# Ion-Specific Interactions between Halides and Basic Amino Acids in Water†

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Ion specific behavior of halides at surfaces of aqueous basic amino acids is unraveled by means of molecular dynamics simulations employing both nonpolarizable and polarizable force fields. Analysis in terms of density plots, cumulative sums, and residence times provides a clear, robust, and quantitative picture of specific ion effects. Small anions like fluoride, but not heavier halides, exhibits strong affinity for positively charged groups in the order guanidinium > imidazolium > ammonium. In contrast, large soft anions such as iodide are weakly attracted to nonpolar regions of the amino acids. Because interactions of halides with positively charged groups exhibit a local character and are not overwhelmingly strong, similar behavior will be observed (in an additive sense) as well at surfaces of hydrated proteins.

## 1. Introduction

Ion specificity beyond simple electrostatics, i.e., different behavior of ions of the same valency has been observed for many processes involving proteins including salting out, denaturation, and enzymatic activity.[1−4] Already in 1888, Hofmeister ordered separately cations and anions (most of them monovalent) according to their ability to salt out egg white proteins.[5] Traditionally, salting out is rationalized in terms of structuring ability of ions on water, those ions possessing it being termed kosmotropes, whereas the others are called chaotropes.[6] Because these terms are often connected with an assumed long-range structuring of water by ions, a safer classification may be in terms of strongly vs weakly hydrated ions.[6] This is particularly relevant since recent experimental studies do not provide evidence for a long-range structuring effect indicating that monovalent ions can organize at best their first hydration shell.[7−9] These observations have shifted the attention from ion−water to ion−protein interactions.[10−12] This is not to say that interactions of ions with water in aqueous solutions are unimportant; nevertheless, the molecular origin of Hofmeister ordering of ions in biochemistry and biophysics has to be searched for also (and maybe primarily) at surfaces of hydrated proteins.

Among atomic monovalent cations, the two biologically most important species are sodium and potassium. In the Hofmeister series they occur next to each other with $Na^+$ having a slightly stronger salting out ability than $K^+$.[5,13] Recently, a higher affinity of sodium over potassium to protein surfaces has been established.[11] It was rationalized in terms of stronger binding of the former cation to the negatively charged side chains of aspartate and glutamate. To a lesser extent, $Na^+$ vs $K^+$ specificity was also due to interactions with amide oxygens at the protein backbone.[11] It was concluded that generic affinity of alkali cations to aqueous proteins can be to a good approximation reduced to binding to the constituting amino acid residues.

It is well-established that salting out is more sensitive to the choice of anions than cations.[14] Among the former, halides provide a series of simple biologically relevant monovalent anions, suitable for a comparative computational study. The behavior of halides at surfaces of hydrated proteins is more complex than that of alkali cations. Although the latter interact only with charged and polar groups, halides can also exhibit affinity for nonpolar surface regions.[15] The principle aim of the present study is to elucidate and quantify interactions of halide anions with basic amino acid residues, i.e., terminated arginine, lysine, and histidine. By means of molecular dynamics (MD) simulations with both polarizable and nonpolarizable potentials we establish ordering of fluoride, chloride, bromide, and iodide in terms of their affinity to positively charged amino acids (i.e., protonated Arg, Lys, and His) and, for comparison, to the neutral (deprotonated) histidine. This allows us to rationalize the Hofmeister series of halides in terms of specific anionic interactions with positively charged and nonpolar regions of the amino acid residues and to provide a molecular picture of generic halide-protein interactions.

## 2. Systems and Methods

Systems under investigation were prepared from scratch using the MD software package Amber8 in the modeling environment Leap.[16] First, a given amino acid was acetylated at the N-terminus and methylated at the C-terminus in order to exclude effects of charged groups not present in proteins. Such a capped amino acid was surrounded by 600 water molecules in a cubic periodic box with side length of 27 Å, modeling thus bulk solvation. Four fluoride, chloride, bromide or iodide anions were then added by one-to-one substitution of randomly chosen water molecules and the system was made electroneutral by putting in three (for Arg, Lys, or protonated His) or four (for deprotonated His) potassium cations. Finally, periodic boundary conditions were applied to this unit cell. The parm99[17] was used for the positively charged Arg, Lys, and His residues, as well as for the neutral deprotonated (at $\varepsilon$ position) histidine, while parameters for halide ions were the same as in our previous studies.[18] Both polarizable and nonpolarizable versions of the force field were used for simulations. In the former case the POL3 water model was employed,[19] while in the latter case we used the SPCE water.[20] A cutoff for nonbonded interactions was varied in test runs between 7.5 and 10 Å with no appreciable effect on presented results. Long-range electrostatic interactions

