# Effect of Ions on the Hydrophobic Interaction between Two Plates

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***Abstract:*** We use molecular dynamics simulations to investigate the solvent mediated attraction and drying between two nanoscale hydrophobic surfaces in aqueous salt solutions. We study these effects as a function of the ionic charge density, that is, the ionic charge per unit ionic volume, while keeping the ionic diameter fixed. The attraction is expressed by a negative change in the free energy as the plates are brought together, with enthalpy and entropy changes that both promote aggregation. We find a strong correlation between the strength of the hydrophobic interaction and the degree of preferential binding/exclusion of the ions relative to the surfaces. The results show that amplification of the hydrophobic interaction, a phenomenon analogous to salting-out, is a purely entropic effect and is induced by high-charge-density ions that exhibit preferential exclusion. In contrast, a reduction of the hydrophobic interaction, analogous to salting-in, is induced by low-charge-density ions that exhibit preferential binding, the effect being either entropic or enthalpic. Our findings are relevant to phenomena long studied in solution chemistry, as we demonstrate the significant, yet subtle, effects of electrolytes on hydrophobic aggregation and collapse.

## I. Introduction

Recognition of salt solutions as central to the regulation of chemical and biological processes goes back more than a century, starting with Hofmeister's[1] experiments on the ability of ions to solubilize/precipitate proteins in 1888. The purpose of this paper is to demonstrate how the hydrophobic interaction between nanoscale nonpolar solutes is modified by the addition of salt to the aqueous solution. This issue has been studied for at least six decades,[2-5] and has led to the notion that the hydrophobic interaction can be either amplified or reduced, depending on the nature of the salt and the size of the solute. A large number of experimental studies[1,6-9] suggest that an increased ionic charge density, that is, the ionic charge per unit ionic volume, amplifies the hydrophobic interaction between nonpolar solutes. This behavior is related to a decline of the solubility in the salt solution versus pure water, and is often referred to as salting-out. The opposite phenomenon is known as salting-in. The strength of the hydrophobic interaction is also sensitive to changes in the concentration of the electrolyte. This is described by the empirical Setschenow equation.[10]

In light of the biological and medical significance of salt solutions, it is not surprising that a great deal of effort has been put into understanding their properties. In his groundbreaking work, Hofmeister[1] ranked ions by their ability to solubilize/precipitate proteins, in what is now known as the Hofmeister series. In many cases, the common $Na^+$ and $Cl^-$ ions mark the separation between salting-out versus salting-in. Hofmeister ascribed salting-out to the ability of salts to "absorb" water, which he pictured as water molecules attached to the ions and thus removed from their role as solvent molecules. Experiments later suggested[6,7] that for salting-out ions, the ion—water interaction is stronger than the water—water interaction, and vice versa for the salting-in ions. More recent work[8,9] has demonstrated that effects of salts on proteins are complicated by the presence of both polar and nonpolar residues, which have opposite effects.

Following Hofmeister's initial study, many authors[3,11-13] have proposed models to explain the effects of electrolytes on the properties of hydrophobes in aqueous solution. An early favorite[3,11,12] relates the extent of volume contraction upon mixing salt and water to the degree of salting-out. A later model by Debye and McAulay[13] represents both the solvent and the hydrophobic solute as continuous media, whose interactions with the ions are determined by their respective macroscopic dielectric constants. With this view, the electric field around the ions pulls the material of higher dielectric constant into the field and pushes the material of lower dielectric constant away. Since the dielectric constant of water is higher than that of most other neutral media, that is, the hydrophobes, the water molecules will cluster around the ions while the less polar

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hydrophobic material is pushed into the distant low field region. While these models are fairly accurate in determining the ability of a particular ion to salt-out, that is, reduce the solubility of the hydrophobes, they are severely deficient in their ability to account for experimental observations of salting-in behavior.[3] According to these models, salting-in should occur when either dissolving salt causes a volume expansion, or the hydrophobic solute has a dielectric constant higher than that of water. However, that is not always the case.[3]

Our primary aim in this paper is to demonstrate a model that predicts salting-in versus salting-out for a given type of salt solution correctly. The key observation is the thermodynamic relationship between the change in the chemical potential of a macromolecule upon the addition of cosolute to the solution and the excess binding/exclusion of that cosolute to the macromolecule. This was formulated by Wyman through his theory of linked-functions.[14,15] However, it can also be derived from the change in the surface tension as a function of the excess amount of cosolute at the interface, a dependency given by the Gibbs adsorption isotherm.[16] Here, excess amount relates to the number of solute molecules in the vicinity of the surface of the macromolecule compared with the number of solutes that would be in the same volume of bulk solution. Tanford later extended Wyman's analysis to incorporate the effect of hydration.[17] He considered the following equilibrium reaction in which two macromolecules, A and B, solvated in aqueous solution react with each other to yield the macromolecule C,

$$A(aq) + B(aq) \rightleftharpoons C(aq) \tag{1}$$
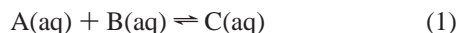
At issue is how the addition of a ligand X will affect the value of the equilibrium constant, $K$, of the reaction. In the limit of infinite dilution of the macromolecules, the change in $K$ as a function of the change in the chemical activity, $a_X$, of the ligand X can be written as,

$$\frac{d \ln K}{d \ln a_X} = \Delta v_X - \frac{n_X}{n_W} \Delta v_W \equiv \Delta v_{X,pref} \tag{2}$$

where $\Delta v_X$ and $\Delta v_W$, are the changes in the number of bound ligand and water molecules that accompany the reaction of the macromolecules (eq 1), respectively. $n_X$ and $n_W$ are the total number of ligand and water molecules, respectively. Here the preferential binding of a ligand X, $\Delta v_{X,pref}$, incorporates the binding/exclusion of the water molecules. The relation expressed in eq 2 can also be obtained from a simple thermodynamic analysis of an equilibrium dialysis experiment.[18] The preferential binding concept has mainly been used to characterize changes in the stability of native structures of proteins with respect to unfolded states induced by the addition of denaturing reagents.[19-23]

Our second motivation is to investigate the effect of salt solutions on the hydrophobic interactions as a function of the

size of the hydrophobic solute, particularly as the hydrophobic solutes approach the nanoscale relevant to biological systems. Other authors[24-27] have investigated the effect of salt on the interaction between small, that is, up to a diameter of 0.37 nm, hydrophobic particles, either by the Widom particle insertion method or by analyzing the radial distribution functions. Recently, we have extended these investigations by studying the spontaneous aggregation of particles with diameter of 0.5 nm in a series of salt solutions.[28] However, it is well-known that the hydrophobic interaction is highly sensitive to the length scale of the particles, especially in the transition region from small to nanoscale.[29] Similarly, we suspect that the effects of salt solutions will be qualitatively different in the small and nanoscale regimes.

