Specific Ion Effects at Protein Surfaces: A Molecular Dynamics Study of BPTI and HRP in Selected Salt Solutions

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Abstract

The distribution of sodium, choline, sulfate, and chloride ions around two proteins, horseradish peroxidase (HRP) and bovine pancreatic trypsin inhibitor (BPTI) is investigated by means of molecular dynamics simulations with the aim to elucidate ion adsorption at the protein surface. Although the two proteins under investigation are very different from each other, the ion distributions around them are remarkably similar. Sulfate is always strongly attached to the proteins, choline shows a significant, but unspecific propensity for the protein surfaces, sodium ions have a weak surface affinity, while chloride has virtually no preference for the protein surface. In mixtures of all four ion species in protein solutions, the resulting distributions are almost a superposition of the distributions of sodium sulfate and choline chloride, except that sodium partially replaces choline close to the proteins. The present simulations support a picture of ions interacting with individual ionic and polar amino acid groups rather than with an averaged protein surface. The results thus show how subtle the so-called Hofmeister and electroselectivity effects are in salt solution of proteins making all simplified interaction models questionable.

Introduction

Ion effects in biological systems have been investigated for more than a century. In the 1880s and 1890s Hofmeister and co-workers published a series of papers in which they showed that salts could be classified according to their salting-in and salting-out behavior against proteins.[1] They found similar series also for other effects in biological systems, e.g., the swelling of tissues. It is remarkable that they forwarded explanations for this series that involve ion-water interactions and that these explanations are qualitatively still valuable.

During the following decades numerous examples of salt classifications similar to the so-called Hofmeister series were discovered, not only for biological systems but also for various interfacial properties such as surface tension.[2] Several questions arise and are often discussed in the literature. Is the Hofmeister series strictly related to interfaces? If so, can a protein be approximated as an entity with a well-defined surface? In most papers where ion-protein effects are studied, the interactions turn out to be highly specific. For example, about a decade ago, Chakrabarti wrote a review in which he analysed the binding of sulfate and phosphate ions in 34 different protein structures.[3] He listed in detail which amino acids are involved in the binding process and even gave the probable geometries of the amino acid-ion configurations and the hydrogen bonds between the ion and the amino acids, as well as between ions and the surrounding water molecules.

The subtleties concerning the balance between different effects emerge from the papers by Arakawa, Timasheff and co-workers.[4-8] In most systems proteins are indeed stabilized by salting-out ions and destabilized by salting-in ions, but sometimes preferential hydration does not stabilize the native structure of proteins, as it would be supposed according to the Hofmeister series. Moreover, these effects

seem to crucially depend on the salt concentration, the nature of the proteins (basic or acidic), the pH values, etc. It should be noted that these authors used techniques such as densitometry and inferred interaction parameters using relatively simple models.Thus, it seems difficult to conclude about general rules. Curtis et al.[9,10] suggest an appealing interpretation of these apparently contradicting results. They forward a competition between Hofmeister and electroselectivity effects. The direct, lyotropic Hofmeister series concerns the structuring of the water molecules around the ions, which in turn leads to a stabilization or destabilization of the proteins by salting-out and salting-in ions, respectively. By contrast, the electroselectivity series reflects the affinity of the ions for an anion-exchange resin. For monovalent ions the electroselectivity series is equivalent to the inverse Hofmeister series.

For sulfate ions the apparently contradictory behavior towards different proteins could be explained as follows: if the sulfate interaction with the protein "surface" is week, sulfate strengthens the water structure and acts as a stabilizing salting-out ion according to the direct lyotropic Hofmeister series. By contrast, if the sulfate ions can bind to the protein, the protein behaves like an anion-exchange resin and the sulfate ion must be classified according to the electroselectivity series. And indeed the electroselectivity series is often found to be the adequate description of ion-protein interactions.[11-13] From the literature data it seems that at low salt concentrations the electroselectivity series is prevailing, whereas at higher salt concentration a competition is found between the two counteracting effects that are at the origin of the two series. This was already discovered and discussed in details in 1911 by T. B. Robertson.[14] Finally, it should be noted that the series are not always linear. In some cases even a "bell-shaped" Hofmeister series was found.[15]