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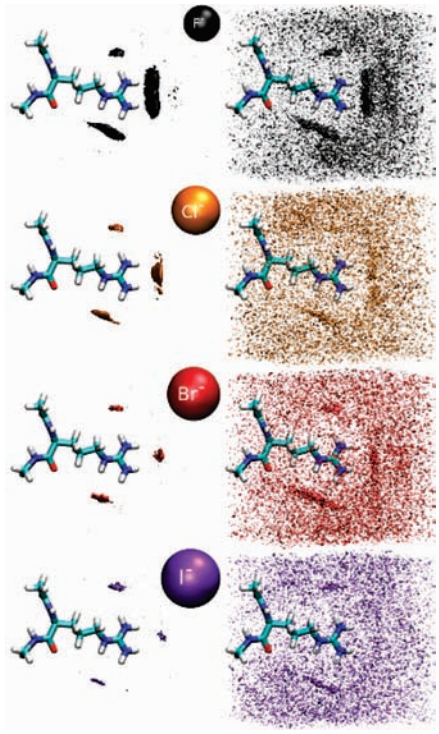

**Figure 1.** Density distribution of fluoride (black), chloride (gold), bromide (red), and iodide (violet) anions around aqueous terminated arginine. Results for nonpolarizable force field (left column) and polarizable force field (right column).

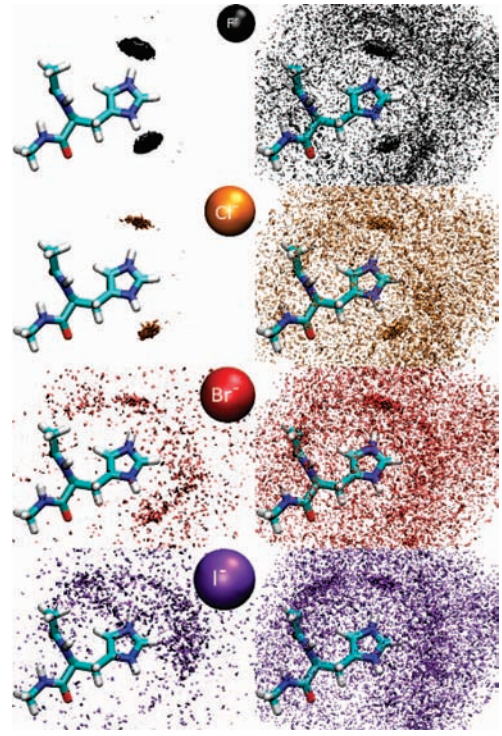

**Figure 3.** Density distribution of fluoride (black), chloride (gold), bromide (red), and iodide (violet) anions around aqueous terminated protonated histidine. Results for nonpolarizable force field (left column) and polarizable force field (right column).

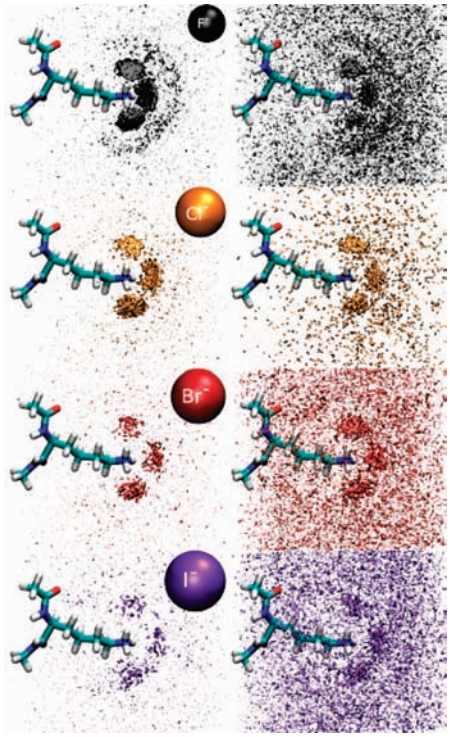

**Figure 2.** Density distribution of fluoride (black), chloride (gold), bromide (red), and iodide (violet) anions around aqueous terminated lysine. Results for nonpolarizable force field (left column) and polarizable force field (right column).

