# Simulation of Osmotic Pressure in Concentrated Aqueous Salt Solutions

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ABSTRACT Accurate force fields are critical for meaningful simulation studies of highly concentrated electrolytes. The ion models that are widely used in biomolecular simulations do not necessarily reproduce the correct behavior at finite concentrations. In principle, the osmotic pressure is a key thermodynamic property that could be used to test and refine force field parameters for concentrated solutions. Here we describe a novel, simple, and practical method to compute the osmotic pressure directly from molecular dynamics (MD) simulation of concentrated aqueous solutions by introducing an idealized semipermeable membrane. Simple models for Na^+, K^+, and Cl^- are tested and calibrated to accurately reproduce the experimental osmotic pressure at high salt concentration, up to the solubility limit of 4−5 M. The methodology is general and can be extended to any type of solute as well as nonadditive polarizable force fields.

SECTION Dynamics, Clusters, Excited States

Osmotic pressure of NaCl solution
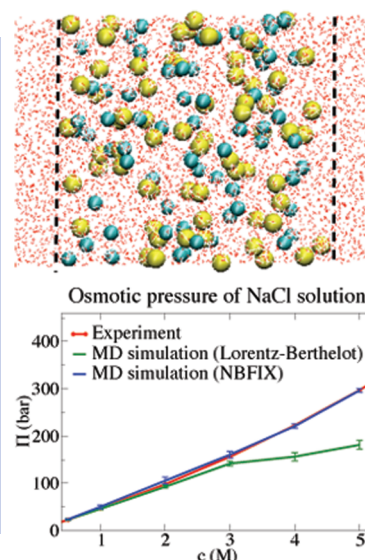
Accurate force fields are critical for meaningful computer simulation studies of highly concentrated solutions. Electrolytes present particular challenges because of the long-range nature and large magnitude of electrostatic interactions. The models widely used in molecular dynamics (MD) simulations of aqueous ionic solutions have generally been developed on the basis of single ion properties, such as the gas phase monohydrate enthalpy and the hydration free energy at infinite dilution,[1−4] and there is no guarantee that the properties of concentrated solutions are accurately represented. For example, excessive ion pairing causing poor solubility and spontaneous crystallization have been noted with the AMBER-99 force fields[5] for NaCl and KCl well below the experimentally determined solubility limit.[3,4] This has been explained by how Åqvist's cation Lennard-Jones (LJ) parameters, developed for the geometric mixing rule, were adapted to the arithmetic mixing rule in the AMBER-99 force field ion pairs.[3,6,7] Clearly, the thermodynamic stability of concentrated solutions depends sensitively on ion pairing propensity. However, a quantitative assessment of computational results against experimental measurements is difficult because available data provide only indirect information about ion pairing. Neutron and X-ray scattering yield the average structure factor of a solution and multiple experiments involving isotopic substitutions as well as extensive analysis are needed to extract individual solvent−solvent, ion−solvent, and ion−ion pair correlation functions.[8−10] The amount of ion pairing can indirectly be related to conductivity measurements,[11] although this involves additional assumptions. Despite the vast literature covering the theory of electrolyte solutions, our knowledge of the range of validity of the available potentials to construct reliable computational models at very high concentrations remains fairly incomplete.

Because it offers a measure of the strength of the effective solvent-mediated interaction between solutes, the osmotic pressure is potentially of great interest to test and refine force fields used in computer simulations of very concentrated solutions. It is one of the key thermodynamic properties that can be measured for a wide range of conditions. However, establishing the formal relationship between the force field used in computer simulations and the osmotic pressure is not completely straightforward. For nonideal solutions, the osmotic pressure is equal to $\Pi = \phi\Pi^*$, where ϕ is the osmotic coefficient defined with respect to the osmotic pressure Π^* for an ideal solution by the Van't Hoff equation $\Pi^* = cRT$ (c is the total concentration of dissociated ionic species and R is the gas constant). Theoretical studies of osmotic coefficients in multicomponent solutions have often relied on statistical mechanical frameworks such as the McMillan−Mayer (MM)[12] and Kirkwood−Buff (KB)[13] theories. Both MM and KB theories provide a formal route relating the thermodynamic properties of solutions by averaging out the solvent, albeit on the basis of a different reference state. MM theory starts by considering infinitely dilute solutions.[12] This makes

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it possible to construct potentials of mean force (PMFs) representing the effective solvent-averaged interactions between the ions, and to develop virial-like expansions that are analogues to the virial coefficients in the theory of nonideal gases. These coefficients are then expressed as many-body interactions using the solvent-averaged effective PMF between ions in the solvent. KB theory proceeds from a generalization of the compressibility equation for a one-component system to multicomponent systems,[13] the properties of which are expressed in terms of integrals over solute–solute pair correlation functions. KB theory requires knowledge of those integrals at multiple solute concentrations, or an assumption of linear behavior, to provide the osmotic pressure or solute activities under specific conditions. Ben-Naim introduced the important concept of the inverse KB theory,[14] where one performs a global optimization to determine the value of the KB integrals (KBIs) needed to match experimental data for solvent mixtures. Application of this method yields experimentally derived KBIs, which can then be utilized as raw target data that one may try to match with simulations based on atomic models. For a comparative discussion of MM and KB, see ref 15.

