k^{(1)}(r_3) - \rho_{ik}^{(2)}(r_1, r_3) \rho_j^{(1)}(r_2) - \rho_{jk}^{(2)}(r_2, r_3) \rho_i^{(1)}(r_1) + 2\rho_i^{(1)}(r_1) \rho_j^{(1)}(r_2) \rho_k^{(1)}(r_3)] dr_1 dr_2 dr_3 \end{aligned} \quad (28)$$

The required integrals expressed as fluctuations are therefore given by,

$$\begin{aligned} \rho_i \rho_j V G_{ij} &= \langle \delta N_i \delta N_j \rangle - \delta_{ij} \langle N_i \rangle \\ \rho_i \rho_j \rho_k V G_{ijk} &= \langle \delta N_i \delta N_j \delta N_k \rangle - \delta_{ij} \langle \delta N_i \delta N_k \rangle - \delta_{ik} \langle \delta N_i \delta N_j \rangle - \delta_{jk} \langle \delta N_i \delta N_j \rangle + 2\delta_{ij} \delta_{jk} \langle N_i \rangle \end{aligned} \quad (29)$$

These fluctuations can then be expressed in terms of a set of distribution functions $g^{(n)}$ for species i, j and k defined with reference to the bulk distributions by,

$$g_i^{(1)}(r_1) = \frac{\rho_i^{(1)}(r_1)}{\rho_i} = 1, g_{ij}^{(2)}(r_1, r_2) = \frac{\rho_i^{(2)}(r_1, r_2)}{\rho_i \rho_j} g_{ijk}^{(3)}(r_1, r_2, r_3) = \frac{\rho_{ijk}^{(3)}(r_1, r_2, r_3)}{\rho_i \rho_j \rho_k} \quad (30)$$

The integrals can then be related to the distribution functions where we have integrated over the position of the central particle and only consider the inter-particle distance $r_{12} = |r_2 - r_1|$,

$$G_{ij} = G_{ji} = 4\pi \int_0^\infty [g_{ij}^{(2)}(r) - 1] r^2 dr$$

$$G_{ijk} = \iint [g_{ijk}^{(3)}(r_{12}, r_{13}) - g_{ij}^{(2)}(r_{12}) - g_{ik}^{(2)}(r_{13}) - g_{jk}^{(2)}(r_{23}) + 2] dr_{12} dr_{13} \quad (31)$$

At this point we have related the particle number fluctuations to integrals over radial distribution functions in the grand canonical ensemble. FT does not require information on the angular distributions for pairs of molecules as these are averaged out in the above expressions. The rdfs correspond to distributions obtained in a solution at the composition of interest, after averaging over all the remaining molecular degrees of freedom. The G_{ij} 's are known as Kirkwood-Buff integrals (KBIs) and are the central components of KB theory.

The KBIs quantify deviations from a random distribution surrounding a central molecule summed over all space. In this respect they are more informative than the particle fluctuation formulation as they can then be decomposed and interpreted in terms of spatial contributions – using computer simulation data, for example. They clearly resemble the integrals one encounters in the study of imperfect gases and the McMillan-Mayer theory of solutions.⁴⁵ However, the KBIs do not form part of a virial expansion and are relevant at any solution composition – not just infinitely dilute solutes in the gas phase or a primary solvent.

Multiplying the KBIs by number densities (concentrations) one obtains an alternative picture of the KBIs. The quantities, $N_{ij} = \rho_j G_{ij}$, are referred to as excess coordination numbers and quantify the change in the number of j molecules observed in an open volume of solution on introduction of a central i molecule, from that observed for the same volume of bulk solution in the absence of the i molecule.⁴² The integrals can be related to the fluctuation densities via,

$$B_{ij} = \rho_i (\delta_{ij} + N_{ij}) = \frac{\langle \delta N_i \delta N_j \rangle}{V}$$

$$\rho_i (\delta_{ij} \delta_{ik} + \delta_{jk} N_{ij} + \delta_{ij} N_{ik} + \delta_{ik} N_{kj} + \rho_j \rho_k G_{ijk}) = \frac{\langle \delta N_i \delta N_j \delta N_k \rangle}{V} \quad (32)$$

The triplet correlations will become important when discussing third derivatives of the thermodynamic potentials that arise when we consider the variations in the KBIs and chemical equilibria with temperature, pressure and composition. Hence, there are two complimentary viewpoints. The first is provided by integrals over molecular distribution functions and quantifies any deviation from a random distribution (Equation 31). The second is provided by particle number fluctuations and describes the degree of correlation between the various particle numbers (Equation 32).

An example of the fluctuating quantities obtained from computer simulation is provided in Figure 3. The results are specifically for a mixture of methanol and water at 300 K and 1 bar at a methanol mole fraction of 0.25, but are merely used here to illustrate the typical behavior observed for many solutions. The rdfs display the usual oscillatory behavior, which

is dampened as one moves further away from the central atom until all rdfs approach unity as the random bulk distribution is reached. The resulting KBIs also display a similar oscillatory behavior as the integration distance is increased. This is both an advantage and disadvantage. Clearly, any structure arising from the different solvation shells is apparent in the KBIs. However, these oscillations extend over many solvation shells, often more than appears in the original rdf due to the r^2 weighting in Equation 31, which can hinder the assignment of a final limiting value. The same oscillatory behavior is not observed by direct simulation of the fluctuations in a spherical region of space of the same volume, but not centered on a particular molecule. The values appear to converge much more rapidly with distance and reasonable estimates can be obtained for spherical radii of as small as 0.5 nm or so. As required, the KBI and fluctuation approaches tend to the same values.

Finally, we note that particle number fluctuations increase, and many properties diverge, as one approaches a critical point. O'Connell and others have shown that one can still apply FT under these circumstances by adopting integrals over direct correlation functions (DCF),^{41,46} and not integrals over the total correlation function as adopted in the standard KBIs.²³ The advantages of the DCF approach include the ability to treat systems close to critical points and a general simplification of the expressions provided above. The disadvantages are that the DCFs cannot be calculated directly, only through the defining Ornstein-Zernike expression, and that they appear to be more difficult to interpret than the KBIs. We will not consider the DCF approach here.

7 Limiting Expressions for the Fluctuating Quantities

In many cases one is interested in a particular fluctuating quantity as the concentration of a species tends to infinite dilution. The limiting behavior of the chemical potential derivatives is such that $\mu_{ij} \rightarrow \delta_{ij}$ as $\rho_j \rightarrow 0$. The behavior of the N_{ij} 's and F_i 's under these conditions is the easiest to follow and is one of the reasons we adopted this particular formulation. It is clear that N_{ij} tends to zero as ρ_j tends to zero. However, the same is not true for N_{ij} and F_i when ρ_i tends to zero. Under these circumstances the following limiting expressions are found,

$$\delta_{ij} + N_{ij}^\infty = \lim_{N_i \rightarrow 1} \frac{\langle N_i N_j \rangle - \langle N_i \rangle \langle N_j \rangle}{\langle N_i \rangle} = \langle N_j \rangle_i - \langle N_j \rangle \quad (33)$$

The interpretation of the above expression is as follows. The first term represents the average number of j molecules observed in a given local volume centered on a single infinitely dilute i particle, compared to the number of j particles observed in the same volume of solution in the absence of the i particle. The exact size of the local volume is unimportant as long as it is large enough to extend beyond the influence of the central i particle. When this condition is met, the difference between the two extensive quantities should be intensive with respect to the local volume. Correspondingly, for the F_i 's we have,

$$F_i^\infty = \lim_{N_i \rightarrow 1} \frac{\langle N_i \epsilon \rangle - \langle N_i \rangle \langle \epsilon \rangle}{\langle N_i \rangle} = \langle \epsilon \rangle_i - \langle \epsilon \rangle \quad (34)$$

which quantifies the difference between the average excess energy in the local region surrounding the i particle and the average excess energy in the same volume of solution in the absence of the i particle. The above expression can be expanded further to give,

$$F_i^\infty = \langle E \rangle_i - \langle E \rangle - \sum_j (\langle N_j \rangle_i - \langle N_j \rangle) H_j^o = \langle E \rangle_i - \langle E \rangle - \sum_j (\delta_{ij} + N_{ij}^\infty) H_j^o \quad (35)$$

which is more convenient and useful for the analysis of computer simulations. Hence, the fluctuations have essentially disappeared and we find only the difference between average properties in various regions of the solution. The value of Δ_m remains a fluctuation representative of the bulk solution under these conditions.

The triplet correlations that appear in later sections have a more interesting limiting behavior. If we define the following fluctuating quantities,

$$N_{ijk} = \frac{\langle \delta N_i \delta N_j \delta N_k \rangle}{\langle N_i \rangle} N_{ij\varepsilon} = \frac{\langle \delta N_i \delta N_j \delta \varepsilon \rangle}{\langle N_i \rangle} N_{i\varepsilon\varepsilon} = \frac{\langle \delta N_i \delta \varepsilon \delta \varepsilon \rangle}{\langle N_i \rangle} \quad (36)$$

and then use a similar approach as above one finds that as ρ_j tends to zero we have,

$$\begin{aligned} N_{jik}^\infty &= N_{jki}^\infty = 0 \\ N_{ijk}^\infty &= \langle \delta N_j \delta N_k \rangle_i - \langle \delta N_j \delta N_k \rangle \\ N_{iik}^\infty &= \langle N_k \rangle_i - \langle N_k \rangle \\ N_{iii}^\infty &= 1 \end{aligned} \quad (37)$$

where $\delta N_j = N_j - \langle N_j \rangle$, etc. The limiting expressions for the fluctuations involving the excess energy can be obtained by a simple substitution of N_j or N_k with ε .

8 Application to Binary Mixtures

8.1 Bulk Thermodynamic Properties in Terms of Local Fluctuating Quantities

Binary mixtures are the most common type of system studied using FT. The expressions for the bulk properties in terms of local fluctuations are of both conceptual interest and also useful for the practical analysis of theoretical or simulation results. If we consider a binary mixture of a solvent (1) and a solute (2) and then define two additional variables,

$$\begin{aligned} \eta_{12} &= |M| = \rho_1(1 + N_{22} - N_{12}) + \rho_2(1 + N_{11} - N_{21}) \\ \zeta_2 &= |NN| = (1 + N_{11})(1 + N_{22}) - N_{12}N_{21} \end{aligned} \quad (38)$$

one finds the following results,

$$\begin{aligned} \mu_{22} &= \frac{\rho_1}{\eta_{12}} \left(\frac{\partial \beta \mu_2}{\partial \ln x_2} \right)_{T,p} = \frac{\rho}{\eta_{12}} \left(\frac{\partial \beta \mu_2}{\partial \ln \rho_2} \right)_{T,p} = \frac{1}{1 + N_{22} - N_{12}} \\ \bar{V}_i &= \frac{1 + N_{jj} - N_{ij}}{\eta_{12}} \\ \bar{H}_i^E &= \rho \bar{V}_i H_m^E + \frac{\rho_j}{\eta_{12}} (F_i - F_j) \\ k_B T \kappa_T &= \frac{\zeta_2}{\eta_{12}} \\ k_B T^2 \alpha_P &= \rho k_B T \kappa_T H_m^E - \phi_1 F_1 - \phi_2 F_2 \\ k_B T^2 C_{P,m} &= \Delta_m + k_B T^2 \alpha_P H_m^E - x_1 F_1 \bar{H}_1^E - x_2 F_2 \bar{H}_2^E \end{aligned} \quad (39)$$

Other chemical potential derivatives can be found by application of the GD expression. All the quantities on the right hand side involve local fluctuations or properties that can be expressed in terms of local fluctuations using a previous expression. In principle, they can be obtained from a single simulation. Hence, one can determine, given a set of fluctuating quantities, all the properties on the left hand side by a stepwise process. The above

expressions can be manipulated further to provide relationships for changes in the various activity coefficients,

$$\begin{aligned}\left(\frac{\partial \ln \gamma_2}{\partial \ln m_2}\right)_{T,p} &= -\frac{\rho_1(N_{22} - N_{12}) + \rho_2(1 + N_{11} - N_{21})}{\eta_{12}} \\ \left(\frac{\partial \ln f_2}{\partial \ln x_2}\right)_{T,p} &= -\frac{\rho_1(N_{22} - N_{12}) + \rho_2(N_{11} - N_{21})}{\eta_{12}} \\ \left(\frac{\partial \ln y_2}{\partial \ln \rho_2}\right)_{T,p} &= -\frac{N_{22} - N_{12}}{1 + N_{22} - N_{12}}\end{aligned}\quad (40)$$

which may be of more interest for certain applications.

