Can Salting-In/Salting-Out Ions be Classified as Chaotropes/Kosmotropes?

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Attempts to understand the Hofmeister Series at the molecular level has yielded numerous hypotheses, many of which refer to the way different salts modify the structural and dynamical properties of water. The most famous, and at the same time the most controversial, is the classification of cosolutes and ions as structure-breakers (chaotropes) or structure-makers (kosmotropes), and their identification as salting-in and salting-out agents, respectively. In this paper, we present results from molecular dynamics simulations correlating the ion-induced changes of the structural and dynamical properties of water and the ability of these ions to alter the magnitude of the hydrophobic interaction. Although most of the properties of water in the salt solutions change monotonically with the ability of the salt to increase/decrease the hydrophobic interaction, none of them is able to predict the transition from salting-in to salting-out, a prediction that was observed by the preferential binding/exclusion analysis. In addition, we find that the use of the terms, kosmotropes and chaotropes, is very misleading since the so-called kosmotropes can actually reduce the structure of water, whereas, the so-called chaotropes can increase the structure of water. Specifically, we find that the ability of the ions to reduce the hydrophobic interaction (a property attributed to chaotropes), correlates with their ability to increase the structure between the water molecules, including the number and strength of hydrogen bonds, and as a consequence, the water−water interaction energy (features attributed to kosmotropes). Nevertheless, the viscosity (as well as the rotational decay rate) of the water molecules decreases (increases) due to weaker binding to the ions. Thus, it is not the ion-induced structural ordering between the water molecules that affect the dynamical properties of water, but the strength of the ion−water interaction. Our results indicate that attempts to understand and predict salt-induced modulation of hydrophobic interactions only through the binary, salt−water, system is not possible.

I. Introduction

Hydrophobic interactions are solvent induced. This is a consequence of the extensive hydrogen bonds network of water rendering it a well-structured liquid. In fact, the two main characteristics of hydrophobic solvation that distinguish it from "normal" solvation, the large negative change of unitary entropy (at room temperature) and large positive change of the heat capacity, can be readily explained by changes in this structure of water.[1−8] An accepted explanation for the large changes in these thermodynamic properties suggests that the water molecules around hydrophobic solutes arrange themself in a quasi-crystalline structure (referred to as an "iceberg") in which there is less randomness.[9−11] These ordered water molecules have entropy that is lower than that of water molecules in the bulk, but approaches the bulk entropy with a rise in temperature.

In salt solution the strength of the attractive interaction between two hydrophobes can increase or decrease, relative to the strength in pure water, depending on the nature of the solvated ions.[12−15] Given the above-mentioned driving force for hydrophobic attraction in terms of changes in the structure of surrounding water molecules, it is very natural to attribute the effect caused by salts to alteration in these structural changes. This has led to the classification of ions as either kosmotropes, structure-makers (from Greek *kosmos* meaning order), or chaotropes, structure-breakers (in Greek *chao* means disorder) depending on their ability to enhance or interrupt, respectively, the structure of the nearby water molecules.[11,16−21] However, a

consensus definition of the structural character that determines whether an ion is a kosmotrope or chaotrope is lacking.[22,21]

Originally the degree of water structuring induced by ions was determined by the increase or decrease in the viscosity of water due to the added salt.[23] Many of the ions that induce salting-out behavior also exhibit an increase in the viscosity of water. The interpertation was that these ions can induce more order in the structure of water, increasing the number of hydrogen bonds and thereby the water−water interaction energy.[24−26] On the other hand, Samoilov proposed a relation between the viscosity and the exchange rate of water molecules in the first solvation shell of the ion with bulk waters.[27] Ions with high ionic charge density, binding surrounding waters stronger than water binds to itself, are characterized by positive activation free energy (of the exchange process) and are termed "positively hydrated". On the other hand, ions with low ionic charge density, that weakly bind surrounding waters, are characterized by negative activation free energy and are termed "negatively hydrated". A related definition was suggested by Collins;[28] small ions of high charge density (kosmotropes) bind water molecules strongly, whereas large monovalent ions of low charge density (chaotropes) bind water molecules weakly relative to the strength of water−water interactions in bulk solution. Thus, the strength of water−water interactions in bulk solution acts as a critical reference energy level in classifying kosmotropic and chaotropic ions. From a thermodynamic point of view, it was suggested to use the change in entropy accompanying ion solvation for the classification of ions as structure-making or -breaking.[11,19,29] Assuming that this entropy

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change can be decomposed into terms due to the ion and due to water, the change in the entropy of water induced by the presence of an ion can be defined.[24,30] Negative values imply water structure-making character of the ion, and vise versa. Another quantity that was considered is the extent of volume contraction upon mixing salt and water, which seemed to be correlated with the degree to which the structure of water was modified,[31] and the degree to which the hydrophobic interactions were strengthened.[13,32,33] Other dynamical and structural properties of water are used for classifying ions as kosmotropes or chaotropes, for example, the rotational correlation time of the water molecules, which can be longer (structure-making) or shorter (structure-breaking) than that in bulk water (see recent review[21] for more details).