As mentioned above, simple models and interaction parameters are often used to describe these very complex and subtle effects.[4,9] A somewhat more advanced

approach is attempted by Ninham, Boström et al.[16] who argue that to the first approximation a protein can be considered as an object having a certain surface and that the interactions between the ions and that surface can be described by electrostatic and dispersion forces. In average, the ions thus "see" a more or less homogeneous surface. Here, specificity comes in mainly through the frequency dependent polarizability of the ions, but important effects such as the granularity of the solvent are neglected. In this way the authors could predict a Hofmeister series both for ions near to proteins and water/air interfaces. However, a simultaneous description of bulk activity coefficients and surface tension based on this model failed.[17] It is interesting that Arakawa and Timasheff[4-8] and Curtis et al.[9,10] also try to make a relation between ion-protein interactions and the surface tensions of ions in aqueous solutions. But in the light of the results of Ref. 17, such an attempt is questionable.

In the present paper we are attempting to shed more light on these questions by considering a molecular level of description. A further motivation comes from a preceding experimental study where we investigated the separate and combined effects of several ions on the enzymatic activity of HRP.[18,19] In the following, molecular dynamics simulations of HRP or BPTI in a periodic box of an aqueous salt solution are presented. We investigate solutions of choline chloride, choline sulfate, sodium chloride, sodium sulfate, as well as their mixture. The simulations provide the distributions of individual ionic species in the vicinity of the protein "surface" and give information about ion specific adsorption to different amino acid residues.

Computational methodology

All molecular dynamics simulations were carried out using the AMBER 8

software package[20] with the PARM99 force field[21]. Polarization interactions were not included in most of the runs for the following reasons: i) the employed protein force field implicitly assumes the use of non-polarizable water and ions, ii) polarization effects were shown to be of minor importance for cations (such as sodium) and multiply charged anions (such as sulfate)[22,23], and iii) inclusion of polarization interactions significantly slows down the calculations. Moreover, test polarizable calculations (see below) showed that the behavior of the ions in the vicinity of the protein did not change qualitatively (if anything the ion propensity for the protein surface increased), indicating that polarization effects are smaller in the aqueous bulk and close to the protein than, e.g., at the air/water interface.[22] After minimization and heating procedures, molecular dynamics (MD) simulations consisted of 0.5 ns equilibration and 1 ns production runs. To check for convergence, representative simulations were extended up to 10 ns. Temperature and pressure were held constant at 300 K and 1 atm.

The BPTI protein structure was downloaded from the PDB database (code 6PTI)[24]. It was protonated according to pH 7 and then solvated in a box of 2184 SPC/E water molecules[25] with approximate dimensions 41 x 45 x 45 Å^3. The resulting net positive charge of the protein (+6) was compensated by 6 chloride anions. A similar procedure was applied to the HRP protein structure (PDB code 1ATJ)[26] that was solvated in a box of 8304 SPC/E water molecules with approximate dimensions 57 x 79 x 68 Å^3. The heme moiety, as well as the two Ca^{2+} cations in crystallographic positions were retained. The remaining protein net charge (+3) was compensated by 3 chloride anions. In both cases, at least 13 Å of water separated the periodic images of the protein.

The following systems were created using combinations of various anions and cations, distributed initially randomly within the simulation cell (concentrations are

approximate and the compensating Cl^- anions are not presented):

A: BPTI or HRP with 0.8 or 0.5 M choline chloride (32 or 70 ion pairs, respectively),

B: BPTI or HRP with 0.8 or 0.5 M sodium chloride (32 or 70 ion pairs, respectively),

C: BPTI or HRP with 0.4 or 0.25 M choline sulfate (32 choline and 16 sulfate ions, or 70 choline and 35 sulfate ions, respectively),

D: BPTI or HRP with 0.5 or 0.25 M sodium sulfate (40 sodium and 20 sulfate ions, or 70 sodium and 35 sulfate ions, respectively),

E: BPTI or HRP with 0.8 or 0.5 M choline chloride and 0.4 or 0.25 M sodium sulfate.

