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A Bulk Water-Dependent Desolvation Energy Model for Analyzing the Effects of Secondary Solutes on Biological Equilibria

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A Bulk Water-Dependent Desolvation Energy Model for Analyzing the Effects of Secondary Solutes on Biological Equilibria

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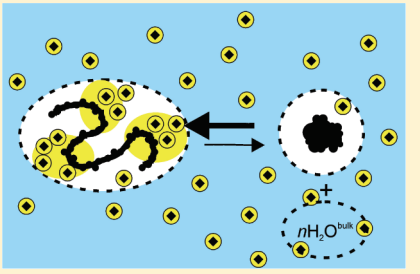
ABSTRACT: A new phenomenological model for interpreting the effects of solutes on biological equilibria is presented. The model attributes changes in equilibria to differences in the desolvation energy of the reacting species that, in turn, reflect changes in the free energy of the bulk water upon addition of secondary solutes. The desolvation approach differs notably from that of other solute models by treating the free energy of bulk water as a variable and by not ascribing the observed shifts in reaction equilibria to accumulation or depletion of solutes next to the surfaces of the reacting species. On the contrary, the partitioning of solutes is viewed as a manifestation of the different subpopulations of water that arise in response to the surface boundary conditions. A thermodynamic framework consistent with the proposed model is used to derive a relationship for a specific reaction, an aqueous solubility equilibrium, in two or more solutions. The resulting equation reconciles some potential issues with the transfer free energy model of Tanford. Application of the desolvation energy model to the analysis of a two-state protein folding equilibrium is discussed and contrasted to the application of two other solute models developed by Timasheff and by Parsegian. Future tabulation of solvation energies and bulk water energies may allow biophysical chemists to confirm the mechanism by which secondary solutes influence binding and conformational equilibria and may provide a common ground on which experimentalists and theoreticians can compare and evaluate their results.



This work introduces a phenomenological model that treats water explicitly as a coreactant and coproduct for any aqueous reaction equilibrium. A key motivating factor for this approach is the fact that water structure is altered at a boundary; i.e., the network of hydrogen-bonded water molecules near a surface or solute is perturbed relative to neat water, and changes in the physical and thermodynamic properties of water are expected to accompany this rearrangement. The presence of altered water structure, adjacent to small solutes and to the surfaces of larger macromolecules, has been documented by many experimental techniques, including nuclear magnetic resonance (NMR),[1,2] light scattering,[3] X-ray adsorption,[4,5] Raman spectroscopy,[6,7] and neutron diffraction.[8−10]

For the hypothetical binding reaction shown in Figure 1, a solvation sphere of perturbed water molecules surrounds each reactant, denoted X and Y, as well as the product, complex XY. In general, the average structure of the water within each sphere is altered relative to the bulk water outside the sphere. Because of changes in hydrogen bonding (enthalpy) and orientational randomness (entropy), the thermodynamic activity of water molecules within a sphere of influence may vary significantly from the activity of water molecules outside the sphere, even though the number density of water molecules may be nearly equal for both subpopulations. The water molecules surrounding X and Y of Figure 1 are shaded differently to emphasize the fact that these water molecules also differ from each other; the structure and thermodynamic properties of the water within each solvation sphere reflect the specific surface chemistry of the reactant. The hydration spheres do not represent separate phases

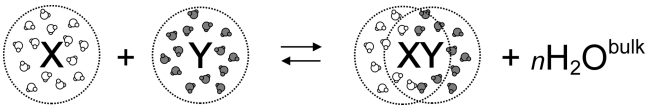


Figure 1. Hypothetical binding reaction. Reactants X and Y are surrounded by a layer of water molecules that differ from the bulk phase, as dictated by the surface chemistry of each reactant. The product of the reaction, complex XY, exposes less surface area to solvent than the sum of the two reactants; thus, a number of bulk water molecules (n) must be included in the balanced reaction.

of liquid in the physical chemistry sense; rather, each hydration layer should be viewed as the solvent's response to a boundary condition. At any given instant in time, water molecules within a sphere of influence may leave to rejoin the bulk population, but a dynamic equilibrium exists such that the total numbers and properties of the water molecules surrounding the reactants and product are relatively constant. Moreover, because the product of this particular binding reaction exposes less surface area than the sum of the reactants, a number of solvation water molecules must be released to the bulk phase (nH$_2$O^{bulk}). For the reaction in Figure 1 $n = 9$, the number of water molecules released from the solvation spheres of the reactants upon formation of complex XY.

For the thermodynamic framework that follows, we define bulk water as all water molecules that are not included within the

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solvation spheres of the reacting species. It is important to note that bulk water is not equivalent to pure neat water in our model. Bulk water includes all perturbed water molecules that reside near secondary solutes or surfaces in contact with the solvent that do not participate directly in the reaction, in addition to the interstitial water between all solutes; in set theory nomenclature, bulk water is the complement to the solvation spheres of the reactants. A freely diffusing water molecule will sample the microenvironment of each locale, and therefore, we suggest that, in a well-mixed system, the number-weighted properties of each subpopulation of water determine the average overall thermodynamic properties of the bulk solvent phase, including the Gibbs free energy. In addition, we propose that a given solute need only perturb a single layer of water to have a significant influence (at high solute concentrations) on any reaction equilibrium that takes place in the same medium. Thus, the presence of long-range water structure, or a global change in the hydrogen bonding network of the solvent, is not pertinent to the validity of the model.