Ion-induced structural change of water has a far reaching significance since it can be used to interpert a wide range of experimental phenomena involving salts.[34−41] The Hofmeister Series,[42] in which the ions are ranked according to their ability to solubilize/precipitate proteins, is probably the most well-known effect attributed to structure-making and -breaking character of ions.[20,24] The interpertation based on the ion's influence on water structure was extended to account for the ranking of ions in their effect on other phenomena. For example, their effect on the formation of secondary and tertiary structures in proteins, on the recognition between proteins, on the cloud points of nonionic surfactants, on polymer swelling, and on the phase boundaries of micellar solutions and lipid bilayers.[43] Apart from specific interactions between ions,[44] it is well accepted that the major contribution to these effects arises from ion-induced modulations of the hydrophobic interactions. A large number of experimental studies suggest that an increased ionic charge density amplifies the hydrophobic interaction between nonpolar solutes.[13] 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.

Many theories have been proposed to explain the salting phenomenon.[13,32,33,45] However, their success is limited to the prediction of the relative effectiveness of the ions as salting-out agents. Furthermore, they fail to account, even qualitatively, for a number of experimental observations, for example, in predicting the transition from salting-out to salting-in behavior.

A thermodynamic relationship exists between the change in the chemical potential of a macromolecule upon the addition of cosolute (ion) to the solution and the excess binding/exclusion of that cosolute to/from the macromolecule. This was formulated by Wyman through his theory of linked-functions,[46] which was later extended by Tanford to incorporate the effect of hydration.[47] Wyman's relation 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,[48] as well as by a thermodynamic analysis of an equilibrium dialysis experiment.[49] This preferential binding concept of the cosolute 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.[50−54]

In a previous work,[55] we carried out molecular dynamics simulations studying the driving forces of solvent mediated attraction between two large hydrophobic surfaces in aqueous salt solutions. We demonstrated 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 interac-

tion, 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.

A question still exists as to whether or not there is a qualitative relation between the ion-induced structural changes of water and the salting-in and salting-out effects. If there is, can these changes be quantified to predict the changes in the magnitude of the hydrophobic interaction and to locate the transition from salting-in to salting-out behavior? Although, skepticism has been raised about this issue,[22] some computational studies[56] found a strong correlation between the extent of water−water hydrogen bonding and experimental solubility data for hydrophobic solutes, leading the authors to conclude that the Hofmeister effect may primarily be a manifestation of salt-induced changes in the water structure. In addition, a theory based on the notion that kosmotropes induce stronger water−water interactions whereas chaotropes induce weaker water−water interactions has been argued to explain the salting effect on hydrophobic aggregation.[25,26]

In this paper, we present results from molecular dynamics simulations correlating the ion-induced changes of structural and dynamical properties of water and the ability of these ions to alter the magnitude of the hydrophobic interaction. The results indicate that most of the properties of water in the salt solutions change monotonically with the ability of the salt to increase/decrease the hydrophobic interaction. However, none of these changes can predict the transition from salting-in to salting-out effect. Nevertheless, as we have already shown elsewhere,[55] the preferential binding/exclusion of the ions to the hydrophobes can explain salting-in and salting-out. In the series of salts we examined, we find that the ability of the ions to reduce the hydrophobic interaction (a property attributed to chaotropes), correlates with their ability to increase (and not decrease as the word chaotrope would imply) the structure between the water molecules, including the number and strength of hydrogen bonds, and as a consequence, the water−water interaction energy. On the other hand, the structure of the water molecules with respect to the ions decreases. As mentioned above, a connection had been made in the literature between proposed changes in the structure of water and changes in measurable dynamical properties,[19,57] and it was assumed that anything that produces more structured water molecules should lead to higher solution viscosities and smaller water rotational decay rates. However, we find that the viscosity of the solution and the rotational decay rate of the water molecules changes due to weaker or stronger ion hydration and not due to strengthening or weakening the interactions between the water molecules. Thus, it is not so much the ion-induced structural ordering between water molecules that affects the dynamical properties of water, as the strength of the ion−water interaction. Taken together, our results indicate that attempts to understand and predict salt-induced modulation of hydrophobic interactions only through the ordering or disordering in binary, salt−water, systems is not possible.

II. Methods

We study the effect of salts on the structural and dynamical properties of water and the correlation of these changes to changes in the magnitude of the hydrophobic interactions. Quantitative data of the effect of salt solutions on the hydrophobic interaction between two nonpolar hydrophobic surfaces

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were reported in our previous work.[55] The trajectories obtained from these simulations can not be used to investigate the intrinsic effect of the ions on the properties of water, since the hydrophobic plates alter the ion distribution differently for different ionic charge density, q. Therefore, in this paper, simulations of the binary systems, consisting of only the salts solvated in water, were performed. In the current simulations the setup of the systems is almost identical to the previous work.[55] For pure water we used 1090 water molecules, 60 of which were replaced by 30 cations and 30 anions in the simulations of salt solutions. As in previous work, all ions considered in this study have the same LJ parameters of $\sigma_{ion} = 0.50$ nm and $\varepsilon_{ion} = 1.00$ kJ/mol. 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. (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 1.62 M, although the molarity varied from 1.31 to 1.46 M because the volume of the solution decreases with increasing q due to electrostriction. In order to check whether the ions in the simulation box experience aggregation or clustering, we plotted the radial distribution function of the cation–anion, anion–anion, and cation–cation pairs (graphs not shown). We found that for all q the limiting values approach 1, indicating that at large distances the ions are randomly distributed and no clustering is taking place. Note that the fact the ions in our simulations do not represent any particular salts does not weaken any of the conclusions we observe in this paper. For example, when we consider whether a specific change in the structure/dynamic property of water is driving the salting-in or salting-out effect, the observation of this salting behavior without the corresponding proposed change rules out this change (in the property of water) as the driving force.