At this point it is worth pausing to remind ourselves what we have accomplished here. The above expressions correspond to thermodynamic properties of solutions that are amenable to experiment. The expressions involve fluctuating quantities for an equivalent open system in which the average particle numbers are equal to the fixed number of particles per unit volume used in the experiment. The same argument holds for the chemical potentials, except in the opposite direction, and for the volume and the pressure, i.e. we are invoking the equivalence of ensembles approach. In addition, the open regions can be thought of as finite local regions in an essentially infinite bulk solvent. Hence, the approach provides information on local structure and fluctuations within the system. These local fluctuations are different from the bulk fluctuations characteristic of isothermal isobaric systems.

Examination of the above expressions clearly demonstrates that, even for binary mixtures, one requires combinations of multiple fluctuating quantities to provide the thermodynamic properties. This illustrates that the thermodynamic properties considered here are rather complex and will be difficult to relate to the underlying microscopic picture of molecular interactions and association. In contrast, focusing on the fluctuating quantities representative of local regions in the solution provides a simpler picture of solution behavior. It is more probable that one can explain the fluctuating quantities – in terms of molecular distributions or interactions, hydrogen bonds, hydrophobic interactions, etc – than one could explain the trends in the excess partial molar enthalpies, for instance. This is the argument we presented in the introduction.

In many cases one is interested in the limiting behavior when the solute concentration approaches zero. The limiting expressions are then,

$$\begin{aligned}\left(\frac{\partial \beta \mu_1}{\partial m_2}\right)_{T,p}^{\infty} &= -1 \left(\frac{\partial \beta \mu_1}{\partial x_2}\right)_{T,p}^{\infty} = -1 \left(\frac{\partial \beta \mu_1}{\partial \rho_2}\right)_{T,p}^{\infty} = -\frac{1}{\rho_1^o} \\ \left(\frac{\partial \beta \mu_2}{\partial m_2}\right)_{T,p}^{\infty} &= \frac{1}{m_2} \left(\frac{\partial \beta \mu_2}{\partial x_2}\right)_{T,p}^{\infty} = \frac{1}{x_2} \left(\frac{\partial \beta \mu_2}{\partial \rho_2}\right)_{T,p}^{\infty} = \frac{1}{\rho_2} \\ k_B T \kappa_T^o &= \frac{1 + N_{11}^o}{\rho_1^o} k_B T^2 \alpha_p^o = -F_1^o k_B T^2 C_{p,m}^o = \Delta_m^o \\ \bar{V}_1^o &= \frac{1}{\rho_1^o} \bar{V}_2^{\infty} = \frac{1 + N_{11}^o - N_{21}^{\infty}}{\rho_1^o} = k_B T \kappa_T^o - G_{12}^{\infty} \\ \bar{H}_1^{E,o} &= 0 \bar{H}_2^{E,\infty} = F_2^{\infty} - F_1^o = k_B T^2 \alpha_p^o + F_2^{\infty} \\ G_{12}^{\infty} &= (\langle N_1 \rangle_2 - \langle N_1 \rangle) V_1^o F_2^{\infty} = \langle \varepsilon \rangle_2 - \langle \varepsilon \rangle\end{aligned}\quad (41)$$

The limiting activity coefficient derivatives are provided by,

$$\begin{aligned}\left(\frac{\partial \ln \gamma_2}{\partial m_2}\right)_{T,p}^{\infty} &= -[1 + \rho_1^o(G_{11}^o + G_{22}^{\infty} - 2G_{12}^{\infty})] \\ \left(\frac{\partial \ln f_2}{\partial x_2}\right)_{T,p}^{\infty} &= -\rho_1^o(G_{11}^o + G_{22}^{\infty} - 2G_{12}^{\infty}) \\ \left(\frac{\partial \ln y_2}{\partial \rho_2}\right)_{T,p}^{\infty} &= -(G_{22}^{\infty} - G_{12}^{\infty})\end{aligned}\quad (42)$$

and can be used to analyze Henry's law, for instance.⁴⁷ The above expressions represent all the thermodynamic quantities typically provided by FT that are relevant for binary solutions. If one has access to the fluctuating quantities then it is possible to predict or rationalize the experimental properties. The main sources of the fluctuations would appear to be computer simulation and integral equation methods.⁴⁸ Hence, one can determine the fluctuating quantities and use this information to make contact with real experimental data. This can thereby provide added confidence in the theoretical results.

8.2 Local Fluctuating Quantities in Terms of Bulk Thermodynamic Properties

Most investigations of closed binary systems have involved the determination and examination of the three KBIs, possibly followed by some description of local composition or preferential solvation, as a function of composition. The fluctuating quantities in terms of experimentally available properties can be obtained from Equation 25. It is more common, however, to express the particle fluctuations in terms of partial molar volumes, the compressibility and chemical potential derivatives, rather than the pcg approach.¹³ Hence, we provide the more common relationships here. The final expressions are,

$$\begin{aligned}\delta_{ij} + N_{ij} &= \rho_j k_B T \kappa_T + \frac{x_j^2}{x_1 x_2} \frac{(1 - \phi_i)(1 - \phi_j)}{\mu_{ij}} \\ F_i &= -k_B T^2 \alpha_P + (\delta_{i1} + N_{i1}) \bar{H}_1^E + (\delta_{i2} + N_{i2}) \bar{H}_2^E \\ \Delta_m &= k_B T^2 C_{P,m} - k_B T^2 \alpha_P H_m^E + x_1 F_1 \bar{H}_1^E + x_2 F_2 \bar{H}_2^E\end{aligned}\quad (43)$$

All the data on the right hand side of the above expressions can be obtained from experiment or involve fluctuating quantities which can be expressed in terms of experimental data.

8.3 Fluctuation Theory Analysis of Experimental Data

To illustrate the usefulness of the above expressions we have analyzed the experimental data for several binary solution mixtures. The results are presented in Figure 4 for a series of small primary alcohols in water at 298 K and 1 bar. The fluctuating quantities obtained from the experimental data using Equation 43 are displayed as fluctuation densities as a function of composition. The three top panels provide KBI related quantities and have been investigated before. The bottom three panels, which all involve excess energy fluctuations, have only become available recently. The data indicate increasing values of B_{22} and B_{11} for the central regions, with a corresponding decrease in the B_{12} value, and an increasing trend with alcohol chain length. The trend with chain length is to be expected, as an increase in hydrophobic character would tend to promote local solute-solute and solvent-solvent self association or aggregation. This is manifested in an increase in the B_{ij} values and, therefore, an increase in the width of the solute-solute particle distribution. The B_{ij} values for water and propanol mixtures display a large peak or inverted peak around $x_2 = 0.2$ (note the scale change in this graph). The next alcohol in the series (n-butanol) phase separates in this region. What is most interesting is that no similar behavior is observable in the raw experimental data. This is usually the case. However, on extracting the particle number

fluctuations, one immediately sees that the solution behavior in this composition region is the most noticeable feature of the mixture and, presumably, deserves the most attention.

The energy fluctuations display similar features. The excess energy-energy fluctuations (Δ_m) for methanol and ethanol mixtures with water are rather simple. The fluctuations vary slowly from those of pure water to those of pure methanol. They are somewhat similar to the values expected for ideal mixtures, and very similar to the values obtained from just the heat capacity term in Equation 43. However, this is not true when one examines the propanol and water mixtures. Clearly, the large particle number fluctuations significantly influence the energy-energy fluctuations in the region of 0.2 mole fraction. The energy fluctuations increase in this region. This is to be expected as the particle fluctuations are the ultimate source of the energy fluctuations, although the energy of each particle can vary significantly. The excess energy-particle number fluctuations also exhibit the same behavior. The values for the mixture of methanol and water are somewhat similar to the ideal values, and to the excess partial molar enthalpies. However, as one moves to ethanol and then propanol the local fluctuations again display interesting features that are not apparent in the experimental data used for extracting these values.

The data in Figure 4 indicate changes in the local properties of solution mixtures. In many cases, this can be expressed in terms of changes in the local composition of the mixture. This has proved to be a very successful approach to understanding solution structure and has led to the concept of preferential solvation.^{49–51} Preferential solvation can be defined by use of the KBIs for both binary and ternary mixtures,^{52,53} although only qualitative results are typically reliable as the size of the local region is generally unknown when examining experimental data. It may be possible to consider the energy fluctuations in a similar way, but we know of no such approach.

Another series of interest is the poly alcohols. A FT analysis of methanol, 1,2-ethanediol, and glycerol with water is presented in Figure 5. Clear trends in the particle number fluctuations are observed as the number of hydroxyl groups is increased. The general trend is towards solutions that mix “better” with water at the local level, as evidenced by the decrease in B_{ii} values. A shift in the positions of the maxima and minima to lower alcohol compositions also occurs. The excess energy-particle number fluctuation densities are more varied and specific trends depend on the composition of interest. The values for 1,2-ethanediol are relatively insensitive to composition, whereas the methanol solutions displayed the largest variation.

A final example is provided by mixtures of acetone as a solute with water or chloroform as a solvent. Again, this probes the effects of changes in the solvent polarity on the fluctuating quantities and their composition dependence. The results are also displayed in Figure 5. A significant degree of local water self association (a large positive B_{11}) is observed for most compositions with acetone. In contrast, mixtures of chloroform and acetone display no such behavior. The acetone and chloroform mixtures are clearly more ideal, whereas aggregation is observed in acetone and water mixtures due to an inability of acetone to donate hydrogen bonds. A similar trend is observed for the excess energy related fluctuations. While these trends are not particularly surprising, FT provides a clear and meaningful way to quantify these effects.

The analysis of experimental data in this form is well established for the KBIs and their related number fluctuation densities.⁹ Alternatively, one can use the KBIs to define and quantify local composition and the degree of preferential solvation of one species by another in solution.^{10,50,52} In contrast, the analysis of the excess energy fluctuations is relatively new and the exact meaning of the variations in the fluctuations still needs to be explored.

What is clear from Figures 4 and 5 is that features not present in the bulk properties become clear in the local fluctuations. The fluctuations in the excess energy provide a measure of the movement of particles in and out of the local region, coupled with how energetic these particles are. The shape of the F_1 and F_2 data curves presented in the previous examples are essentially mirror images of each other. This probably reflects the fact that (on average) a fluctuation of one of the molecules will tend to increase the excess energy of the local region, while a change in the number of the other type of particle will therefore decrease the excess energy of the local region. This is simply related to the differences in the single particle excess energies. This issue will become clearer following the results presented in Section 10.

8.4 Osmotic Systems

Most biological processes occurring under cellular conditions involve systems that are open to some form of matter exchange. Osmotic systems also provide a way to study open systems and are the foundation for the McMillan-Mayer (MM) theory of solutions.^{45,54,55} Binary osmotic systems of a solute in a primary solvent typically employ a virial expansion for the osmotic pressure (Π) in terms of the solute number density such that,

$$\beta \Pi = \sum_{i \geq 1} \frac{1}{i} B_i \rho_2^i \quad (44)$$

where $B_1 = 1$ and several terms (2–5) are typically required in the sum. The above equation can be applied to directly fit the experimental data using the virial coefficients (B_n) as fitting constants. Experimental data concerning solute-solute interactions can then be obtained from B_2 . Although higher order osmotic virial coefficients are not normally required for biological solutes, due to the low solute concentrations involved, this is not the case for smaller and/or more soluble solutes.

In contrast to the traditional MM approach, the resulting FT related expressions can easily be applied at any concentration in any multicomponent system. Kirkwood and Buff recognized the possible uses of their theory for osmotic systems in their original paper.⁶ O'Connell and coworkers have since used KB theory to probe the exact relationships between osmotic virial coefficients and other thermodynamic properties of solution mixtures.⁵⁶ More recently, KB theory has been used to directly rationalize osmotic pressure data and equilibria in open and closed systems.^{31,57}

In this section, we outline how KB theory can be used to understand osmotic systems. One of the advantages of using KB theory is that the solution thermodynamics can be formulated in terms of integrals, which have a well defined physical significance. This is also true of the MM theory of osmotic systems, but then one is restricted to an interpretation in terms of distributions at infinite dilution in the primary solvent. This restriction is not required by KB theory, although MM theory is obtained, as expected, under infinite dilution conditions. Temperature will be assumed to be constant throughout this section.