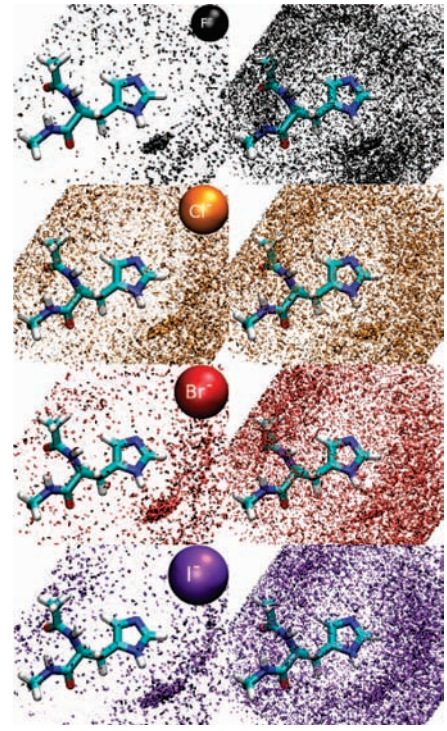

**Figure 4.** Density distribution of fluoride (black), chloride (gold), bromide (red), and iodide (violet) anions around aqueous terminated deprotonated histidine. Results for nonpolarizable force field (left column) and polarizable force field (right column).

were accounted for using the particle mesh Ewald procedure.[21] All bonds involving hydrogen atoms were constrained using the SHAKE algorithm.[22]

A trajectory can be used for sampling purposes only if the system is in thermodynamic equilibrium. To have such a system for propagation, the following steps were taken. First, the potential energy of the prepared system was minimized using 1000 steps of a steepest descent minimizer in order to prevent close contacts during the subsequent dynamics. Next, random velocities were generated according to the Maxwell−Boltzmann

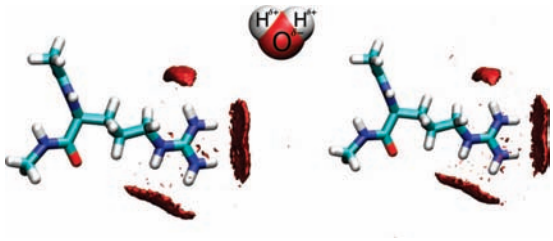

**Figure 5.** Density distribution of water oxygens (red) around aqueous terminated arginine. Results for nonpolarizable force field (left column) and polarizable force field (right column).

velocity distribution for initial temperature of 10 K. Temperature was then gradually increased to 300 K and isotropic pressure of 1 atm was applied to the unit cell. The emerging canonical ensemble was then equilibrated for 500 ps. Finally, propagation of such a system in thermodynamic equilibrium served for sampling of the phase space. The time step was 1 fs, the coordination output was saved every 1 ps, and the propagation time was 50 ns, which provided 50 000 frames for analyzing purposes. Such a simulation length was sufficient to provide convergence of averaged quantities. This was checked by performing significantly longer (100 ns for polarizable potential and up to 500 ns for nonpolarizable force field) for arginine with fluoride.

A most detailed analysis of the resulting trajectories is provided by density plots. This is done by first reorienting the analyzed trajectory with respect to the positively charged side chain group of the amino acid. Next, density maps of halide anions and water molecules were generated by plotting (dotting) out the positions of halides and water in every frame. To visualize results of this analysis, maps for chosen isodensity values, 0.006 for ions and 0.125 for water (the number of the latter species being 150 times higher), were produced. This 3D information was further reduced to 1D by evaluating distribution functions and cumulative sums (the latter being the integral of the former) for ions in the vicinity of the amino acids. Finally contact times, i.e., fractions of the simulation time (in percent) a given type of ion spent in the vicinity (i.e., within the region of the first peak of the distribution function) of the charged group of the amino acid were determined.