The resulting trajectories were analyzed in terms of the occurrence of different ions in the vicinity of the protein using scripts developed in our group for the VMD program.[27] The number of ions located within a specified distance of the protein was counted for a range of distances, providing information about ionic distribution and affinity of ions to the protein as a whole. This analysis provided the cumulative sum plots (dashed lines in Figs. 1-5). A cumulative sum is the total number of ions of a given type that can be found within a certain distance of the protein, with zero distance corresponding to the protein surface. In the limit of a large distance, the cumulative sum reaches the total number of ions of a given type in the simulation cell. Differentiation and subsequent normalization (division by the total number of ions of a given type) of these cumulative sums provides the distribution curves (solid lines in Figs. 1-5).

A detailed analysis of specific interactions of ions with different types of amino acid residues was also performed. To this purpose the total length of contacts between individual protein residues and different ions was extracted. The contact was defined as a situation where an ion is found within 3.5 Å of a given protein residue.

The surfaces of the HRP and BPTI proteins were compared by means of their amino acid content. The solvent accessible surface area (SASA) analysis was

performed using a spherical probe with a radius of 1.5 Å. All amino acids with SASA larger than 50 $Å^2$ were taken into account. Note that the higher the SASA value the more the residue is exposed to the surrounding aqueous medium and ions.

All trajectories were checked in terms of energetics and root mean square deviations (RMSD) from X-ray structures. Energy profiles of production runs indicated well equilibrated systems. The RMSD of the final structures relative to the starting (X-ray) geometries were found to be around 1.3 Å (backbone only), or 2.3 Å (all atoms). For HRP the heme moiety and both calcium cations retained their initial positions during the simulations.

Results

Distribution functions and cumulative sums

In the following, the term "protein surface" is frequently used. By this we understand all protein atoms that are accessible to the surrounding aqueous solution. For simplicity, we also employ the term "ion binding to the protein", which in this context does not imply any chemical bond but rather an affinity of a particular ion for the protein surface due to electrostatic and van der Waals interactions. The distribution of ions around the BPTI and HRP proteins for all five solutions described in the previous section was analyzed. Cumulative sums as well as distribution functions (i.e., differentiated cumulative sums) are displayed in Figures 1 to 5. In order to show the distribution functions and the cumulative plots on a single plot, the former were scaled up by a constant factor.

It can be clearly seen from the ion distribution plots for systems with choline chloride (systems A; Fig. 1), that choline exhibits certain affinity to the protein

surface (see the peaks of the distribution function at 2.2 Å), both for BPTI and HRP. A diffuse second layer of the choline cations can be observed at approximately 5 Å. Note that, despite the fact that both proteins are positively charged, there is practically no preferential binding of Cl^- anions to the protein surface.

Interestingly, when sodium replaces choline as counter cation to chloride (systems B), virtually no preference of ions for the protein surface is observed in the case of BPTI (Fig. 2a). Na^+ cations exhibit a certain surface preference for HRP (Fig. 2b) which is, however, weaker than the preference for choline.

The situation changes dramatically when sulfate is used as a counter anion for choline instead of chloride (systems C; Fig. 3). The choline signal remains roughly the same as for the choline chloride solution, however, sulfate exhibits a very strong affinity for the protein surface, which is reflected in the first ion peak at 1.8 Å. As in the previous simulations, no strong surface preference of the Cl^- anions, which compensate the positive charge of the proteins, is observed (distribution curves for chloride are not presented in order not to obscure the plots).

In a sodium sulfate solution (systems D; Fig. 4), sulfate again exhibits a strong propensity for the protein surface, while Na^+ shows a relatively weak preference for the surface. As before, the charge compensating chloride anions are found to have no preference for the surface and their distribution curves are omitted to simplify the plots.