Most of the fundamental theoretical investigations of the osmotic properties of electrolyte solutions have been based on the primitive model, in which the solvent molecules are represented by a featureless continuum. Theories such as the mean spherical approximation (MSA)[16,17] or the hypernetter chain (HNC) theory[18] were used to derive pair correlation functions needed to calculate ϕ. The molecular nature of water was taken into account using the multicomponent reference interaction site model HNC (RISM-HNC) theory.[19] Going beyond integral equations using the frameworks provided by MM and KB theories in the context of explicit solvent simulations, however, proves difficult, as the osmotic coefficient primarily concerns the solvent-averaged effective forces between the ions. In practice, to help bridge the different levels of representation, most computer simulations of osmotic properties have adopted the following two-step strategy: effective ion–ion pair PMFs are computed from all-atom simulations with explicit solvent in a first stage, those are then used, in a second stage, as "input" according to MM or KB theories. The latter stage, which involves only effective PMFs with no explicit solvent, has been carried out by Monte Carlo[20] MD,[21] or even integral equations.[22] However, the usefulness of a two-stage computational strategy based on effective PMFs becomes limited at very high concentrations, where accuracy and reliability is most needed. For instance, additional assumptions are needed to account for the effect of high salt concentration on the dielectric permittivity of the bulk water solvent[21] or to construct multi-ion PMFs on the basis of a superposition approximation.[20] A completely different approach consists of calculating the KBIs directly using computer simulations.[23–30] By directly comparing those with experimentally derived KBIs, Smith and co-workers have sought to improve potential functions and develop a "KB" force field.[23–27]

While MM and KB theories play a central role in statistical mechanical formulations of the thermodynamic properties of mixtures, they do not offer a simple computational route to

Table 1. Ion LJ Parameters

ion	$\frac{1}{2}R^i_{min}$ (Å)	E^i_{min} (kcal/mol)
Na^{+a}	1.36375	−0.0469
Na^{+b}	1.41075	−0.0469
K$^+$	1.76375	−0.0870
Cl$^-$	2.27000	−0.1500

[a] Parameterization of Na$^+$ in 1994 from ref 2. [b] Reparameterization of Na$^+$ in 2008 from ref 39.

Table 2. Modified Ion–Ion LJ Parameters

	R^{ij}_{min} Å	
ion pair	combination rule	NBFIX
Na^{+a} − Cl$^-$	3.633	3.676
Na^{+b} − Cl$^-$	3.680	3.731
K$^+$−Cl$^-$	4.033	4.081

[a] Parameterization of Na$^+$ in 1994 from ref 2. [b] Reparameterization of Na$^+$ in 2008 from ref 39.

estimate the osmotic pressure of a concentrated solution. An accurate treatment of osmotic pressure, a basic thermodynamic property of solutions, is of great importance for the proper modeling of a wide range of physiological[31] and physical[32–36] phenomena. Ideally, one would like to dispose of a simple and transparent computational scheme to calculate the osmotic pressure. Such a scheme would also be an extremely useful tool for the validation and refinement of the force field used in all-atom simulations.

Here we present a simple and practical method for accurately computing the osmotic pressure directly from all-atom MD simulations of concentrated aqueous solutions. The basic idea is to introduce "virtual" walls to represent the effect of ideal semipermeable membranes, separating a high concentration region from a pure water region. The walls keep the ions confined, but the water molecules are allowed to pass freely, permitting an equalization of their chemical potential throughout the entire system. The mean force per unit area exerted on the ions by the virtual walls during the simulations can be directly related to the osmotic pressure. This method bears some similarities to ideas developed by Murad,[32–34,37] where two regions were separated by a simple model of an actual semipermeable membrane model, and the osmotic pressure was calculated from the mean force necessary to tether the semipermeable membrane in place.

We initially focus our attention on the monovalent salts NaCl and KCl, which are among the most abundant ions. In the following, the method and the simulation systems are described in detail. Then, the osmotic pressure is used to test and refine the CHARMM parameters for K$^+$, Na$^+$, and Cl$^-$. The osmotic pressure of NaCl and KCl aqueous solution are calculated over a wide range of concentrations (up to 5 M) and compared with the experimental values.[38] The study shows that the osmotic pressure is a powerful route for validating and parameterizing atomistic force fields. It depends sensitively on ion pair interactions, in agreement with previous studies.[20,21] The method is general and can be extended to
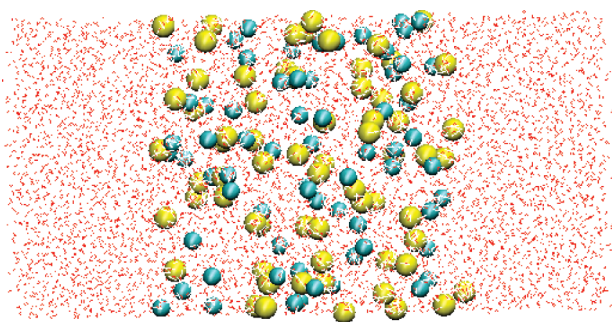
Figure 1. The equilibrium configuration for the simulation of a 1 M NaCl solution. Sodium ions are in yellow, chloride ions are in green, and TIP3P is in red.

any type of solute as well as nonadditive polarizable force fields.