## 3. Results

Density plots showing distributions of halide ions around aqueous positively charged terminated amino acids (Arg, Lys, and His), averaged over 50 ns MD simulations, are presented in Figures 1−3. For comparison, analogous distributions in the vicinity of neutral (deprotonated) histidine are provided in Figure 4. The picture emerging from our simulations is clear and robust. Namely, fluoride exhibits a strong affinity of the positively

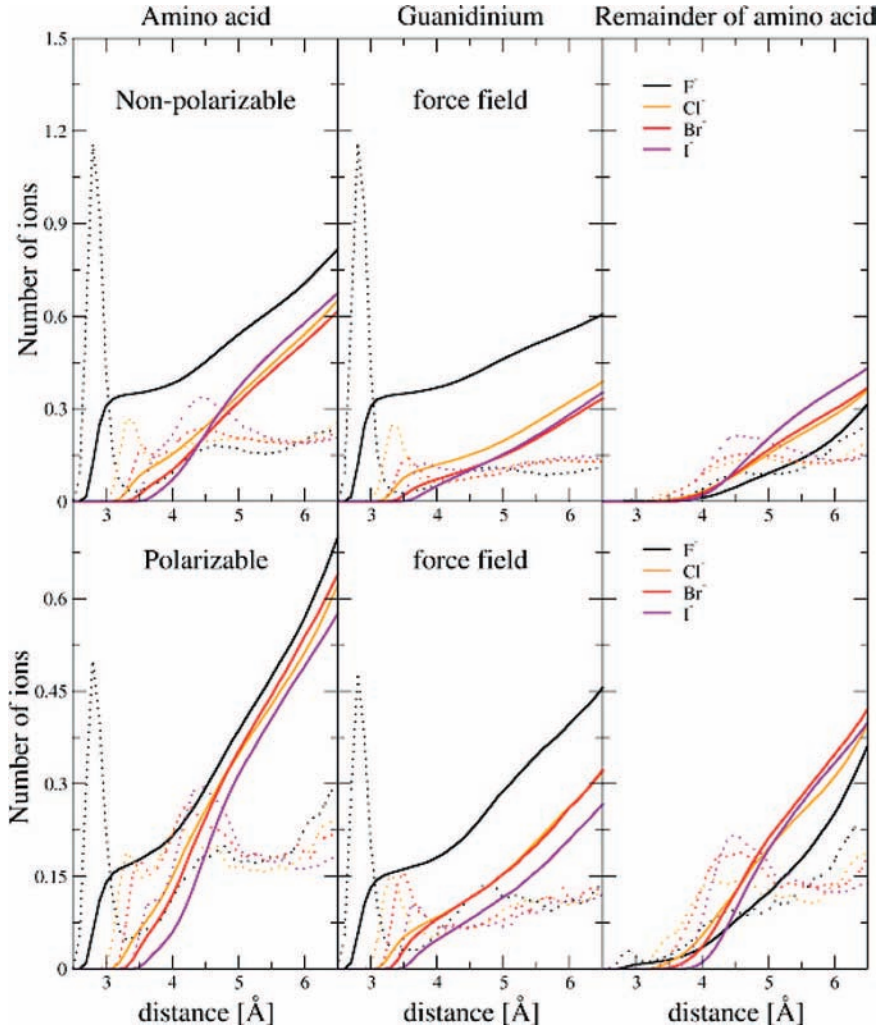


**Figure 6.** Cumulative sums and their derivatives (i.e., distributional functions) for halide anions in the vicinity of aqueous terminated arginine, its guanidinium group, and the remainder.