The application of KB or fluctuation theory to binary osmotic systems provides expressions for derivatives of the osmotic pressure in terms of a series of integrals and the solute number density. The first derivative can be obtained directly from Equations 2 and 17 and is given by,

$$\beta \left(\frac{\partial \Pi}{\partial \rho_2} \right)_{\beta, \mu_1} = \frac{1}{1 + N_{22}} \quad (45)$$

Clearly, ideal osmotic behavior requires either a small solute concentration or $G_{22} = 0$ for all compositions. A tendency for solute self association ($G_{22} > 0$) would result in a lower than ideal ($\beta\Pi^{\text{id}} = \rho_2$) osmotic pressure as the solute concentration is increased, and *vice versa*. An expression for the second derivative has also been provided and can be written,

$$\beta \left(\frac{\partial^2 \Pi}{\partial \rho_2^2} \right)_{\beta, \mu_1} = - \frac{G_{22} + \rho_2 (G_{222} - G_{22}^2)}{(1 + N_{22})^3} \quad (46)$$

Both derivative expressions apply at any solute concentration. The second derivative of the osmotic pressure involves triplet solute correlations in the form of G_{222} . Taking derivatives of the right hand side of Equation 45 and equating with the right hand side of Equation 46 also provides an expression for the derivative of G_{22} with respect to solute concentration at constant T and solvent chemical potential,

$$G'_{22} = \left(\frac{\partial G_{22}}{\partial \rho_2} \right)_{\beta, \mu_1} = \frac{G_{222} - 2G_{22}^2}{1 + N_{22}} \quad (47)$$

Hence, if $G_{222} = 2G_{22}^2$ for all compositions, then the value of G_{22} will be independent of composition, whereas one requires $G_{222} = 0$ for ideal systems. However, when $G_{222} > 2G_{22}^2$, then G_{22} will tend to increase with composition and *vice versa*. When G_{22} is independent of composition, one finds that $\beta\Pi = G_{22}^{-1} \ln(1 + \rho_2 G_{22})$.

The limiting values of the KB integrals and their derivatives can be expressed in terms of the osmotic virial coefficients,

$$\begin{aligned} G_{22}^{\infty} &= -B_2 G_{222}^{\infty} = 3B_2^2 - B_3 \\ G_{22}^{\infty'} &= G_{222}^{\infty} - 2(G_{22}^{\infty})^2 = B_2^2 - B_3 \end{aligned} \quad (48)$$

The above expressions are necessarily equivalent to those of MM theory, except for the fact that we have not inferred the superposition approximation for the triplet potential of mean force to simplify and evaluate B_3 . The above relationships lead to the following osmotic pressure expansion,⁶

$$\beta \Pi = \rho_2 - \frac{1}{2} G_{22}^{\infty} \rho_2^2 - \frac{1}{3} [G_{222}^{\infty} - 3(G_{22}^{\infty})^2] \rho_2^3 + \dots \quad (49)$$

which provides the B_2 and B_3 coefficients in terms of KB integrals. Hence, MM theory is obtained from FT theory when the required derivatives are evaluated at infinitely dilute solute concentrations.

8.5 Cosolvent Effects on Surface Tension

The distribution of solutes at the solution/vapor interface has important consequences in chemistry and chemical engineering.^{58,59} Consequently, a variety of experimental and theoretical approaches have been used to help understand surface adsorption or exclusion. It is well established that an increase in the surface tension of a solution due to the addition of a solute indicates exclusion of that solute from the interface region, and *vice versa*. Unfortunately, it has been difficult to fully quantify such structural and thermodynamic changes using computer simulation data so that they may be compared with experimental data on surface adsorption. A recent application of FT to this problem has provided a solution to this problem.²²

Analysis of the GD expressions for the surface and bulk solution regions provides a differential for the surface tension (γ_s) of a liquid-vapor interface.⁶⁰ From this one can obtain the standard Gibb absorption expression for the change in surface tension on addition of a cosolvent,

$$\left(\frac{\partial \gamma_s}{\partial x_i}\right)_{\beta,p} = - \sum_{j>1} \Gamma_{sj} \left(\frac{\partial \mu_j}{\partial x_i}\right)_{\beta,p} \quad (50)$$

where the sum is over all components except for the primary solvent, and one could use any concentration derivative on both sides of the expression. The chemical potential derivative is a property of the bulk solution away from the interface, while the Γ_{sj} values quantify the surface adsorption of j molecules relative to the primary solvent. For a simple binary mixture this reduces to,

$$\left(\frac{\partial \gamma_s}{\partial x_2}\right)_{\beta,p} = - \Gamma_{s2} \left(\frac{\partial \mu_2}{\partial x_2}\right)_{\beta,p} \quad (51)$$

The activity derivative must be positive for stable solutions and hence the direction of the surface tension change is dictated by the sign of Γ_{s2} . The activity derivative is provided by FT as indicated in Equation 39. The surface adsorption can be related to the number of molecules within the interface region, or in terms of surface probability distributions by,

$$\Gamma_{sj} = A^{-1} (\langle N_j \rangle_s - m_j \langle N_1 \rangle_s) = \rho_j \int_0^\infty [g_j^s(z) - g_1^s(z)] dz \quad (52)$$

where z is the direction perpendicular to the surface of area (A). The integration over $g_j - g_1$ should be performed to a distance at which the integral remains unchanged, i.e. $g_j = g_1 = 1$, which may involve many solvation shells away from the surface. This type of formulation has proved useful in obtaining a consistent picture of solute effects on surface tension as provided by computer simulation.²²

8.6 Force Fields for Molecular Simulation

Fluctuation theory provides information on local fluctuations in solution and may be used to characterize the properties of mixtures. The fluctuations or integrals provided by FT are also relatively easy to obtain from computer simulation. Clearly, the results obtained from a computer simulation should agree with the experimentally extracted fluctuations providing that the description of the intermolecular interactions, i.e. the force field, is sufficiently accurate and one has adequate sampling of the solution distributions. Therefore, one can use FT results to test and improve the quality of force fields used for molecular simulation.^{61,62} We have taken this approach to help develop new, hopefully improved, force fields for the simulation of liquid mixtures.^{61,63}

Force fields, especially in the biomolecular simulation area, have traditionally been developed with the aid of quantum calculations.^{64,65} The calculations involve properties of molecules in the gas phase. Unfortunately, these properties change in the condensed phase. This is, of course, a rather well known effect. However, exactly how one has to adjust the description of intermolecular interactions to account for the role of molecular polarization in liquids is still unknown. Furthermore, many force fields only test the resulting models using properties of pure liquids. This is a necessary, but not sufficient test, to establish the quality of a force field. More recently, thermodynamic data such as the solvation free energies of solutes have been used to improve the quality of many force fields.⁶⁶ Unfortunately, this quantity does not depend on an accurate description of the solute-solute interactions. We have found that relatively poor agreement with the KBIs is observed for many solution

mixtures.^{63,67,68} This clearly indicates an incorrect balance between solute-solute, solute-solvent, and solvent-solvent distributions which arises due to an incorrect description of the interactions. The main problem appears to be related to the choice of effective charges characterizing the polarity of a molecule or ion.

In an attempt to improve current force fields, we have designed a set of solute-solvent models that specifically aim to reproduce the experimentally derived KBIs, and thereby ensure a reasonable balance in the molecular distributions. These are termed Kirkwood-Buff derived Force Field (KBFF) models. Several recent reviews highlight our progress in this area.^{69,70} In developing these new models our main focus has been on modifying the charge distributions assigned to the solutes and solvents. This has led to a series of models for small solutes representative of the functional groups found in peptides and proteins, together with various cosolvents of biological interest. The result is a complete force field for the simulation of peptides and proteins based on the KBFF approach (<http://kbff.chem.k-state.edu>). It is hoped that these models provide a significant degree of improvement for the accurate computer simulation of biomolecular systems. Others have taken a similar approach for small solutes.^{62,71}

As an example of the quality of data that can be obtained from a computer simulation, we present the results of a FT analysis of methanol and water mixtures in Figure 6. The simulations employed the KBFF model for methanol,⁷² the SPC/E model for water,⁷³ and were performed in periodic unit cells of 6 nm for simulation times of 50 ns with the same protocols as our other recent studies.⁷⁴ The simulations were then analyzed to determine the fluctuating quantities and compared with the analysis performed for Figure 3. The particle number and energy fluctuations are very well reproduced with all the same observed trends. The agreement is quantitative for most compositions. The models did not perform so well for the particle-energy fluctuations. This is somewhat surprising considering the excess enthalpy of mixing is very reasonable, although not perfect,⁷² compared to many other methanol models. It appears that the partial molar enthalpies, which are related to derivatives of the excess enthalpy of mixing are more sensitive indicators of the quality of a particular model. Nevertheless, the particle-energy fluctuations are of the appropriate magnitude. This provides some confidence in the quality of the distributions provided in Section 10.

9 Ternary Mixtures

9.1 Bulk Thermodynamic Properties in Terms of Local Fluctuating Quantities

Ternary systems are much more complicated than binary systems. The explicit expressions for the chemical potential derivatives, partial molar volumes and compressibility become rather cumbersome. Experimental data is also much less common. However, there are many interesting effects that involve ternary systems. Also, we shall see that considerable simplification is obtained when the solute is at infinite dilution. It will prove convenient to define the following set of variables,⁷⁵

$$A_i = 1 + \rho_i (G_{ii} + G_{jk} - G_{ij} - G_{ik}) \quad (53)$$

where i, j, and k can be 1, 2 or 3 and we note that,

$$\eta_{123} = |M| = \rho_1 A_2 A_3 + \rho_2 A_1 A_3 + \rho_3 A_1 A_2 \quad (54)$$

and,

$$\eta_{ij} = \rho_i A_j + \rho_j A_i = \rho_i (1 + N_{jj} - N_{ij}) + \rho_j (1 + N_{ii} - N_{ji}) \quad (55)$$

which is a generalization of Equation 38. The following expressions are then obtained for ternary systems,

$$\begin{aligned} \mu_{13} &= -\frac{\rho_3 A_2}{\eta_{123}} \mu_{23} = -\frac{\rho_3 A_1}{\eta_{123}} \mu_{33} = \frac{\eta_{12}}{\eta_{123}} \\ \bar{V}_1 &= \frac{(1 + N_{22} - N_{12})(1 + N_{33} - N_{13}) - (N_{32} - N_{12})(N_{23} - N_{13})}{\eta_{123}} \\ k_B T \kappa_T &= \frac{\zeta_3}{\eta_{123}} \\ \zeta_3 &= |NN| = (1 + N_{11})(1 + N_{22})(1 + N_{33}) - (1 + N_{11})N_{23}N_{32} - (1 + N_{22})N_{13}N_{31} - (1 + N_{33})N_{12}N_{21} + 2N_{12}N_{23}N_{31} \end{aligned} \quad (56)$$

The other chemical potential derivatives and partial molar volumes can then be obtained via a simple index change. Unfortunately, there are many equivalent alternative expressions for the thermodynamic properties. Other, arguably more useful, forms can be obtained for the chemical potential derivatives if one uses the notation,⁴²

$$N_{ij}^+ = N_{ij} + m_j(1 + N_{11} - N_{i1} - N_{j1}) \quad (57)$$

where i and $j > 1$. This expression is essentially the elements of the matrix used by Kirkwood and Buff in their original study.⁶ Consequently,

$$\begin{aligned} \mu_{23} &= -\frac{N_{23}^+}{(1 + N_{22}^+)(1 + N_{33}^+) - N_{23}^+ N_{32}^+} \\ \mu_{33} &= \frac{1 + N_{22}^+}{(1 + N_{22}^+)(1 + N_{33}^+) - N_{23}^+ N_{32}^+} \end{aligned} \quad (58)$$

However, one loses the symmetry inherent in the former expressions. In either case, the properties related to the energy fluctuations are then provided by,

$$\begin{aligned} \bar{H}_i^E &= \rho \bar{V}_i H_m^E + F_1 \mu_{i1} + F_2 \mu_{i2} + F_3 \mu_{i3} \\ k_B T^2 \alpha_P &= \rho k_B T \kappa_T H_m^E - \phi_1 F_1 - \phi_2 F_2 - \phi_3 F_3 \\ k_B T^2 C_{P,m} &= \Delta_m + k_B T^2 \alpha_P H_m^E - x_1 F_1 \bar{H}_1^E - x_2 F_2 \bar{H}_2^E - x_3 F_3 \bar{H}_3^E \end{aligned} \quad (59)$$

and complete the primary expressions provided by FT for ternary mixtures.

