# On the Molecular Origins of Volumetric Data

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We use a statistical thermodynamic approach and a simple thermodynamic model of hydration to examine the molecular origins of the volumetric properties of solutes. In this model, solute−solvent interactions are treated as a binding reaction. The free energy of hydration of the noninteracting solute species coincides with the free energy of cavity formation, while the free energy of solute−solvent interactions is given by the binding polynomial. By differentiating the relationship for the free energy of hydration with respect to temperature and pressure, one obtains the complete set of equations describing the thermodynamic profile of hydration, including enthalpy, entropy, volume, compressibility, expansibility, and so forth. The model enables one to rigorously define in thermodynamic terms the hydration number and the related concept of hydration shell, which are both widely used as operational definitions in experimental studies. Hydration number, $n_h$, is the effective number of water molecules solvating the solute and represents the derivative of the free energy of hydration with respect to the logarithm of water activity. One traditional way of studying hydration relies on the use of volumetric measurements. However, microscopic interpretation of macroscopic volumetric data is complicated and currently relies on empirical models that are not backed by theory. We use our derived model to link the microscopic determinants of the volumetric properties of a solute and its statistical thermodynamic parameters. In this treatment, the partial molar volume, $V°$, of a solute depends on the cavity volume, hydration number, and the properties of waters of hydration. In contrast, the partial molar isothermal compressibility, $K°_T$, and expansibility, $E°$, observables, in addition to the intrinsic compressibility and expansibility of the cavity enclosing the solute, hydration number, and the properties of waters of hydration, contain previously unappreciated relaxation terms that originate from pressure- and temperature-induced perturbation of the equilibrium between the solvated solute species. If significant, the relaxation terms may bring about a new level of nonadditivity to compressibility and expansibility group contributions that goes beyond the overlap of the hydration shells of adjacent groups. We apply our theoretical results to numerical analyses of the volume and compressibility responses to changes in the distribution of solvated species of polar compounds.

## 1. Introduction

Water continues to be an enigmatic solvent; the physicochemical parameters of water and hydration in aqueous solutions remain at the center of attention of experimental and theoretical scientists.[1−3] The significance of insight emerging from such studies is augmented by the fact that water is the only solvent in which life is known to have originated and be supported. It is widely accepted that water plays a major role in modulating the equilibrium of virtually every reaction of the chemistry of life, including folding and binding events in proteins and nucleic acids. In these reactions, water is not a passive viewer, but it is an active and, often, the decisive player.[4] Despite the acknowledgment of this notion, the tools available for hydration studies are limited and often provide only indirect insight, while interpretation of experimental data is far from straightforward.

Among the experimental methods employed in hydration studies, volumetric techniques are characterized by high sensitivity and nonselectivity. However, the microscopic interpretation of volumetric observables largely relies on intuitive empirical relationships that do not provide the level of sophistication required for gaining robust, quantitative insight into the

nature, amount, and intensity of solute−solvent interactions. The majority of current schemes of microscopic rationalization of thermodynamic data rely explicitly or implicitly on the notion of the group additivity of measured macroscopic observables. The additivity principle, which implies that an atomic group exhibits the same contribution, independent of its microenvironment, is highly attractive, since it substantially simplifies microscopic interpretation of experimental data. Moreover, it frequently offers the only way to interpret data. However, upon closer inspection, many thermodynamic parameters become nonadditive.[5] Subtle changes in hydration due to changes in solute composition or conformation may cause disproportionate alterations of volumetric properties, in particular, compressibility and expansibility. However, simple models currently used for interpreting volumetric data in terms of hydration fail to explain the observed nonlinear volumetric responses to hydration changes.

In this work, we present a simple yet rigorous model of hydration that can be adapted for microscopic interpretation of a wide range of thermodynamic variables describing solute−solvent interactions. In this model, hydration is treated as a binding reaction between the solute and solvent molecules, while the free energy of solute−solvent interactions is described by

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the binding polynomial. With this model, we explore the link between the statistically thermodynamically defined parameters of solute−solvent interactions and the volumetric characteristics of a solute. Furthermore, we examine the origins of nonadditivity of volumetric observables. Notably, the combination of the theoretical results presented here with the observed nonadditivity of experimental volumetric parameters of solute yields a new level of understanding of the extent and nature of solute−solvent interactions, in particular, about the heterogeneity and distribution of the solvated solute species. In general, theoretical insight provided by the results of this study leads to additional criteria for developing improved schemes of microscopic interpretation of macroscopic volumetric data.

## 2. Statistical Thermodynamics of Solvation

The thermodynamics of solvation is traditionally described in terms of vapor-to-solution transfer experiments. The chemical potential of a solute in the ideal gas state (vapor) phase is given by the expression[6−8]

$$\mu_g = \mu^\circ_g + RT \ln[S_g] \qquad (1)$$

where $\mu^\circ_g(T) = RT \ln(\Lambda^3 q^{-1})$ is the standard chemical potential of solute in the vapor phase; $q$ is the internal partition function, which includes rotational, vibrational, electronic, and nuclear partition functions of a single solute molecule; $\Lambda = h/(2\pi m k_B T)^{1/2}$ is the so-called momentum partition function or the thermal de Broglie wavelength of solute molecules; $h$ is the Planck constant; $k_B$ is the Boltzmann constant; $m$ is the mass of each of the molecules contained in $\Lambda^3$; and $[S_g] = N/V$ is the number density or molar concentration of solute. For a given solute, the values of $\Lambda$ and $q$ are considered to be the same in the vapor and solution phases.