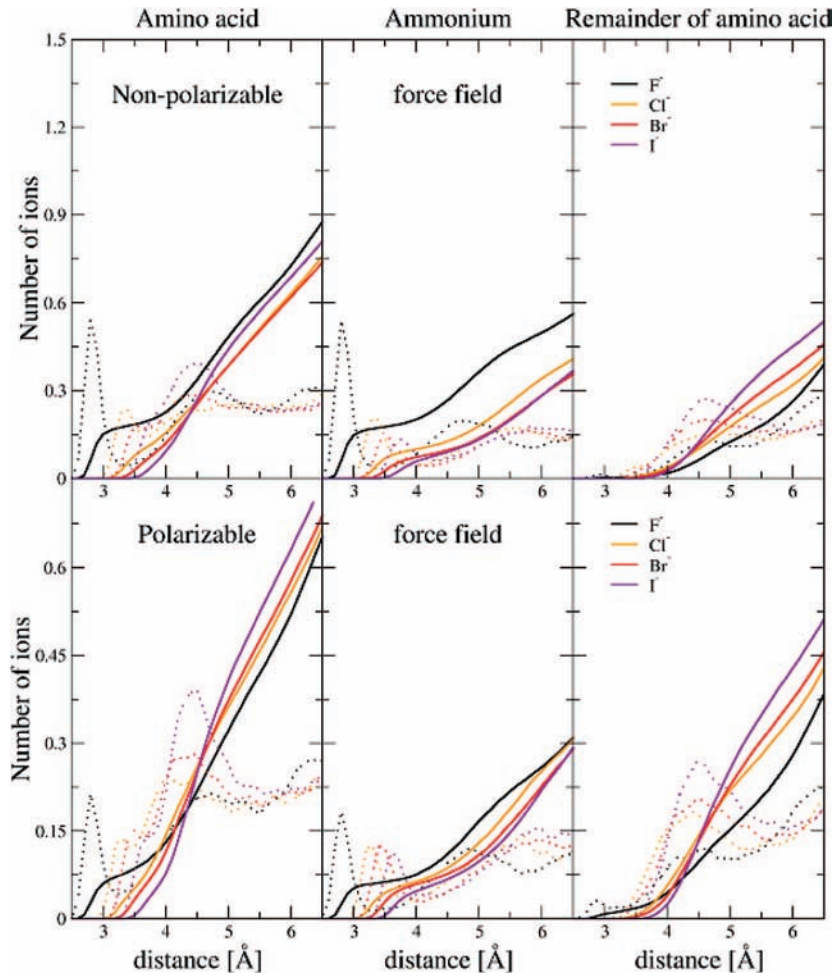


**Figure 7.** Cumulative sums and their derivatives (i.e., distributional functions) for halide anions in the vicinity of aqueous terminated lysine, its ammonium group, and the remainder.

charged side chain groups (i.e., guanidinium, ammonium, or imidazolium), whereas the interaction of heavier halides with these groups is significantly weaker. Although $F^-$ binds exclusively to the cationic groups, $Br^-$ and $I^-$ exhibit a certain affinity also for nonpolar regions of the terminated amino acids. Additionally, halides interact with the acidic hydrogen of the amide group. Qualitatively, ion specificity among halides is present in simulations with both nonpolarizable and polarizable force fields; quantitatively, the ion−amino acid binding is stronger in the former case.

The behavior of halide anions in the vicinity of neutral deprotonated histidine (Figure 4) is very different from the situation for charged amino acids (Figures 1−3). The neutral amino acid is not able to attract significant amounts of halide anions from the aqueous solution, with the only region of appreciable interaction with anions being the acidic imidazole hydrogen.

It is instructive to compare distributions of halide anions around positively charged amino acid residues to that of the water solvent. To this end, Figure 5 shows water density plot around arginine, the picture being similar also for the remaining positively charged amino acids. Note that the shape of water distribution closely resembles that of fluoride; however, the affinity for the positively charged groups is almost an order of magnitude weaker (as deduced from the isodensity values). Water molecule is isoelectronic to fluoride and has a comparable size. The pattern of affinity of its oxygen toward positively charged groups is, therefore, similar to that of $F^-$. Nevertheless, the water molecule bears only a dipole, whereas fluoride has a full charge, which explains the difference in the strengths of interactions with cationic groups. Finally, note that the results for water densities around positively charged amino acid side chain groups are very similar for nonpolarizable and polarizable force fields.

The 3D information provided in the density plots can be reduced to 1D by integrating over angular coordinates, which provides radial distributions of ions around charged and other groups of the amino acids. These distributions can be then integrated yielding cumulative sums of ions within a certain distance from the amino acid. The cumulative sums and derivatives thereof (i.e., unnormalized distribution functions) for halides in the vicinity of aqueous Arg, Lys, and protonated as well as deprotonated His are shown in Figures 6−9. Fluoride is the only halide that exhibits a strong peak in the distribution functions, corresponding to an appreciable enhancement of this ion next to a positively charged amino acid. A decomposition to contributions from different parts of the amino acid shows that this enhancement of $F^-$ is exclusively due to interactions with the positively charged side chain groups. Also in terms of cumulative sums, fluoride is the clear winner with heavier halides exhibiting a very weak affinity for investigated amino acids. Moreover, for heavier halides ($Br^-$ and $I^-$ in particular) this small affinity is shared by both charged and nonpolar parts of the amino acids.