The size effect can be realized by considering the change in the way *pure water* responds to the presence of two nanoscale hydrophobic surfaces versus two small hydrophobic particles. If the nanoscale hydrophobic surfaces are brought into close contact while facing each other, at some critical distance the confined water between them will spontaneously exit the region.[30-36] This is an example of a microscopic drying transition, in which the water confined between the two plates becomes thermodynamically unstable in its liquid phase and evaporates. Nevertheless, the drying transition disappears below a critical size of the hydrophobic surface[37] and above a critical value of the plate-water attractive interactions.[38,39] These factors have recently been shown to play a crucial role in the way multidomain proteins self-associate; the existence of a dewetting transition was found to depend on the geometry and chemical composition of the interacting regions.[40,41] In the present study, we explore nanoscale hydrophobic surfaces that firmly exhibit a drying transition. Since association of biomolecules is primarily driven by hydrophobic interactions, our results are relevant to many biophysical and biochemical processes.

The outline of the paper is as follows. In the first subsection of the Results and Discussion section, we calculate the potential of mean force (PMF) between two nanoscale hydrophobic plates solvated in aqueous salt solutions as a function of the charge density of the ions. In the second subsection we analyze the thermodynamics of the plate association process. We find that an increase of the hydrophobic interaction (salting-out), is a purely entropic effect. In contrast, a reduction of the hydrophobic

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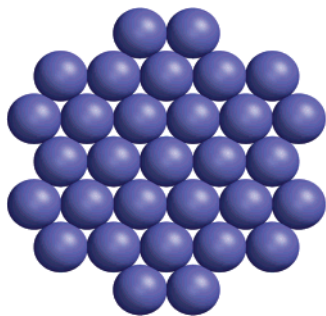
**Figure 1.** The geometry of the atoms composing the hydrophobic plate used in this study. The approximate diameter of this disklike shape is 2.1 nm.

interaction (salting-in) can be caused by either entropic or enthalpic effect. In the third subsection we analyze the binding/exclusion of the ions and water molecules to the hydrophobic surfaces. In particular, we calculate, $\Delta v_{\text{ions,pref}}$, the preferential binding/exclusion of the ions to the hydrophobic plates as defined in eq 2. Our results display a strong correlation between the strength of the hydrophobic interaction and $\Delta v_{\text{ions,pref}}$. Specifically, we find that preferential binding induces a decrease (salting-in), while preferential exclusion induces an increase (salting-out), of the hydrophobic interaction. In the fourth subsection we discuss the mechanism by which salting-in and salting-out take place. Finally, in the conclusion section we compare these results with our previous study on small hydrophobic particles.[28] We observe significant size effects, as small particles do not display a monotonic relation between the strength of the hydrophobic interaction and the degree of preferential binding/exclusion.

## II. Methods

We study the effects of salt solutions on the hydrophobic interaction between two nonpolar, hydrophobic surfaces. Each surface is represented by a plate of 31 single-layer atoms, arranged in a triangular lattice with a bond length of 0.32 nm. The shape of the plate is disklike, with a diameter of about 2.1 nm (see Figure 1). The Lennard-Jones (LJ) parameters of the plate atoms are $\sigma_{\text{plt}} = 0.40$ nm and $\epsilon_{\text{plt}} = 0.50$ kJ/mol. In comparison to a methane molecule, $\sigma_{\text{plt}}$ is 18% larger, while $\epsilon_{\text{plt}}$ is 60% smaller. Nevertheless, the magnitude of the attraction between the water molecules and this plate is very similar (as evidenced by the water density profiles) to that of water and a hydrocarbon monolayer described at the atomic level.[35] During simulations, the positions of the plate atoms are held fixed, interactions between atoms on the same plate are excluded, and the orientation of the two plates with respect to each other is parallel and in-registry. In the simulations with pure water, we used 1090 water molecules, 60 of which were replaced by 30 cations and 30 anions in the simulations of salt solutions. All ions considered in this study have the same LJ parameters of $\sigma_{\text{ion}} = 0.50$ nm and $\epsilon_{\text{ion}} = 1.00$ kJ/mol. The value of $\sigma_{\text{ion}}$ is 13% larger than for a chloride ion. To represent salts with different ionic charge density, the magnitude of the cationic and anionic charges were varied in lockstep, from 0.50 to 1.40 $e$, such that the solution was electrically neutral. (In this study, the variation of the charge density of the ions was performed by allowing the charge to assume a range of noninteger values, while keeping the ionic diameter fixed. In nature, however, the ionic charge density is varied by changing the charge of the ions in integer increments, while the diameter of the ions is not quantized.) All of the ions have a mass of 40.0 amu. The salt concentration is fixed at 1.43 molal, although the molarity fluctuated in the range 1.20–1.35 M as a result of variations in the volume of the solutions.

We used the molecular dynamics (MD) package GROMACS version 3.1.4[42] to perform all of the computer simulations, with a time step of

0.002 ps. We chose the SPC/E model of water to calculate the interactions of the water molecules.[43] Their bond distances and angle were constrained using the SETTLE algorithm.[44] The system was maintained at a constant temperature of 300 K and pressure of 1.0 bar.[45] The electrostatic forces were evaluated by the particle-mesh Ewald method (with grid spacing of 0.12 nm and quadratic interpolation) and the LJ forces by a cutoff of 1.0 nm.