Again, the case of an infinitely dilute solute deserves some attention. The infinitely dilute expressions can be presented in various forms. The relationships obtained from Equations 56 and 59 are given by,

$$\begin{aligned} \mu_{23}^\infty &= \left(\frac{\partial \ln \gamma_2}{\partial \ln m_3} \right)_{T,p}^\infty = -\frac{\rho_1 \rho_3 (G_{23}^\infty - G_{21}^\infty)}{\eta_{13}} - \phi_3 \\ \left(\frac{\partial \ln \gamma_2}{\partial m_2} \right)_{T,p}^\infty &= -\rho_1 (G_{22}^\infty + G_{31}^\infty - G_{21}^\infty - G_{23}^\infty) - \frac{\rho_1 A_1 A_3}{\eta_{13}} \\ \bar{V}_2^\infty &= \frac{(1 + N_{11} - N_{21}^\infty)(1 + N_{33} - N_{13}) - (N_{31} - N_{21}^\infty)(N_{13} - N_{23}^\infty)}{\eta_{13}} = k_B T \kappa_T - N_{21}^\infty \bar{V}_1 - N_{23}^\infty \bar{V}_3 \\ \bar{H}_2^{E,\infty} &= \rho \bar{V}_2^\infty H_m^E + F_1 \mu_{21}^\infty + F_2^\infty + F_3 \mu_{23}^\infty = k_B T^2 \alpha_P + F_2^\infty - N_{21}^\infty \bar{H}_1^E - N_{23}^\infty \bar{H}_3^E \end{aligned} \quad (60)$$

The second form for the volumes and enthalpies was obtained from Equation 18 and is often more useful than the full expressions.

9.2 Local Fluctuating Quantities in Terms of Bulk Thermodynamic Properties

Inversion of the formulas for ternary mixtures to provide the fluctuating quantities is significantly more complicated for the particle number fluctuations. If one is simply interested in obtaining numerical values for the KBIs, then the matrix expression provided in Equation 25 can be solved directly using the experimental data as input. However, many applications require specific expressions for the various fluctuating quantities. The particle-energy and energy-energy expressions arise directly from Equations 18. The particle-particle expressions can be obtained from the matrix relation indicated by Equation 25, or by other approaches to provide,⁴⁴

$$\begin{aligned} 1+N_{11} &= \rho_1 k_B T \kappa_T + \rho_1 \frac{(\mu_{33} \bar{V}_2 - \mu_{23} \bar{V}_3) \phi_2 + (\mu_{22} \bar{V}_3 - \mu_{32} \bar{V}_2) \phi_3}{\mu_{22} \mu_{33} - \mu_{23} \mu_{32}} \\ N_{12} &= \rho_2 k_B T \kappa_T + \rho_2 \frac{(\mu_{13} \bar{V}_3 - \mu_{33} \bar{V}_1) \phi_2 + (\mu_{32} \bar{V}_1 - \mu_{12} \bar{V}_3) \phi_3}{\mu_{32} \mu_{13} - \mu_{12} \mu_{33}} \\ F_i &= -k_B T^2 \alpha_P + (\delta_{i1} + N_{i1}) \bar{H}_1^E + (\delta_{i2} + N_{i2}) \bar{H}_2^E + (\delta_{i3} + N_{i3}) \bar{H}_3^E \\ \Delta_m &= k_B T^2 C_{P,m} - k_B T^2 \alpha_P H_m^E + x_1 F_1 \bar{H}_1^E + x_2 F_2 \bar{H}_2^E + x_3 F_3 \bar{H}_3^E \end{aligned} \quad (61)$$

The expressions for other particle fluctuations can be obtained via simple index changes. Unfortunately there are many equivalent expressions for the particle fluctuations that correspond to various replacements of the chemical potential derivatives in the numerator and denominators using the GD expressions.

9.3 Equilibrium Dialysis

Our initial studies in the area of FT were motivated by a desire to better understand the effects of cosolvents on the properties of peptides and proteins.^{76,77} In particular, how could one analyze the results of a computer simulation to make contact with the experimental data?⁷⁸ The most direct and useful experimental data for this appears to be provided by equilibrium dialysis experiments.⁷⁹ Here, the effect of a protein on the distribution of a cosolvent and solvent across a membrane, impermeable to the protein, is measured in terms of changes in the solution density or cosolvent molality. An expression for the resulting preferential interaction parameters provided from the experimental data is available directly from Equation 19 and provides,⁸⁰

$$\Gamma_{23} = \left(\frac{\partial m_3}{\partial m_2} \right)_{T, \mu_1, \mu_3} = \frac{N_{23} - m_3 N_{21}}{1 + N_{22} - N_{12}} \quad (62)$$

which for the usual case of an infinitely dilute protein reduces to,

$$\Gamma_{23}^\infty = \rho_3 (G_{23}^\infty - G_{21}^\infty) = \langle N_3 \rangle_2 - m_3 \langle N_1 \rangle_2 \quad (63)$$

Hence, the value of the preferential interaction parameter is a measure of changes in the local distribution of the cosolvent and water surrounding the protein solute. Positive values occur when the cosolvent/solvent ratio in the vicinity of the protein exceeds that of the bulk solvent (m_3), and *vice versa*. The effects include both direct binding of the cosolvent to the protein together with any possible changes in the cosolvent and solvent distributions at larger distances from the protein surface. The parameters are composition dependent and under high cosolvent concentrations the protein may denature. The parameters then

correspond to a weighted binding to each form present in the solution. Assuming a two state transition one has,

$$\Gamma_{23}^{\infty} = f_D \Gamma_{D3}^{\infty} + f_N \Gamma_{N3}^{\infty} \quad (64)$$

where f_D and f_N are the fraction of denatured and native protein, respectively. We will see in Section 11.4 how these values can be used to provide exact relationships for understanding cosolvent denaturation of proteins under isothermal isobaric conditions.

There are a variety of other expressions related to Equation 62 where the compositions are measured by molarities or even mole fractions.^{18,81,82} Furthermore, different experiments, isopiestic distillation for instance, may probe slightly different conditions. These expressions have been summarized in the literature.⁷⁵

9.4 Cosolvent Effects on Solute Solubility

Cosolvents are well known to affect the solubility of solutes in solution. KB theory has been used to rationalize the effects of cosolvents on the solubility of sparingly soluble solutes in terms of intermolecular distributions. Hall and O'Connell were the first to suggest the use of KB theory to study changes in solubility in ternary systems with a finite solute solubility.^{14,42} In particular, KB theory has been applied to study low solute solubility in water by deriving KB based expressions for Henry's constant.⁴⁷ These studies typically used closed ensembles, sparingly soluble solutes, with solubilities and cosolvent concentrations expressed using mole fractions. Hall,⁴² and more recently Mazo,¹⁶ recognized that the most appropriate ensemble for these studies was one where T, P, and the chemical potential of the solute are held constant.

For isothermal studies one can express changes in the saturated solute molarity (S_2) in terms of the pcP,

$$d \ln S_2 = -\beta d\mu_2^* \quad (65)$$

An expression for the effect of a cosolvent on the solubility of the solute is then provided by FT after using Equation 93 and then eliminating one of the activity derivatives using the GD relation. The final expression is then,⁸³

$$\left(\frac{\partial \ln S_2}{\partial \rho_3} \right)_{\beta, p, \mu_2} = \frac{G_{23} - G_{21}}{1 + N_{33} - N_{13}} \quad (66)$$

and is valid for any solvent, solute and cosolvent concentration. This is essentially the same type of effect as described by the equilibrium dialysis data (see Section 9.3). Hence, a preferential binding of the cosolvent to the solute drives an increase in solubility. For sparingly soluble solutes the expression remains the same, but the denominator becomes a property of the binary solvent and cosolvent mixture only. The salting out constant (K_{23}) is typically associated with the slope of this saturation curve at infinite dilution of both the solute and the cosolvent. In this case one finds,

$$K_{23} = \left(\frac{\partial \ln S_2}{\partial \rho_3} \right)_{\beta, p, \mu_2}^{\infty} = \lim_{\rho_3 \rightarrow 0} G_{23}^{\infty} - G_{21}^{\infty} \quad (67)$$

There are many variations of salting out constants that have appeared depending on the solute and/or cosolvent concentration units, the ensemble, and the type of correlation functions that are employed.^{17,84,85} They can all be expressed in terms of fluctuations.⁸³

10 Particle Number and Energy Distributions in Solution

The fluctuating quantities outlined in the previous sections are related to the moments of the particle number and energy probability distribution $P = P(N_1, N_2, \dots, E \text{ or } \epsilon)$. For systems far away from a critical point a multivariate Gaussian distribution in the energy and particle numbers is often assumed. Hence, one can write,

$$P(Y) = \frac{1}{(2\pi)^{(n_c+1)/2} \sqrt{|\Sigma|}} \exp \left(-\frac{1}{2} (Y - \langle Y \rangle)^T \Sigma^{-1} (Y - \langle Y \rangle) \right) \quad (68)$$

where Y is a vector of the form $Y = (N_1, N_2, \dots, E \text{ or } \epsilon)$ and Σ is a symmetric correlation matrix of the form,

$$\begin{pmatrix} \langle \delta N_1 \delta N_1 \rangle & \langle \delta N_1 \delta N_2 \rangle & \dots & \langle \delta N_1 \delta E \rangle \\ \langle \delta N_2 \delta N_1 \rangle & \langle \delta N_2 \delta N_2 \rangle & \dots & \langle \delta N_2 \delta E \rangle \\ \vdots & \vdots & \ddots & \vdots \\ \langle \delta E \delta N_1 \rangle & \langle \delta E \delta N_2 \rangle & \dots & \langle \delta E \delta E \rangle \end{pmatrix} \quad (69)$$

This matrix is clearly related to the matrices provided earlier. In this section we investigate the above multidimensional distribution in more detail. It should be noted that various previous studies along this line have appeared in the literature. Kirkwood and Goldberg provided an elegant analysis of light scattering in multicomponent systems, which focused on composition fluctuations using a multivariate Gaussian approach.⁸⁶ More recently, Mazo has analyzed the eigenvalues of the B matrices for a series of binary systems and observed that most systems, over large composition ranges, can be described by B matrices that contain a large eigenvalue and a small eigenvalue, to the extent that the smaller eigenvalue can almost be neglected when attempting to understand the system properties.^{87,88} We will relate the current findings to these results. We could find no such analysis that included the particle-energy and energy-energy distributions.

Using computer simulations one can extract not only the number of particles of each type in a local region of space, but also the energy of that region. This latter quantity requires a pairwise decomposition of intermolecular interactions. Fortunately, this is the approach adopted by most (even polarizable) force fields for liquid simulations. In Figure 7 we provide the number and energy distributions for methanol and water mixtures. It is immediately clear that the distributions are not Gaussian in nature. Furthermore, while the energy distribution appears to be essentially symmetrical, the N_1 and N_2 probability distributions display significant asymmetry. The fact that there are differences between the assumed Gaussian distribution and the true distribution was recognized some time ago.⁸⁹ Fortunately, it appears that the second moments are the same in both cases.

In Figure 8 we provide scatter plots for the particle number and energy distributions obtained from simulations of methanol and water mixtures. It is clear that the data are correlated, i.e. when N_1 is smaller than the average then N_2 is larger than the average. This leads to particle number correlations with negative slopes. This correlation also appears in the energy probability distributions, but the slope can have either sign. In this system the energy of the region (E) tends to become lower in energy when fluctuations increase the number of water molecules, and *vice versa*. This is a result of the more favorable average energy per molecule for water compared to methanol.