The chemical potential of a solute in solution is given by the equation[7−9]

$$\mu_l = \mu^\circ_g + W(s/l) + RT \ln[S_l] = \mu^\circ_l + RT \ln[S_l] \quad (2)$$

where $[S_l]$ is the molar concentration of solute in solution; $\mu^\circ_l = W(s/l) + RT \ln(\Lambda^3 q^{-1})$ is the standard chemical potential of a solute in the solution phase; and $W(s/l) = \mu^\circ_l - \mu^\circ_g$ is the average Gibbs energy of interaction of a solute with solvent. Importantly, $W(s/l)$ may reflect both attractive and repulsive interactions between the solute and solvent molecules.

The reversible work required to add a solute molecule to the system consists of two contributions.[7−9] The first contribution, referred to as the pseudochemical potential, $\mu^*_l = W(s/l) + RT \ln(q^{-1})$, is the change in Gibbs free energy associated with introducing the solute at a fixed position in the liquid. The second contribution is the liberation free energy, $RT \ln(\Lambda^3[S_l])$, which represents the work related to removing the constraints imposed by fixing the position of the molecule. The liberation free energy is generally negative and reflects a gain in the momentum partition function, the accessibility of the entire volume of the system, and the assimilation of the newly added molecule when it is released from the fixed position.

Solvation is defined as the process of transferring a solute molecule from a fixed position in an ideal gas phase into a fixed position in the liquid phase.[6−8] Assuming that the internal partition function, $q$, of a solute is not affected by solvent, the Gibbs energy of solvation, $\Delta G^*$, is the difference in the pseudochemical potentials of a solute in the liquid and gas phases:
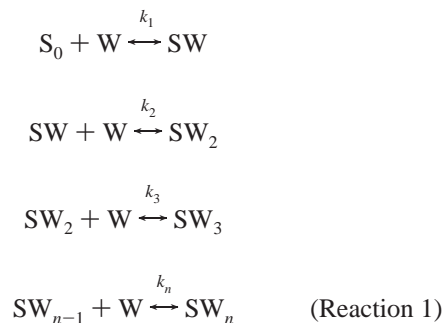
$$\Delta G^* = \mu^*_l - \mu^*_g = W(s/l) \qquad (3)$$

Given that the momentum partition function of a solute, $\Lambda^3$, is independent of the environment, one obtains the following expression by equating eqs 1 and 2:[7−9]

$$\Delta G^* = -RT \ln([S_l]/[S_g]) \qquad (4)$$

where $[S_g]$ and $[S_l]$ are, respectively, the molar concentrations of a solute in the ideal gas and solution phases existing in equilibrium.

To determine the solute concentration in solution, $[S_l]$, solute−solvent interactions can be presented as a binding reaction:

$$S_0 + W \overset{k_1}{\longleftrightarrow} SW$$

$$SW + W \overset{k_2}{\longleftrightarrow} SW_2$$

$$SW_2 + W \overset{k_3}{\longleftrightarrow} SW_3$$

$$SW_{n-1} + W \overset{k_n}{\longleftrightarrow} SW_n \qquad \text{(Reaction 1)}$$

where $S_0$ signifies noninteracting solute in solution; W stands for water as solvent; $SW_i$ denotes the hydrated complex containing $i$ interacting solvent molecules; and $n$ is the maximum amount of potential binding sites. This approach and a similar binding scheme has been used in the quasi-chemical approach to solution theory developed by Pratt and colleagues.[10,11]

In reaction 1, the total concentration of a solute in solution is given by the binding polynomial[12]

$$[S_l] = \sum_{i=0}^{n} [SW_i] = [S_0](1 + K_1 a_W + K_1 K_2 a_W{}^2 +$$

$$K_1 K_2 K_3 a_W{}^3 + \ldots + \prod_{j=0}^{n} K_j a_W{}^j) = [S_0] \sum_{i=0}^{n} (a_W{}^i \prod_{j=0}^{i} K_j) =$$

$$[S_0] \sum_{i=0}^{n} [a_W{}^i \exp(-\Delta G_i/RT)] \quad (5)$$

where $\Delta G_i = -RT \ln(\prod_{j=0}^{i} K_j)$; $K_j = [SW_j]/([SW_{j-1}]a_W)$ is the association constant for the $j$th bound water molecule; $a_W$ is the solvent activity; and $K_0 = 1$. Note that the binding constant $K_j$ is contributed by the disruption of preexisting solvent−solvent interactions that are required to initiate new solute−solvent interactions. Consequently, $K_j$ may be larger than 1 (attractive interactions as in hydration of charged and polar groups), smaller than 1 (repulsive interactions as in hydration of nonpolar groups), or equal to 1 (no interactions). In this context, no interactions means the energetic equivalence of solute−solvent and solvent−solvent interactions.

A change in free energy associated with the transfer of solute from the vapor to the solution phase (the free energy of hydration) is given by the relationship

$$\Delta G^* = -RT \ln([S_l]/[S_g]) = -RT \ln([S_0]/[S_g] \sum_{i=0}^{n}$$

$$(a_W{}^i \prod_{j=0}^{i} K_j)) = -RT \ln([S_0]/[S_g]) - RT \ln(\sum_{i=0}^{n} (a_W{}^i \prod_{j=0}^{i} K_j))$$

$$(6)$$

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Note that $[S_0]$ represents the concentration of those solute molecules that, while being in solution, do not appreciably interact with surrounding water molecules. By definition, the free energy of hydration of such molecules is given by the unfavorable free energy of cavity formation, $G_C$.[7,8,13] Consequently, one can substitute $G_C = -RT \ln([S_0]/[S_g])$ in eq 6 and obtain the following:

$$\Delta G^* = G_C + G_I = G_C - RT \ln(\sum_{i=0}^{n} (a_W^{\ i} \prod_{j=0}^{i} K_j)) = G_C -$$

$$RT \ln(\sum_{i=0}^{n} [a_W^{\ i} \exp(-\Delta G_i/RT)]) \quad (7)$$

The first term, $G_C$ (the cavity contribution), reflects the free energy of creating a cavity of suitable size at a fixed position in the solvent due to thermal fluctuations in the spatial distribution of solvent molecules. The second term, $G_I = -RT \ln(\sum_{i=0}^{n}[a_W^{\ i} \exp(-\Delta G_i/RT)])$ (the contribution due to solute–solvent interactions) is given by the binding polynomial of solute–solvent interactions and reflects the formation of nonspecific (van der Waals interactions) and specific (hydrogen bonding and/or charge–dipole interactions) solute–solvent interactions.[7,8] In other words, $G_I$ reflects the reversible work associated with imparting the cavity, the charge distribution, and the polarizability to simulate the real solute.[13] The interaction component of the free energy of hydration can be presented as the sum $G_I = G_V + \sum_i G_S^i + \sum_{i,j} G_S^{i,j} + \sum_{i,j,k} G_S^{i,j,k} + ....$[7,8] In this representation, the term $G_V$ represents the free energy of van der Waals interactions occurring between the solute and solvent molecules, while the higher terms reflect specific (electrostatic and hydrogen bonding) solute–solvent interactions. The $\sum_i G_S^i$ term describes the hydration of independently hydrated functional groups, while $\sum_{i,j} G_S^{i,j}$, $\sum_{i,j,k} G_S^{i,j,k}$, and higher terms represent the solvation free energies of pair-correlated, triple-correlated, and higher correlated solvent-exposed functional groups that may result in the formation of cooperative water networks at the solute surface and other nonlinear hydration effects. One manifestation of such effects is the appearance of tightly bound water molecules that can be detected by X-ray and NMR. In the "binding model" of hydration outlined by eq 7, the more tightly bound water molecules are characterized by larger values of $K_j$. Stated somewhat differently, in the binding model, structurally ordered water molecules that can be detected by X-ray and NMR correspond to a subpopulation of tightly bound water molecules around the solute exhibiting large values of $K_j$. In fact, it has been proposed that the high solubility of globular protein and the driving force of protein–protein recognition is related to correlated water molecules.[14–17]

**2.1. Thermodynamic Parameters of Hydration.** By differentiating eq 7 with respect to temperature and pressure, one may obtain the complete thermodynamic profile of hydration (enthalpy, entropy, heat capacity, volume, etc.). In particular, the enthalpy, $\Delta H^*$, entropy, $\Delta S^*$, and volume, $\Delta V^*$, of hydration are given by the expressions

$$\Delta V^* = (\Delta G^*/\partial P) = V_C + \sum_{i=0}^{n} \alpha_i \Delta V_i = V_C + \langle \Delta V \rangle \quad (8)$$

$$\Delta H^* = -RT^2[\partial(\Delta G^*/RT)/\partial T]_P = H_C + \sum_{i=0}^{n} \alpha_i \Delta H_i = H_C +$$

$$\langle \Delta H \rangle \quad (9)$$

$$\Delta S^* = -[\partial(\Delta G^*/\partial T)]_P = S_C + R \ln(\sum_{i=0}^{n} [a_W^{\ i}$$

$$\exp(-\Delta G_i/RT)]) + \langle \Delta H \rangle/T \quad (10)$$

where $\alpha_i = [SW_i]/[S_l] = a_W^{\ i} \prod_{j=0}^{i} K_j / \sum_{i=0}^{n}(a_W^{\ i} \prod_{j=0}^{i} K_j)$ is the fractional composition of the solvated species $SW_i$; $V_C = (\partial G_C/\partial P)_T$ is the volume of the cavity enclosing the solute; $H_C = -RT^2[\partial(G_C/RT)/\partial T]_P$ is the enthalpy of cavity formation; $S_C = -(\partial G_C/\partial T)_P = (H_C - G_C)/T$ is the entropy of cavity formation; $\Delta V_i = (\partial \Delta G_i/\partial P)_T$; and $\Delta H_i = -RT^2[\partial(\Delta G_i/RT)/\partial T]_P$.

**2.2. Hydration Number.** Differentiation of eq 7 with respect to $\ln a_W$ yields the effective number of water molecules that bind to the solute in equilibrium (the hydration number), $n_h$:

$$-[\partial(\Delta G^*/RT)/\partial \ln a_W]_T \approx -[\partial(G_I/RT)/\partial \ln a_W]_T =$$

$$\sum_{i=0}^{n} i\alpha_i = n_h \quad (11)$$

Inspection of eq 11 reveals that the hydration number represents the slope of the dependence of the hydration free energy on the logarithm of water activity in solution. With this definition, $n_h$ represents an objective thermodynamic measure of the size of the hydration shell and the number of affected water molecules.