The potential of mean force (PMF) between the two plates was computed from the mean force acting on each of the plates.[46,47] Then the mean force acting between the plates along their axis of separation was integrated as a function of the distance between the plates, $d$, to yield the free energy profile. Since the forces acting on the plates perpendicular to the axis of separation averaged to zero, as required in equilibrium, we believe that our results are converged. As the PMF represents only relative values, it was shifted such that the free energy of the states at the largest separations correspond to zero. For each salt, we performed between 53 and 56 simulations with different values of $d$, ranging from 0.36–1.44 nm. At each distance, the system was equilibrated for 2.0 ns and data collected for 5.0 ns. At points where the force converged slowly (around the wetting/drying transition), the data collection stage was extended for an additional 5.0 ns. The error in the quantities obtained from the simulations was estimated using the block averaging method.[48]

## III. Results and Discussion

**Potentials of Mean Force.** Figure 2 displays the PMF (free energy profile) of the hydrophobic plates, solvated in salt solutions and in pure water, as a function of the distance between their planes, $d$. The curves show that the depth of the potential well, at $d = 0.41$ nm, increases monotonically with the charge density of the ions. (The largest contribution of the bare (direct) interaction between the plates to the PMF is $-69.7$ kJ/mol, at the minimum of the curve.) We observe that the minimum of the curve for pure water is very similar to that of salt solution with ion charges of $|q| = 0.90$ $e$ (curve not shown). Thus, salting-out of the hydrophobic plates is observed for ions with charge density higher than 0.90 $e$, while salting-in is observed for ions with charge density lower than 0.90 $e$. (We use the term salting-out when the stability of the associated state in the salt solution is greater than the stability in pure water and vice versa for the term salting-in.)

In the curves for $|q| = 0.50$ and 0.60 $e$, a second, shallow, minimum is observed at $d \simeq 0.9$ nm. This is because on bringing the plates from large distances to contact, first the water molecules are expelled, leaving the ions in the interplate region. The ions remain in the interplate region for a range of values of $d$ (yielding the shallow minimum at $d \simeq 0.9$ nm) and only at a smaller distance, corresponding to the maxima at $d = 0.80$ and 0.83 nm for $|q| = 0.50$ and 0.60 $e$, respectively, do the ions exit the region between the plates. In Table 1, we indicate the interplate distances at which the water molecules and ions leave the interplate region for different values of ionic charges $|q|$.

Figure 3 shows snapshots of the simulation box, for a salt solution with $|q| = 1.00$ $e$, at different interplate distances. The

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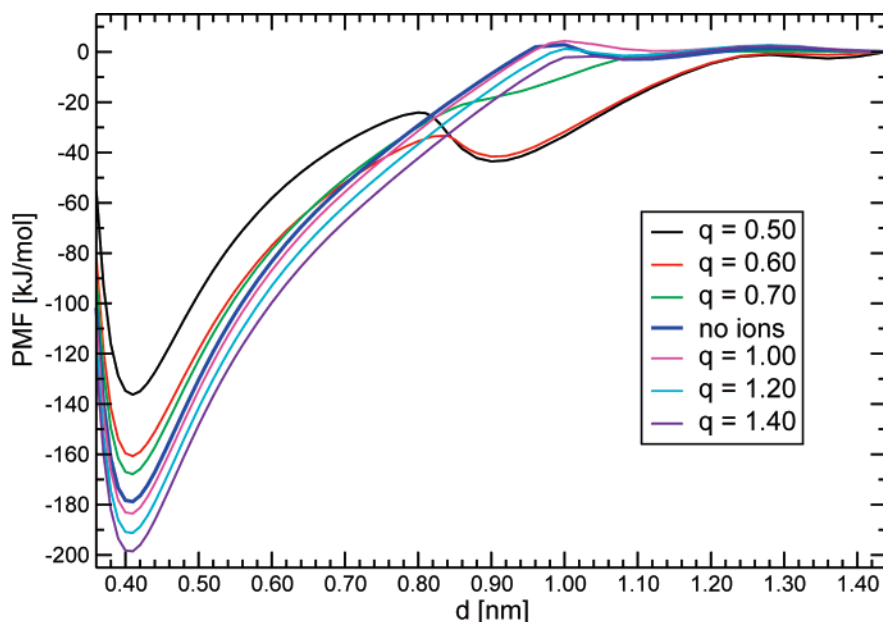
**Figure 2.** The potential of mean force between the hydrophobic plates as a function of the distance between their planes, *d*, plotted for few salt solutions (at concentration of 1.43 molal) with different charges of the ions. The planes of the two plates are parallel and in-registry with respect to each other. Note that at *d* = 0.90 and 0.71 nm the ions and the water molecules, respectively, can just fit (based on the LJ diameters) into the gap between the plates.

**Table 1.** The Interplate Distances, *d* (nm), at Which the Water Molecules and the Ions Leave the Region between the Plates during the Association Process for Different Values of Ionic Charges

| ionic charge [*e*] | water | ions |
|---|---|---|
| $0.50 \leq |q| \leq 0.65$ | 1.15−1.36 | 0.82−0.86 |
| $0.70 \leq |q| \leq 0.85$ | 1.00−1.10 | 0.89−1.12 |
| $0.90 \leq |q| \leq 1.10$ | 0.98−1.00 | 1.14−1.28 |
| $1.20 \leq |q| \leq 1.40$ | 1.00 | 1.32−1.40 |

center frame, corresponding to *d* = 0.96 nm, is close to the critical distance of the drying transition. Water in the liquid phase is unstable inside the region between the two plates, even though the gap of width ∼0.56 nm can accommodate at least two layers of water.