The results follow the trends described by Mazo for his analysis of particle-particle fluctuations.⁸⁷ In particular, fluctuations in the various particle numbers are related through Equation 18. This indicates that the fluctuation in the number of N_1 and N_2 particles are

related to the respective partial molar volumes and the compressibility. For liquids approaching zero compressibility, albeit an impossible situation, the fluctuations are simply given by,

$$k_B T \kappa_T = \langle \delta N_1 \delta N_1 \rangle \langle N_1 \rangle^{-1} \bar{V}_1 + \langle \delta N_1 \delta N_2 \rangle \langle N_1 \rangle^{-1} \bar{V}_2 \approx 0 \quad (70)$$

and therefore suggests that $\delta N_1 \bar{V}_1 \approx -\delta N_2 \bar{V}_2$ on the average. This simply states that, for an incompressible fluid, exchanges of particle numbers occur in order to maintain the same total volume.

Diagonalization of the above matrix provides a series of eigenvalues and eigenvectors – three in the case of a binary solution. The eigenvalues correspond to the magnitude of the principle components of the various fluctuations. The eigenvectors form the components of a 3x3 rotation matrix, which transform the orthogonal fluctuating quantities into the observed results (Equation 69). To our knowledge, general expressions for the eigenvectors and eigenvalues have not been obtained. However, for binary systems one can diagonalize the number fluctuation matrix as illustrated below, and thereby partially diagonalize the full fluctuation matrix.

The above type of analysis was generalized by Kirkwood and Goldberg for particle number fluctuations.⁸⁶ They observed that one could decouple the composition and density fluctuations by transformations of the form,

$$\begin{aligned} \xi_V &= \sum_i N_i \bar{V}_i \\ \xi_i &= N_i - m_i N_1 \\ \xi_H &= E - \sum_i N_i \bar{H}_i = \epsilon - \sum_i N_i \bar{H}_i^E \end{aligned} \quad (71)$$

The final transformation was prompted by our recent work.^{32,37} Using these transformations one can show that,

$$\begin{aligned} \langle \xi_V \rangle &= V \langle \xi_2 \rangle = 0 \langle \xi_H \rangle = -pV \\ \langle \delta \xi_V \delta \xi_V \rangle &= V k_B T \kappa_T \langle \delta \xi_H \delta \xi_H \rangle = k_B T^2 C_p \\ \langle \delta \xi_2 \delta \xi_2 \rangle &= \frac{\langle N_2 \rangle}{\mu_{22}} \langle \delta \xi_V \delta \xi_H \rangle = -V k_B T^2 \alpha_p \\ \langle \delta \xi_V \delta \xi_2 \rangle &= \langle \delta \xi_H \delta \xi_2 \rangle = 0 \end{aligned} \quad (72)$$

It should be noted that the first and last transformations in Equation 71 can also be used to transform the Gibbs ensemble expressions for the compressibility, thermal expansion and heat capacity (Equation 11) into the expressions provided in Equation 72.³⁷ For binary systems the transformations provided in Equation 71 generate a diagonal correlation matrix for the particle number fluctuations. One of the eigenvalues is related to the compressibility of the system, while the other is related to a single chemical potential derivative.⁸⁶ The former is the small eigenvalue from the analysis performed by Mazo.⁸⁷ However, the matrix displayed in Equation 69 is not diagonal using the above transformations, due to the cross correlation in the energy and volume transformations. Furthermore, even the number fluctuation matrices for ternary or higher component systems are not diagonal with the above transformations.

Application of the above transformations to computer simulation data is also provided in Figures 7 and 8. The ξ_H and ξ_V probability distributions provided in Figure 7 are very close to symmetrical, although far from Gaussian in character. Furthermore, the ξ_V distribution in

Figure 7 is no closer to being Gaussian or symmetrical than the individual particle number distributions. In Figure 8 one observes that the collective particle number (ξ_2) and density (ξ_V) fluctuations are clearly orthogonal, as are the collective particle number and energy fluctuations (ξ_H), although the excess energy and volume fluctuations still present a degree of correlation related to the thermal expansion coefficient. In principle, one can fully diagonalize the above matrix of fluctuations. However, the corresponding transformations are unknown, even for binaries, and it is not clear that this is even required. The partially diagonalized matrix of number fluctuations was satisfactorily used by Kirkwood and Goldberg to study light scattering in multicomponent systems,⁸⁶ and hence it appears that other potential applications may be viable with a partial diagonalization.

In Figure 9 we compare the fluctuations in particle, energy, and excess energy obtained from simulation for the linear alcohols in water. The number-number fluctuations all display a negative slope, as indicated above, the magnitude of which increases with alcohol size and composition. The former trend is also a consequence of the approximate relationship provided by Equation 70. The reason for the latter trend is less obvious. The energy-number distributions also vary in magnitude and shape as one changes composition and system. The change in slope for the energy-alcohol number distributions is understandable. On average the local energy will become more unfavorable as the number of alcohol molecules in the region increases (pure water has a higher energy density than the pure alcohols in our models), while the pure solutions will display a negative slope if there is a net cohesive energy.

Also shown in Figure 9 are the fluctuations in the excess energy of the region. It is clear that distributions involving the excess energy and the particle number are almost orthogonal. This is due to the fact that the transformation to the excess energy is numerically very close to the transformation of Equation 71. The difference involves the excess enthalpies, which are typically small in magnitude compared to the pure enthalpies. Interestingly, it is only in the excess energy distributions that one observes features that may help explain the changes observed in the experimental fluctuations in the region of $x_2 = 0.2$ for propanol. The change in shape and character of the simulated distributions appears to suggest that, at low propanol compositions, the energy fluctuations increase substantially and become much less correlated with either particle number fluctuation. It is precisely these types of features which may help to deepen our understanding of changes in solution properties with composition. Clearly, additional work needs to be performed on more systems to fully appreciate this type of behavior. In our opinion, the combination of computer simulation and FT will play a major role in this area.

11 Molecular Association and Conformational Equilibria

11.1 General Background

Fluctuation theory has been applied to the study of chemical, conformational and association equilibria. O'Connell and coworkers have provided general expressions for reactive systems.⁹⁰ Ben-Naim derived expressions for the effect of a cosolvent on association equilibria in a primary solvent using an alternative approach where the chemical equilibrium conditions were imposed on the usual multicomponent KB expressions.⁹¹ Several studies have used expressions derived for thermodynamically independent infinitely dilute solutes to study the equilibrium between two infinitely dilute forms.^{81,92} More recently, we have derived general expressions that can be applied to interpret real experimental data for complex systems in a variety of ensembles.^{31,32} Here, we will focus on the simple case of molecular association, which can also be applied to conformational changes such as protein denaturation. The process can be described as the formation of an aggregate (A) formed from n monomers (M) of a solute (2) described by an equilibrium constant K such that,

$$nM \rightarrow AK = \frac{\rho_A}{\rho_M^n} N_2 = N_M + nN_A f_M = \frac{N_M}{N_2} f_A = \frac{nN_A}{N_2} \quad (73)$$

for which the equilibrium conditions are $\mu_A = n\mu_M$ and $d\mu_A = nd\mu_M$. We note that the equilibrium constant is expressed in terms of the actual concentrations, and not by concentrations that approximate the corresponding activities, and that the value of K could be made dimensionless by the inclusion of a standard concentration factor – although this has no effect on the following results. FT can be applied to the above situation to help interpret changes in the equilibrium constant due to changes in pressure, temperature, or the composition of the surrounding solution. From Equation 17 we find that,

$$d\ln K = -(F_A - nF_M)d\beta + \sum_i (N_{Ai} - nN_{Mi})(d\beta\mu_i - H_i^\circ d\beta) \quad (74)$$

after application of the equilibrium condition. The summation extends over all thermodynamically independent components. The fluctuations involve the species A, M and 2, whereas species 2 is the only thermodynamically independent component. The fluctuating quantities involving these three species are related by the expressions,

$$N_{M2} = 1 + N_{MM} + nN_{MA}N_{A2} = n + nN_{AA} + N_{AM} \quad (75)$$

which can be obtained from the original fluctuation expressions for the solute (2) by a simple substitution of $N_2 = nN_A + N_M$.³¹ The expression for $d\ln K$ provides the starting point for an investigation of changes in the equilibrium due to pressure, temperature and composition.

11.2 First Derivatives of the Equilibrium Constant

Several expressions can be obtained directly from Equation 74. Taking derivatives with respect to pressure with temperature and the independent compositions fixed provides,

$$\left(\frac{\partial \ln K}{\partial p}\right)_{\beta, \{N\}} = \beta \sum_i (N_{Ai} - nN_{Mi}) \bar{V}_i = \beta \left[\frac{\langle \delta N_A \delta \xi_V \rangle}{\langle N_A \rangle} - n \frac{\langle \delta N_M \delta \xi_V \rangle}{\langle N_M \rangle} \right] \quad (76)$$

Derivatives with respect to temperature with pressure and the independent compositions fixed provides,

$$\left(\frac{\partial \ln K}{\partial \beta}\right)_{p, \{N\}} = -(F_A - nF_M) + \sum_i (N_{Ai} - nN_{Mi}) \bar{H}_i^E = - \left[\frac{\langle \delta N_A \delta \xi_H \rangle}{\langle N_A \rangle} - n \frac{\langle \delta N_M \delta \xi_H \rangle}{\langle N_M \rangle} \right] \quad (77)$$

Finally, changes in composition with temperature and pressure fixed give,

$$\left(\frac{\partial \ln K}{\partial \rho_j}\right)_{\beta, p, \{m\}'} = \sum_i (N_{Ai} - nN_{Mi}) \mu_{ij}' \quad (78)$$

where a prime on the chemical potential derivative is used to indicate a derivative of μ_i with respect to ρ_j . All three expressions are exact and can be applied to solutions with any number of components at any concentrations. The terms on the right hand side represent fluctuating quantities or properties which can be expressed in terms of fluctuating quantities. They are equivalent to the standard volume change, and standard enthalpy change, when it is safe to replace activities by concentrations in the expression for K . A comparison with the expressions provided in Equation 93 indicates that the terms on the right hand side are

equivalent to differences in the pcg, volume and enthalpy between the two forms. The same approach can also be applied to study equilibria in other ensembles.^{31,32}

11.3 Second Derivatives of the Equilibrium Constant

Second derivatives of the equilibrium constant can also be expressed in terms of local fluctuating quantities. Our previous work provided relationships most suitable for the analysis of computer simulation data. A similar approach for the experimentally relevant expressions is rather complicated. However, the expressions can be greatly simplified by using the transformations provided in Section 10. This leads to the following general expressions,³²

$$\begin{aligned}
 \left(\frac{\partial^2 \ln K}{\partial p^2} \right)_{\beta, \{N\}} &= \beta^2 [N_{AVV} - N_{AV}^2 - n(N_{MNV} - N_{MV}^2)] - \beta \sum_i (N_{Ai} - nN_{Mi}) \bar{V}_i \bar{\kappa}_{T,i} \\
 \left(\frac{\partial^2 \ln K}{\partial \beta^2} \right)_{p, \{N\}} &= (N_{AHH} - F_{AH}^2) - n(N_{MHH} - F_{MH}^2) - k_B T^2 \sum_i (N_{Ai} - nN_{Mi}) \bar{C}_{p,i} \\
 \left(\frac{\partial^2 \ln K}{\partial p_k^2} \right)_{\beta, p, \{m\}'} &= \sum_{i,j} \mu'_{ik} \mu'_{jk} [N_{Aij} - N_{Ai} N_{Aj} - n(N_{Mij} - N_{Mi} N_{Mj})] + \sum_i (N_{Ai} - nN_{Mi}) \mu'_{ikk} \\
 \left(\frac{\partial}{\partial \beta} \left(\frac{\partial \ln K}{\partial p} \right) \right)_{\beta, \{N\}} &= -\beta [N_{AVH} - F_{AH} N_{AV} - n(N_{MVH} - F_{MH} N_{MV})] + \sum_i (N_{Ai} - nN_{Mi}) \bar{V}_i (1 - T \bar{\alpha}_{p,i})
 \end{aligned} \tag{79}$$

We note that there is no assumption of thermodynamic independence for A and M used during the derivation of the above expressions, only that 2 is thermodynamically independent. The above expressions contain third derivatives of the Gibbs energy involving triplet fluctuations, which we have attempted to simplify with the following notation:

$$\begin{aligned}
 N_{ijk} &= \frac{\langle \delta N_i \delta N_j \delta N_k \rangle}{\langle N_i \rangle} & N_{ijH} &= \frac{\langle \delta N_i \delta N_j \delta \xi_H \rangle}{\langle N_i \rangle} & N_{iHH} &= \frac{\langle \delta N_i \delta \xi_H \delta \xi_H \rangle}{\langle N_i \rangle} \\
 N_{ijV} &= \frac{\langle \delta N_i \delta N_j \delta \xi_V \rangle}{\langle N_i \rangle} & N_{iVH} &= \frac{\langle \delta N_i \delta \xi_V \delta \xi_H \rangle}{\langle N_i \rangle} & N_{iVV} &= \frac{\langle \delta N_i \delta \xi_V \delta \xi_V \rangle}{\langle N_i \rangle} \\
 F_{iH} &= \frac{\langle \delta N_i \delta \xi_H \rangle}{\langle N_i \rangle} \\
 N_{2jk} &= N_{Mjk} + n N_{Ajk} \\
 N_{A2k} &= N_{Ak2} = N_{AMk} + n N_{AAk} & N_{M2k} &= N_{Mk2} = N_{MMk} + n N_{MAk} \\
 N_{A22} &= N_{AMM} + 2n N_{AMA} + n^2 N_{AAA} & N_{M22} &= N_{MMM} + 2n N_{MMA} + n^2 N_{MAA}
 \end{aligned} \tag{80}$$

The subsequent relationships were obtained in the same manner as before and are useful for determining the behavior of the fluctuations for infinitely dilute solute concentrations. The partial molar compressibilities, thermal expansion coefficients, and heat capacities can also be expressed in terms of fluctuations. We have argued that the last term in each of the above expressions should be small for protein denaturation equilibria in a single solvent.³² However, this might not be generally true.