Finally, both proteins were solvated in a mixture of aqueous choline chloride and sodium sulfate (systems E; Fig. 5). For BPTI, the strongest affinity to the surface can again be observed for sulfate anions. Although the shapes of curves for choline and Na^+ are somewhat different, it is safe to say (also according to the cumulative sum) that close to the protein, both of them are populated roughly equally. Situation is slightly differenr for HRP, where sulfate still exhibits a very high affinity to the

protein surface, however, the highest peak belongs to sodium cations. Note that sodium is the only ion for which BPTI and HRP do not show the same behavior. Finally, chloride anions do not exhibit any strong propensity for the surface in either case.

To check for convergence of the present results we extended the simulation length for the systems E by an order of magnitude to a total of 10 ns. The results from the long simulations are displayed in Figs. 5c and 5d. It can be seen that the plots are very similar to those corresponding to 1 ns simulations (Figs. 5a and 5b). Namely, the cumulative sums are within the statistical uncertainity the same for the short and long runs (the only exception being sodium in HRP, where the deviation is slightly larger).

Ion binding patterns

For choline, rather unspecific binding patterns were observed. As expected, these cations were found in the vicinity of the negatively charged ASP and GLU residues, but a substantial portion of the contacts could be assigned also to ARG, CYS, GLN, GLY, LEU, MET, PRO, PHE, SER, or TYR amino acids. The second cation used in our simulations, Na^+, showed some preference for ASP, ASN, GLU, and GLN. In accord with the ion distribution results presented above, contact times of choline (~30-70 % of the trajectory) are slightly larger than contact times of sodium cations (~30-50 % of the trajectory), i.e., choline has a higher affinity (also due to the larger variety of aminoacids it tends to interact with) to the protein surface than sodium and when adsorbed, it tends to remain at the surface for a longer time. Note that even large contact times do not necessarily imply long contiguous ion-protein interactions. The usual pattern is rather that of many binding and unbinding events during the simulation.

Chloride shows only a very weak preference for ARG and LYS residues, interactions with other amino acids are non-specific. This again corresponds with the observed low overall affinity of the Cl^- anions to the protein surface. Contact times – 10–25 % of the trajectory, are significantly smaller than values observed for sulfate (see below).

Sulfate ions show generally the strongest preference for ARG and LYS and exhibit very long contact times (~ 50-80 % of the trajectory). After careful inspection we also observed several contacts of sulfate with negatively charge residues that could be assigned to interactions mediated by Na^+ cations. However, these played only minor role in the overall statistics.

For BPTI, numerous single residue contacts between sulfate and ARG or LYS are observed. Moreover there is a strong contact between a sulfate and binding sites comprising of (TYR10, ARG39, and LYS41) or (ARG20, TYR35, and LYS46) residues.

The same analysis carried out for the HRP protein shows that sulfate ions preferentially bind not only to ARG and LYS (that are, however, the most common binding sites), but also to SER and THR. Apart from single residue contacts, sulfates are for a substantial portion of the simulation time located in some of the following binding sites: (ASN 231, LYS232, SER269, THR270, and GLN271) – see Fig. 6a, (ASN16 and ARG19), (ARG27 and SER28), (ASP150 and ARG153), (ASN198 and THR200, LYS241) - see Fig. 6b, and (ASN158 and ARG159), (VAL23 and ARG27) or (THR293 and GLN294).

Some of arginines and lysines were found to have interactions with two sulfates at the same time for a significant portion of the trajectory. The analysis also revealed that the interactions with backbone NH groups (e.g., BPTI - ASP3, ILE18, ALA40 and ARG42; HRP - LEU2, THR270, GLN271) play only minor role for

sulfate binding.

Extending the simulation length by an order of magnitude (up to 10 ns) almost did not change the results. Contact times of ions dropped slightly (choline ~30-50 %, sodium ~15-30 %, sulfate ~30-80 %, chloride ~5-20 % of the trajectory). These findings further indicate the convergence of the present results.