**Thermodynamics of Salting-In and Salting-Out.** A measure of the liquid induced plate association is given by the free energy difference, $\Delta G$, of the following process:

$$P(aq) + P(aq) \rightleftharpoons P_2(aq) \qquad (3)$$
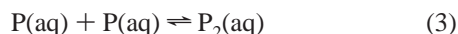
where $P_2(aq)$ is the associated state at *d* = 0.41 nm and 2P(aq) is the dissociated state at *d* = 1.44 nm. (For simplicity, we have

only considered the in-registry configuration. However, it is likely that out-of-registry alignment of the plates is more stable in the contact configuration.) The value of $\Delta G$, calculated from the difference between the value of the PMF of these two states, as a function of the charge of the ions is shown in Figure 4a. We observe a significant increase in the magnitude of the hydrophobic interaction as the charge density of the ions increases. (It is possible that a minimum in the strength of the hydrophobic interaction (thus, a maximum in the value of $\Delta G$) will be observed in the range of ionic charges $|q|$ between 0 and 0.5 *e*, given the strong hydrophobic character of the ions.) The change in the potential energy of the whole system for the association process, $\Delta E$, is shown in Figure 4b. Since the difference in the system energy is obtained by subtracting a large number from a similar large number, the accuracy of $\Delta E$ is quite low. To this end, the simulations representing the associated (*d* =0.41 nm) and dissociated (*d* =1.44 nm) states were extended to a total time of at least 45.0 ns for each value of $|q|$. The corresponding change in the entropy of the system, $\Delta S$, derived from the relation $\Delta G = \Delta H - T\Delta S$, is shown in
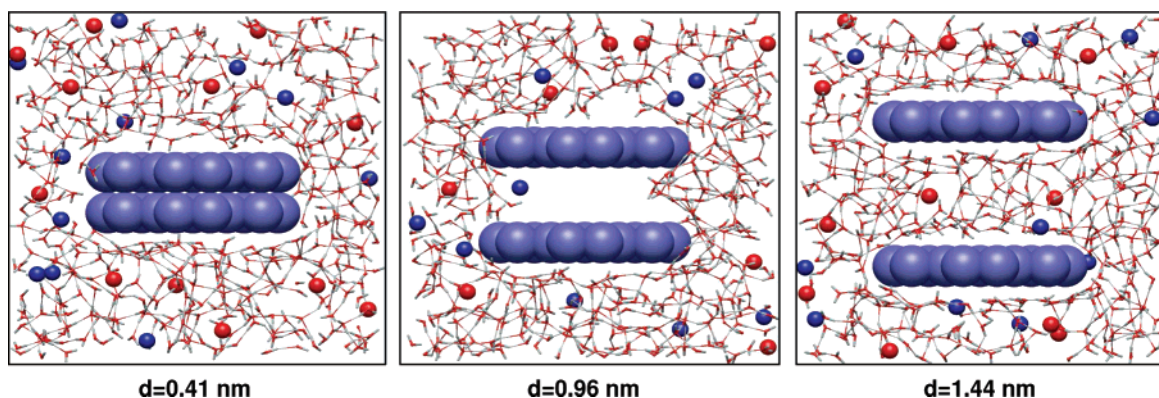


**Figure 3.** Snapshots of the simulation box, for salt solution with $|q|$ = 1.00 *e*, at different values of the interplate distance. For clarity, only a slice (with a width of 1.6 nm) of the box, perpendicular to the plates planes is shown. The plate atoms are depicted in purple, positive ions in red, and negative ions in blue. The hydrogen−bond network of the water molecules is shown by thin lines. The sizes of the different atoms are not plotted according to their excluded volume.
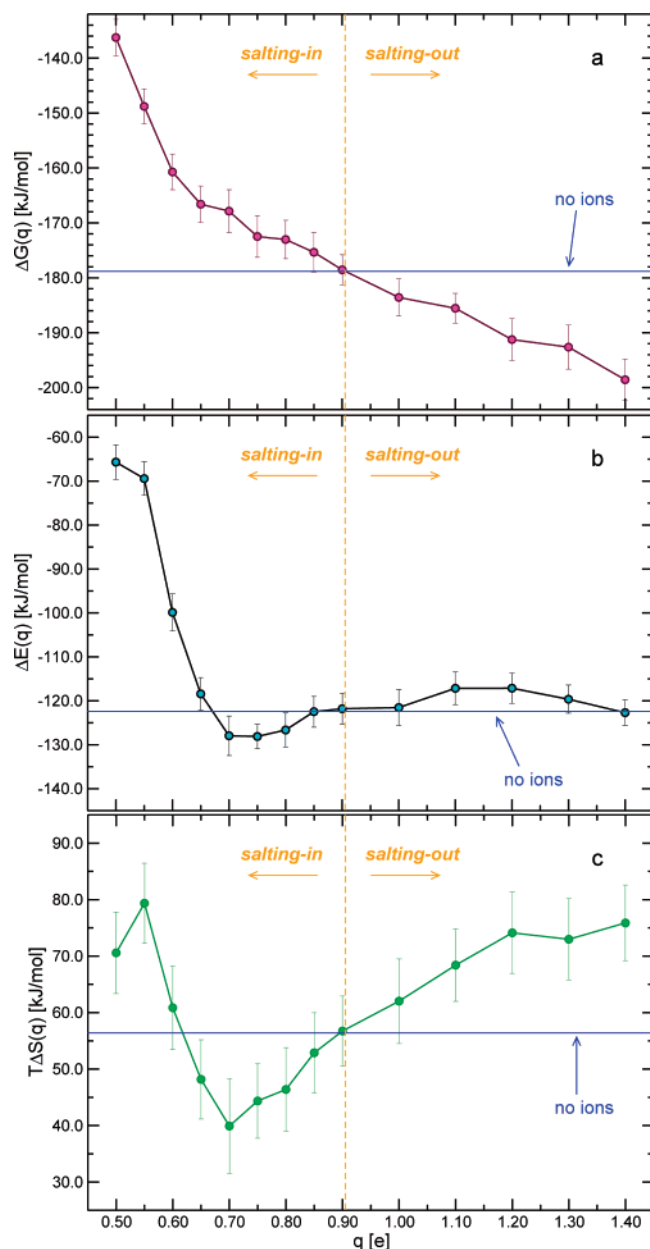
**Figure 4.** Thermodynamics of the association process of the two hydrophobic plates solvated in salt solutions as a function of the charge of the ions: (a) the free energy difference, (b) the potential energy difference, and (c) the entropy difference, $T\Delta S$. The values obtained for pure water are indicated by horizontal lines.

Figure 4c. The change in enthalpy, $\Delta H$, is approximated by the change in energy, since the volume change between the two states is negligible. To obtain the solvent induced contribution to the free energy and enthalpy changes, the value of the direct (bare) potential energy change between the plates ($-69.7$ kJ/mol) has to be subtracted from the value of $\Delta G$ and $\Delta H$, respectively.