We used the molecular dynamics (MD) package GRO-MACS version 3.3.3[58] 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.[59] Their bond distances and angle were constrained using the SETTLE algorithm.[60] 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.

We performed two sets of simulations, in both pure water and salt solutions. In the first set, simulations of 12 ns long in which the coordinates were saved every 2 ps were conducted. All structural properties were calculated from these simulation trajectories. In this case, the system was maintained at a constant temperature of 300 K and pressure of 1.0 bar using the Berendsen weak coupling of the velocities and volume (with a relaxation time of 0.1 and 1.0 ps), respectively.[61]

In the second set of simulations of pure water and salt solutions (the initial configuration was taken from the last frame of the first set), 2 ns long trajectories were generated and the coordinates and velocities were saved every 0.1 ns. These simulations were used for the calculation of dynamical properties, that is, the solution viscosities and the rotational autocorrelation functions of the water molecules. It was suggested[62] that in order to minimize the effect of the thermostat on the correlation functions, a slower relaxation time should be
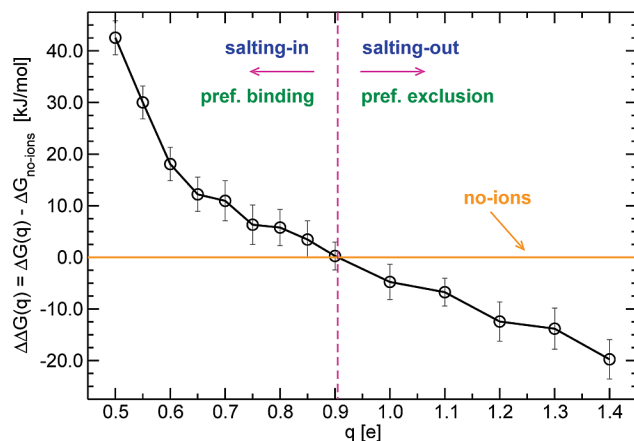

Figure 1. Difference between the free energy change, for the association process of two hydrophobic plates, in aqueous salt solution ($\Delta G(q)$) and in pure water ($\Delta G_{\text{no-ions}}$) as a function of the ionic charge density, q. Salting-in is induced by the preferential binding of the ions to the plates, whereas salting-out by preferential exclusion. Results were taken from a previous simulation study.[55]

employed (2.5 ps). Therefore, a few simulations (for pure water, $q = 0.5$, 0.9, and 1.40 e$^-$) were performed in the NVT ensemble with the slow thermostat where the coupling time was 2.5 ps. We found that the viscosity and the rotational correlation function were almost identical to the simulations with the barostat and the fast thermostat.

The potential energy between two neighboring water molecules was calculated as the sum of the LJ and electrostatic interactions. Two water molecules were considered to be neighbors if the distance between their oxygen atoms was smaller than 0.33 nm, which is the first minimum of the oxygen–oxygen radial distribution function. The hydrogen bond energy between two water molecules was calculated in the same way but with an additional condition that the donor–hydrogen–acceptor connectivity angle is larger than a cutoff value of 150°. These two conditions were also applied for the calculation of the number of hydrogen bonds per water molecule.

The viscosity was obtained from the transverse-current autocorrelation functions.[62,63] The dependence of the viscosity on the plane wave vector, k, is extrapolated to $k = 0$ to obtain the bulk viscosity. This is achieved using the expansion,

$$\eta(k) = \eta(0)(1 - ak^2) + O(k^4) \tag{1}$$

where the odd powers of k are ignored due to symmetry arguments.

III. Results and Discussion

In a previous study[55] we presented computational results on the effect of different salts on the strength of the hydrophobic interaction. The system composed of two large hydrophobic plates, each with a disk-like shape of about 2.1 nm in diameter. Salts with high ionic-charge density strengthen, whereas salts with low ionic-charge density weaken, the magnitude of the attractive interaction between the two plates relative to the magnitude in pure water. The amplitude of the effect was large, as can be seen in Figure 1. This figure (taken from the results of our previous work[55]) displays the change in the free energy for the association process in salt solution relative to pure water, $\Delta\Delta G = \Delta G(q) - \Delta G_{\text{no-ions}}$, as a function of the ionic charge density of the salt. The preferential binding of the ions to the hydrophobic plates was shown to strongly correlate with the
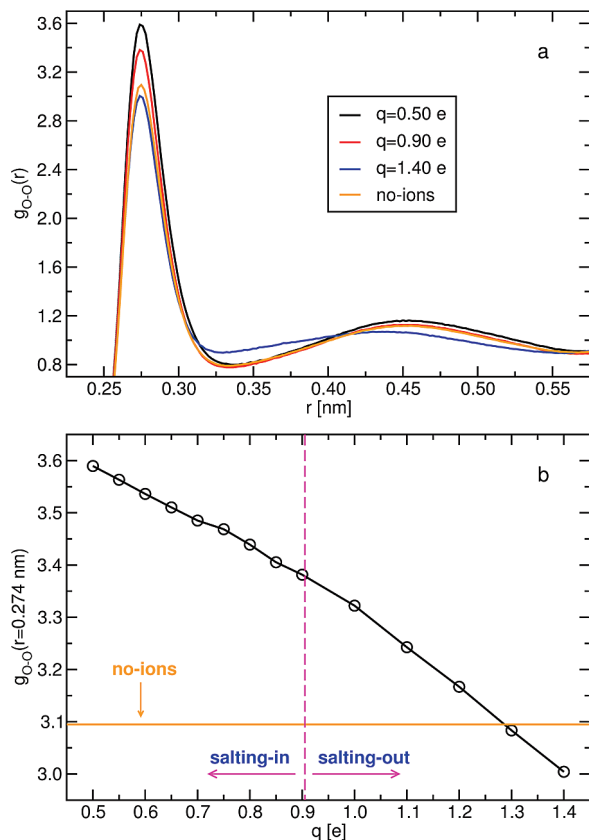
Figure 2. (a) Radial distribution function of the oxygen atoms (of the water molecules) for $q = 0.50$, 0.90, 1.40 e$^-$, and for pure water. (b) Maximum value of $g(r)$ (in all cases located at $r_{max} = 0.274$ nm) as a function of q.