It is worth noting that eq 11 represents the theoretical basis for the osmotic stress technique widely used in hydration studies.[18,19] For an arbitrary reaction (A + B ↔ AB), the binding constant of the reaction can be presented as consisting of two components: the binding constant in the gaseous phase, $K_g$, and the hydration contribution,[17]

$$K_b = \exp(-\Delta G^\circ/RT) = K_g \exp(-\Delta\Delta G^*/RT) \quad (12)$$

where $K_g = \exp(-\Delta G^g/RT) = [(\Lambda_A^3 q_A^{-1})(\Lambda_B^3 q_B^{-1})/(\Lambda_{AB}^3 q_{AB}^{-1})] \exp[-\Delta U(AB)/RT]$; $\Delta U(AB)$ is the free energy of direct interactions between A and B in the complex AB; $\Delta\Delta G^* = W(AB/l) - W(A/l) - W(B/l)$ is the net change in free energy of hydration. In fact, $\Delta\Delta G^*$ represents the desolvation penalty of burial of atomic groups within the functional interface between the reacting species in the complex AB.

Comparison of eqs 11 and 12 reveals that $(\partial \ln K_b/\partial \ln a_W)_T = [\partial(-\Delta\Delta G^*/RT)/\partial \ln a_W]_T = n_h(AB) - n_h(A) - n_h(B) = \Delta n_h$, the effective number of water molecules released from or taken up by the hydration shells of the interacting species. This relationship enables one to determine the amount of water molecules released from or taken up by the hydration shells of the reacting species from osmotic stress measurements in which the binding constant is determined as a function of water activity.[18,19]

## 3. Partial Molar Volume, Expansibility, and Compressibility

**3.1. Partial Molar Volume.** The partial molar volume, $V^\circ$, of a solute is the first pressure derivative of its chemical potential, $\mu_l$. Differentiating eq 2 with respect to pressure and taking into account eq 7, one obtains the following relationship for $V^\circ$:

$$V^\circ = [\partial \Delta G^*/\partial P]_T + RT(\partial \ln[S_l]/\partial P)_T = (\partial G_C/\partial P)_T +$$

$$(\partial G_I/\partial P)_T + \beta_{T0}RT = V_C + \sum_{i=0}^{n} \alpha_i \Delta V_i + \beta_{T0}RT = V_C +$$

$$\langle \Delta V \rangle + \beta_{T0}RT \quad (13)$$

where $\beta_{T0}$ is the coefficient of isothermal compressibility of the solvent.

Note that $\Delta V_i = V_{SWi} - iV_W - V_C$, where $V_{SWi}$ is the partial molar volume of the $SW_i$ complex (a solute associated with $i$ water molecules), and $V_W$ is the partial molar volume of water. Comparing eq 13 with $V° = V_C + V_I + \beta_{T0}RT$, the conventional equation used for interpretation of partial molar volume data,[20,21] reveals that interaction volume, $V_I$, is given by $V_I = \langle \Delta V \rangle = \sum_{i=0}^{n} \alpha_i V_{SWi} - n_h V_W - V_C = \langle V_{SW} \rangle - n_h V_W - V_C$, where $\langle V_{SW} \rangle$ is the ensemble average volume of the solvated complex. [Note that eq 13 effectively reduces to $V° = \langle V_{SW} \rangle - n_h V_W + \beta_{T0}RT$.]

The $V_C$ and $V_I$ terms of eq 13 reflect the volume change due to the addition of a solute molecule at a fixed position in the solvent, while the $\beta_{T0}RT$ term originates from the availability of the entire volume of the solution to the solute. The cavity volume, $V_C$, is made up of the intrinsic volume of the solute, $V_M$, and the thermal volume, $V_T$. The latter has three components: steric, vibrational, and structural. The steric component reflects the imperfect packing of solute and solvent molecules in the solution. The vibrational component results from thermally induced mutual vibrational motions of solute and solvent molecules and, theoretically, should subside to zero at 0 K. The structural component reflects the open (tetrahedral) structure of water and related packing effects around solute molecules.

Interaction volume, $V_I = \langle \Delta V \rangle$, represents the volume effect of solute−solvent interactions and predominantly originates from hydrogen bonding and electrostriction, although the molecular origins of the two processes are different. Hydrogen bonding has a partially covalent character, which leads to partial interpenetration of the electron shells of interacting solute and solvent molecules with a concomitant reduction in their effective van der Waals volumes and, as a result, in the cavity volume of the solute. Such a reduction in the effective size of a pair of hydrogen-bonded solute and solvent molecules is reflected in a decrease in the partial molar volume of a solute as manifested in the negative sign of its interaction volume. On the other hand, electrostriction, which results from interactions between charged solute groups and water dipoles, causes rearrangement of water molecules, orienting them along the electrostatic field of the solute. This, in turn, increases the packing efficiency of the affected water molecules with the resulting diminution of the partial molar volume of the solute.

**3.2. Partial Molar Isothermal Compressibility.** We will concentrate below on partial molar isothermal compressibility, $K°_T$, although the partial molar adiabatic compressibility, $K°_S$, is more frequently used in experimental studies. The relationship between $K°_T$ and $K°_S$ is as follows: $K°_T = K°_S + (T\alpha^2/\rho_0 c_{P0})(2E°/\alpha_0 - C°_P/\rho_0 c_{P0})$, where $\rho_0$ is the density of the solvent; $\alpha$ is the coefficient of thermal expansion of the solvent; $c_{P0}$ is the specific heat capacity at constant pressure of the solvent; $E°$ is the partial molar expansibility of a solute; and $C°_P$ is the partial molar heat capacity of a solute.[22] Because of the small value of $\alpha_0$ and the large value of $c_{P0}$ of water, the difference between $K°_T$ and $K°_S$ in aqueous solutions is not large. Therefore, the results and conclusions obtained below for partial molar isothermal compressibility can be applied to partial molar adiabatic compressibility. The partial molar isothermal compressibility, $K°_T$, of a solute is the negative pressure derivative of its partial molar volume, $V°$:

$$K°_T = -(\partial V°/\partial P)_T = K_C - \sum_{i=0}^{n} \alpha_i(\partial \Delta V_i/\partial P)_T - \sum_{i=0}^{n} (\partial \alpha_i/\partial P)_T \Delta V_i - (\partial \beta_{T0}/\partial P)_T RT \quad (14)$$

where $K_C = -(\partial V_C/\partial P)_T$ is the intrinsic compressibility of the cavity containing solute. Note that $\sum_{i=0}^{n} \alpha_i(\partial \Delta V_i/\partial P)_T = -\sum_{i=0}^{n} \alpha_i \Delta K_i = -\langle \Delta K \rangle$; $(\partial \alpha_i/\partial P)_T = (\alpha_i/RT)(\sum_{i=0}^{n} \alpha_i \Delta V_i - \Delta V_i) = (\alpha_i/RT)(\langle \Delta V \rangle - \Delta V_i)$; while $\sum_{i=0}^{n} (\partial \alpha_i/\partial P)_T \Delta V_i = (\langle \Delta V \rangle^2 - \sum_{i=0}^{n} \alpha_i \Delta V_i^2)/RT = (\langle \Delta V \rangle^2 - \langle \Delta V^2 \rangle)/RT$. Consequently, eq 14 transforms into the following relationship:

$$K°_T = K_C + \langle \Delta K \rangle + (\langle \Delta V^2 \rangle - \langle \Delta V \rangle^2)/RT - (\partial \beta_{T0}/\partial P)_T RT \quad (15)$$

Since $\Delta K_i = K_{SWi} - iK_W - K_C$ (where $K_{SWi}$ is the partial molar isothermal compressibility of the $SW_i$ complex, and $K_W$ is the partial molar isothermal compressibility of bulk water), $\langle \Delta K \rangle = \sum_{i=0}^{n} \alpha_i \Delta K_i = \sum_{i=0}^{n} \alpha_i K_{SWi} - n_h K_W - K_C = \langle K_{SW} \rangle - n_h K_W - K_C$, where $\langle K_{SW} \rangle$ is the ensemble average compressibility of the solvated complex. The term $\langle \Delta K \rangle$ predominantly reflects the compressibility effect of direct solute−solvent interactions. It results from the solute-induced change in the partial compressibility of water of hydration, as well as a change in the compressibility of the cavity enclosing a solute upon turning on solute−solvent interactions, e.g., hydrogen bonding. The fluctuation term, $(\langle \Delta V^2 \rangle - \langle \Delta V \rangle^2)/RT$, which has never been considered in volumetric investigations, reflects the relaxation increase in compressibility due to the pressure-induced perturbation of the hydration equilibrium, i.e., the distribution of solvated solute species. This term becomes substantial under the conditions of significant heterogeneity of solvated solute species (with respect to $\alpha_i$) and nonzero values of $\Delta V_i$. Note that the heterogeneity of solute species reflects interplay between water activity and the solute−solvent association constants $K_j$ in eq 15.

It needs to be pointed out that the relaxation term $(\langle \Delta V^2 \rangle - \langle \Delta V \rangle^2)/RT$ in eq 15 is distinct from the differential relaxation contribution to compressibility of water of solute hydration and bulk water. The latter represents the intrinsic property of water molecules solvating a particular atomic group and is not related to the change in the number of such water molecules. This feature is reflected in the partial molar adiabatic compressibility of water of hydration and is contained in the term $\langle \Delta K \rangle$ of eq 15 (for more information, see ref 23). In contrast, the $(\langle \Delta V^2 \rangle - \langle \Delta V \rangle^2)/RT$ term reflects a pressure-induced change in the number of affected water molecules.

**3.3. Partial Molar Expansibility.** The partial molar expansibility, $E°$, of a solute is the temperature derivative of $V°$:

$$E° = (\partial V°/\partial T)_P = (\partial V_C/\partial T)_P + \sum_{i=0}^{n} \alpha_i(\partial \Delta V_i/\partial T)_P + \sum_{i=0}^{n} (\partial \alpha_i/\partial T)_P \Delta V_i + R[\beta_{T0} + T(\partial \beta_{T0}/\partial T)_P] \quad (16)$$

where $E_C = (\partial V_C/\partial T)_P$ is the intrinsic expansibility of the cavity containing solute.

Note that $\sum_{i=0}^{n} \alpha_i(\partial \Delta V_i/\partial T)_P = \sum_{i=0}^{n} \alpha_i \Delta E_i = \langle \Delta E \rangle$; $(\partial \alpha_i/\partial T)_P = (\alpha_i/RT^2)(\Delta H_i - \sum_{i=0}^{n} \alpha_i \Delta H_i) = (\alpha_i/RT^2)(\Delta H_i - \langle \Delta H \rangle)$; $\sum_{i=0}^{n} (\partial \alpha_i/\partial T)_P \Delta V_i = (\sum_{i=0}^{n} \alpha_i \Delta H_i \Delta V_i - \langle \Delta H \rangle\langle \Delta V \rangle)/RT^2 = (\langle \Delta H \Delta V \rangle - \langle \Delta H \rangle\langle \Delta V \rangle)/RT^2$.[2] Consequently, one obtains the following expression:

$$E° = E_C + \langle \Delta E \rangle + (\langle \Delta H \Delta V \rangle - \langle \Delta H \rangle\langle \Delta V \rangle)/RT^2 + R[\beta_{T0} + T(\partial \beta_{T0}/\partial T)_P] \quad (17)$$