As a starting point, it is convenient to dissect the free energy expression for a general reaction in an aqueous medium into two components, one from the traditional reactants and products and one from rearrangement of the solvent, water. For the hypothetical binding reaction in Figure 1

$$\Delta G^{\mathrm{rxn}} = G^{\mathrm{XY}} - (G^{\mathrm{X}} + G^{\mathrm{Y}}) + \Delta G^{\mathrm{H_2O}} \quad (1)$$

In the case of chemical reactions, where covalent bonds are broken and/or formed, the solvent contribution to the total free energy change of the reaction may be negligible. In the case of conformational equilibria or binding equilibria, however, water may play a decisive role and dictate the position of the equilibrium for the overall reaction. Consequently, $\Delta G^{\mathrm{H_2O}}$ may be extremely important in biological systems where nearly all reactions are mediated by changes in conformation of, or binding to, macromolecules. In general, any aqueous equilibrium that involves exposure or burial of a surface in contact with water is subject to significant hydration effects. In the binding reaction depicted by Figure 1, for which the product exposes less surface than the reactants, $\Delta G^{\mathrm{H_2O}}$ may be termed the desolvation energy of the reaction. For binding or conformational equilibria in general, the value of $\Delta G^{\mathrm{H_2O}}$ is dependent on the average free energy of the bulk water because a subset of bulk water molecules participate in the reaction. In notation form, the desolvation energy term for a binding equilibrium or for a conformational (intramolecular binding) equilibrium may be expressed as follows:

$$\Delta G_{\mathrm{i}}^{\mathrm{H_2O}} = \hat{n}C(\bar{G}_{\mathrm{i}}^{\mathrm{bulk}} - \bar{G}^{\mathrm{solv}}) \quad (2)$$

where $\hat{n}$ represents the moles of displaced water per mole of reactant, C is the concentration of the reactant of interest in moles per mole of bulk water, $\bar{G}_{\mathrm{i}}^{\mathrm{bulk}}$ represents the average free energy of the bulk water per mole of bulk water in a given solution i, and $\bar{G}^{\mathrm{solv}}$ is the average free energy of the perturbed water per mole of perturbed water in the solvation sphere of the reactant that is displaced upon product formation. The value of $\bar{G}^{\mathrm{solv}}$ is a function of the surface chemistry of the reactants, and the value of $\bar{G}_{\mathrm{i}}^{\mathrm{bulk}}$ is a function of all solutes and surfaces that contact the solvent in solution i. Thus, in this model, the value of $\bar{G}_{\mathrm{i}}^{\mathrm{bulk}}$ is dependent on the solution, whereas $\bar{G}^{\mathrm{solv}}$ is a solution-independent value. If the concentration of the reacting species is relatively small compared to the concentration of secondary solutes, then bulk water properties may be estimated from reactant-free solutions, consistent with our working definition of bulk water.

THEORY

One of the most elegant and experimentally convenient ways to study the effects of solutes (and water) on reaction equilibria is to measure the maximum solubility of a given compound in multiple solutions. Because establishing a saturation equilibrium involves exposure or loss of a molecular surface in contact with the bulk aqueous phase, the change in the free energy of water in this system is highly analogous to the change in the free energy of water for a binding equilibrium.

Classical Approach for Solubility Equilibria. Consider the following dissolution reaction for a specific reactant, Φ, in equilibrium between the solid state and the aqueous state:

$$\Phi_{(\mathrm{s})} \leftrightarrow \Phi_{(\mathrm{aq})}$$

One begins by measuring the solubility of Φ in two different aqueous solutions at a constant temperature and pressure. For example, solution A may be neat water and solution B may contain a 1.0 M solute of interest. Because the two solutions, A and B, are in equilibrium with the identical solid, $\Phi_{(\mathrm{s})}$, the chemical potentials of the solute in each solution (μ_{i}) are thought to be equal. This equilibrium relationship has been used as a starting point to develop the following set of equations:

$$\mu_{\mathrm{A}} = \mu_{\mathrm{B}} \quad (3)$$

$$\mu_{\mathrm{A}}^{\circ} + RT \ln a_{\mathrm{A}} = \mu_{\mathrm{B}}^{\circ} + RT \ln a_{\mathrm{B}} \quad (4)$$

$$\mu_{\mathrm{A}}^{\circ} + RT \ln(C_{\mathrm{A}}\gamma_{\mathrm{A}}) = \mu_{\mathrm{B}}^{\circ} + RT \ln(C_{\mathrm{B}}\gamma_{\mathrm{B}}) \quad (5)$$

$$\mu_{\mathrm{B}}^{\circ} - \mu_{\mathrm{A}}^{\circ} = RT \ln\left(\frac{C_{\mathrm{A}}}{C_{\mathrm{B}}}\right) + RT \ln\left(\frac{\gamma_{\mathrm{A}}}{\gamma_{\mathrm{B}}}\right) \quad (6)$$

where μ_{i}° is defined as the standard chemical potential of $\Phi_{(\mathrm{aq})}$ at infinite dilution in a specific solution i (subscript A or B), a_{i} is thermodynamic activity of $\Phi_{(\mathrm{aq})}$, γ_{i} denotes the activity coefficient of $\Phi_{(\mathrm{aq})}$, and C_{i} is the saturation concentration of $\Phi_{(\mathrm{aq})}$ in the corresponding solution. The last term in eq 6, containing the activity coefficients, is often ignored, although the importance and experimental measurement of activity coefficients are both matters of concern.[11] The concentration term in eq 6, however, is easily calculated and is often denoted as the apparent transfer free energy, $\Delta G_{\mathrm{tr}}^{\mathrm{app}}$.

$$\Delta G_{\mathrm{tr}}^{\mathrm{app}} = RT \ln\left(\frac{C_{\mathrm{A}}}{C_{\mathrm{B}}}\right) \quad (7)$$

Transfer free energies based on solubility measurements have been reported in numerous papers dating back to the works of Tanford,[12−15] Jencks,[16−18] and Robinson.[19,20] More recently, Bolen and co-workers have applied the Tanford transfer model to their investigations of the physical mechanisms by which osmolytes mediate their favorable effects on protein folding and stability.[11,21−23]