Figure 3. (a) Number of hydrogen bonds per water molecule. (b) Average potential energy between two water molecules (filled green squares) and the average hydrogen bond energy (open black circles), as a function of the ionic charge density of the salt solution, as well as in pure water. Error estimation using the block averaging method resulted in error bars that are smaller than the symbol size in the graph.

salting-in effect ($\Delta\Delta G(q) > 0$), whereas the preferential exclusion with salting-out ($\Delta\Delta G(q) < 0$). The point of zero preferential binding/exclusion coincided with the transition point from salting-in to salting-out, $|q| = 0.905$ e$^-$. Can a change in a structural or dynamical property of water be used as a predictive descriptor for the change in the strength of the hydrophobic interactions?

There is enough evidence indicating that, to some extent, salts modify the structure of water. It seems reasonable to assume that the magnitude of these changes decay with the distance from the ions. However, at what distance is the structure of water no longer affected? There are conflicting reports in the literature about this issue. For example, a femtosecond two-color mid-infrared spectroscopy study on the reorientation dynamics of water molecules argued that ions do not enhance or break the hydrogen bond network of water outside their first solvation shell.[64] On the other hand, arguments for a much longer range of perturbations by the ions has been discussed in details.[21] For this reason, where possible, we analyze the structural and dynamical properties of water in the entire solution and do not restrict it only to the first and/or second solvation shells around the ions.

1. Correlation of the Salting-Effect to Structural Changes of Water. Figure 2a presents the radial distribution function (RDF), $g_{O-O}(r)$, between the oxygen atoms of the water molecules for $|q| = 0.50$, 0.90, and 1.40 e$^-$, as well as in pure water. For the salting-in ions (usually termed chaotropes), there is enhancement of the structure between the oxygen atoms of the water molecules (a property usually attributed to kosmotropes). The maximum value of $g_{O-O}(r)$ (observed at $r = 0.274$ nm in all salt solutions and in pure water) is plotted in Figure
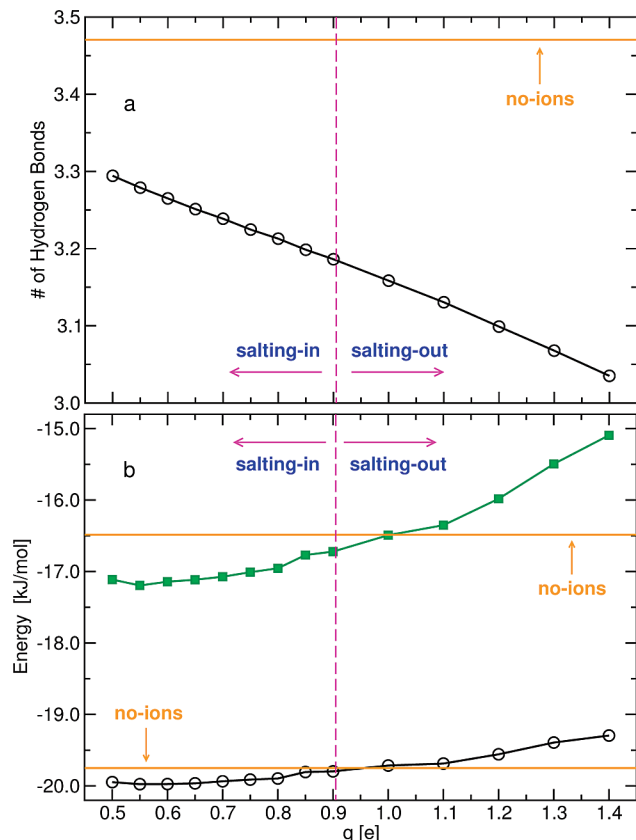
2b as a function of the ionic charge density, q. The maximum of $g_{O-O}(r)$ increases as q decreases. The value for pure water intersects the curve at $|q| = 1.29$ e$^-$, quite far from the transition point at $|q| = 0.905$ e$^-$ to go from salting-in to salting-out as defined from $\Delta\Delta G$. The same trends are observed with the maximum value of the second peak of the oxygen−oxygen RDF, as well as, with the first and second peaks of the oxygen−hydrogen and hydrogen−hydrogen RDF's (figures are not shown). Thus, from any structural standpoint that can be derived from the radial distribution functions between the water molecules, we find that the structure of water is more ordered the stronger are the ions as salting-in agents.