11.4 The Infinitely Dilute Solute Case

A system of common interest involves the solute at infinite dilution in a primary solvent. In this case the above relationships simplify to provide,

$$\begin{aligned}\left(\frac{\partial \ln K}{\partial p}\right)_{\beta, m_2}^{\infty} &= \beta(G_{A1}^{\infty} - nG_{M1}^{\infty}) \\ \left(\frac{\partial \ln K}{\partial \beta}\right)_{p, m_2}^{\infty} &= -(F_A^{\infty} - nF_M^{\infty}) \\ \left(\frac{\partial \ln K}{\partial \rho_3}\right)_{\beta, p}^{\infty} &= \frac{G_{A3}^{\infty} - G_{A1}^{\infty} - n(G_{M3}^{\infty} - G_{M1}^{\infty})}{1 + N_{33} + N_{13}}\end{aligned}\quad (81)$$

where the last expression is restricted to the case of a single cosolvent (3). The above equations are particularly useful for the analysis of biological systems where the solute is often at very low concentrations. They represent the effects on the equilibrium constant in terms of local fluctuations surrounding each form present in the equilibrium. The last expression for protein denaturation by a cosolvent can be written as,

$$\left(\frac{\partial \ln K}{\partial \rho_3}\right)_{\beta, p}^{\infty} = \frac{1}{\rho_3} \frac{\Gamma_{D3}^{\infty} - \Gamma_{N3}^{\infty}}{1 + N_{33} - N_{13}} \quad (82)$$

and provides a fluctuation based expression for the m-value.^{93,94} The solution stability requirements ensure that the denominator is positive. Indeed, for many denaturants it appears that the denominator can be considered to be essentially unity either due to the low cosolvent concentrations involved and/or the close to ideal nature of the cosolvent solution in the case of urea. Hence, the direction and magnitude of the denaturation effect is determined by the preferential interaction coefficients. If a cosolvent displays a larger preferential coefficient with the denatured state, then the equilibrium is shifted towards the denatured state on increasing the cosolvent concentration. The fact that the m-value is observed to be constant for many proteins denatured by urea and guanidinium chloride indicates that $G_{D3}^{\infty} - G_{N1}^{\infty}$ is essentially constant. The binding to both forms can then be isolated using equilibrium dialysis data and Equation 64.

Cosolvent denaturation is typically envisioned as a consequence of surface binding of the denaturant. This simple picture has been extended to include the exchange with water on cosolvent binding. However, the use of simple binding models appears to be somewhat simplistic and does not convey the full range of possibilities. Equation 82, on the other hand, provides a rigorous description of the cosolvent effect in terms of the local composition around each form of the protein. This includes both direct binding together with longer range effects, which are often referred to as changes in the “structure” of water.

Second derivatives of the equilibrium constant for an infinitely dilute solute are also of interest. This has proved to be a problem for the analysis of computer simulation data concerning the properties of proteins. In particular, the expressions usually provided for these quantities involve the application of fluctuation based formulas for bulk properties (Equation 11), applied to each form of the solute.^{95–97} This is incorrect on two levels. First, a solute, even the size of a protein, cannot be formally treated as a system. Second, the expressions treat the two forms of the solute as independent species, which they are not. Fluctuation theory makes neither approximation. The effect of pressure on the equilibrium becomes,

$$\left(\frac{\partial^2 \ln K}{\partial p^2}\right)_{\beta, \{N\}}^{\infty} = \beta(V_A^* - nV_M^*)\kappa_{T,1}^o + \beta^2[\langle \delta V_A^* \delta V_A^* \rangle - n\langle \delta V_M^* \delta V_M^* \rangle - (1-n)\langle \delta N_1 \delta N_1 \rangle (V_1^o)^2] \quad (83)$$

In the above expression we have defined an instantaneous infinite dilution pseudo protein volume given by $V^* = -(N_1 - \langle N_1 \rangle) V_1^o$, where N_1 is the instantaneous number of solvent molecules in the local region for each member of the ensemble and the average is for the same volume of bulk solution (see Equation 41). Hence, the solute volume in either form is provided by the number of solvent molecules in the local volume surrounding that form, compared to the number in the same volume of bulk solution. The “compressibility” of the reaction is given (as expected) by fluctuations in the solute volumes - as long as these are measured using the number of local solvent molecules. This is the second term in the above expression. The first term takes into account the changes in the solution compressibility on increasing the pressure. The last term is important, except for the $n = 1$ case, and removes the fluctuations due to solvent molecules moving in and out of the local volume, independent of any fluctuation in the solute volume.

The corresponding expression for the second temperature derivative takes a similar form and is given by,

$$\left(\frac{\partial^2 \ln K}{\partial \beta^2} \right)_{p, \{N\}}^{\infty} = k_B T^2 \rho_1^o (V_A^* - n V_M^*) C_{p,m,1}^o + \langle \delta \varepsilon \delta \varepsilon \rangle_A - n \langle \delta \varepsilon \delta \varepsilon \rangle_M - (1-n) \langle \delta \varepsilon \delta \varepsilon \rangle \quad (84)$$

where we have an instantaneous excess energy given by $\delta \varepsilon = \varepsilon - \langle \varepsilon \rangle_{A/M}$. Hence, the “heat capacity” associated with the equilibrium is related to differences in the excess energy fluctuations in the local regions surrounding both forms of the solute. The second derivative effect for a cosolvent on the equilibrium can be expressed by,

$$\left(\frac{\partial^2 \ln K}{\partial \beta_3^2} \right)_{\beta, p, m_2}^{\infty} = (V_A^* - n V_M^*) \phi_1^{-1} \mu'_{33} + (\Gamma_{A3}^{\infty} - n \Gamma_{M3}^{\infty}) \mu'_{333} + [\langle \delta \Gamma \delta \Gamma \rangle_A - n \langle \delta \Gamma \delta \Gamma \rangle_M - (1-n) \langle \delta \Gamma \delta \Gamma \rangle] (\mu'_{33})^2 \quad (85)$$

where we have an instantaneous preferential interaction parameter $\Gamma = N_3 - m_3 N_1$ and have used derivatives of the GD equation to provide $\mu'_{133} = -m_3 \mu'_{333} - (\rho_1 \phi_1)^{-1} \mu'_{33}$ for a binary solution of a solvent and cosolvent. The fact that this derivative is usually zero for protein denaturation suggests that fluctuations in the preferential interaction parameter are similar for both native and denatured proteins.

A full characterization of the equilibrium requires the cross derivative between temperature and pressure perturbations. This is provided by the following expression,

$$\left(\frac{\partial}{\partial \beta} \left(\frac{\partial \ln K}{\partial p} \right)_{\beta, m_2}^{\infty} \right)_{p, m_2}^{\infty} = -(V_A^* - n V_M^*) (1 - T \alpha_{p,1}^o) - \beta [\langle \delta N_1 \delta \varepsilon \rangle_A - n \langle \delta N_1 \delta \varepsilon \rangle_M - (1-n) \langle \delta N_1 \delta \varepsilon \rangle] V_1^o \quad (86)$$

As expected, it involves terms that resemble the expression for the thermal expansion coefficient, except for the local region surrounding each solute form. The second derivative expressions have only recently appeared in the literature,³² but they provide a rigorous description of the corresponding effects and it is envisioned they will also provide a solid foundation for the interpretation of the effects of temperature, pressure and composition on the equilibrium constant.

12 The Effects of Temperature, Pressure and Composition on Local Fluctuations

The local fluctuations or KBIs are dependent on temperature and pressure in addition to composition, although the composition dependence appears to be the most significant. In

Section 5.1 we illustrated how one can use derivatives of the average particle numbers and energy to provide expressions involving fluctuating quantities. The same approach can be applied to the resulting fluctuations. Substituting the fluctuations for X in Equation 15 one can determine the required derivatives.³² The simplest expressions involve the B_{ij} 's and provide,

$$\begin{aligned} \left(\frac{\partial B_{ij}}{\partial \beta} \right)_{p, \{N\}} &= - \frac{\langle \delta N_i \delta N_j \delta \xi_H \rangle}{V} \\ \left(\frac{\partial B_{ij}}{\partial p} \right)_{\beta, \{N\}} &= \beta \frac{\langle \delta N_i \delta N_j \delta \xi_V \rangle}{V} \\ \left(\frac{\partial B_{ij}}{\partial x_l} \right)_{p, \beta} &= \frac{1}{x_l(1-x_l)V} \sum_k \langle \delta N_i \delta N_j \delta N_k \rangle \mu_{kl} \end{aligned} \quad (87)$$

for any number of components. The first two expressions have been simplified by using the transformations for ξ_V and ξ_H provided in Equation 71. Some of the chemical potential derivatives can also be eliminated using the GD expression if desired. All the above derivatives involve triplet correlations. It is immediately apparent, therefore, that if the distribution of particle numbers and excess energy were truly characterized by a multivariate Gaussian, then the above derivatives would all be zero. Hence, the observed variations of the B_{ij} 's with temperature, pressure and composition probe the nature of the underlying true (non-Gaussian) distribution.

In principle, the experimental data required to compute the above derivatives is available for many systems. However, we know of very few (experimental or simulation) studies in this direction for all but infinitely dilute solutes.^{98,99} The most relevant is a recent analysis of the composition dependence of the osmotic pressure for binary systems,⁵⁷ where the expressions are somewhat simpler to develop. It is highly probable that such an analysis is numerically challenging. Given the sensitivity of the KBIs to the quality of the activity data, the additional derivatives required for the triplet correlations could be problematic. Furthermore, it is unlikely that the KBIs vary dramatically with pressure, unless one examines very high pressures. The interpretation of the above derivatives is also complicated by the presence of many terms, although all the terms could be determined.

The last expression in Equation 87 does, however, provide some insight in to the nature of the composition dependence of the B_{ij} 's. For many binary mixtures one observes maxima and minima in the B_{ij} 's as a function of composition. Using the GD expression it is possible to show that this maxima or minima occurs when $N_{1ij} = N_{2ij}$. The significant changes of the B_{ij} s with composition observed in many systems suggest the particle number fluctuations cannot be Gaussian or symmetric in nature.

Matteoli and Lepori have investigated the experimental data for aqueous methanol and ethanol mixtures as a function of temperature.⁹⁸ The alcohol KBIs (G_{22}) are essentially independent of temperature until one reaches low solute concentrations. In this region the G_{22} values systematically increase with temperature for both alcohols. This clearly indicates a positive sign for the $\langle \delta N_2 \delta N_2 \delta \xi_H \rangle$ triplet fluctuation at low alcohol concentrations according to Equation 87. However, the small changes of the properties with temperature suggests that the distribution must be close to symmetric in nature.