Although solubility experiments can be highly informative, there are some unresolved or debatable issues associated with the classical approach described above, namely, (i) the proper measurement and application of activity coefficients for aqueous reactions and (ii) the additivity of transfer free energies obtained

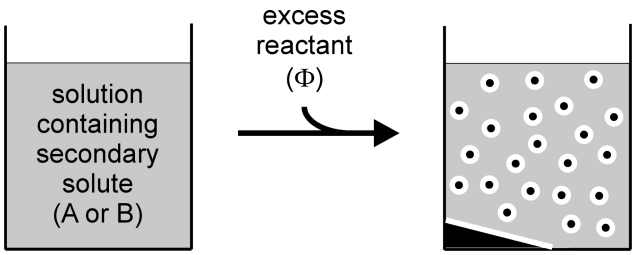

Figure 2. Solubility experiment with model compound Φ. The free energy of the bulk water, shaded gray, is a function of the chemistry and concentration of the secondary solute. The white halo surrounding each molecule of Φ, to the right of the reaction arrow, indicates a new subpopulation of water that originated from the bulk water pool upon dissolution.

from the solubility of small model compounds for the purpose of estimating the thermodynamic properties of a larger macromolecule. Another question relevant to this work is how transfer free energies are related to desolvation energies.

It has been noted in the literature that the choice of concentration units for eq 7 will influence the value and sometimes even the sign of the apparent transfer free energy. Bolen and coworkers have argued that any unit of concentration may be employed (molarity, molality, or mole fraction), as long as a correction factor is included such that solutions A and B are treated on the same experimental basis.[23] In the derivation that follows, concentrations are assumed to be normalized to the total moles of water in the solution. It is a trivial calculation to convert molar solute concentrations to this basis if an accurate density value is known for the corresponding solution.

Bulk Water-Dependent Desolvation Energy Model. Below, we consider how the equations describing the solubility of Φ might change if water is treated as a coreactant and coproduct of the saturation equilibrium. In the new framework, the solubility equilibrium is described as follows:

$$\Phi_{(s)} + \hat{n}H_2O^{bulk} \leftrightarrow \Phi_{(aq)} + \hat{n}H_2O^{solv}$$

where $\hat{n}$ is the number of perturbed water molecules in the hydration sphere surrounding a molecule of Φ. The solvation water molecules (H_2O^{solv}) must originate from the preexisting pool of bulk water (H_2O^{bulk}). In Figure 2, the bulk water is shaded gray, Φ is black, and the sphere of solvation water is represented by the white "halo" surrounding each molecule of dissolved solute on the right side of the diagram. The secondary solutes also induce a sphere of perturbed water molecules, but this subpopulation is combined with the interstitial water to define the bulk phase, depicted as a solid color in Figure 2.

As employed in the development of eqs 2−7 of the classical approach, solubility measurements in two different solutions may be equated through the chemical potential of the solid state. In the new thermodynamic treatment, however, the participation of solvent is taken into account explicitly by including terms for the bulk water and for the solvation shell of the reactant. We begin by writing the individual chemical potential expressions for the equilibrium reaction in each solution, using subscripts to designate solutions A and B as before:

$$\mu_A^{\Phi_{(s)}} + N_A\overline{\mu}_A\text{bulk} = \mu_A^{\Phi_{(aq)}} + N_A\overline{\mu}_A\text{solv} \quad (8)$$

$$\mu_B^{\Phi_{(s)}} + N_B\overline{\mu}_B\text{bulk} = \mu_B^{\Phi_{(aq)}} + N_B\overline{\mu}_B\text{solv} \quad (9)$$

where $\overline{\mu}_i^{bulk}$ and $\overline{\mu}_i^{solv}$ refer to the chemical potentials of the water molecules that move between the bulk phase and the solvation sphere of a single molecule of Φ and N_i refers to the total number of water molecules that solvate all molecules of $\Phi_{(aq)}$ at equilibrium. The bar above the chemical potentials for the two solvent terms is a reminder that these potentials refer to a specific subset of water molecules. By equating the chemical potential of the solid phase in each solution $[\mu_A^{\Phi_{(s)}} = \mu_B^{\Phi_{(s)}}]$ and by substituting the corresponding expressions from eqs 8 and 9, we obtain

$$\mu_A^{\Phi_{(aq)}} - \mu_B^{\Phi_{(aq)}} = N_A(\overline{\mu}_A^{bulk} - \overline{\mu}_A^{solv}) - N_B(\overline{\mu}_B^{bulk} - \overline{\mu}_B^{solv}) \quad (10)$$

Next we digress from the classical approach by treating the standard chemical potential of the reactant, $\mu^{o,\Phi}$, as a solution-independent parameter. This assumption is in accord with the concept that the surface chemistry of Φ defines a boundary condition that dictates the properties of the solvation sphere. This treatment also implies that $\overline{\mu}_A^{solv} = \overline{\mu}_B^{solv} = \overline{\mu}^{solv}$. In essence, this approach is equivalent to treating the activity coefficient of the reactant, γ^{Φ}, as a constant for all aqueous solutions. We believe this thermodynamic framework is valid because the system is more fully defined; deviations from ideality, as defined by the existing conventions of physical chemistry, are embedded in the chemical potential terms for the two subpopulations of water. By implementing the assumptions described above and by substituting the corresponding activity expression for $\mu_i^{\Phi_{(aq)}}$ into eq 10, we derive the following:

$$\mu^{o,\Phi} + RT\ln a_A^{\Phi} - (\mu^{o,\Phi} + RT\ln a_B^{\Phi}) = N_A(\overline{\mu}_A^{bulk} - \overline{\mu}_A^{solv}) - N_B(\overline{\mu}_B^{bulk} - \overline{\mu}_B^{solv}) \quad (11)$$

which simplifies to

$$RT\ln(a_A^{\Phi}/a_B^{\Phi}) = N_A(\overline{\mu}_A^{bulk} - \overline{\mu}^{solv}) - N_B(\overline{\mu}_B^{bulk} - \overline{\mu}^{solv}) \quad (12)$$