It is expected that this structural ordering of the water molecules will be correlated with the degree of hydrogen bonding and interaction energy between the water molecules. Figure 3a displays the average number of hydrogen bonds per water molecule. Kosmotropes (high charge density ions) are salting-out agents and are usually assumed to be structure-makers, but we find that they decrease the extent of hydrogen bonding between the water molecules over the chaotropes (low charge density ions). In fact, there is an increase in the degree of hydrogen bonding with the ability of the ions to act as salting-in agents and reduce the magnitude of the hydrophobic interactions (low charge density ions which are normally referred to as chaotropes). This may happen in part because the larger the charge, the more strongly hydrated will be the ion, therebye reducing the number of hydrogen bonds made by the hydrated waters, and concomitantly decreasing the water−water inter-

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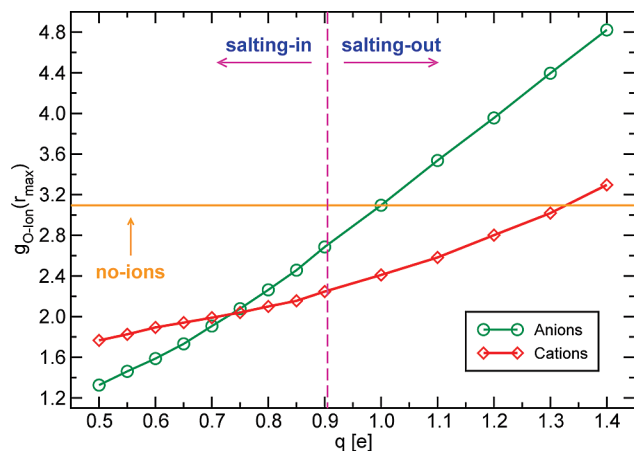*J. Phys. Chem. B, Vol. 114, No. 1, 2010* **647**

Figure 4. Maximum value, observed at the first peak, of the (oxygen atoms) water–ions (anions and cations) radial distribution function. For pure water, the maximum value of the oxygen–oxygen radial distribution function is indicated. Note that the difference in the ion–water structure induced by salting-in and salting-out ions is much larger for anions than for cations.

acton energy. This behavior has been reported by Hribar et al.[65] using the Mercedes Benz model for water in two dimensions. In their simulations they observed only salting-out behavior for all salts studied, which were still referred to as kosmotropes. It was argued that these ions cause strong electrostatic ordering of nearby waters. Indeed, the radial distribution function between the ions and the water molecules is more structured as the charge density of the ions increases. We show this in Figure 4 where the maximum (in all cases observed at the first peak) of the radial distribution function between the oxygen atom of water and the anions, as well as the cations, is plotted. In both cases, there is an increase in the structure of the hydration complex with the salting-out ability of the ions. Note, however, that the anions have a larger ordering effect on the water molecules than the cations do. This can explain why the Hofmeister Series is dominated by anions; for a given change in ionic radius, anions induce larger changes than cations.[20] An explanation for this behavior is likely to be related to the asymmetry in the charge distribution around the water molecule. Collins and Washabaugh suggested that the strength of hydration depends on the amount of charge transfer from the ions to the solvent molecules. Since the oxygen atom of water is very electronegative, it is easier to accept negative charge from anions than positive charge from cations.[20] However, since our classical simulations do not allow charge transfer between particles and the effect is still observed, this explanation is probably not accurate. From the analysis of the interaction energy between the different particles in our system, we find that anions interact with the water molecules stronger than cations do. For example, for $|q| = 1.40$ e⁻, the energy (electrostatic and LJ) between cations and waters is −403.4 kJ per mole cation, whereas the corresponding anions–waters interaction energy is −655.5 kJ per mole anion. This asymmetry in the interaction energy of anions and cations with the water molecules is due to the ability of anions to approach the positive charge distribution around the two hydrogen atoms closer than the ability of cations to approach the negative charge distribution around the oxygen atom. The difference in the interaction energy of the anions and cations with the water molecules decreases with $|q|$.

Nevertheless, identifying high charge density ions as kosmotropes is very misleading since the original classification of structure-makers and structure-breakers was attributed to changes in the structure of water, and from this point of view

the high charge density ions disorder the structure of water *vis à vis* water (as shown in Figure 2 and Figure 3). This issue was raised in the review article by Ball,[22] and we agree that future reference of salts as kosmotropes or chaotropes should be avoided. Recently, Thomas and Elcock[56] argued that the salt-induced modulations of the hydrophobic interaction are primarily manifestation of salt-induced changes in the water structure. From our study, we indeed see that there is an inverse qualitative correlation between the salt-induced strengthening of the hydrophobic interaction and the degree of hydrogen bonding, or structuring, of the water molecules. However, the values of these structural properties for pure water can not predict the transition from salting-in to salting out. The strength of our simulations is that in addition to salting-out we also observe salting-in behavior, which was not the case in the work of Hribar et al.[65] and that of Thomas and Elcock.[56] The difference is that in their simulations they used small hydrophobic particles, which tend to exhibit more salting-out behavior. In this case, the binding of low charge density ions (at low concentration of salt and hydrophobic solute) can increase the propensity for solute aggregation since these ions behave like hydrophobes, effectively increasing the concentration of hydrophobic particles in the system, forming a "micelle-like" structure.[15]