The other primary fluctuating quantities can also be affected by temperature, pressure and composition. The variations in the F_i 's are provided by,

$$\begin{aligned} \left(\frac{\partial \rho_i F_i}{\partial \beta}\right)_{p,\{N\}} &= -\frac{\langle \delta N_i \delta \varepsilon \delta \xi_H \rangle}{V} + \frac{k_B T^2}{V} \sum_j \langle \delta N_i \delta N_j \rangle C_{p,m,j}^o \\ \left(\frac{\partial \rho_i F_i}{\partial p}\right)_{\beta,\{N\}} &= \beta \frac{\langle \delta N_i \delta N_j \partial \xi_V \rangle}{V} - \frac{1}{V} \sum_j [\langle \delta N_i \delta N_j \rangle V_{m,j}^o (1 - T \alpha_{p,j}^o)] \quad (88) \\ \left(\frac{\partial \rho_i F_i}{\partial x_l}\right)_{p,\beta} &= \frac{1}{x_l(1-x_l)V} \sum_j \langle \delta N_i \delta N_j \delta \varepsilon \rangle \mu_{jl} \end{aligned}$$

whereas the variation in Δ_m is provided by,

$$\begin{aligned} \left(\frac{\partial \rho \Delta_m}{\partial \beta}\right)_{p,\{N\}} &= -\frac{\langle \delta \varepsilon \delta \varepsilon \delta \xi_H \rangle}{V} + \frac{2k_B T^2}{V} \sum_i \langle \delta N_i \delta \varepsilon \rangle C_{p,m,i}^o \\ \left(\frac{\partial \rho \Delta_m}{\partial p}\right)_{\beta,\{N\}} &= \beta \frac{\langle \delta \varepsilon \delta \varepsilon \delta \xi_V \rangle}{V} - \frac{2}{V} \sum_i [\langle \delta N_i \delta \varepsilon \rangle V_{m,i}^o (1 - T \alpha_{p,i}^o)] \quad (89) \\ \left(\frac{\partial \rho \Delta_m}{\partial x_l}\right)_{p,\beta} &= \frac{1}{x_l(1-x_l)V} \sum_i \langle \delta \varepsilon \delta \varepsilon \delta N_i \rangle \mu_{il} \end{aligned}$$

However, we know of no studies that have attempted to characterize these variations.

For binaries solutions it is possible to introduce the definition of ξ_2 to simplify the above expressions. A further simplification is provided if one considers the activity derivative. The final expressions are then,

$$\begin{aligned} \left(\frac{\partial B_{ij}}{\partial \beta \mu_2}\right)_{p,\beta} &= \frac{\langle \partial N_i \partial N_j \delta \xi_2 \rangle}{V} \\ \left(\frac{\partial \rho_i F_i}{\partial \beta \mu_2}\right)_{p,\beta} &= \frac{\langle \partial N_i \partial \varepsilon \delta \xi_2 \rangle}{V} \quad (90) \\ \left(\frac{\partial \rho \Delta_m}{\partial \beta \mu_2}\right)_{p,\beta} &= \frac{\langle \delta \varepsilon \delta \varepsilon \delta \xi_2 \rangle}{V} \end{aligned}$$

Hence, the activity dependence of the fluctuating quantities probes the nature of the correlation between the transformed particle number fluctuation and the original fluctuating quantities.

13 Analysis of Computer Simulation Data

It is clear that computer simulations represent one of the better approaches to determining the local fluctuations. There are, however, some technical difficulties which can arise during a typical analysis. We discuss some of these issues here. Most evaluations of the KBIs have used the integration approach, in contrast to the actual particle number fluctuations. Furthermore, as the vast majority of simulations are performed for closed periodic systems, one is naturally limited to performing the integration out to some cutoff distance from the particle of interest. It is then assumed that that the rdfs are essentially unity beyond this distance. In practice, deciding exactly where to truncate the integral can be rather subjective. In our previous studies we usually plot the KBIs as a function of integration or cutoff distance. Hence, one can define distance dependent KBIs (and even distance dependent thermodynamic functions) such that,

$$G_{ij}(R) \approx 4\pi \int_0^R [g_{ij}^{(2)}(r) - 1] r^2 dr \quad (91)$$

A plot of $G_{ij}(R)$ can be very informative. In favorable cases, the integral converges and one observes a limiting constant value for the KBI. More typically, there can be significant statistical noise that obscures the real limiting behavior. The situation can be improved by simulating relatively large systems (> 6 nm box length) for times in excess of 10 ns. However, this approach is not always productive.

Several studies have investigated this problem with a variety of suggested solutions.^{39,100,101} The problem seems to be particularly acute when determining the isothermal compressibility, especially if this property is plotted as a function of the integration distance. Slowly converging oscillatory behavior is commonly observed. The isothermal compressibility obtained from simple integration should be considered questionable at best. Fortunately, the situation is significantly improved when examining the partial molar volumes and chemical potential derivatives. This is probably related to the fact that the partial molar volume and chemical potential derivative expressions only involve differences in the KBIs. In the majority of our previous simulations, we have chosen to extract the final KBI values after averaging the integrals over a short range of distances (typically a full solvation shell) where the integrals appear to be converging.¹⁰² This is a subjective approach. However, we have tested the values of the resulting properties with other approaches – typically finite difference compressibilities or partial molar volumes obtained from the simulated densities – and found good agreement.⁷² This provides some confidence in the above approximation. An investigation of the activity of urea and water mixtures has also been performed using thermodynamic integration techniques,¹⁰³ and provides excellent agreement with our own KBI determined values. However, care should always be taken to ensure that reasonable estimates for the KBIs are obtained.

A slightly different approach is usually adopted for the particle-energy (F_i) and energy-energy (Δ_m) fluctuating quantities. First, to evaluate these properties one has to determine the energy of a given volume of the solution. This is possible if one can assign energies to each particle in the system. Single particle potential energies can be determined by assigning one half of the potential energy for the interaction between each pair of molecules.³⁵ This is even possible for models that include explicit polarization. In principle, the internal energy should include all intramolecular contributions and the kinetic energy of the molecules. In practice, this may be somewhat awkward to achieve. It may be safe to assume the intramolecular potential energies cancel in determining the value of ε , i.e. they are the same in the mixture and the pure liquids. The kinetic energy contribution to the local energy cancels in the calculation of the F 's such that one can use just the potential energies for both the instantaneous internal energies (E) and the reference pure solvent values. The kinetic energy contribution to the $\beta^2\Delta_m$ values does not cancel. For the nonlinear molecules studied here this contribution is simply 3 and arises from the three translational and three rotational degrees of freedom. The intramolecular kinetic energy contribution is more problematic as high frequency vibrations will not contribute to the heat capacity at normal temperatures. However, heavier molecules at higher temperatures could provide a significant contribution. Classical simulations would provide the equipartition values at all temperatures for all non-constrained vibrational degrees of freedom. A series of random origins can then be chosen and the instantaneous energy and number of particles determined as a function of the volume of a local region centered at each origin. The fluctuations observed after averaging over the trajectory provide values of F_i and Δ_m as a function of local volume (sphere radius). A graph of F_i or Δ_m as a function of sphere radius should tend to a constant value for radii large enough to approach the bulk solution distributions (see Figure 3).

Another issue arises when determining the KBIs, or local distributions, around infinitely dilute solutes. An example of this includes our study of the solvent and cosolvent distributions around a single protein solute.¹⁰⁴ As the simulations involve finite systems, it

is sometimes necessary to adjust the bulk distribution (m_3) during the analysis. For instance, the ratio of cosolvent to solvent molecules in the bulk region might not be equal to the ratio of total molecules used in the simulation when cosolvent and solvent molecules can exchange with other molecules in the local vicinity of the protein. This is especially true for small systems and/or large values of Γ_{23} . In this case the value of the preferential binding should be adjusted so that,

$$\Gamma_{23}^{\infty} = \langle N_3 \rangle_2 - m_3 \langle N_1 \rangle_2 = \langle N_3 \rangle_2 - \frac{N_3^o - \langle N_3 \rangle_2}{N_1^o - \langle N_1 \rangle_2} \langle N_1 \rangle_2 \quad (92)$$

where the zero superscript indicates the total number of molecules in the system. Hence, as molecules move in or out of the local region the bulk ratio is correspondingly adjusted. This adjustment may appear small but is magnified by the fact that $\langle N_1 \rangle_2$ may be very large for biological solutes.

Finally, we note that the problem of choosing a reference energy/enthalpy for the energy fluctuations is not actually required for the analysis of computer simulation data. One can, in fact, determine the H_i values themselves. Hence, they can be directly used in expressions such as that provided by the energy transformation in Equation 85. This has the advantage that the expressions for many of the properties described here become much simpler with this formulation. More details can be found in the literature.³²

14 Pseudo Chemical Potentials, Volumes and Enthalpies

As mentioned previously, the pseudo chemical potential and related properties can be used to analyze experimental data concerning a variety of processes – transfer free energies, etc.¹⁰ For many applications it is advantageous to use general fluctuation based expressions for the pseudo chemical potential, volume and enthalpy directly. From Equation 18 one finds,

$$\begin{aligned} \mu_{ik}^* &= - \sum_j N_{ij} \mu_{jk} = - \sum_{j>1} (N_{ij} - m_j N_{i1}) \mu_{jk} = - \sum_{j>1} \Gamma_{ij} \mu_{jk} \\ V_i^* &= - \sum_j N_{ij} \bar{V}_j \\ H_i^{*,E} &= H_i^* - H_i^{*,o} = k_B T^2 (\alpha_p - \alpha_{p,i}^o) + F_i - \sum_j N_{ij} \bar{H}_j^E \end{aligned} \quad (93)$$

after taking the appropriate derivatives. These expressions can also be used directly for the study of changes in chemical equilibria (see Section 11.2).

15 Ideal Solutions

The properties of ideal solutions are of interest for several reasons. First, the KBIs are not typically zero for solutions exhibiting ideal behavior. Second, they provide a good reference point for many properties of real solutions. Third, in cases where one does not possess the relevant experimental data, one can assume ideal behavior as a (hopefully) reasonable form of approximation. Solution ideality is generally thought to arise when the different species correspond to molecules of a similar size, and for which the intermolecular interactions are also of a similar magnitude. However, ideal behavior also depends on the concentration scale of interest. Ideal solutions defined using the most common (LR scale) activities are characterized by $\beta \mu_i = \ln x_i$ and zero excess enthalpies and volumes of solution over the whole composition range. This is also referred to as symmetric ideal (SI) behavior. Such solutions then obey the following relationships,¹⁰⁵

$$\begin{aligned}
 G_{ij} &= k_B T \kappa_T - V_i^o - V_j^o + S_{n_c} \quad S_{n_c} = \sum_k \rho_k (V_k^o)^2 \\
 \Delta G_{ij} &= G_{ii} + G_{jj} - 2G_{ij} = 0 \\
 \Delta_m &= k_B T^2 C_{p,m} \quad F_i = -k_B T^2 \alpha_p \\
 V_m &= \sum_k \chi_k V_k^o \quad \kappa_T = \sum_k \phi_k \kappa_{T,k}^o \quad \alpha_p = \sum_k \phi_k \alpha_{p,k}^o \quad C_{p,m} = \sum_k \chi_k C_{p,m,k}^o
 \end{aligned} \tag{94}$$

where the sum is over all the components and the expressions are valid for all i,j, combinations. Other relationships exist for ideality using different concentration scales. For ideal behavior on the molality scale, one requires $\beta \mu_i = \ln m_i$, which is provided when,

$$G_{ij} = k_B T \kappa_T - V_i^o - V_j^o + S_{n_c} - \delta_{1i} \delta_{1j} \rho_1^{-1} \tag{95}$$

with V_1^o set to zero. While for ideal behavior on the molarity scale one requires $\beta \mu_i = \ln \rho_i$, for which one must therefore have,

$$G_{ij} = k_B T \kappa_T - V_o \tag{96}$$

Here, all the volumes of the pure components must be equal. This is clearly an impossible situation and will be of little practical use. However, some solution mixtures do approach this type of behavior over a reasonable concentration range,¹⁰⁶ although this is due to the similar values for the G_{ij} and certainly not because they are the same size and or character. Finally, we note that ideal osmotic solutions (molarity scale) are observed when all G_{ij} 's are zero for all compositions. Again, we argue that the simplicity of this latter situation, compared to Equations 94–96 for example, is a consequence of the fact that the system is open to matter exchange.