The values of $\Delta G$, $\Delta H$, and $\Delta S$ shown in Figure 4 for pure water indicate that the driving force for plate association is caused by both a negative enthalpy change and a positive entropy change. The solvent induced contribution to $\Delta H$ is about $-52.7$ kJ/mol. The value of $T\Delta S$ is 56.4 kJ/mol. Thus, the magnitudes of the favorable enthalpy and entropy changes that drive the association process in pure water are similar. The negative value of the solvent induced $\Delta H$ is largely a result of

unfavorable water−plate interactions. Moreover, it has been suggested[49] that the positive value of $\Delta S$ in pure water is caused by an increase in the number of ordered water molecules around the hydrophobic plates at separation. Both, the enthalpic and entropic contributions also exist, significantly, in salt solutions. However, the effect of the ions is reflected in a change of the magnitudes of these contributions relative to pure water.

Figure 4 indicates that the value of $|q|$ for which $\Delta G(q)$ is the same as in pure water (the transition for salting-in to salting-out) is $|q| \simeq 0.90\ e$. Furthermore, the value of $\Delta H$ (and, thus, $\Delta S$) at $|q| = 0.90\ e$ is very similar to that in pure water. Therefore, we will compare the effect of the ions on $\Delta H$ and $\Delta S$ with respect to the corresponding values in pure water.

In the salting-out regime, $|q| > 0.90\ e$, the magnitude of $\Delta E$ (which is negative) is smaller than that in pure water. Therefore, enthalpy *is not* the driving force for salting-out (strengthening the hydrophobic interaction). On the other hand, in this range of $|q|$, the magnitude of $\Delta S$ (which is positive) is larger than that in pure water. This indicates, that the driving force for salting-out (induced by high charge density ions) is entropic. The driving force for salting-in depends on whether the charge density of the ions is medium or low, yielding two distinct regimes. In the first salting-in regime, $0.70 \leqslant |q| < 0.90\ e$, the magnitude of $\Delta H$ is larger, while the magnitude of $\Delta S$ is smaller, than that in pure water. Therefore, salting-in (weakening the hydrophobic interaction) in this regime (induced by medium charge density ions) is also driven predominately by an entropic effect. In contrast, in the second salting-in regime, $0.50 \leqslant |q| \leqslant 0.65\ e$, the magnitude of $\Delta H$ is smaller, while the magnitude of $\Delta S$ is mostly larger, than that in pure water. This reveals that the driving force for salting-in in this regime (induced by low charge density ions) is enthalpic.

**Preferential Binding/Exclusion of the Ions.** Equation 2 predicts a correlation between the values of $\Delta G$ displayed in Figure 4a and the preferential binding/exclusion of the ions to hydrophobic surfaces:

$$\mathrm{d}(\Delta G) = -RT\Delta v_{\text{ions,pref}}\, \mathrm{d}\ln a_{\text{ions}} \qquad (4)$$

The densities of the cations, anions and water molecules around the hydrophobic plates are shown in Figure 5. We chose to perform the analysis when the plates are in contact, that is, at $d = 0.41$ nm. (The contact distance in a LJ system is usually the point where the interparticle potential crosses zero, i.e., at $d = 0.40$ nm. In this study, the contact distance corresponds to the minimum of the PMF.) The top panel displays a projection of the cations and anions onto the *yz*-plane of the simulation box for salting-in ions ($|q| = 0.50\ e$), "neutral" ions that do not cause a change in the hydrophobic interactions ($|q| = 0.90\ e$), and salting-out ions ($|q| = 1.40\ e$). The projections are superpositions of 1000 frames and shows a slab with a thickness of 1.50 nm along the *x*-axis. The projected slabs contain an average of 23, 22, and 34% of the total ions for $|q| = 0.50$, 0.90, and 1.40 $e$, respectively. (For $|q| = 0.90$ and 1.40 $e$ the number of cations, found inside the slabs, was very similar to the number of anions. For $|q| = 0.50\ e$ there were 3% more anions than cations.) The middle panel of Figure 5 displays the analogous projections for the water molecules. In this case, the projections are superpositions of 50 frames. From this figure it