Because the traditional approach to solution thermodynamics does not provide a means for addressing distinct subsets of solvent molecules, the water-related terms $\overline{\mu}_i^{bulk}$ and $\overline{\mu}^{solv}$ in eq 12 require special consideration. We propose to substitute the average Gibbs free energy for the chemical potential of each of these subpopulations of water, in units of energy per mole of the corresponding water population. These free energy values must be expressed relative to a standard state, but the standard state value will cancel out within the two differences that appear on the right-hand side of eq 12. Again, we stress that the free energy of the bulk water represents an average of all the subpopulations of water that coexist in a given solution, excluding the hydration shell of the reactants. Substitution of Gibbs free energies for chemical potentials in eq 12 leads to the following expression:

$$RT\ln(a_A^{\Phi}/a_B^{\Phi}) = N_A(\overline{G}_A^{bulk} - \overline{G}^{solv}) - N_B(\overline{G}_B^{bulk} - \overline{G}^{solv}) \quad (13)$$

where $\overline{G}_i^{bulk}$ and $\overline{G}^{solv}$ are identical to the desolvation energy terms in eq 2. From stoichiometry considerations, we also recognize

$$N_i = \hat{n}C_i^{\Phi} \quad (14)$$

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where $\hat{n}$ is the moles of affected water per mole of reactant Φ and C_i^{Φ} is the saturation concentration of Φ in moles per total moles of water in solution i. By applying eq 14 to N_A and N_B and replacing the thermodynamic activities of the reactant in eq 13 with the corresponding concentrations and activity coefficients, we obtain

$$RT \ln \left(\frac{C_A^{\Phi}\gamma_A^{\Phi}}{C_B^{\Phi}\gamma_B^{\Phi}} \right) = \hat{n}C_A^{\Phi}(\bar{G}_A^{\text{bulk}} - \bar{G}^{\text{solv}}) - \hat{n}C_B^{\Phi}(\bar{G}_B^{\text{bulk}} - \bar{G}^{\text{solv}}) \quad (15)$$

As discussed in the preceding section, the activity coefficient of the reactant may be treated as a constant because the system is more fully defined:

$$\gamma_A^{\Phi} = \gamma_B^{\Phi} = \gamma^{\Phi} \quad (16)$$

Thus, the activity coefficients offset in eq 15, leading to our final expression:

$$RT \ln \left(\frac{C_A^{\Phi}}{C_B^{\Phi}} \right) = \hat{n}C_A^{\Phi}(\bar{G}_A^{\text{bulk}} - \bar{G}^{\text{solv}}) - \hat{n}C_B^{\Phi}(\bar{G}_B^{\text{bulk}} - \bar{G}^{\text{solv}}) \quad (17)$$

or substituting the relationship given by eq 2, we obtain

$$RT \ln \left(\frac{C_A^{\Phi}}{C_B^{\Phi}} \right) = \hat{n}(C_A^{\Phi}\Delta\bar{G}_A^{H_2O} - C_B^{\Phi}\Delta\bar{G}_B^{H_2O}) \quad (18)$$

where $\Delta\bar{G}_i^{H_2O}$ denotes the molar desolvation energy change in solution i.

Equations 17 and 18 reveal that measuring the solubility concentrations of a model compound in two or more solutions is not adequate for determining the desolvation energy of the compound in either solution. However, if $\hat{n}$ and $\bar{G}_i^{\text{bulk}}$ can be obtained by other experimental methods, then eq 17 allows one to obtain $\bar{G}^{\text{solv}}$, as needed to calculate $\Delta\bar{G}_i^{H_2O}$ by eq 2.

Importantly, eq 18 is similar in form to a more general equation derived by Ben-Naim for a solute, s, in equilibrium between any two phases:[24]

$$kT \ln \left(\frac{\rho_s^{\alpha}}{\rho_s^{\beta}} \right)_{eq} = \Delta G_s^{*\beta} - \Delta G_s^{*\alpha} \quad (19)$$

where k is the Boltzmann constant, ρ_s^{α} and ρ_s^{β} are the number density concentrations of s in phases α and β, respectively, and $\Delta G_s^{*\alpha}$ and $\Delta G_s^{*\beta}$ are the corresponding solvation Gibbs free energies of s in each phase (for example, see eq 7.30 of ref 24). Because eqs 17 and 18 were derived for two liquid phases in equilibrium with the same solid phase, Ben-Naim's general expression should be applicable to this system. If one notes that Ben-Naim defined the free energy term in the solvation direction, as opposed to the desolvation direction, the two nomenclatures can be related as follows:

$$\Delta G_s^{*\alpha} = -\Delta G_A^{H_2O} = -\hat{n}C_A^{\Phi}\Delta\bar{G}_A^{H_2O} \quad (20)$$

Thus, the right-hand sides of eqs 17−19 are all equivalent, and the formalism of Ben-Naim provides an excellent corroboration of ideas about solvation thermodynamics, one developed from the viewpoint of an experimentalist (eqs 17 and 18) and one developed from the viewpoint of a theoretician (eq 19). Equation 17 may be seen as a significant advance in the application of Ben-Naim's formalism because it breaks down the solvation−desolvation energy into two components, $\bar{G}_i^{\text{bulk}}$ and $\bar{G}^{\text{solv}}$, and because it includes a parameter that accounts for the effect of secondary solutes on bulk water, $\bar{G}_i^{\text{bulk}}$.