The interaction term is given by $\langle \Delta E \rangle = \sum_{i=0}^{n} \alpha_i \Delta E_i = \sum_{i=0}^{n} \alpha_i E_{SWi} - n_h E_W - E_C = \langle E_{SW} \rangle - n_h E_W - E_C$. This term represents the expansibility effect of direct solute−solvent

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interactions and reflects an alteration of the expansibility in the cavity and a solute-induced change in the partial expansibility of waters of hydration. The relaxation term, $(\langle\Delta H\Delta V\rangle - \langle\Delta H\rangle\langle\Delta V\rangle)/RT$,[2] reflects an increase in expansibility due to the temperature-induced perturbation of the distribution of solvated species. As in the case of compressibility, this term is significant if there is a wide distribution of solvated solute species and if the values of $\Delta V_i$ and $\Delta H_i$ are not zero. Also note that the term $(\langle\Delta H\Delta V\rangle - \langle\Delta H\rangle\langle\Delta V\rangle)/RT^2$ is distinct from the differential relaxation contribution to the expansibility of water of solute hydration and bulk water that is contained in the term $\langle\Delta E\rangle$ of eq 17.

**3.4. Nonadditivity of Volumetric Observables.** Current approaches of microscopic rationalization of volumetric data frequently rely explicitly or implicitly on the notion of group additivity. Analysis of the literature reveals that the principle of additivity provides highly reliable estimates of the partial molar volumes of low molecular weight compounds based on the information on their constituent atomic groups and the respective group contributions.[24,25] On the other hand, the partial molar compressibility and expansibility observables are characterized by significantly poorer additivity of group contributions. Notably, nonlinear responses (nonadditivities) may be manifested in both the magnitude and the sign of the compressibility or expansibility contribution of the group in question.[25]

Nonadditivity of volumetric parameters may result from the overlap of adjacent atomic groups and/or unaccounted intramolecular interaction within a solute. On the other hand, the relaxation terms $(\langle\Delta V^2\rangle - \langle\Delta V\rangle^2)/RT$ and $(\langle\Delta H\Delta V\rangle - \langle\Delta H\rangle\langle\Delta V\rangle)/RT^2$ in eqs 15 and 17 may become a new source of nonadditivity and, hence, add a new level of complexity to the interpretation of the compressibility and expansibility observables. These terms originate from the pressure- or temperature-induced modulation of the extent of solute hydration and are principally nonlinear. As such, the relaxation contributions result in nonlinear responses of the compressibility and expansibility observables to hydration changes. In contrast to compressibility and expansibility, volume lacks any relaxation term and, therefore, exhibits better group additivity compared to its pressure and temperature derivatives. To elaborate on this notion, we consider below the simplest case when each bound water molecule causes the same change in volume, $\Delta V$, compressibility, $\Delta K = -(\partial\Delta V/\partial P)_T$, expansibility, $\Delta E = (\partial\Delta V/\partial T)_P$, and enthalpy, $\Delta H$. In this scenario, one can write $\Delta V_i = i\Delta V$, $\Delta K_i = i\Delta K$, $\Delta E_i = i\Delta E$, and $\Delta H_i = i\Delta H$. The partial molar volume, $V^\circ$, isothermal compressibility, $K^\circ_T$, and compressibility, $E^\circ$, of a solute are given by the relationships

$$V^\circ = V_C + \sum_{i=0}^{n}\alpha_i i\Delta V + \beta_{T0}RT = V_C + n_h\Delta V + \beta_{T0}RT \quad (18)$$

$$K^\circ_T = K_C + n_h\Delta K - \sum_{i=0}^{n}[i\Delta V(\alpha_i/RT)(\sum_{i=0}^{n}\alpha_i i\Delta V - i\Delta V)] -$$

$$(\partial\beta_{T0}/\partial P)_T RT = K_C + n_h\Delta K - (\Delta V^2/RT)[(\sum_{i=0}^{n}i\alpha_i)^2 -$$

$$\sum_{i=0}^{n}i^2\alpha_i] - (\partial\beta_{T0}/\partial P)_T RT = K_C + n_h\Delta K + (\Delta V^2/RT)$$

$$(\sum_{i=0}^{n}i^2\alpha_i - n_h^2) - (\partial\beta_{T0}/\partial P)_T RT \quad (19)$$

$$E^\circ = E_C + \sum_{i=0}^{n}\alpha_i i\Delta E + \sum_{i=0}^{n}[(\partial\alpha_i/\partial T)_P i\Delta V] + R[\beta_{T0} + T$$

$$(\partial\beta_{T0}/\partial T)_P] = E_C + n_h\Delta E + (\Delta H\Delta V/RT^2)(\sum_{i=0}^{n}i^2\alpha_i - n_h^2) +$$

$$R[\beta_{T0} + T(\partial\beta_{T0}/\partial T)_P] \quad (20)$$

Inspection of eqs 18 to 20 reveals that the hydration contribution to the partial molar volume, $V_h$, of a solute depends solely on the effective number of affected water molecules, $n_h$, independent of how the solvated species are distributed. On the other hand, the hydration contributions to the partial molar isothermal compressibility, $K^\circ_T$, and expansibility, $E^\circ$, in addition to $n_h$ depend on the distribution of solvated species as reflected in the set of $\alpha_i$ values. The relaxation terms, $K_{rel} = (\Delta V^2/RT)(\sum_{i=0}^{n}i^2\alpha_i - n_h^2)$ and $E_{rel} = (\Delta H\Delta V/RT^2)(\sum_{i=0}^{n}i^2\alpha_i - n_h^2)$, in eqs 19 and 20 reflect nonlinear responses of the compressibility and expansibility observables to hydration changes. The situation becomes especially dramatic when the relaxation and nonrelaxation terms exhibit different signs. In this case, the nonadditivity may be manifested not only in the magnitude but also in the sign of the compressibility and expansibility group contributions of the same atomic group in different microenvironments. An interesting implication that results from eqs 18 to 20 is that the nonadditivity of the compressibility and expansibility contributions coupled with the additivity of the volume contribution of an atomic group suggests heterogeneity of the solute species with respect to the extent of their hydration.