In the following, we provide all details about the simulation methodology and force field used in the present study. The parameters of the LJ 6−12 potential for K^+ and Cl^- are taken from ref 2. A reparameterization of Na^+, optimized to yield the relative bulk solvation energy of K^+ and Na^+ close to the experimental value of 17.5 kcal/mol, is also considered.[39] The TIP3P water potential[40] as modified for the CHARMM force field[41] is used. All the LJ parameters for ions used in this study are listed in Table 1. All the atomic systems were constructed and equilibrated using the program CHARMM.[42,43] The NaCl or KCl solution models at different concentrations were initially built by randomly replacing a number of water molecules by ions, within an equilibrated cubic box of 3840 TIP3P water molecules (unit cell length = 48 Å). The number of ions within the box was chosen according to the molar scale (i.e., concentrations were set from the number of ions per unit of volume of the simulated system). This was followed by a short minimization and a 200 ps equilibrium simulation at constant volume and temperature (300 K) with a Langevin thermostat[44] with periodic boundary conditions. The geometry of the TIP3 water molecules was constrained using the SHAKE algorithm.[45] Then, the ionic solution was joined with a copy of the pure water cubic box to assemble an orthorhombic system approximately 96 Å. The water molecules overlapping near the junction between the solution and pure water region were deleted, followed by a short equilibration with fixed ions. The system, comprising a salt solution and a pure water region, is shown in Figure 1. The z axis is aligned with the long axis of the orthorhombic system, and the system is centered on the salt solution region ($z = 0$).

A flat-bottom half-harmonic planar restraint was applied to the ions at $z = \pm 24$ Å to keep them inside the central region corresponding to the salt solution; a force constant of 10 kcal/mol/Å^2 was used. No restraint was applied to the water molecules, and they can move freely throughout the periodic system. The virtual walls were imposed via the MMFP GEO facility of the CHARMM program,[43] which can be used for setting up various type of geometric restraining potentials on selected atoms. The entire system was subjected to NPT dynamics using the constant pressure−temperature module of CHARMM with a leapfrog integrator with the time step of 1 fs. The temperature was set at 300 K using Hoover

temperature control.[46] The extended system pressure algorithm was employed with constant normal pressure ($P = 1$ atm) and constant cross-sectional area in the XY plane; the size of the system was allowed to change only in the z direction.[47] After 500 ps, the resulting configurations were used to pursue the MD simulations with the program NAMD.[48] The virtual walls restraints were setup via a Tcl script in NAMD. The MD simulations were generated at constant volume and at 300 K constant temperature (NVT) using Langevin dynamics with a friction of 0.5 ps^{-1}.[48] The long-range electrostatic forces were calculated with the particle-mesh Ewald.[49] Six NaCl solution systems (concentration 0.5, 1, 2, 3, 4, and 5 M) and five KCl solution systems (0.5, 1, 2, 3, and 4 M) were constructed as described above. After additional equilibration of 1 ns, 10 independent 1.5 ns production simulations with different initial velocities were carried out for each system. The z-coordinate of ions were saved every 0.1 ps from the last 1 ns of the trajectories for analysis. The osmotic pressure is calculated directly from $\langle F_{wall} \rangle / A$, where A is the (constant) cross-sectional area of the system (48×48 Å^2) and F_{wall} is the instantaneous force exerted by one wall onto the ions. The mean force is calculated from $\langle F_{wall} \rangle = k(1/N)\sum_N\sum_i(|z_i - z_{wall}|)$, where $|z_i| > |z_{wall}|$, k is the force constant (10 kcal/mol/Å^2), N is number of steps, and i is the index of ions. The mean force was averaged over the two half-harmonic walls.

We now go over the main results. Figure 2a,b show the calculated osmotic pressure for the NaCl and KCl solutions using those models. The CHARMM parameters for K^+, Na^+, and Cl^- are able to reproduce the experimental osmotic pressure accurately up to 1 M. This is consistent with a previous study, where no excessive ion pairing and solubility problems was observed in MD simulations of KCl at 1 M.[50] The results are close to the experimental values for NaCl solutions below 3M and for KCl solutions below 2 M. Above this concentration, the values obtained from MD simulations drop significantly, and the deviation from the experimental value increases with concentration. This discrepancy is caused, at least in part, by the excess of ion pairing at high concentrations (see below). To verify that the simulation system was sufficiently large to avoid any finite-size artifacts, calculations with a larger system were carried out using the same simulation protocol. The larger system was prepared by combining the same cubic 5 M NaCl solution box (48^3 Å^3) with a pure solvent box that is 3 times longer in the z direction ($48 \times 48 \times 144$ Å^3). The osmotic pressure obtained from the larger system is 186 ± 12 bar, in excellent agreement with the result from the smaller system (181 ± 9 bar; see Figure 2a).