Quantitatively, fluoride interacts most strongly with the guanidinium group of Arg, followed by the imidazolium group

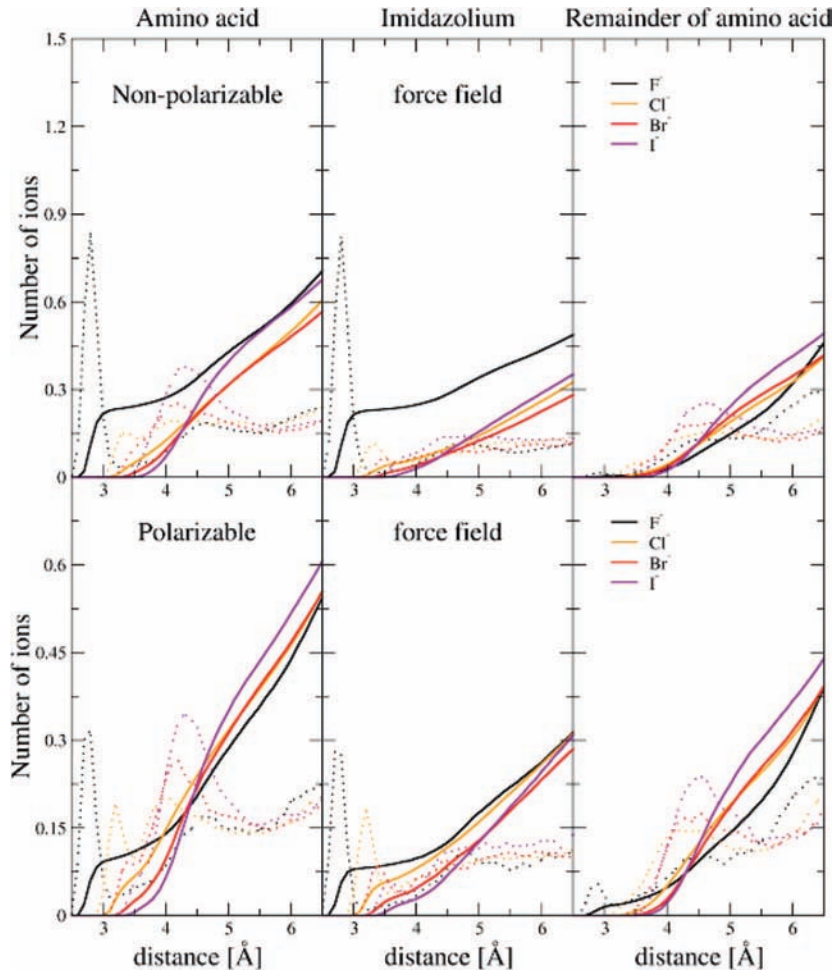


**Figure 8.** Cumulative sums and their derivatives (i.e., distributional functions) for halide anions in the vicinity of aqueous terminated protonated histidine, its imidazolium group, and the remainder.

of (protonated) His, and the ammonium group of Lys. This affinity is present for both nonpolarizable and polarizable potentials being, however, weaker by about a factor of 2 in the latter case. Compared to positively charged residues, the interaction of halides with neutral deprotonated His is much weaker and, consequently, ion specificity is significantly reduced, too.

Information obtained from simulations can be further reduced to 0D by monitoring the residence times of different halides in the vicinity of a given amino acid (defined as the region of the first peak of the 1D distribution functions). These residence times, expressed as fractions (percents) of the total simulation time are presented in Tables 1−4. For nonpolarizable force fields, fluoride is the clear winner among the halides for all positively charged amino acid residues, with the dominant part of the halide−amino acid affinity being due to the charged side chain groups. Upon including the polarization term interaction of $F^-$ with amino acids is reduced. Such a reduction is not necessarily present for heavier halides, where affinity for nonpolar parts of the amino acid surface can come into play and is enhanced by polarization effects. This leads to a situation where the overall affinity for the amino acid surface can be higher for iodide than for fluoride, which is caused by a sizable propensity of the former ion for the interface between water and nonpolar groups when using a polarizable force field.

Finally, we have compared the performance of the present potential for fluoride with another widely used force field for this anion.[23] The results presented in Figure 10 show for both nonpolarizable and polarizable calculations a small decrease of the affinity of $F^-$ for arginine, which is connected with the slightly larger van der Waals size of the second fluoride model. Nevertheless, even this larger fluoride is still significantly smaller than chloride (as it should be) and continues to exhibit the strongest affinity among the halides for the positively charged amino acid residues.