(49) Frank, H. S.; Evans, M. W. *J. Chem. Phys.* **1945**, *13*, 507−532.
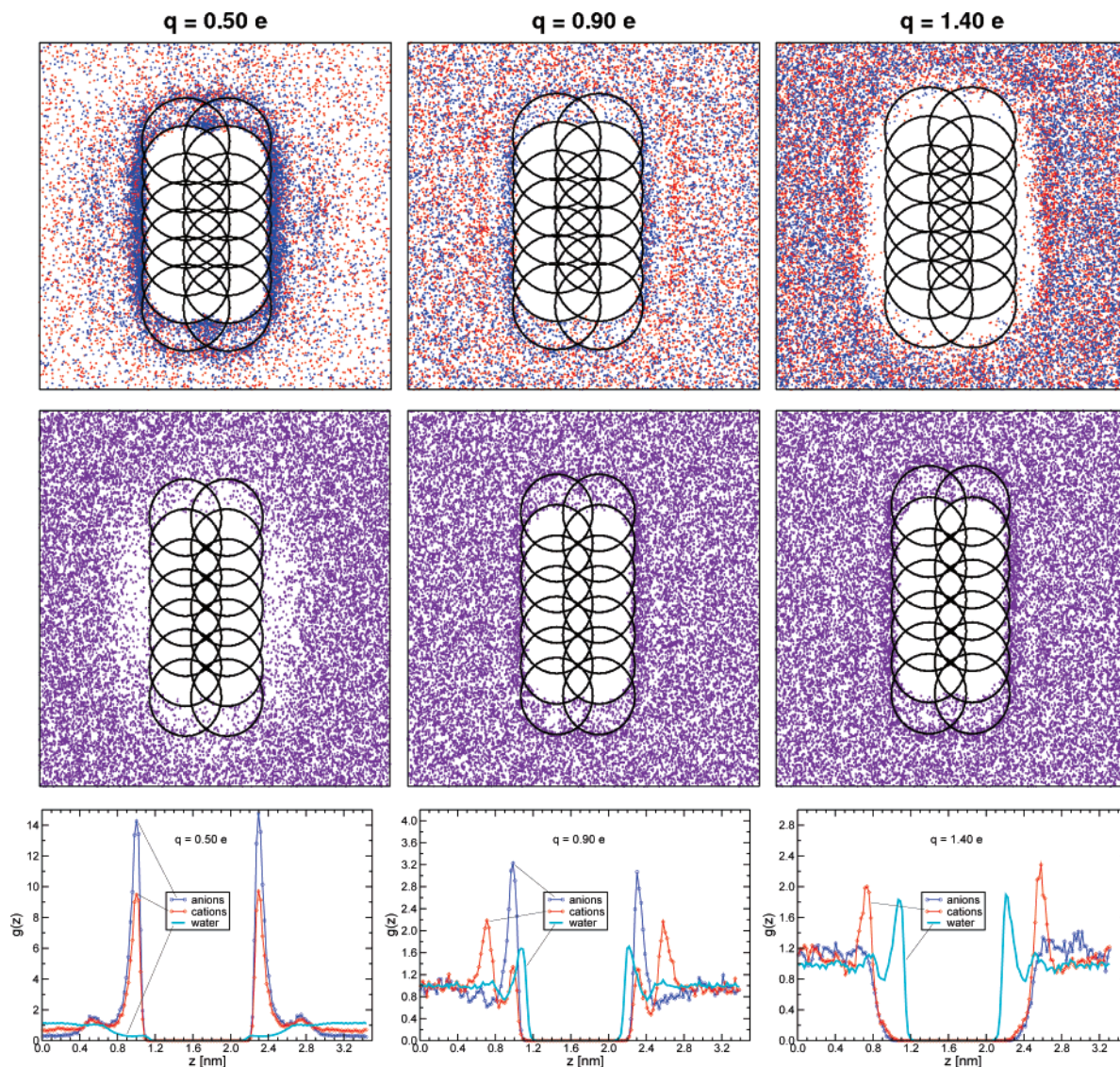
**Figure 5.** The top panels represent the projection of the simulation box onto the *yz*-plane (perpendicular to the plates planes) of the anions (blue dots) and cations (red dots) for $|q| = 0.50$, 0.90, and 1.40 *e* in the associated state ($d = 0.41$ nm). For clarity, the projection shows a slab with thickness of 1.50 nm along the *x*-axis of 1000 frames. Since the ions are represented by dots, the drawn radius of the circles representing the plate atoms include the LJ radius of the plate atoms and the LJ radius of the ions. The middle panels show the same as the top panels but for the water molecules using 50 frames. Here the drawn circles of the plate atoms include the LJ radius of the plate atoms and the LJ radius of the oxygen atom of the water molecules. The lower panels show the distribution function, $g(z)$, of the anions, cation, and water (in the associated state) that are found in a cylinder along the *z*-axis with a diameter of 1.8 nm. The distributions are normalized by the random distribution.

is clear that salting-in behavior (reduction of the hydrophobic interaction) is associated with preferential binding of the ions to the hydrophobic plates. Namely, an increase in the density of the ions and a depletion of water around the plates. In contrast, salting-out behavior (amplification of the hydrophobic interaction) is associated with preferential exclusion of the ions to the hydrophobic plates. Thus, there is a decrease in the density of the ions and a slight increase in the density of water (see below) next to the plates.

The lower panel of Figure 5 displays the one-body density function for the anions, cations, and water molecules along the *z*-axis of the simulation box. (The distributions are normalized by the uniform density, i.e., the number of ions divided by the free volume outside the exclusion zone of the plates.) Only particles inside a cylinder along the *z*-axis of the simulation box with a radius (in the *xy*-plane) of 0.90 nm are considered

in these density plots. The distributions confirm the results presented in the upper and middle panels, i.e., that low charge density ions display preferential binding while high charge density ions display preferential exclusion. For $|q| = 1.40$ *e*, the first layer around the plates is almost completely void of ions, while for $|q| = 0.50$ and 0.90 *e*, the first layer around the plates contains more anions than cations, indicating a negative surface potential. This is in agreement with experimental measurements of the surface potential at the air−water interface of aqueous solutions of electrolytes with comparable size of anions and cations,[50] as well as NMR studies of the self-association of hydrophobic particles in salt solutions.[51,52] This effect is probably caused by the asymmetry of the charge distribution of the water molecules.

(50) Jarvis, N. L.; Scheiman, M. A. *J. Phys. Chem.* **1968**, *72*, 74−78.

To quantify the degree of preferential binding/exclusion of the ions to the hydrophobic plates we calculate the value of $\Delta v_{\text{ions,pref}}$ by
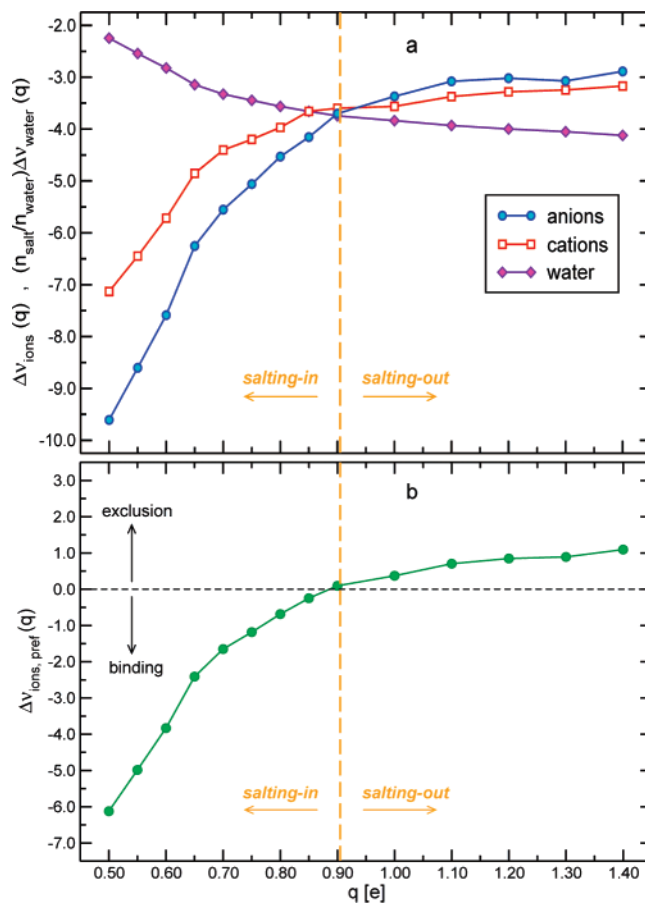
$$\Delta v_{\text{ions,pref}} = (\Delta v_{\text{cations}} + \Delta v_{\text{anions}})/2 - \frac{n_{\text{salt}}}{n_{\text{water}}} v_{\text{water}} \quad (5)$$