The LJ parameters for K^+, Na^+, and Cl^- were previously optimized to reproduce the hydration free energies in infinitely dilute solution.[2,51] Those models are part of the CHARMM PARAM27 force field,[41] which is developed on the basis of the TIP3P water potential.[40] By default, the LJ parameters used for the cation−anion pair interaction, E_{min}^{ij} and R_{min}^{ij}, are generated via the Lorentz−Berthelot combining rule[52] from the LJ parameter as $E_{min}^{ij} = (E_{min}^{ii}E_{min}^{jj})^{1/2}$ and $R_{min}^{ij} = (R_{min}^{ii} + R_{min}^{jj})/2$. It is possible to override the simple combination rule and impose specific pairwise LJ parameters for the ion pair. Practically, this is done via the NBFIX keyword in the
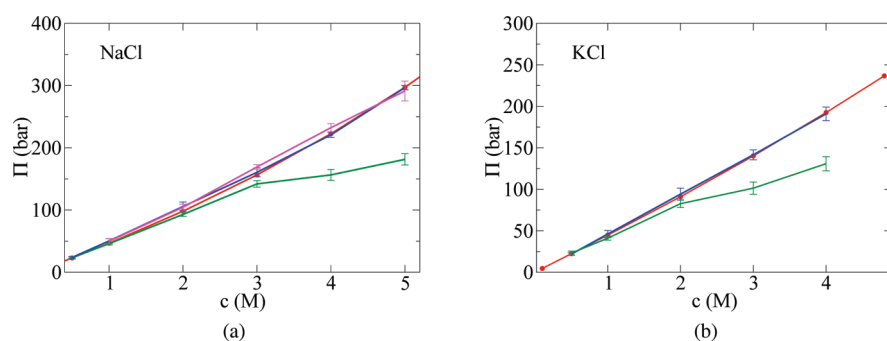
Figure 2. The osmotic pressure as a function of the NaCl (a) or KCl (b) salt concentration. The red line is the experimental values; green line is obtained from MD simulations with the Lorentz–Berthelot combination rule; blue line and magenta line are from simulations with adjusted R_{min} LJ parameters using NBFIX. In panel a, the blue line is the original Na$^+$ (1) from ref 2, and the magenta line is the reparameterized Na$^+$ (2) from ref 39. The errorbars are calculated from 10 separate trajectories of 1 ns.
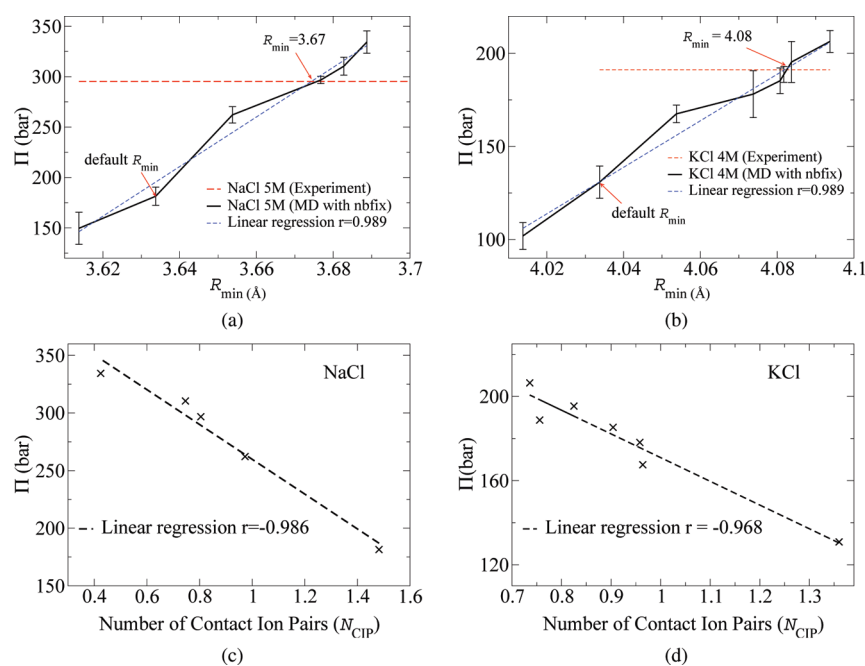

Figure 3. Osmotic pressure as a function of R_{min} scaled to reproduce the experimental data for 5 M NaCl and 4 M KCl solutions (a and b). The error bars are calculated from five separate trajectories of 1 ns. Osmotic pressure as a function of the number of CIPs in 5 M NaCl solution (c). The sample points correspond to the scaled R_{min} in panel a: from left to right, R_{min} is 3.688, 3.682, 3.676, 3.653, and 3.633 Å. Osmotic pressure as a function of the number of CIPs in 4 M KCl solution (d). The sample points correspond to the scaled R_{min} in panel b: from left to right, R_{min} is 4.093, 4.083, 4.081, 4.080, 4.073, 4.053, and 4.033 Å. The original Na$^+$ (1) from ref 2 is used for analysis.

parameter file that is read by NAMD and CHARMM.[43] The NBFIX keyword enables the user to override the standard combination rule for selected pairs of atom types. In the following, we use NBFIX to alter the value of R_{min}^{ij} between cation and anion to explore the impact on ion pairing and the osmotic pressure for NaCl and KCl solutions at different concentrations. For the sake of simplicity, we chose to leave the E_{min}^{ij} unchanged and modify only the LJ radius R_{min} of the anion–cation pair because the latter has a direct impact on the very strong cation–anion Colombic interaction.