The relaxation contributions to $K^\circ_T$ and $E^\circ$ correlate with the volume change $\Delta V$. For nonpolar groups, the value of $\Delta V$ and, consequently, the values of $K_{rel}$ and $E_{rel}$, are close to zero.[21] Therefore, nonpolar groups generally exhibit good group additivity. On the other hand, polar groups, for which $\Delta V$ values are large and negative, may be highly "nonadditive" with respect to their compressibility and expansibility contributions. In agreement with this notion, the compressibility contribution of polar (e.g., hydroxyl) groups varies from highly negative to highly positive values ($-5.5 \times 10^{-4}$ to $3.8 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ at 25°) depending on the proximity and the nature of neighboring groups.[25-27] Charged groups, for which $\Delta V$ is also large and negative, are studied less extensively; however, given the large magnitude of $\Delta V$, one cannot exclude the possibility that charged groups also may exhibit relaxation-based nonadditivity. As noted above, the necessary condition of the relaxation terms $K_{rel}$ and $E_{rel}$ and the related nonadditivity to be significant is substantial heterogeneity of solute species with respect to the extent of their hydration. To clarify this point, we present below numerical analyses of volume and compressibility responses to changes in distribution of hydrogen-bonded species of water and some polar groups.

*3.4.1. Water in Water.* The maximum number of hydrogen bonds per water molecule, $n$, is equal to four, while the volume change associated with hydrogen bond formation, $\Delta V$, has been estimated to be between $-1.6$ and $-2.6$ cm$^3$ mol$^{-1}$.[21] In the analysis below, we consider $\Delta V$ to be $-2.5$ cm$^3$ mol$^{-1}$ (the exact value of $\Delta V$ is not essential for the qualitative analysis we present below). At room temperature, ~20% of hydrogen bonds in water are broken; hence, $n_h = \sum_{i=0}^{n}i\alpha_i \sim 3.2$. We consider here some arbitrary distribution schemes of $\alpha_i$ in which the fraction of saturated water molecules ($i = 4$) gradually decreases and which all result in an $n_h$ of 3.2. Scheme 1 is given by $\alpha_0 = 0.2$, $\alpha_1 = 0$, $\alpha_2 = 0$, $\alpha_3 = 0$, and $\alpha_4 = 0.8$; scheme 2 is given by $\alpha_0 = 0$, $\alpha_1 = 0.2$, $\alpha_2 = 0$, $\alpha_3 = 0.2$, and $\alpha_4 = 0.6$;

scheme 3 is given by $\alpha_0 = 0$, $\alpha_1 = 0.1$, $\alpha_2 = 0.1$, $\alpha_3 = 0.3$, and $\alpha_4 = 0.5$; scheme 4 is given by $\alpha_0 = 0$, $\alpha_1 = 0$, $\alpha_2 = 0.1$, $\alpha_3 = 0.6$, and $\alpha_4 = 0.3$; and scheme 5 is $\alpha_0 = 0$, $\alpha_1 = 0$, $\alpha_2 = 0$, $\alpha_3 = 0.8$, and $\alpha_4 = 0.2$. The values of $V_I = \sum_{i=0}^{n}\alpha_i i\Delta V$ for distribution schemes 1, 2, 3, 4, and 5 are all $-8.0$ cm$^3$ mol$^{-1}$, respectively, while the relaxation components of compressibility, $K_{rel}$, are $6.5 \times 10^{-4}$, $3.4 \times 10^{-4}$, $2.4 \times 10^{-4}$, $0.9 \times 10^{-4}$, and $0.4 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$, respectively.

Thus, changes in the distribution of hydrogen-bonded water species, which are not reflected in volume, may bring about significant changes in compressibility. On the basis of high-frequency ultrasonic velocity measurements, the relaxation component of the compressibility of water has been estimated to be $\sim$70% ($\sim$5 $\times$ 10$^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$) at 25 °C.[28] Thus, our estimates of $K_{rel}$ for distributions 1 and 2 are not unreasonable and close to experimental observations.

*3.4.2. Hydroxyl Groups.* Hydroxyl groups may form up to three hydrogen bonds with their solvating water molecules. The effective number of solute–solvent hydrogen bonds, $n_h$, can be found by dividing the interaction volume, $V$, of $-$OH groups by $-2.5$ cm$^3$ mol$^{-1}$, the volume change associated with one solute–solvent hydrogen bond formation. The interaction volume contribution, $V_I$, of a hydroxyl group ranges from $-4$ to $-7.5$ cm$^3$ mol$^{-1}$ depending on the neighboring groups.[21] Thus, $-$OH groups can effectively form 1.6 (4/2.5) to 3 (7.5/2.5) hydrogen bonds with water molecules. If $n_h$ is 3 (which implies that $\alpha_0 = 0$, $\alpha_1 = 0$, $\alpha_2 = 0$, and $\alpha_3 = 1$), the relaxation component of compressibility, $K_{rel}$, is zero. This situation is observed, for example, for the hydroxyl group of serine side chain (in zwitterionic serine) that exhibits a $V_I$ of $-7$ cm$^3$ mol$^{-1}$, while its compressibility contribution is highly negative and equal to $-5.5 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$.[20,26] In the absence of the relaxation component, the compressibility contribution of a serine $-$OH group is mainly determined by $n_h\Delta K$ [see eq 19]. With $n_h = 3$, the value of $\Delta K$ is $-1.8 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ ($-5.5 \times 10^{-4}$/3). Below we will use this value as the compressibility change accompanying the formation of a hydrogen bond.