16 Electrolyte Solutions

Electrolyte solutions initially appeared somewhat troublesome for KB theory.^{7,8} It is generally assumed that the number of cations surrounding an anion must result in charge neutrality for the local region. These relationships are known as the electroneutrality conditions and can be written,

$$\begin{aligned}
 z_+ + z_+ \rho_+ G_{++} + z_- \rho_- G_{+-} &= 0 \\
 z_- + z_- \rho_- G_{--} + z_+ \rho_+ G_{+-} &= 0 \\
 z_+ \rho_+ G_{i+} + z_- \rho_- G_{i-} &= 0 \\
 z_+ \rho_+ F_+ + z_- \rho_- F_- &= 0 \\
 n_+ z_+ + n_- z_- &= 0
 \end{aligned} \tag{97}$$

for a salt ($M_{n_+}^{z_+} X_{n_-}^{z_-}$) that generates a total of $n_{\pm}$ ions containing n_+ and n_- cations and anions in solution with charges of z_+ and z_- , respectively, and where the index i refers to any nonelectrolyte component. It was shown quite early that if one treats a salt solution as a mixture where the cations, anions and solvent are all treated as independent variables, then application of the electroneutrality conditions leads to a singular number fluctuation matrix which appears to invalidate KB theory.⁷ The simplest way to avoid this problem is to not treat the anion and cation concentrations as independent variables. The ion concentrations cannot, after all, be varied independently. This leads to either a treatment in terms of salt “molecules” (s), or as a collection of indistinguishable ions (2), the latter being more convenient for comparison with simulation data. The latter approach also agrees with the more formal treatment of Kusalik and Patey.⁸

More recently, Ben-Naim has argued that KB theory is perfectly valid for salt solutions and that one can in principle treat the anions and cations as independent variables.¹⁰ His argument lies in the fact that the KBIs are defined for a grand canonical ensemble and therefore the electroneutrality constraints represent an unnecessary restriction on the corresponding distributions. Furthermore, he argues one could, in principle, vary the anion and cation concentrations independently, but unfortunately the data is simply not available. In light of the absence of the required data it seems reasonable to invoke the electroneutrality expressions and to treat the anion and cation concentrations as dependent variables. A simple substitution of $N_2 = N_+ + N_-$ then provides relationships between distributions obtained using the indistinguishable ion notation (2) and the individual cation and anion distributions,

$$\begin{aligned} G_{ss} &= \frac{1-n}{\rho_2} + G_{22} & G_{si} &= G_{2i} \\ G_{22} &= \left(\frac{n_+}{n_{\pm}}\right)^2 G_{++} + \left(\frac{n_-}{n_{\pm}}\right)^2 G_{--} + \frac{n_+ n_-}{n_{\pm}} (G_{+-} + G_{-+}) \\ G_{2i} &= G_{i2} = \frac{n_+}{n_{\pm}} G_{i+} + \frac{n_-}{n_{\pm}} G_{i-} \\ F_2 &= \frac{n_+}{n_{\pm}} F_+ + \frac{n_-}{n_{\pm}} F_- \end{aligned} \quad (98)$$

They above expressions do not assume electroneutrality and simply represent an index change. Subsequent application of the electroneutrality conditions leads to a series of relationships between the fluctuating quantities,

$$\begin{aligned} G_{22} &= -\frac{1}{\rho_2} + G_{+-} & G_{+-} &= -\frac{1}{\rho_+} + G_{++} \\ \frac{1}{\rho_+} + G_{++} &= \frac{1}{\rho_-} + G_{--} \\ G_{i2} &= G_{i+} = G_{i-} & F_2 &= F_+ = F_- \end{aligned} \quad (99)$$

Consequently, there is only one unique or independent fluctuating quantity involving the salt species, the others being related by the above expressions. This results in the following expressions being used to analyze the experimental data,

$$\begin{aligned} m_2 &= n_{\pm} m_s & \rho_2 &= n_{\pm} \rho_s & n_{\pm} \bar{V}_2 &= \bar{V}_s \\ \mu_{22} &= 1 + \left(\frac{\partial \ln \gamma_{\pm}}{\partial \ln m_s} \right)_{p,T,\{m\}'} & \mu_{ss} &= n_{\pm} \mu_{22} & n_{\pm} \bar{H}_2^E &= \bar{H}_s^E \end{aligned} \quad (100)$$

where $\gamma_{\pm}$ is the mean ion molal activity coefficient.

17 Summary and Future Directions

In the previous sections we have attempted to provide a relatively comprehensive summary of the theory and applications of a general fluctuation approach to understanding the behavior of solutions. It is quite clear that many of the above expressions for “normal” thermodynamic quantities appear to be quite complex. We are arguing that this complexity arises due to the constraints implied by the typical thermodynamic conditions of constant temperature, pressure, and composition. In contrast, a simpler picture of solutions emerges when one isolates the particle number and energy fluctuations corresponding to local regions of the solution, i.e. a microscopic constant volume and temperature region open to all matter exchange, for which the average pressure, energy, and number of particles corresponds to that of the experimental macroscopic conditions. It seems clear to us that attempting to understand the characteristics of solutions using properties of these open local regions is much simpler than focusing on the much larger bulk properties. Nevertheless, a simple picture of solution mixtures remains elusive.

At this stage it appears that a general FT of solutions, together with association and conformational equilibria, is essentially complete. The expressions provided here are exact and can be applied to any stable solution mixture containing any number of components at any concentration. In particular, we envision the application of FT to biological systems to continue, especially in the area of conformational equilibria. The natural coupling of FT and computer simulation holds great promise for future study. The ability to express changes in the equilibrium constant in terms of local properties has led to expressions for the enthalpy, heat capacity changes, etc, which have previously been treated in a somewhat ad hoc manner, but are now available through a rigorous approach. Hopefully, this will provide significant insight into biological equilibria such as protein denaturation and peptide aggregation.

It is well known that many non-equilibrium effects that are observed in the presence of a perturbation can be related to fluctuations observed in the absence of that perturbation, i.e. to equilibrium properties. It seems that the local fluctuations described here could be of future use in understanding many perturbing effects on solutions. We anticipate future work in this area which will further advance the applications of a general fluctuation theory of solutions.

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Figure 1.

A schematic illustration of the local versus bulk representations of solution behavior. Both the local and bulk regions are characterized by the same average pressure and temperature. However, in the bulk region the number of particles is fixed and the chemical potentials are obtained as ensemble averages. In the relatively small local region molecules are free to enter or leave in an effort to maintain a set of constant chemical potentials, determined by the bulk composition, which acts as a matter bath.

Figure 2.

A general scheme for obtaining local fluctuating quantities from bulk thermodynamic properties. See text for the definition of the symbols. All properties are composition dependent.

Figure 3. Radial distribution functions, Kirkwood-Buff integrals, particle-excess energy and excess energy-energy fluctuations obtained from a simulation of methanol (2) and water (1) mixtures ($x_2 = 0.25$) at 300 K and 1 bar. The properties are displayed as a function of distance (r) and integration distance or sphere radius (R) in nm. The KBIs obtained from integration of the rdfs correspond to the oscillating curves (thick lines) in the bottom left panel.

Figure 4.

A fluctuation theory analysis of water (1) and alcohol (2) mixtures as a function of composition at 298 K and 1 bar. The panels correspond to methanol (left), ethanol (center), and n-propanol (right) mixtures, respectively. Experimental data were taken from the literature,^{107–111} and analyzed using Equation 43. The data are displayed as number-number (B_{ij}), number-excess energy ($\rho_i F_i$), and excess energy-energy ($\rho \Delta_m$) fluctuation densities in units of mol/L. Solid lines correspond to the experimental analysis and the dashed lines to the symmetric ideal values (see Section 15). The dotted lines appearing on the number-excess energy and excess energy-energy fluctuation plots correspond to the experimental $\beta \rho_i \bar{H}_i^E$ and $\rho C_{p,m}/kB$ data, respectively.

Figure 5.

A fluctuation theory analysis of water (1) and alcohol (2) mixtures (left panel) and solvent (1) and acetone (2) mixtures (right panel) as a function of composition at 298 K and 1 bar. The left panel contains data for methanol (solid lines), 1,2-ethanediol (dotted lines), and glycerol (dashed lines). The right panel contains data for the solvents water (solid lines) and chloroform (dashed lines). Experimental data was taken from the literature,^{99,107–110,112–122} and analyzed using Equation 43. The data are displayed as number-number (B_{ij}), number-excess energy ($\rho_i F_i$), and excess energy-energy ($\rho \Delta_m$) fluctuation densities in units of mol/L.

Figure 6.

A fluctuation theory and computer simulation analysis of water (1) and methanol (2) mixtures as a function of composition at 298 K and 1 bar. Experimental data (solid lines) was taken from the literature and analyzed using Equation 43 (see Figure 4 caption). The simulated data (closed circles) were determined from simulations using the KBFF model for methanol,⁷² together with the SPC/E water model,⁷³ using procedures outlined previously.⁷⁴ The data are displayed as number-number (B_{ij}), number-excess energy ($\rho_i F_i$), and excess energy-energy ($\rho \Delta_m$) fluctuation densities in units of mol/L.

Figure 7.

Number, energy, and excess energy probability distributions for methanol (2) and water (1) mixtures at 300 K and 1 bar obtained from computer simulation. The distributions correspond to local spherical regions with a radius of 1.5 nm. The raw data are displayed in the top panel, while the transformed quantities of Equation 71 are displayed in the bottom panel. The probabilities are in arbitrary units. Energies and ξ_H are in units of kJ/mol, with ξ_V in units of L/mol. Only the intermolecular energies are used in the analysis. The solid, dotted, and dashed lines correspond to methanol mole fractions of 0.25, 0.50 and 0.75, respectively.

Figure 8.

Number-number and number-energy distributions for methanol (2) and water (1) mixtures at 300 K and 1 bar obtained from computer simulation. The distributions correspond to local spherical regions with a radius of 1.5 nm. In the top panel, the reduced number, $\delta N'_i = \delta N_i / \langle N_i \rangle$, and energy, $\delta E' = \delta E / |\langle E \rangle|$, fluctuations are provided. In the bottom panel, the transformed quantities of Equation 71 are displayed as reduced properties $\delta \xi'_2 = \delta \xi_2 / \langle N_2 \rangle$, $\delta \xi'_V = \delta \xi_V / V$, and $\delta \xi'_H = \delta \xi_H / |\langle E \rangle|$. All axes range from -1 to $+1$ with lines drawn through $x = 0$ and $y = 0$. Only the intermolecular energies are used in the analysis.

Figure 9.

Number-number and number-energy distributions for the alcohol (2) and water (1) mixtures at 300 K and 1 bar obtained from computer simulation. The distributions correspond to local spherical regions with a radius of 1.5 nm. Data are displayed for mixtures of water with methanol (top panel), ethanol (center panel) and n-propanol (bottom panel) as a function of alcohol mole fraction. The reduced number, $\delta N'_i = \delta N_i / \langle N_i \rangle$, energy $\delta E' = \delta E / |\langle E \rangle|$, and excess energy $\epsilon' = \delta \epsilon / |\langle \epsilon \rangle|$ fluctuations are provided. All axes range from -1 to $+1$, with the exception of $\delta \epsilon'$ the axis which ranges from -10 to 10 for methanol and from -30 to 30 for ethanol and n-propanol. Lines are drawn through $x=0$ and $y=0$ for reference. Only the intermolecular energies are used in the analysis.

Table 1

Schematic relationship between second derivatives of the Gibbs free energy

	βG		
	$\beta\mu_i = \partial / \partial N_i$	$V = \partial / \partial p$	$H = \partial / \partial \beta$
$\partial / \partial N_j$	μ_{ij}	V_j^-	H_j^-
$\partial / \partial p$	V_i^-	κ_T	α_p
$\partial / \partial \beta$	H_i^-	α_p	C_p

Table 2

First and second derivatives in the Grand Canonical ensemble

	$\beta p V$		
	$\langle N_i \rangle = \partial / \partial \mu_i$	$-\langle E \rangle = \partial / \partial \beta$	$\beta p = \partial / \partial V$
$\partial / \partial \mu_j$	$\langle \delta N_i \delta N_j \rangle$	$-\langle \delta E \delta N_j \rangle$	$\langle N_j \rangle / V$
$\partial / \partial \beta$	$-\langle \delta E \delta N_i \rangle$	$\langle \delta E \delta E \rangle$	$\langle E \rangle / V$
$\partial / \partial V$	$\langle N_i \rangle / V$	$\langle E \rangle / V$	0