The purpose of varying R_{min}^{ij} is to determine an optimal value yielding a model able to reproduce the experimental osmotic pressure accurately for a wide range of concentrations. A systematic exploration of the Na$^+$–Cl$^-$ LJ radius was first carried out for the 5 M NaCl solution, which has the largest

deviation between simulation and experimental data. Figure 3a shows that the osmotic pressure is very sensitive to the R_{min} value. The linear regression gives the optimized R_{min} value at 3.676 Å, only 0.043 Å larger than the default value of 3.633 Å from the combination rule. However, this small change is sufficient to shift the electrostatic energy between Na$^+$ and Cl$^-$ by 1 kcal/mol in vacuum (from -91.5 kcal/mol to -90.5 kcal/mol). The optimized R_{min} is then used to calculate the osmotic pressure for different concentrations. The blue line in Figure 2a fits the experimental data very well in the 0.5–5 M concentration range. Using the same method, we calibrated the R_{min} for the new parameter of Na$^+$.[39] The default R_{min} for the new Na$^+$–Cl$^-$ pair is 3.680 Å. The optimized R_{min} value 3.731 Å gives a good fit with the experimental data (Figure 2a). For the KCl solution, the

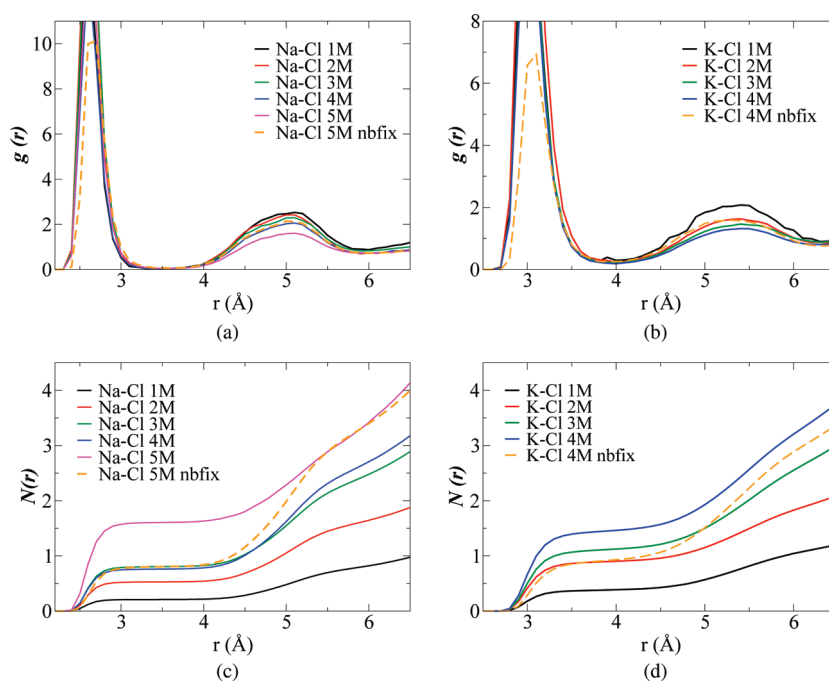**Figure 4.** Na^+-Cl^- and K^+-Cl^- radial distribution functions for different salt concentrations (a and b). The height of the first peak is not shown here for clarity. Na^+-Cl^- and K^+-Cl^- coordination numbers for different salt concentrations (c and d). The $N(r)$ and $g(r)$ obtained from adjusted R_{min} are shown by the orange dashed line. The original Na^+ (1) from ref 2 is used for analysis.

4 M system was used to optimize the LJ radius of K^+ with Cl^-. A good fit with experimental osmotic pressure for 4 M KCl aqueous solution is achieved by increasing R_{min} by 0.048 Å (Figure 3b). Using this optimized R_{min} 4.081 Å (default 4.033 Å) to calculate the osmotic pressure for other concentrations, Figure 2b shows that the refined parameters can reproduce the experimental data very well for a large range of KCl concentrations (from 0.5 to 4 M). The default LJ parameters and the NBFIX values for Na^+-Cl^- and K^+-Cl^- interactions are summarized in Tables 1 and 2.

The relationship between ion pairing and the osmotic pressure is confirmed by plotting the osmotic pressure (Π) against the number of contact ion pairs (CIPs), N_{CIP}, as a function of R_{min} (Figure 3c). N_{CIP} is calculated by integrating the Na^+-Cl^- radial distribution function up to the first minimum. The determination of the first minimum in the ion–ion pair correlation function is partly arbitrary, and values of 3.5 Å for Na^+ and 3.7 Å for K^+ were chosen for the sake of the analysis. The Na^+-Cl^- radial distribution function was calculated from 1 ns trajectories and averaged over five trajectories. While longer simulations may decrease the statistical uncertainties, the figure shows that increasing R_{min} decreases N_{CIP} This change in N_{CIP} is directly correlated with an increase in the osmotic pressure. Likewise, the linear relationship between the osmotic pressure and N_{CIP} for the KCl solution is shown in Figure 3d.