The hydroxyl group of the amino acid serine can be characterized as "correlated"; water molecules simultaneously interact with more than one polar group. Such polar groups have been defined as "closely located".[26] On the other hand, hydroxyl groups in alkyl alcohols represent a typical example of uncorrelated, independently hydrated polar groups (such groups have been characterized as "single'" polar groups[26]). In alkyl alcohols, the $V_I$ value exhibited by terminal $-$OH groups is $-4.6$ cm$^3$mol$^{-1}$, while the compressibility contribution is positive and equal to $3.8 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$.[21,26] The latter suggests the presence of a large positive relaxation component. On the basis of the $V_I$ value, the effective number of solute–solvent hydrogen bonds, $n_h$, is $\sim$1.8 (4.6/2.5). We consider here different distribution schemes of $\alpha_i$ that all result in an $n_h$ of 1.8: scheme 1 is given by $\alpha_0 = 0.4$, $\alpha_1 = 0$, $\alpha_2 = 0$, and $\alpha_3 = 0.6$; scheme 2 is given by $\alpha_0 = 0.2$, $\alpha_1 = 0.1$, $\alpha_2 = 0.4$, and $\alpha_3 = 0.3$; and scheme 3 is given by $\alpha_0 = 0.1$, $\alpha_1 = 0$, $\alpha_2 = 0.9$, and $\alpha_3 = 0$. The values of $V_I = \sum_{i=0}^{n}\alpha_i i\Delta V$ for all the combinations is $-4.5$ cm$^3$ mol$^{-1}$, while the relaxation compressibility, $K_{rel}$, for distribution schemes 1, 2, and 3 are $5.4 \times 10^{-4}$, $2.9 \times 10^{-4}$, and $0.9 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$, respectively. With $\Delta K$ of $-1.8 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ and $n_h\Delta K$ of $-3.2 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ ($-1.8 \times 10^{-4} \times 1.8$), the compressibility contribution of the hydroxyl group in an aliphatic alcohol is equal to $2.2 \times 10^{-4}$, $0.3 \times 10^{-4}$, and $-2.3 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ for distribution schemes 1, 2, and 3, respectively. Note that

distribution scheme 1, in which significant populations of saturated and non-hydrogen-bonded solute species exist in equilibrium, yields a compressibility contribution of $2.2 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ that is in qualitative agreement with the experimental value of $3.8 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$.

*3.4.3. Peptide Group.* The contributions to $V_I$ and $K_I$ of a peptide group are $-8.3$ cm$^3$ mol$^{-1}$ [21] and $0.5 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$,[29] respectively. The peptide group may form up to four hydrogen bonds with their solvating water molecules. The effective number of solute–solvent hydrogen bonds, $n_h$, can be found by dividing the interaction volume, $V_I$, of $-$OH groups by $-2.5$ cm$^3$ mol$^{-1}$. Thus, a peptide group effectively forms 3.3 (8.3/2.5) hydrogen bonds with water molecules. The nonrelaxation contribution to the compressibility of the peptide group, $n_h\Delta K$, is $-5.9 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ ($-1.8 \times 3.3$). From $K_I = n_h\Delta K + K_{rel} = 0.5 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$, the relaxation contribution, $K_{rel}$, is very large and equal to $6.4 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$. A close value of $5.8 \times 10^{-4}$ cm$^3$ mol$^{-1}$ bar$^{-1}$ can be obtained, for example, from the distribution scheme $\alpha_0 = 0.175$, $\alpha_1 = 0$, $\alpha_2 = 0$, $\alpha_3 = 0$, $\alpha_4 = 0.825$. Such cooperativity (the presence of species that are either saturated with or completely devoid of hydrogen bonds) is interesting and may suggest crosstalk between the amide and carbonyl moieties of the peptide group.

## 4. Concluding Remarks

We use a statistical thermodynamic approach to model solute hydration. In this approach, solute–solvent interactions are treated as a binding reaction. The free energy of hydration of the noninteracting solute species coincides with the free energy of cavity formation, while the free energy of solute–solvent interactions is given by the binding polynomial. With this model, we derive a complete set of equations describing the thermodynamic profile of solute hydration (enthalpy, entropy, volume, compressibility, expansibility, etc.). The model enables one to rigorously define in thermodynamic terms the hydration number, a widely used operational definition in experimental studies. Hydration number, $n_h$, is the effective number of water molecules solvating the solute and represents the derivative of the free energy of hydration with respect to the logarithm of water activity.

The partial molar volume, $V°$, of a solute depends on the cavity volume, the hydration number, and the properties of waters of hydration. In addition to these contributions, the partial molar isothermal compressibility, $K°_T$, and expansibility, $E°$, contain relaxation terms that originate from pressure- and temperature-induced perturbation of the equilibrium between the solvated solute species. In essence, the latter represents the pressure- or temperature-induced perturbation of the extent of solute hydration and depends on the distribution of the solvated species. The relaxation terms represent a heretofore unappreciated source of nonadditivity of the compressibility and expansibility group contributions that goes beyond the overlap of hydration shells of adjacent groups. We present numerical analyses of volume and compressibility responses to changes in the distribution of solvated species for some polar compounds.

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