The decrease in the number of hydrogen bonds between the water molecules with the increase of the ionic charge (or equivalently the salting-out character) of the ions also implies a decrease in the strength of the water–water attractions. Figure 3b, shows the potential energy between two water molecules as a function of q. This water–water interaction energy becomes more attractive for salting-in ions and less attractive for salting-out ions, in contrast to what is proposed for chaotropes and kosmotropes.[25,26] In addition, Figure 3b shows that the strength of the hydrogen bond also become stronger as q is decreased. In both cases, the intersection of the curve with the value for pure water is not far from the transition between salting-in and salting-out behavior. However, the saturation of the curve observed at low q while there is a sharp change in the hydrophobic interaction for these value of q (see Figure 1) preclude the identification of these energetic terms (by themselves) as descriptors for the salting effect. Note that the water–water interaction energy behaves opposite to the Samoilov activation free energy describing the strength of ion hydration.[27] The later is larger the stronger the ion as salting-out agent due to the stronger ion–water interactions,

One of the earliest theories of the salting effect was the "internal pressure" model[33] based on Tait's equation and Tammann's hypothesis. It states that water in salt solution behaves like water under a pressure greater than the external pressure. This was inspired by the observation that the extent of volume contractions upon the dissolution of a salt in water was related to an increase in the salting-out ability of the salt.[32] The physical interpertation given was that in order to insert into the salt solution hydrophobic particles, an excess work must be done against the ion–water forces that is proportional to this change in volume.[13,66] Figure 5 displays the volume of the system as a function of q. As observed experimentally, the stronger the salting-out character of the salt the larger the volume contraction. The volume of the pure water solution is much smaller than that of all salt solutions. This is not surprising as the LJ diameter of the cations and anions is 0.5 nm, larger than the diameter of a water molecule. Thus, these results indicate that even though the mixing of water and salt in our simulation leads to a volume increase, these salts can, either, salt-in or salt-out hydrophobic particles. Note that the curve of the volume
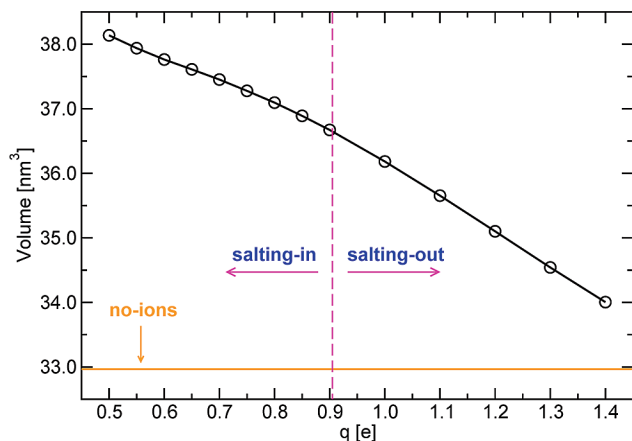
Figure 5. The volume of the simulation box as a function of the ionic charge density of the salt solution, as well as in pure water.

has two slopes and the switch occurs around the transition point. This is probably a result that at high charge density it is the electrostatic interaction that attracts the waters to the ions, whereas at low charge density it is the van der Waals repulsive potential that pushes the water away from the ions.

2. Correlation of the Salting-Effect to Dynamical Changes of Water. It has been known for a long time that the viscosity of water is modified in salt solutions compared to that in pure water. For example, CsI reduces, whereas NaF increases, the value of the viscosity.[21] In dilute aqueous salt solutions (lower than 0.3 M) the viscosity can be expanded as a function of the concentration, c,

$$\eta/\eta_0 = 1 + A\sqrt{c} + Bc + \dots \qquad (2)$$

where η_0 is the viscosity of pure water.[23] The coefficient A is expected to be positive for all strong electrolytes and zero for nonelectrolytes. On one hand, the Jones−Dole B coefficient is suggested to be positive for kosmotropic ions and negative for chaotropic ions.[19,67] As the ionic charge density increases the absolute value of B increases as well. For example,[21] for the alkali ions Li^+, Na^+, K^+, Rb^+, and Cs^+, the corresponding values of B are $+0.146$, $+0.085$, -0.009, -0.033, and -0.047; and for the halide ions F^-, Cl^-, Br^-, and I^-, the corresponding values of B are $+0.107$, -0.005, -0.033, and -0.073. In Figure 6a we present an extrapolation of $\eta(k)$, from finite values of k to the macroscopic viscosity $k = 0$, (see the Methods section for the form of the fit function) for the simulations with $|q| = 0.50$ and 1.40 e$^-$. The value of $\eta(k = 0)$ as a function of q is displayed in Figure 6b. As experimentally found the viscosity increases with the charge density of the ions. The intersection of the curve with the value of pure water ($|q| = 0.86$ e$^-$) in not too far from the transition between salting-in and salting-out behavior. However, the saturation of the viscosity at small values of q points to the fact that there is no strong correlation with the values of $\Delta\Delta G$, since at low values of q a strong decrease in the magnitude of the hydrophobic interaction is observed (see Figure 1). The fact that the salting-out ions induces a stronger effect on the dynamics of the water molecules was also observed experimentally.[68]