The effect of concentration on ion pairing is investigated by comparing the cation–anion radial distribution functions among different concentrations. In Figure 4a, the first peak at 2.6 Å corresponds to the Na^+-Cl^- CIPs, which is in good agreement with the previous simulation using the same force field and water model.[53] The second peak at 5.0 Å is the solvent separated ion pairs (SSIPs). The height of the second

peak is lower with increasing salt concentrations, which means the probability of the formation of SSIPs decreases with increasing salt concentration. The height of the first peak is not shown here for clarity, but the integration of the first peaks is shown in the coordination number profile $N(r)$ (Figure 4c). The value of $N(r)$ at the first minimum of $g(r)$ corresponds to the N_{CIP}. Figure 4c shows that the probability of the formation of CIPs increases with concentration. The N_{CIP} in 1 M NaCl solution is in agreement with a previous MD study.[21] The $g(r)$ of the 5 M NaCl solution using the optimized R_{min} is also shown in Figure 4a (orange dashed line). Comparing with the same concentration using default R_{min}, the height of first peak decreases, and the second peak increases. This suggests that the calibration of R_{min} affects the balance between CIP and SSIP configurations, reflecting complex many-body effects in the highly concentrated ionic solutions. The coordination numbers $N(r)$ in Figure 4c also show that the optimized R_{min} decreases the N_{CIP} The K^+-Cl^- radial distribution functions in Figure 4b show the first peaks at 3.0 Å for K^+-Cl^- CIP, and the second peaks at 5.3 Å for SSIP, which is in agreement with the previous work.[50] Similar concentration effects on K^+-Cl^- ion pairing are shown in Figure 4b,d.

In conclusion, we have presented a simple and practical computational method to calculate the osmotic pressure of concentrated ionic solutions directly from all-atom MD simulations. The results show that the nonpolarizable CHARMM force field for K^+, Na^+, and Cl^- without adjustment is able to reproduce the experimental osmotic pressure for concentrations up to 2 M. Small changes of the cation–anion pairwise LJ parameter R_{min}, less than 0.05 Å relative to the default value obtained from the Lorentz–Berthelot combining rule, yield

atomic models of NaCl and KCl aqueous solution able to reproduce the experimental osmotic pressure at a wide range of concentrations, up to the solubility limit. In the case of the Na^+ and Cl^-, the modified pairwise LJ parameter shifts the energy of the cation−anion pair only by 1 kcal/mol in vacuum (from −91.5 kcal/mol to −90.5 kcal/mol). It might be possible to reach the same goal via a global optimization of all the LJ parameters while strictly enforcing the standard combination rule, although this would not yield a fundamentally better force field. Ultimately, the LJ 6−12 potential is only an approximation to the quantum mechanical cation−anion interactions, and the combination rule is merely a convenient shortcut for generating pairwise parameters between unlike atoms. This investigation shows the relationship between ion pairing and the osmotic pressure at high concentrations. In all cases, excessive ion pairing at high concentrations is clearly associated with an underestimated osmotic pressure.

Calibration and validation of force fields for highly concentrated aqueous salt solution is crucial for meaningful simulations of a wide range of physiological and physical systems. Force fields parametrized from single ion properties at infinite dilution[1−4] do not necessarily yield accurate models of concentrated electrolyte solutions. For example, excessive ion pairing was observed in the AMBER-99 force field,[5] leading to an onset of crystallization well below the experimental solubility limit.[3,4] Ion−ion interactions can be calibrated to properly represent the effective solvent-mediated interactions by monitoring the osmotic pressure of concentrated ionic solutions.

The osmotic pressure simulation method described here is general and can be extended to any type of solutes as well as nonadditive polarizable force fields. Work is under way to utilize this method for the validation and refinement of force field parameters for a variety of ionic, polar, and nonpolar solutes and denaturants.

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REFERENCES

(1) Åqvist, J. Ion Water Interaction Potential Derived from Free Energy Perturbation Simulations. *J. Phys. Chem.* **1990,** *94,* 8021–8024.

(2) Beglov, D.; Roux, B. Finite Representation of an Infinite Bulk System: Solvent Boundary Potential for Computer Simulations. *J. Chem. Phys.* **1994,** *100,* 9050–9063.

(3) Chen, A. A.; Pappu, R. V. Parameters of Monovalent Ions in the AMBER-99 Forcefield: Assessment of Inaccuracies and Proposed Improvements. *J. Phys. Chem. B* **2007,** *111,* 11884–11887.

(4) Joung, I. S.; Cheatham, T. E. Determination of Alkali and Halide Monovalent Ion Parameters for Use in Explicitly Solvated Biomolecular Simulations. *J. Phys. Chem. B* **2008,** *112,* 9020–9041.

(5) Wang, J.; Cieplak, P.; Kollman, P. A. How Well Does a Restrained Electrostatic Potential (RESP) Model Perform in Calculating Conformational Energies of Organic and Biological Molecules? *J. Comput. Chem.* 2000, *21,* 1049−1074.

(6) Hess, B.; van der Vegt, N. F. A. Cation Specific Binding with Protein Surface Charges. *Proc. Natl. Acad. Sci. U.S.A.* **2009,** *106,* 13296–13300.