DISCUSSION

The general goal of this work is to quantify the energetic role of water in aqueous reactions, especially binding and conformational equilibria. In brief, the thermodynamic framework presented here for analyzing the effects of solutes on aqueous equilibria differs from other approaches in the literature in two significant ways. (i) The desolvation energy model includes an explicit thermodynamic consideration for the bulk water and for the water of solvation of the reactants, and (ii) the model treats the free energy of bulk water as a variable that depends on the concentration and surface chemistry of all solutes in contact with the solvent, including secondary solutes that do not participate directly in the reaction of interest.

Historically, the outcome of adding a secondary solute to a solubility reaction mixture has been termed "salting in" if the saturation concentration of the model compound is increased and "salting out" if the saturation concentration is decreased.[25,26] With regard to macromolecular structure, salting-in reagents generally correspond to denaturing solutes and salting-out reagents correspond to solutes that stabilize the compact, folded state. Although it has been implied by others that all bulk water molecules, including the interstitial subpopulation between solute molecules, must exhibit altered structure if the effects of solutes on equilibria are related to changes in bulk water properties,[27,28] this assumption may be incorrect. Because water molecules are in a dynamic equilibrium between multiple subpopulations of differing energy, as defined by the chemical boundaries of all solutes and surfaces in contact with the solvent, it seems reasonable to treat the thermodynamics of bulk water in a well-mixed system as a number-weighted average of all of the subpopulations. The absence of a global disruption in the hydrogen bond network of bulk water[4,7,29] does not preclude the possibility that solute effects are mediated through changes in the average properties of the water.

If solvent properties are determined by the average of the various surface-induced subpopulations, then addition of a secondary solute will always shift the free energy of bulk water toward the energy of the solute's hydration shell. When the solute concentration is in the molar range, a large fraction of the total water molecules may be influenced by the surface chemistry of the solute. As calculated by Marcus, the average center-to-center ion spacing for a homogeneously dispersed solution of spherical ions may be expressed as $0.940c^{-1/3}$ nm, where c is the salt concentration in molarity.[30,31] After subtraction of the radii of the anion and cation, there may be space for only a few water molecules between ions in solutions at >1.0 M because of this geometrical constraint. Thus, at solute concentrations of ≥1 M, the free energy of bulk water is expected to be significantly altered because a large fraction of all water molecules is located within the first solvation shell of a solute molecule.

Others have stated that there is no correlation between solute-induced water structure and its effect on specific aqueous equilibria,[6,27,32] but none of the studies cited in these papers relate their results to the key parameter, the Gibbs free energy of the bulk water as a function of solute chemistry, $\bar{G}_i^{\text{bulk}}$.

Presumably, bulk water energies can be determined as a function of solute identity and concentration and reported relative to neat water under standard state conditions, $\Delta\bar{G}_i^{o,bulk}$.

With regard to the highly cited calorimetry work of Pielak and co-workers,[32] we note that the measured heats obtained by pressure perturbation represent $\Delta\Delta S$ values. That is, the thermodynamic framework for pressure perturbation analysis is developed from the second law of thermodynamics,[33] and the measured quantity may be viewed as the change in entropy of solution i between two pressures, P_1 and P_2, relative to a reference cell containing neat water that is subject to the same pressure change; in notation form, pressure perturbation measures $\Delta(\Delta S_{P_1}^{P_2})^i_{H_2O}$. Typically, pressure perturbation data are related to changes in the thermal coefficient of expansion, as developed from the second law of thermodynamics using pressure−volume relationships.[33] Unfortunately, the energetic contribution of $P\Delta V$ is expected to be negligible relative to changes in the enthalpy and entropy of bulk water upon addition of a solute, and thus, it is not surprising that the thermal coefficient of expansion, or any parameter derived from the thermal coefficient of expansion, does not correlate with solute-specific effects on protein stability.[32] The lack of correlation does not imply that water structure is unimportant; rather, it points to the confusion that arises when one focuses on any solute-dependent parameter other than the change in the free energy of the solvent. A more insightful quantity would be the difference in entropy between water in solution i and neat water at the same pressure and temperature, $\Delta\bar{S}_i^{o,bulk}$, which could be used in calculation of $\Delta\bar{G}_i^{o,bulk}$. Unfortunately, direct experimental assessment of $\Delta\bar{S}_i^{o,bulk}$ does not appear to be achievable with current calorimetry instrumentation.

With regard to the issue of whether changes in water structure are important, we agree with Ricci, Soper, and co-workers who state that the phrases "structure maker" and "structure breaker" are highly misleading with regard to how water interacts with solutes on a molecular level.[8] The terms kosmotrope, for solutes that bind water strongly inducing order, and chaotrope, for solutes that bind water weakly inducing chaos, are equally confounding and sometimes misused in the literature. For example, cations with a lower charge density, such as Cs^+ and $N(CH_3)_4^+$, should be termed chaotropes even though these ions tend to have a favorable, stabilizing influence on protein structure.[34] Perhaps better adjectives would be "Gibbsophilic" and "Gibbsophobic" to denote a solute that decreases and increases the free energy of water, respectively, because all solutes alter the structure of water relative to the structure found in neat solution and because changes in the free energy of bulk water may underlie many of the effects of secondary solutes on reaction equilibria.

The idea that the free energy of bulk water is a variable and may dictate conformational equilibria has been suggested previously for proteins confined to the pores of a silica matrix,[34] and recent computational studies have applied this concept to the confinement of a protein in a nanotube[35] and the confinement of a protein in the aqueous compartment of the bacterial chaperonin, GroEL.[36] The GroEL work links changes in the interior surface of the chaperone to changes in the free energy of neighboring water molecules, which, in turn, alters the favored conformational state of the confined protein. An important corollary of the desolvation energy model is the concept that the solvent's contribution to the hydrophobic effect is quantifiable for a given reaction but dependent on all species present in the solution. It has been suggested that the strength of the hydrophobic effect in the crowded environment of living cells is adjusted by nature to enhance,[34] or perhaps to diminish,[37] macromolecular stability relative to the values obtained experimentally in dilute solutions.