where $n_{\text{salt}} = (n_{\text{cations}} + n_{\text{anions}})/2$. In this representation, the activity that appears in eq 4, $a_{\text{ions}}$, is the activity of the salt, an average over the cations and anions. To estimate the difference in the number of bound particles to the plates in the association process (eq 3), we integrated the density functions shown in the lower panel of Figure 5. The integration, over the cylindrical volume element, along the z-axis was performed from $z = 0.30$ to $z = 3.00$ nm (the center of the plates is at $z = 1.65$ nm). The boundaries of the integration capture the particles that are present in the first two layers away from the plates. For larger distances the distribution of the ions is homogeneous (bulk). The difference in the number of bound particles in the association process is the negative value of this integral. This is because in the association process part of the plates' surface area in contact with solution is eliminated.

Figure 6a displays the values of $\Delta v_{\text{cations}}$, $\Delta v_{\text{anions}}$, and $\Delta v_{\text{water}}$ that accompany the association process as a function of the ionic charge of the salt solutions. All three curves intersect around $|q| = 0.90\ e$, the transition from salting-in to salting-out. It is evident that the behavior of the ions is opposite to that of water, and the asymmetry between cations and anions found in Figure 5 is also displayed here. In Figure 6b we plot the value of $\Delta v_{\text{ions,pref}}$ as a function the charge density of the ions. Negative values of $\Delta v_{\text{ions,pref}}$ indicate preferential binding of the ions relative to the binding of water and are clearly associated with salting-in. (The sign of $\Delta v_{\text{ions,pref}}$ arises from considering the association process (eq 3).) Positive values of $\Delta v_{\text{ions,pref}}$ indicate preferential hydration, that is, preferential binding of water and exclusion of the ions, and are associated with salting-out. Thus, we find that the transition between salting-in and salting-out occurs when $\Delta v_{\text{ions,pref}}$ is zero.

In Figure 7, the free energy of the plate association, $\Delta G$, is plotted against the values of $\Delta v_{\text{ions,pref}}$. We observe a strong relation between the extent of preferential binding/exclusion of the ions relative to the plates and the magnitude of the hydrophobic interaction, as measured by $\Delta G$. Three regimes, identified by three different slopes of the curve, are easily identified: (i) the salting-out regime, $\Delta v_{\text{ions,pref}} > 0$ induced by preferential exclusion of high-charge-density ions, (ii) a salting-in regime, $-2.5 \lesssim \Delta v_{\text{ions,pref}} < 0$, caused by weak preferential binding of medium-charge-density ions, and (iii) another salting-in regime, $\Delta v_{\text{ions,pref}} \lesssim -2.5$, caused by preferential binding of low-charge-density ions. The different slopes represent the different dependency the chemical activity of the salt has on the ionic charges.

**Mechanism of Salting-In and Salting-Out.** As we showed earlier the driving force for plate association in pure water is due to both the negative change of $\Delta H$ and the positive change of $\Delta S$ that accompany reaction eq 3. The corresponding value of $\Delta G$ can decrease or increase in salt solutions, yielding either salting-out or salting-in, respectively. On the basis of the results

(51) Holz, M. *J. Mol. Liq.* **1995**, *67*, 175−191.
(52) Sacco, A.; de Cillis, F. M.; Holz, M. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2089−2092.

**Figure 6.** (a) The change in the number of "bound" anions, cations, and water molecules that accompany the association process ($\Delta v_{\text{anions}}$, $\Delta v_{\text{cations}}$, and $\Delta v_{\text{water}}$) as a function of the ionic charge of the salt solutions. A particle is defined as bound if it lies within the first two layers away from the plates. The change in the number of water molecules is normalized by $n_{\text{salt}}/n_{\text{water}}$. The values of all curves are negative because in the association process part of the plates' surface area in contact with solution is eliminated. (b) The preferential binding of the ions, $\Delta v_{\text{ions,pref}}$ (defined by eq 5), as a function of their charge density. Note that for the association process, positives values of $\Delta v_{\text{ions,pref}}$ indicate preferential binding of water and exclusion of the ions relative to the hydrophobic surface and vice versa for negative values.
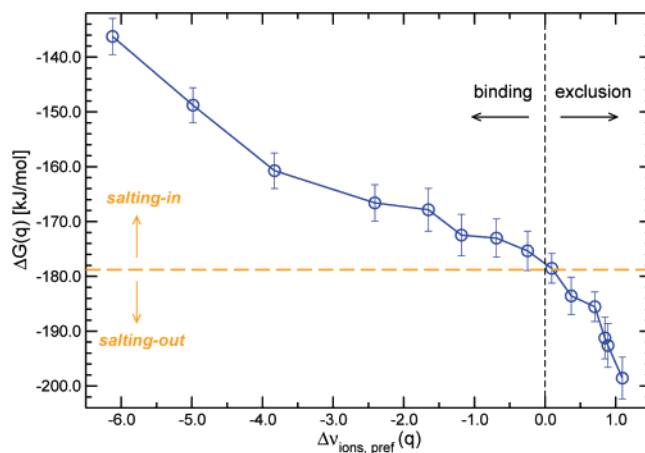


**Figure 7.** The free energy difference of the association process, $\Delta G(q)$, as a function of the preferential binding of the ions, $\Delta v_{\text{ions,pref}}$. Preferential binding of the ions to the hydrophobic surface is associated with salting-in while preferential exclusion is associated with salting-out.

obtained in this study (the thermodynamics and preferential binding analyses), we will demonstrate how the addition of salt to the aqueous solution can change the enthalpic and entropic

contributions of the association process. This permits us to determine the mechanisms by which salting-out and salting-in take place.