## 4. Discussion and Conclusions

Detailed analysis based on present simulations provides a clear quantitative picture of ion-specific interactions of halides with basic amino acid residues. On one hand, fluoride exhibits an appreciable affinity for positively charged groups, while that of heavier halides is much weaker. Among the investigated side chain groups the order of binding to $F^-$ is guanidinium > imidazolium > ammonium > (neutral) imidazole. For ammonium, the ion-pairing preference of small over large halides is also supported by measurements of osmotic and activity coefficients (interestingly, the ordering of halides reverses upon alkylation of the ammonium cation).[24] On the other hand, iodide and to a lesser extent also bromide and chloride are weakly attracted to nonpolar regions of amino acids. This ion-specific behavior in the vicinity of basic amino acids is qualitatively the same in simulations employing both nonpolarizable and polarizable force fields, with ion-pairing being stronger in the former and ion affinity for nonpolar regions in the latter case.

Interactions with positively charged groups are of a local character and are not overwhelmingly strong—even fluoride

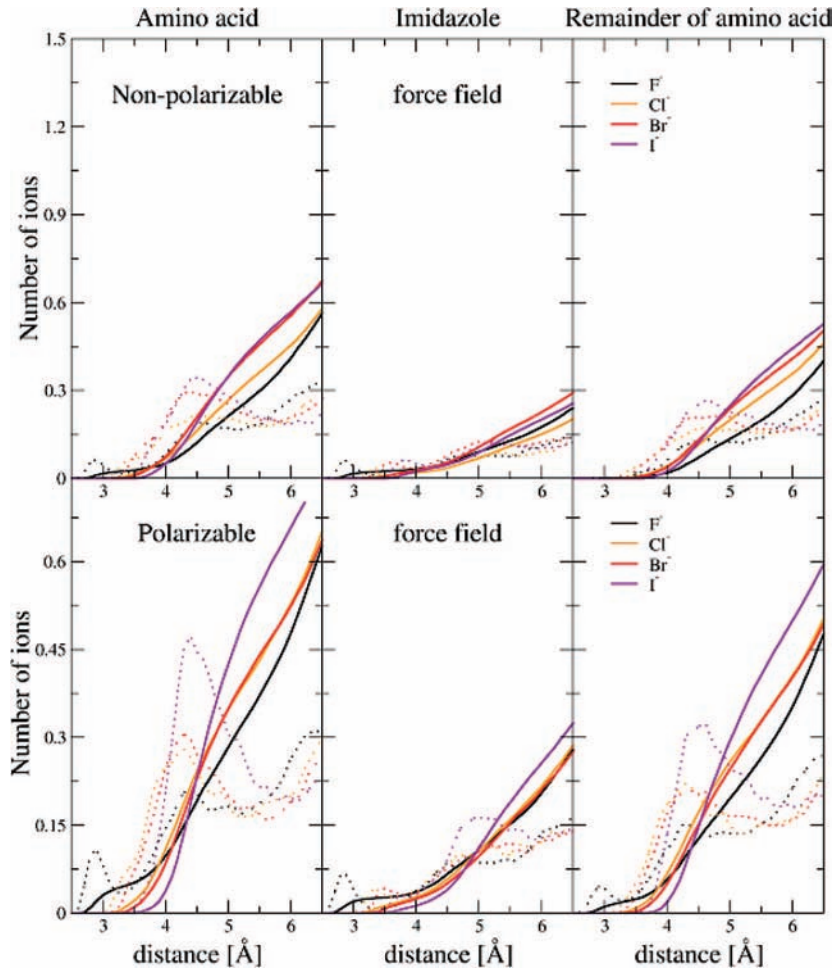


**Figure 9.** Cumulative sums and their derivatives (i.e., distributional functions) for halide anions in the vicinity of aqueous terminated deprotonated histidine, its imidazole group, and the remainder.