We choose to calculate the self-time correlation functions of the orientation of the water molecules by a unit vector perpendicular to the molecular plane and by the unit vector along the dipole vector. The corresponding rotational autocorrelation functions, $C_{rot}(t)$, were calculated using the first Legendre polynomial $P_1(x)$, normalized so that $C_{rot}(t = 0) = 1$. All

rotational correlation functions we obtained fitted best to a stretched exponential form (Figure 7a),

$$C_{rot}(t) = A \exp[-(k_{rot}t)^\beta] \qquad (3)$$

where A is the amplitude, k_{rot} is the decay rate constant, and β is the stretched parameter. The normalization of the function at $t = 0$ leads to $A = 1$, so only k_{rot} and β are used as fit parameters. The fit of $C_{rot}(t)$ was performed between $t = 1.0$ ps until $C_{rot}(t) < 0.001$. The results show that for both correlation functions the rotational decay rate increases as the ionic charge density, q, decreases. However, it is more pronounced for the rotation of the normal to the plane vector (Figure 7, panels b and c). This general feature of the rotational correlation functions is in agreement with results obtained by NMR proton relaxation rates.[21] Thus, as with the viscosity, the rotational dynamics of the water molecules in salt solutions is affected more by the change in the ion−water interaction than by the change in water−water interactions induced by the ions. In our case the decay rate in pure water is much larger than in any of the salt solution investigated (for both rotations), and no prediction on the transition point between salting-in and salting-out behavior using these dynamical properties of water can be made. Previous computer simulation studies on the effect of NaCl and KCl on the rotational dynamics of water also found that the presence of these ions induces a slowing down in the dynamics.[69] Nevertheless, in some cases these salts (depending on the salting-effect measured) are considered to be at the transition between salting-in and salting-out,[70] indicating again the lack
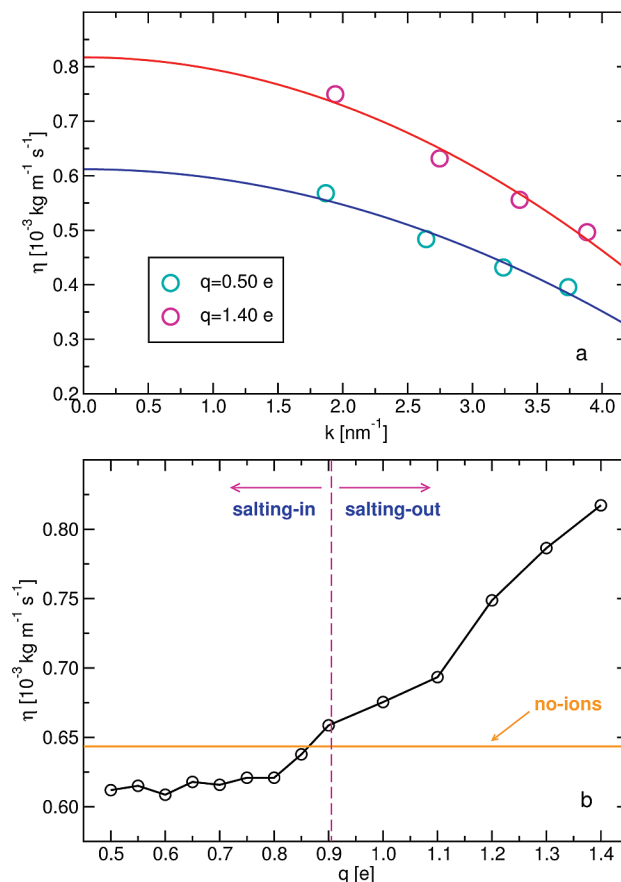

Figure 6. (a) A fit of viscosity, obtained using transverse current autocorrelation functions, as a function of the wave-vector k, for the ionic charge density, $q = 0.50$ and 1.40 e$^-$. Empty circles are the simulation data points and solid lines are the fit to the function: $\eta(k) = \eta(0) - A \cdot k^2$. (b) The viscosity as a function of q.
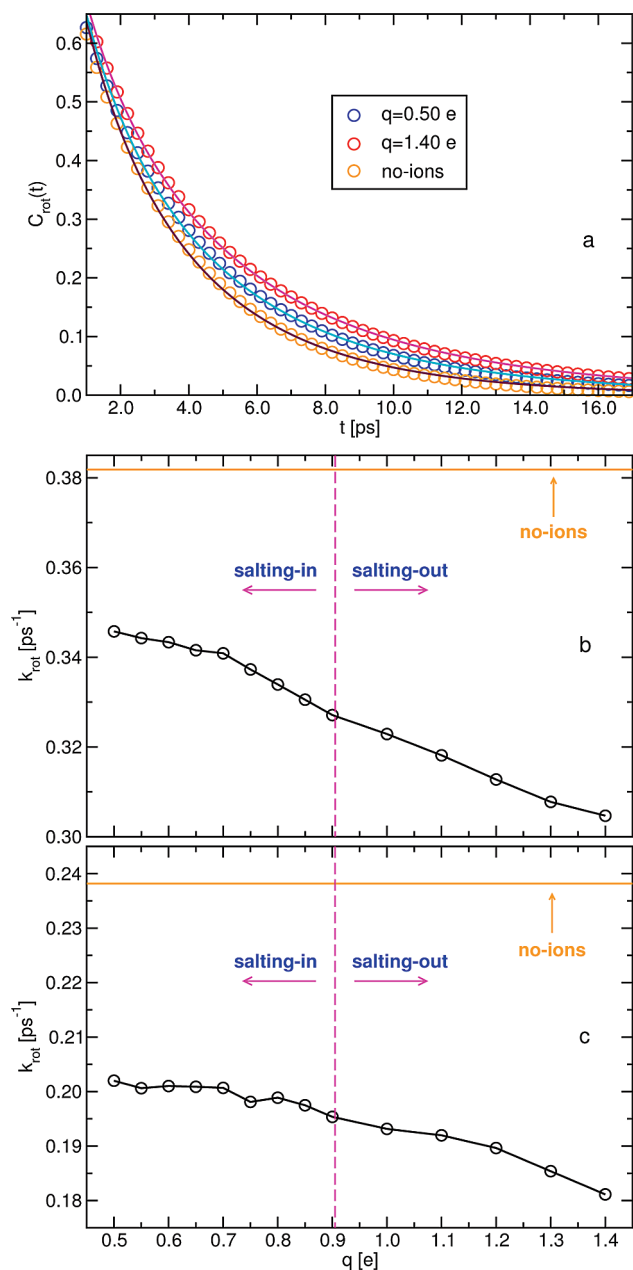
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Figure 7. (a) A fit of the (normal to the plane) rotational autocorrelation function to a stretched exponential form for $q = 0.50, 1.40$ e$^-$, as well as, for pure water. Empty circles are the simulation data points and solid lines are the fit to the stretched exponential function: $C_{rot}(t) = A \exp[-(k_{rot} t)^\beta]$. The decay rate, k_{rot}, as a function of q for the rotation of the (b) perpendicular to the plane, and (c) dipole moment, vectors of the water molecule.