(7) Marlow, G. E.; Pettitt, B. M. Simulations of the Bis-penicillamine Enkephalin in Sodium Chloride Solution: A Parameter Study. *Biopolymers* (*Pept. Sci.*) **2001,** *60,* 134–152.

(8) Enderby, J. E. Neutron Scattering from Ionic Solutions. *Annu. Rev. Phys. Chem.* **1983,** *34,* 155.

(9) Leberman, R.; Soper, A. K. Effect of High Salt Concentrations on Water Structure. *Nature* **1995,** *378,* 364–366.

(10) Neilson, G. W.; Mason, P. E.; Ramos, R.; Sullivan, D. Neutron and X-ray Scattering Studies of Hydration in Aqueous Solution. *Philos. Trans. R. Soc. London, A* **2001,** *359,* 1595–1591.

(11) Altenberger, A. R.; Friedman, H. L. Theory of Conductance and Related Isothermal Transport Coefficients in Electrolytes. *J. Phys. Chem.* **1983,** *78,* 4162–4173.

(12) McMillan, W. G.; Mayer, J. E. The Statistical Thermodynamics of Multicomponent Systems. *J. Chem. Phys.* **1945,** *13,* 276–305.

(13) Kirkwood, J. G.; Buff, F. P. The Statistical Mechanical Theory of Solutions. I. *J. Chem. Phys.* **1951,** *19,* 774–777.

(14) Ben Naim, A. Inversion of Kirkwood−Buff Theory of Solutions: Application to Water−Ethanol System. *J. Phys. Chem.* **1977,** *67,* 4884–4890.

(15) Cabezas, H.; O'Connell, J. P. Some Uses and Misuses of Thermodynamic Models for Dilute Liquid Solutions. *J. Chem. Phys.* **1993,** *32,* 2892–2904.

(16) Lebowitz, J. L.; Percus, J. K. Mean Spherical Model for Lattice Gases with Extended Hard Cores and Continuum Fluids. *Phys. Rev.* **1966,** *144,* 251.

(17) Triolo, R.; Grigera, J. R.; Blum, L. Simple Electrolytes in the Mean Spherical Approximation. *J. Phys. Chem.* **1976,** *80,* 1858–1861.

(18) Allnatt, A. R. Integral Equations in Ionic Solution Theory. *Mol. Phys.* **1964,** *8,* 533.

(19) Pettitt, B. M.; Rossky, P. J. Alkali Halides in Water: Ion−Solvent Correlations and Ion−Ion Potentials of Mean Force at Infinite Dilution. *J. Chem. Phys.* **1986,** *84,* 5836–5844.

(20) Lyubartsev, A. P.; Laaksonen, A. Osmotic and Activity Coefficients from Effective Potentials for Hydrated Ions. *Phys. Rev. E* **1997,** *55,* 5689–5696.

(21) Hess, B.; Holm, C.; van der Vegt, N. F. A. Osmotic Coefficients of Atomistic NaCl (aq) Force Fields. *J. Chem. Phys.* **2006,** *124,* 164509.

(22) Krienke, H.; Vlachy, V.; Ahn-Ercan, G.; Bako, I. Modeling Tetraalkylammonium Halide Salts in Water: How Hydrophobic and Electrostatic Interactions Shape the Thermodynamic Properties. *J. Phys. Chem. B* **2009,** *113,* 4360–4371.

(23) Chitra, R.; Smith, P. E. Molecular Association in Solution: A Kirkwood−Buff Analysis of Sodium Chloride, Ammonium Sulfate, Guanidinium Chloride, Urea, and 2,2,2-Trifluoroethanol in Water. *J. Phys. Chem. B* **2002,** *106,* 1491–1500.

(24) Weerasinghe, S.; Smith, P. E. A Kirkwood−Buff Derived Force Field for the Simulation of Aqueous Guanidinium Chloride Solutions. *J. Chem. Phys.* **2004,** *121*, 2180–2186.

(25) Weerasinghe, S.; Smith, P. E. A Kirkwood−Buff Derived Force Field for Methanol and Aqueous Methanol Solutions. *J. Phys. Chem. B* **2005,** *109*, 15080–15086.

(26) Kang, M.; Smith, P. E. A Kirkwood−Buff Derived Force Field for Amides. *J. Comput. Chem.* **2006,** *27*, 1477–1485.

(27) Pierce, V.; Kang, M.; Aburi, M.; Weerasinghe, S.; Smith, P. E. Recent Applications of Kirkwood−Buff Theory to Biological Systems. *Cell Biochem. Biophys.* **2008,** *50*, 1–22.

(28) Rösgen, J.; Pettitt, B. M.; Bolen, D. W. Uncovering the Basis for Nonideal Behavior of Biological Molecules. *Biochemistry* **2004,** *43*, 14472–14484.

(29) Kokubo, H.; Rösgen, J.; Bolen, D. W.; Pettitt, B. M. Molecular Basis of the Apparent near Ideality of Urea Solutions. *Biophys. J.* **2007,** *93*, 3392–3407.

(30) Rösgen, J.; Pettitt, B. M.; Bolen, D. W. An Analysis of the Molecular Origin of Osmolyte-Dependent Protein Stability. *Protein Sci.* **2007,** *16*, 733–743.