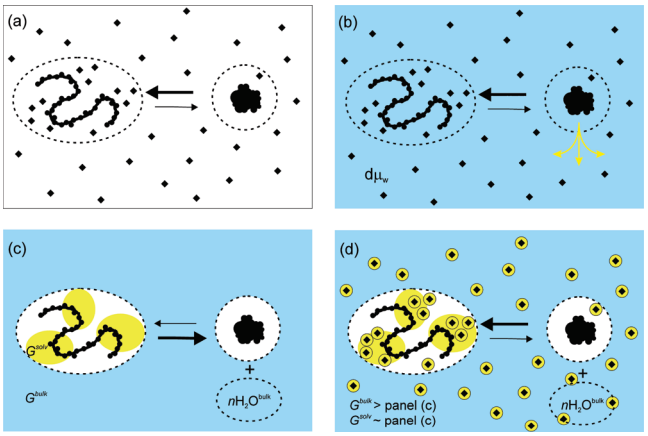

Figure 3. Comparison of models for a secondary solute that destabilizes the folded protein structure. The macromolecule on the left side of each panel represents the ensemble of unfolded states; the molecule on the right represents the compact native state, and the small black diamonds represent the secondary solute. (a) In the Timasheff model of preferential interactions, protein unfolding is attributed to the accumulation of solute near the surface of the unfolded state; subpopulations of water are not a consideration in the thermodynamic framework as depicted by a lack of color shading. (b) In the Parsegian model of osmotic stress, the folded state is disfavored because of the lower density of solute around the folded state relative to the bulk phase; water surrounding the folded state is prone to move to the bulk phase to equalize the solute concentrations in both locales (yellow arrows). (c) The bulk water-dependent desolvation energy model provides a rationale for the contribution of water in the absence of secondary solutes; a fraction of the solvation water surrounding the unfolded state, shaded yellow, is released to the bulk phase upon folding. If the desolvation energy is negative, i.e., $n(\bar{G}^{bulk} - \bar{G}^{solv}) < 1$, then the free energy change of water favors the folded state as indicated by the bold reaction arrow. (d) In the presence of a destabilizing solute, the desolvation energy is more positive because the average free energy of the bulk water is increased by the free energy of the solute hydration spheres, as represented by the yellow ring around each black diamond. Solute molecules are shown to partition to the unfolded state as in panel a, but the solute molecules partition specifically to the yellow regions of higher-energy water, which is expected to exist near hydrophobic residues and amide groups of the backbone.

Other Solute Models. Effects of solutes on biological equilibria are most frequently interpreted using the model of preferential interactions, as developed by Timasheff, or using the osmotic stress model of Parsegian and co-workers. Both of these models attribute the effects of solutes to a nonhomogeneous distribution of the solute between the reactant surface and bulk phase, and both models are limited to analyzing changes in equilibria; i.e., neither model addresses the set point of a given equilibrium in the absence of secondary solutes. Details regarding the application of each model, in addition to the transfer free energy approach, have been reviewed elsewhere.[38]

Within the framework of the desolvation energy model, unequal solute distributions are viewed as a consequence of the existence of two or more energetically distinct subsets of water; accumulation or depletion of a solute near a surface is expected to occur, but it is not viewed as a driving force in altering reaction equilibria. In Figure 3, the preferential interaction model

and osmotic stress model are compared to the desolvation energy model for the case of a two-state protein folding reaction, a focal point for much of the following discussion.

In the Timasheff model, water is designated as component 1, the reacting species is component 2, and the secondary solute is termed the cosolvent or component 3.[39,40] The Timasheff approach emphasizes the weak and nonspecific binding of component 3 to component 2 using an interaction parameter defined as $\delta\mu_2/\delta m_3$, where μ_2 is the chemical potential of the reacting species and m_3 is the molal concentration of the secondary solute. Although Timasheff states that water is treated explicitly in the thermodynamic framework, the formalism is incomplete; there is no consideration for the effect of component 2 or 3 on the chemical potential of water. In the notation of Timasheff, $\delta\mu_1/\delta m_3$ is ignored in the thermodynamic analysis, and all solute effects are interpreted as the consequence of direct binding events at the surface of component 2. In Figure 3a, this concept is depicted by a higher concentration of solute molecules surrounding the unfolded state relative to the folded state, indicating a preferential interaction that favors the unfolded protein.

In the osmotic stress model of Parsegian and co-workers,[41,42] water activity is rightfully treated as a variable. However, only one parameter is used to characterize all water molecules in the system, $d\mu_w$, the change in chemical potential of water upon addition of a solute. Thus, as in the model of preferential interactions, coexisting subpopulations of water that differ in free energy due to the chemistry of the protein or solute surface are not acknowledged in the model of osmotic stress (see Figure 3b). Only the numbers of water molecules next to the surface of a macromolecule are allowed to fluctuate, and the output of this model, as given by the number of excess water molecules next to one conformation or the other, is a different value for each chemically distinct solute employed to probe the same system.

Another potential issue for the osmotic stress approach is the means by which the key parameter, $d\mu_w$, is obtained. Experimental measurements that rely on a colligative property, such as osmotic pressure or vapor pressure lowering, may be proportional to changes in the free energy of the bulk water, but such measurements do not reveal the sign of the free energy change. For example, a 1 M solution of any salt, X, on one side of a semipermeable membrane will yield an osmotic pressure value similar to that of another 1 M solution of salt, Y, regardless of whether each salt increases or decreases the free energy of water; neat water on one side will want to pass through the membrane and dilute the solute on the other side such that the average free energy of water approaches equality on both sides. Thus, changes in osmotic pressure may offer only a scalar quantity proportional to the absolute value of the change in the free energy of bulk water. The same argument applies to vapor pressure measurements because the pure component (in this case, a water molecule in the vapor phase) will want to partition to the liquid phase, regardless of the direction in which the average free energy of bulk water is altered because of the presence of an aqueous solute. For these reasons, one may question the utility of activity coefficients obtained via osmotic measurements.