Salting-out, which is an entropic effect, can be described as follows. As the charge density of the ions increases ($|q| > 0.90$ $e$), there is greater tendency for the ions to form hydration complexes that reside away from the hydrophobic surfaces (thus, the ions exhibit preferential exclusion). This decreases the available volume, and thus the configurational entropy, of each of the species in solution. In the plate−plate association process, part of the surface area of the hydrophobic plates in contact with the solution is eliminated, leading to an increase in the entropy change. Hence, in order to maximize the entropy the association process becomes more favorable.

Salting-in is induced by preferential binding and can be of either entropic or enthalpic origin. The strength of the water−ion interactions decreases as the charge density of the ions is reduced. When the water−ion interaction becomes smaller than the water−water interaction, the ions will preferentially bind to the hydrophobic plates. The binding of the ions to the plates can affect the entropy in two opposite ways. First, because of the binding (loss of translational motion and preferential exclusion of water from the plates), the entropy change of the association process will increase (as less ions can bind to the plates and there is larger available volume for the water molecules when the plates are in contact). However, the results shown in Figure 4c indicate a decrease in the entropy in this region. Second, the binding of the ions reduces the concentration of the ions in the bulk. We observe that low-charge-density ions increases the ordering of the water molecule around them (analyses not shown but will be presented in future publication). Thus, the binding of the ions to the plates causes the entropy change of the association process to decrease (since in the associated state there are more ions in solution). These opposing contributions result in the minimum observed at $|q| = 0.70$ $e$ in the curve of $\Delta S(q)$ in Figure 4c. In the region $0.70 \leq |q| < 0.90$ $e$, the entropy decreases with increasing binding. This will result in a smaller magnitude of $\Delta G$, that is, weaker hydrophobic interaction. Thus, the mechanism of salting-in induced by medium-charge-density ions and driven by entropic effect, $0.70 \leq |q| < 0.90$ $e$, is due to an increase in the number of ordered water molecules (around low-charge-density ions in the bulk) that accompany the association process.

As the charge density of the ions decreases further, $0.50 \leq |q| < 0.70$ $e$, the binding of the ions is stronger and the loss of their translational motion become the dominant factor. Therefore, the change in entropy of the association process increases as the charge density of the ions decreases. This is not the driving force of salting-in as an increase in $\Delta S$ induces stronger hydrophobic interactions. In this regime, the driving force of salting-in induced by low-charge-density ions is enthalpic and the mechanism is as follows. Consider a hydrophobic plate solvated in salt solution. Then, the binding of low-charge-density ions to the hydrophobic plates reduces the enthalpic penalty arising from the unfavorable ion−water and plate−water interactions. Consequently, this reduces also the surface tension of the plate−water interface (the ions behave as "surfactants"). Therefore, the binding of the ions cause a reduction in the magnitude of the favorable enthalpy change for association process (as is shown by the sharp increase of $\Delta H$ in the region,

$0.50 \leq |q| \leq 0.70$ $e$). As a result, the association process becomes less favorable with reducing $|q|$.

## IV. Conclusion

In this study, we have investigated how ions of various charge densities influence the magnitude of the solvent-mediated attraction and drying between two nanoscale hydrophobic surfaces. To accomplish this, we have used molecular dynamics simulations to calculate the potential of mean force between two nanoscale hydrophobic surfaces in aqueous salt solutions as a function of the ionic charge per unit ionic volume (charge density). The attraction is expressed by a negative change in the free energy as the plates are brought together. For pure water, both the enthalpy and entropy changes promote aggregation, roughly, in equal contributions. We find excellent correlation between the strength of the hydrophobic interaction and the degree of preferential binding/exclusion of the ions relative to the surfaces. An increased hydrophobic interaction relative to pure water, referred to as salting-out, is a purely entropic effect associated with ions of high charge density. These ions exhibit preferential exclusion by forming strong hydration complexes far away from the hydrophobic plates. A decreased hydrophobic interaction relative to pure water, referred to as salting-in, is associated with ions of medium and low charge density that exhibit preferential binding. The salting-in effect can have either entropic or enthalpic origin. The weak binding of medium-charge-density ions induces an increase in the number of ordered water molecules in solution and, therefore, a decrease in the entropy change for the association process. On the other hand, low-charge-density ions are nearly hydrophobic and tend to adhere to the hydrophobic surfaces. This strongly reduces the magnitude of the enthalpy of the plate dimerization and induces salting-in behavior.

These findings differ significantly from our recent study on the effect of electrolytes on the propensity for aggregation of small hydrophobic *particles*.[28] In that case, we did not observe a monotonic relationship between the degree of preferential binding/exclusion and the magnitude of the hydrophobic interaction. Rather, at the concentration of ions employed in the current study, called low concentration in our previous study, we found that ions of low and high charge density always led to an *increase* in hydrophobic aggregation. Only in a region of charge density, where the strength of the ion−water interactions are comparable to the water−water interactions (i.e., for medium-charge-density ions), was there no change in the propensity to aggregate relative to pure water. We also discovered that the physical mechanism behind the increased aggregation induced by the low-charge-density ions was the ability of the ions to adsorb to the surface of the hydrophobic particle aggregate, thus forming a "micelle-like" structure with reduced surface tension. As these micelle-like structures can be soluble in water, we believe that the electrolyte can increase the solubility and propensity for aggregation at the same time. For high-charge-density ions, the mechanism for aggregation of small hydrophobic particles is purely entropic, as demonstrated in the present study.

It would seem that there is little connection between the effects of electrolytes on nanoscale hydrophobic surfaces and

small hydrophobic particles. However, in our previous work,[28] where we also studied ions at *high* concentration, we found that ions with low charge density reduce the propensity for aggregation of small hydrophobic particles. We believe that this is the only regime of reduced aggregation. Although we did not pursue simulations of high-charge-density ions at high concentration, we speculate this regime is also associated with amplified aggregation.

In conclusion, we have demonstrated the unique effects of electrolytes on the hydrophobic properties of solutes of various sizes, ranging from small nanoscale particles to large nanoscale surfaces.

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