of correlation between the changes in the structure of water and the salting phenomenon.

Taken together, we find that the observed slowing down in the dynamical properties of water with the increase of the salting-out character of the ions is not due to the ion-induced structural ordering between the water molecules, but is due to an increase in the strength of the ion−water interaction. For example, that the viscosity increases with the salting-out character of the ions is probably due to a larger effective diameter of the diffusing particles (ion−water complexes).

IV. Conclusions

In this paper we correlated the change in the strength of the hydrophobic interaction induced by salts to changes in the

structure and dynamics of the water molecules. A monotonic change in these properties is found with increasing the salting-in/salting-out ability of the ions, however we could not identify one property that can predict the change in the strength of the hydrophobic interactions. In contrast to previous studies of small hydrophobic solutes where only salting-out is observed, we observe in this paper both salting-in and salting-out effects. This allowed us to test whether the changes in the structural or dynamical properties can predict the transition from salting-in to salting-out behavior. We found that none of the properties of water we examined could be used to predict the transition point. This means that it is not possible to indicate whether a particular salt will induce salting-in or salting-out just from these properties of the salt solution in the absence of solute. However, in a previous work, we were able to make such prediction based on analysis of preferential binding of the ions to the hydrophobes.[55] Despite the popularity of the structure-making/breaking hypothesis, the failure to predict the salting effect from only the water−salt system should not be taken as a surprise. This is because, experimentally, there are examples of particular salts that can salt-out one solute but salt-in another.[13] Therefore, the salting effect must depend also on the properties of the solute, in addition to the properties of the salt and water. In fact, it turns out that salting-in behavior is enhanced for larger and more polar solutes.[13] How can this be rationalized? The salting effect is linked to preferential binding through the Wyman/Tanford equation. Furthermore, it is possible to link the preferential binding to the energetics governing the solute−salt−water system. This was done in a recent study[71] of the salting-in (denaturing) behavior of urea, where we established a relation between the magnitude of the urea−solute, relative to water−solute, binding energy and the change in the free energy difference for dissociating a hydrophobe dimer (salting-in). The stronger this difference in binding energy the larger the dissociation (salting-in). The behavior and mechanism of action of the neutral urea molecule is analogous to that of low charge density ions. These ions have stronger binding energy to large polar solutes than to small hydrophobes, and hence, the enhanced salt-in effect. This difference in binding energy represent the requirement to include the ternary, solute-salt-water, system in order to characterize the salting effect.

As mentioned above, it is the solute−water and ion−water interaction strength that determines the salting effect (i.e., the effective interaction between the solute particles). When the solute particles are hydrophobes, their strength of interaction with the water molecules is weak and the ion−water interaction dominates the energetics of the ternary system. This might be the reason why some theories and observations found that the characterization of the ion-induced changes of the water properties can be a good measure (descriptor) of the salting effect. This is true when the prediction of the salting-out power is described on a relative, and not on an absolute, scale[13] (i.e., relative to another salting-out agent), and for one particular solute, where the different salting behavior of small and large hydrophobes,[15,55] does not need to be taken into account. Since many aspects of the salting phenomena (in protein aggregation, secondary/tertiary structure, micelles, and lipid bilayers) are driven partially or completely by hydrophobic interactions, ion/cosolute-induced water structure has been offered as the driving force for these effects in many fields of science. This is unfortunate, because when using the terms chaotropes or kosmotropes for the ions, while actually observing a decrease or increase in the hydrophobic interactions, the actual changes in the structure between the water molecules induced by these

ions is opposite to what the name of these terms implies. In addition, the interpertation of the changes in the dynamical properties of the water molecules is not due to changes in the water structure but mainly governed by the ion—water interactions.

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