(31) King, L. S.; Kozono, D.; Agre, P. From Structure to Disease: the Evolving Tale of Aquaporin Biology. *Nat. Rev. Mol. Cell Biol.* **2004,** *5*, 687–698.

(32) Murad, S. A Computer Simulation of the Classic Experiment on Osmosis and Osmotic Pressure. *J. Chem. Phys.* **1993,** *99*, 7271–7271.

(33) Murad, S.; Powles, J. G.; Holtz, B. Osmosis and Reverse Osmosis in Solutions: Monte Carlo Simulations and van der Waals One-Fuild Theory. *Mol. Phys.* **1995,** *86*, 1473–1483.

(34) Paritosh, F.; Murad, S. Molecular Simulations of Osmosis and Reverse Osmosis in Aqueous Electrolyte Solutions. *AIChE J.* **1996,** *42*, 2984–2986.

(35) Kalra, A.; Garde, S.; Hummer, G. Osmotic Water Transport through Carbon Nanotube Membranes. *Proc. Natl. Acad. Sci. U.S.A.* **2003,** *100*, 10175–10180.

(36) Zhu, F.; Tajkhorshid, E.; Schulten, K. Collective Diffusion Model for Water Permeation through Microscopic Channels. *Phys. Rev. Lett.* **2004,** *93*, 224501.

(37) Powles, J. G.; Murad, S.; Holtz, B. A Novel Osmotic Pressure Route to the Activity Coefficient of a Molecule in a Solution. *Chem. Phys. Lett.* **1995,** *245*, 178–182.

(38) Robinson, R. A.; Stokes, R. H. *Electrolyte Solutions*; Dover Publications, Inc: Mineola, NY, 2002.

(39) Noskov, S. Y.; Roux, B. Control of Ion Selectivity in LeuT: Two Na$^+$ Binding Sites with Two Different Mechanisms. *J. Mol. Biol.* **2008,** *377*, 804–818.

(40) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* **1983,** *79*, 926–935.

(41) MacKerell, J. A. D.; Banavali, N.; Foloppe, N. Development and Current Status of the CHARMM Force Field for Nucleic Acids. *Biopolymers* **2000,** *56*, 257–265.

(42) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM − A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. *J. Comput. Chem.* **1983,** *4*, 187–217.

(43) Brooks, B. R.; Brooks, C. L., III; Mackerell, A. D., Jr.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S.; Caflisch, A.; Caves, L.; Cui, Q.; Dinner, A. R.; Feig, M.; Fischer, S.; Gao, J.; Hodoscek, M.; Im, W.; Kuczera, K.; Lazaridis, T.; Ma, J.; Ovchinnikov, V.; Paci, E.; Pastor, R. W.; Post, C. B.; Pu, J. Z.; Schaefer, M.; Tidor, B.; Venable, R. M.; Woodcock, H. L.; Wu, X.; Yang, W.; York, D. M.; Karplus, M. CHARMM: The Biomolecular Simulation Program. *J. Comput. Chem.* **2009,** *30*, 1545–1614.

(44) Allen, M. P.; Tildesley, D. J. *Computer Simulations of Liquid*; Oxford University Press, Inc.: New York, 1987.

(45) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. Numerical Integration of the Cartesian Equation of Motions of a System with Constraints: Molecular Dynamics of *n*-Alkanes. *J. Comput. Chem.* **1977,** *23*, 327–341.

(46) Hoover, W. H. Canonical Dynamics: Equilibirum Phase-Space Distributions. *Phys. Rev. A* **1985,** *31*, 1695–1697.

(47) Feller, S. E.; Zhang, Y. H.; Pastor, R. W.; Brooks, B. R. Constant Pressure Molecular Dynamics Simulation − The Langevin Piston Method. *J. Chem. Phys.* **1995,** *103*, 4613–4621.

(48) Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kalé, L.; Schulten, K. Scalable Molecular Dynamics with NAMD. *J. Comput. Chem.* **2005,** *26*, 1781–1802.

(49) Darden, T.; York, D.; Pedersen, L. Particle-Mesh Ewald − An *N* log(*N*) Method for Ewald Sums in Large Systems. *J. Chem. Phys.* **1993,** *98*, 10089–10092.

(50) Im, W.; Roux, B. Ions and Counterions in a Biological Channel: A Molecular Dynamics Simulation of OmpF Porin from *Escherichia coli* in an Explicit Membrane with 1 M KCl Aqueous Salt Solution. *J. Mol. Biol.* **2002,** *319*, 1177–1197.

(51) Roux, B. Valence Selectivity of the Gramicidin Channel: A Molecular Dynamics Free Energy Perturbation Study. *Biophys. J.* **1996,** *71*, 3177–3185.

(52) Stone, A. *International Series of Monographs in Chemistry: The Theory of Intermolecular Forces*; Clarendon Press: Oxford, 1996; Vol. 32.

(53) Patra, M.; Karttunen, M. Systematic Comparison of Force Fields for Microscopic Simulations of NaCl in Aqueous Solutions: Diffusion, Free Energy of Hydration, and Structural Properties. *J. Comput. Chem.* **2004,** *25*, 678–689.