The bulk water-dependent desolvation energy model, in contrast to the two models discussed above, provides a rationale for the role of hydration in protein folding equilibria in the absence, as well as in the presence, of secondary solutes, as depicted in panels c and d of Figure 3. This model views all solutes as presenting a boundary condition that must be satisfied by the solvent. The chemistry of the solute surface defines the boundary condition, and water structure rearranges to meet this condition and to minimize the total free energy of the system. In the specific case of a two-state protein folding equilibrium, one may envision three or more subpopulations of water, depicted in Figure 3c as follows: the bulk water (blue), the subpopulation next to largely polar residues of the folded state (white), and water next to the unfolded state that must include at least two subsets, the same waters found next to the folded state (already defined as white) and the waters of hydrophobic or backbone solvation that are expected to be higher in energy (yellow ellipsoid regions). When the protein folds, the high-energy waters of solvation are released to the bulk phase because the hydrophobic residues and backbone amides are no longer in contact with the solvent. The contribution of this desolvation process to the overall reaction may be calculated with eq 2. In the presence of a thermodynamically unfavorable secondary solute (Figure 3d), the bulk water free energy is increased by the perturbed water structure in the hydration shells around each small solute molecule (shaded yellow to indicate a free energy higher than that of interstitial water). The change in bulk water will make the desolvation energy more positive and thereby weaken the driving force for folding. If the unfolded state persists, the secondary solutes will partition between the two subpopulations of water until the thermodynamic advantage of overlapping the high-energy hydration shells of the solute with the high-energy subset of protein hydration molecules is countered by the loss of solute entropy. In the case of a favorable secondary solute that decreases the free energy of bulk water, the scenario is reversed; the driving force for adopting the folded conformation is enhanced because the change in the free energy of desolvation is more negative.

Recently, the Timasheff model has been extended by Pegram and Record, leading to the development of a salt ion partitioning model that relates solute partitioning to the accessible surface area (ASA) of the reacting species.[43,44] Although correlations between partition coefficients, ASA, and surface chemistry are apparent, these correlations are also compatible with the bulk water-dependent desolvation energy model. The ASA and surface chemistry of a reacting species are the two primary factors that determine the solvation energy of the molecule ($n\bar{G}^{\,solv}$), as used in defining the desolvation energy in eq 2. Pegram and Record state that "the various effects of different ions on water structure...are not the direct origins of thermodynamic effects on biomolecular processes",[44,45] yet they acknowledge the existence of a 6 Å thick hydration layer of perturbed water structure at the air−water interface.[44] These two positions seem incongruous; if a nonpolar surface defined by air can induce a subpopulation of water with properties different from those of the bulk phase, then should not smaller solutes be able to do the same, albeit on a lesser scale?

Dialysis equilibrium experiments provide ample evidence that solute partitioning occurs,[39,40] but the desolvation energy model, as presented here, leads one to question (i) whether partitioning is a driving force that contributes to changes in reaction equilibria and (ii) whether direct contacts between solute and reacting species, i.e., preferential interactions, are necessary for partitioning to occur. If one acknowledges that a solute molecule may induce a solvation shell of water that differs in energy from other subpopulations of water, then partitioning of solutes may be rationalized as a solvent energy

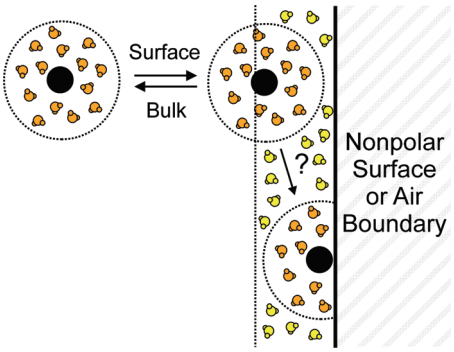

Figure 4. Microscopic view of solute partitioning to a nonpolar surface. If a solute (dark sphere) induces a shell of water structure (gold molecules) with a free energy higher than the average value of the bulk phase, then the solute is likely to partition to other surfaces that induce a layer of water with a higher free energy (yellow), as expected to occur near interfaces like air, hydrocarbons, and unfolded proteins, and as depicted by the vertical boundary line. An overlap between the hydration sphere of the solute and the hydration layer at the interface reduces the total free energy of water in the system and serves as the thermodynamic driving force for partitioning (top, equilibrium arrow). In contrast to other models, partial desolvation of the solute is not required to explain solute-specific partitioning to the interfacial region (lower reaction arrow).

minimization problem. As shown in Figure 4, a solute that induces water structure with a free energy higher than the average bulk value will partition to surfaces that induce a stable layer of water of similarly high energy. In other words, Gibbsophobic solutes, such as perchlorate, thiocyanate, and iodide ions, should partition to the air−water interface (or to surfaces characterized as hydrophobic) because partitioning will reduce the total number of high-energy water molecules in the system that are subject to the boundary conditions of the solute and interface. A Gibbsophilic solute that induces water structure with a free energy lower than the average bulk value will not partition to a high-energy surface because the two competing boundary conditions cannot be met simultaneously. Thus, both solute exclusion and solute accumulation are dependent on the solvation shell of the solute and on the free energy of the boundary layer of water at the site of partitioning.

This microscopic view of partitioning does not require that solute molecules ever exist in a partially desolvated state at the boundary (Figure 4). The Timasheff approach, on the other hand, must invoke partial desolvation of the solute to explain solute-specific effects on reaction equilibria[46] because this model assumes direct binding of the solute to the surface of the reacting species and because this model dismisses the effect of solutes on the thermodynamic properties of water.