Discussion

BPTI and HRP are very different proteins both in size, shape, and in their amino acid sequences, with solvent accessible surface areas of 4052 and 13447 $Å^2$, respectively (see Tab. 1 for the comparison of the amino acid content of their surfaces). The respective protein surfaces contain 31 amino acids (of the total number of 57 BPTI residues) and 114 amino acids (of the 306 HRP residues), respectively. Nevertheless, the average ion distributions around the surfaces of the two proteins are very similar to each other. This is a striking result, which would seem to be in favor of a simplified protein surface model. However, interactions of ions with amino acids are specific, especially for sulfate ions. These specificities cannot be explained within a solvent-averaged model neither with simple electrostatic nor with additional dispersion interactions. The question is how "special" these specificities are. He and Quicho found a fundamental role of SER in a sulfate-binding protein[28] (see also Ref. 29). In our case there are sulfate-SER interactions in HRP, however, for BPTI there is a high sulfate propensity to the surface as well although no SER is at the protein surface. We, therefore, conclude that sulfate has several options to bind and that different groups of amino acids can serve as appropriate anchor sites. Of course, sulfate ions have also preferential contacts with the basic amino acids ARG and LYS and to a lesser extent to the less basic residues. In contrast to Chakrabarti,[3] who

postulated that sulfate ions should in general have a strong affinity to the peptide NH, we found that, although such interactions definitely exist, they are not dominant.

Note that sulfate-protein binding was also found in other systems.[13,14] As Robertson wrote,[14] sulfate can inhibit protein precipitation at low concentration, while enhancing precipitation at high concentration. This seems to be consistent with our results showing that a certain number of sulfate ions can bind to the protein. If the concentration is higher, the excess sulfate ions remain highly hydrated in the solution, thus favoring the salting-out of the protein. This argumentation is also in agreement with that forwarded by Curtis et al.,[9] who stated that at low concentrations these ions are salting-in, while at high concentrations they are salting-out.

Due to its lower charge chloride binding to the protein surface is weaker than that of sulfate. But electrostatics cannot be the whole story, since there is no strong attraction of chloride even though the proteins bear an overall positive charge. This is a further argument against a simplified protein surface model. Note that the weak interaction between chloride and proteins is in good agreement with the "Hofmeister neutral" effect of chloride on enzymatic activities.[21] Compared to the stronger ion binding of sulfate, behavior of chloride ions is also in accord with the electroselectivity series, as discussed in Refs. 11-13.

Concerning the cations, the propensity of Na^+ for the surface is rather low and is mainly limited to acidic residues. We note, however, that Na^+ can interact with basic amino acids (LYS and ARG) via sulfate ions. The role of sodium as a "Hofmeister neutral" ion for enzymatic activity is thus confirmed[3]. Choline binds more strongly, but non-specifically. The fact that choline is not a "Hofmeister neutral" cation is well known from literature. Choline chloride can increase[30] or decrease[18] enzymatic activities much more than NaCl. In our recent experimental work[18] it was shown that for the enzymatic activity of HRP choline had an antagonistic role to

sulfate and that the change of pH due to the presence of ions could not completely explain the effect. Whereas sulfate influenced the enzymatic activity via changes in the pH of the buffer solution, choline caused larger changes in the activity than could be inferred from the modification of the bulk pH. It is clear from the present simulations that choline and sulfate act on very different sites on the proteins. A possible rationalization of the experimental observations is that sulfate, although showing a strong interaction with the enzyme surface, tends to be far away from the active site of the enzyme, whereas choline, although weakly binding, is in a much closer contact with the active site, which contains more hydrophobic residues. Due to its partially hydrophobic character it is not surprising that choline interacts with hydrophobic amino acids.

The observed unspecific choline binding to HRP or BPTI seems to be different from that in molecules having specific choline receptors[31] or binding pockets[32]. In these cases, choline is supposed to bind strongly to aromatic amino acids via cation-π-interactions with electron rich aromatic rings. Although in the present simulations some loose contacts between choline and aromatic amino acids were found, they are not dominant. However, one should keep in mind that the simulations did not include a proper quantum mechanical description of the cation-π-interactions and, therefore, such interactions in the real systems cannot be excluded. But it is clear that these phenomena cannot be considered in solvent-averaged models. A further interesting effect may play a role: Garel *et al.*[33] argued that besides cation-π-interactions and hydrophobic interactions "the degree of freedom of the bound substrate may also play a significant role. A relatively loose association appears to be more favorable than a tight lock"[33]. This would be another argument for a rather unspecific binding of choline, although the contact times of choline with the surfaces are longer than those of Na^+.