**TABLE 1: Residence Times (in percent of the total trajectory) of Halides in the Vicinity of Terminated Arginine and Its Guanidinium Group**

| | amino acid nonpolarizable | guanidinium nonpolarizable | amino acid polarizable | guanidinium polarizable |
|---|---|---|---|---|
| fluoride | 34 | 34 | 17 | 16 |
| chloride | 10 | 9 | 8 | 6 |
| bromide | 7 | 6 | 8 | 7 |
| iodide | 10 | 6 | 8 | 5 |

**TABLE 2: Residence Times (in percent of the total trajectory) of Halides in the Vicinity of Terminated Lysine and Its Ammonium Group**

| | amino acid nonpolarizable | ammonium nonpolarizable | amino acid polarizable | ammonium polarizable |
|---|---|---|---|---|
| fluoride | 17 | 17 | 8 | 6 |
| chloride | 10 | 8 | 7 | 5 |
| bromide | 8 | 6 | 8 | 5 |
| iodide | 13 | 7 | 11 | 5 |

**TABLE 3: Residence Times (in per cent of the total trajectory) of Halides in the Vicinity of Terminated Protonated Histidine and Its Imidazolium Group**

| | amino acid nonpolarizable | imidazolium nonpolarizable | amino acid polarizable | imidazolium polarizable |
|---|---|---|---|---|
| fluoride | 24 | 23 | 11 | 8 |
| chloride | 7 | 5 | 8 | 6 |
| bromide | 6 | 3 | 6 | 4 |
| iodide | 11 | 4 | 10 | 3 |

**TABLE 4: Residence Times (in percent of the total trajectory) of Halides in the Vicinity of Terminated Deprotonated Histidine and Its Imidazole Group**

| | amino acid nonpolarizable | imidazole nonpolarizable | amino acid polarizable | imidazole polarizable |
|---|---|---|---|---|
| fluoride | 2 | 2 | 5 | 2 |
| chloride | 3 | 1 | 3 | 2 |
| bromide | 5 | 2 | 5 | 2 |
| iodide | 8 | 3 | 7 | 2 |

anions, which exhibit the strongest interaction, frequently exchange positions in the vicinity of the amino acid and in the bulk. Therefore, additivity can be invoked and analogous ion-specific behavior of halides is also being observed at surfaces of aqueous proteins and models thereof.[15,25] The overall halide effect there is than a net result of direct ion-pairing with positively charged side chains, where small anions like fluoride win, and affinity for nonpolar regions of protein surface, which is larger for big soft anions, such as iodide. These ions show an affinity for hydrophobic interfaces due to their size (cavitation effect) and polarizability.[26] As a recently studied example of halide−protein interactions, association and solubility of a highly positively charged protein lysozyme in aqueous alkali halide solutions has been quantified by experiments [27−29] and simulations.[25] Both approaches show a stronger effect of heavier halides (bromide or iodide) over a smaller halide (chloride) on lysozyme association. Present calculations allow interpreting this observation in terms of the affinity of heavier halides for

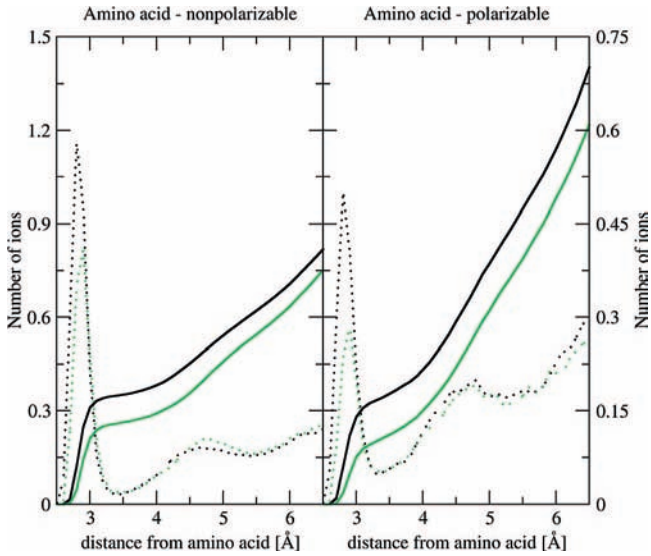


**Figure 10.** Comparison of the presently employed (black) and another widely used (green) model of fluoride. Cumulative sums and their derivatives (i.e., distributional functions) for fluoride in the vicinity of aqueous terminated arginine employing both nonpolarizable and polarizable potentials.

nonpolar surface patches of lysozyme overwhelming the effect of direct ion pairing preferred by smaller halides.

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