Use of Transfer Free Energies. As mentioned earlier, transfer free energies have been used to estimate the thermodynamic properties of macromolecules formed from smaller building blocks. For example, glycine derivatives have been used to model the backbone of a protein, and small peptides or free amino acids have been used to model the individual residues of a protein. However, it is important to note that the principle of additivity, as applied to energy values, is a major issue for both experimental and computational biology.[23,47,48] Comparing the desolvation model-derived relationship for a solubility equilibrium (eq 17) to the classical definition of the apparent transfer free energy (eq 7), we obtain the following expression:

$$\Delta G_{tr}^{app} = \hat{n}C_A^{\Phi}(\bar{G}_A^{bulk} - \bar{G}^{solv}) - \hat{n}C_B^{\Phi}(\bar{G}_B^{bulk} - \bar{G}^{solv}) \quad (21)$$

Thus, the apparent transfer free energy is equal to the difference in desolvation energies weighted by the corresponding saturation concentrations. Taking this analysis one step further and applying the desolvation energy expression in eq 2 to the transfer of molecule Φ between two solutions, A and B, at the same concentration, C^{Φ}, we obtain the following:

$$\Delta\Delta G^{H_2O} = \hat{n}C^{\Phi}(\bar{G}_A^{bulk} - \bar{G}^{solv}) - \hat{n}C^{\Phi}(\bar{G}_B^{bulk} - \bar{G}^{solv}) \quad (22)$$

which simplifies to

$$\Delta\Delta G^{H_2O} = \hat{n}C^{\Phi}(\bar{G}_A^{bulk} - \bar{G}_B^{bulk}) \quad (23)$$

Comparison of eqs 21 and 22 reveals that the apparent transfer free energy is equal to the change in desolvation energy, $\Delta\Delta G^{H_2O}$, only at the limit of $C_A^{\Phi} \rightarrow C_B^{\Phi}$, corresponding to the limit of $\Delta G_{tr}^{app} \rightarrow 0$ by eq 7. Thus, we question the utility of apparent transfer free energies for quantifying solute-specific effects on aqueous equilibria. Also, we note that application of eq 17 does not rely on the measurement of activity coefficients and, therefore, bypasses some key issues associated with the Tanford transfer model. Regardless of one's interpretation of transfer free energy values, the biophysical goal should be a table of desolvation energies as defined by eq 2 and not a table of $\Delta\Delta G^{H_2O}$ values as defined by eq 22 or 23. We assert that the desolvation energy term, $\Delta G_i^{H_2O}$, is the proper parameter for quantifying the total change in the desolvation energy of a macromolecule from the additive contributions of smaller model compounds.

Concluding Remarks. A new phenomenological model for interpreting the effects of solutes on aqueous reaction equilibria that emphasizes the role of water has been described. The desolvation energy model champions the idea that all binding events are accompanied by release of water molecules to the bulk phase and, therefore, one must account for a subset of bulk water in any rigorous thermodynamic framework. The desolvation energy model also recognizes that the free energy of the bulk water is a function of all solutes and surfaces in contact with water. Secondary solutes that increase the free energy of water will result in a more positive desolvation energy and weaken the driving force for binding events, whereas solutes that decrease the free energy of bulk water will result in a more negative desolvation energy and enhance the driving force for binding.

The desolvation energy model acknowledges that partitioning of secondary solutes may occur, but partitioning is viewed as a manifestation of the true driving force, i.e., the existence of subpopulations of water with differing energies. An exception to this hypothesis is the case in which a specific solute binds directly to a specific functional group on the surface, thereby altering the free energy of the solvation shell in addition to altering the bulk water energy. For example, perchlorate anions may bind specifically to the primary ammonium group of lysine residues,[49,50] and certain cations may bind favorably to the face of aromatic amino acids.[51] These specific interactions effectively alter the chemistry of the surface in contact with water and, consequently, alter the desolvation energy in the vicinity of the binding event. In summary, all solutes have the potential to alter aqueous reaction equilibria through their effects on bulk water, whereas a unique

subset of solutes may also influence reaction equilibria by specific binding interactions. In the latter case, solute binding leads to a change in $\bar{G}^{solv}$ that alters the equilibrium due to a change in the desolvation energy (eq 2). Thus, observed shifts in equilibria due to specific solute binding interactions may also be explained as a change in the bulk water-dependent desolvation energy without invoking accumulation and/or depletion of the solute near the surface as a driving force.

Future research on hydration phenomena should focus on determining the desolvation energy of water for many surfaces, including the surfaces of biological molecules. Although obtaining the free energy of bulk water as a function of different secondary solutes should be experimentally feasible, acquiring the second parameter for calculation of the desolvation energy, which is the solvation free energy of the surface of interest, may be more problematic. Although it is clear from the literature that much effort has gone into the experimental determination of water structure at various surfaces, much less effort has gone into evaluating the linkage between changes in water structure and changes in the free energy of water. It is unfortunate that solubility experiments do not directly reveal the energetic contribution of the solvation shell, $n\tilde{G}^{solv}$, as required for tabulating $\Delta G_i^{H_2O}$. However, assuming the free energy of bulk water can be obtained as a function of solute chemistry and concentration, solubility experiments in two or more solutions may allow one to back out the value of $\bar{G}^{solv}$ using eq 17, as derived in this work. Calorimetry techniques may also lead to experimental measures of the desolvation energy for many solutes and model compounds, but in silico methods should greatly expedite this process and facilitate the analysis of complex macromolecular surfaces that contain multiple functional groups in the proximity. Computational techniques that employ inhomogeneous fluid solvation theory to assess the relative energies of individual water molecules as a function of surface chemistry and surface morphology appear to be an important step in the right direction.[52,53]

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