In this context, another point seems worth discussing. In a recent paper[15] it was argued that both cosmotropic ions (e.g., sulfate) and chaotropic ones (such as choline) can have significant, but antagonistic influences on the active site of an enzyme. In the case of NADHase a cosmotropic ion may lead to a higher rigidity of the active site, resulting in a reduced activity of the enzyme, whereas chaotropic ions (in the NADH oxidase example, e.g., thiocyanate) has a similar consequence, but for an opposite reason - they may induce too high flexibility of the active site, and thus a decrease in the affinity of the enzyme for the substrate. Such a dynamical consideration is still a speculation that goes beyond the scope of the present simulations. Nevertheless, this argument would explain the high efficiency of choline to reduce the superactivity of HRP induced by the presence of sulfate.

Finally, we consider protein-ion interactions in the mixture of choline chloride with sodium sulfate around the two investigated proteins. In the case of the BPTI solution, the mixture mainly behaves like a superposition of the sodium sulfate and choline chloride solutions, except that choline is partially replaced by sodium close to the protein surface. This effect is similar, although more pronounced in the salt mixture of the HRP solution. After careful analysis, this behavior can be assigned to the partial replacement of choline cations near the ASP, ASN, GLU, and GLN residues by sodium cations (ASN 46, 153, 211, 267; ASP 7, 131, 149, 181; GLN 244, 293 for HRP and ASP 2, GLU 48 for BPTI). Changes for other types of amino acid residues are rather non-specific and do not significantly influence the overall ion distributions. It is also important to note that only some choline cations are replaced by Na^+; in some cases the choline binding stays intact or both cations exhibit contacts with the same residue in the course of the trajectory. Due to a decreased probability of choline binding to these highly polar residues, the relative preference of choline for nonpolar or hydrophobic residues slightly increases.

The influence of the different ions on the enzymatic activity of HRP cannot be fully explained from the present simulations. To do this, it would be necessary to consider all substrates, the influence of the ions on the substrates, the dynamics of substrate binding and buffer solutions with different pH values, and all this at a quantum mechanical level to properly describe bond breaking and making, a task that is impossible today. However, we have learned that ions bind quite individually and independently of the other ions on specific sites of the protein, as can be seen in the snapshots of the proteins with the surrounding ions (water molecules are omitted for clarity) given in Fig. 7. In this respect, the ion-protein interactions resemble more bulk interactions between different solute species than interactions between ions and (charged) surfaces. If this is general (what we cannot definitely conclude from the two single protein systems considered here), care must be taken not to transfer ion-surface interaction models to proteins.

To consider this point in more details, we also performed for a mixture of sodium sulfate and choline chloride simulations of the solution/vapor (rather than solution/protein) interface. In these calculations we explicitely included polarization interactions, since these interactions were recently found to be of a crucial importance for a proper description of ions at the air/water interfaces[35]. For a consistent comparison with the situation at a protein surface, we also rerun the simulations of BPTI in the mixture of both salts including polarizability into the force field.[36] The resulting ion distributions around BPTI are given in Fig. 8. It can be clearly seen, that results obtaine using a non-polarizable (Fig. 5a) and polarizable (Fig. 8) force field are very similar to each other. If anything, the inclusion of polarization results in a small increase of the surface peaks of all ions, however, the relative order of the peaks is retained.

The results concerning the solution/vapor interface together with a detailed

discussion will be published elsewhere.[34] Briefly, we mention here that the ion distributions are significantly different from those for the solution/protein interface. Namely, chloride exhibits an affinity for the solution/vapor interface, while choline is weakly repelled from the surface. Sodium is repelled and sulfate is very strongly repelled from the solution/vapor interface. Thus, the relative surface preferences of the studied ions are opposite at the solution/vapor and solution/protein interfaces. It follows from the present simulations that the latter should not be viewed as an interface between a high dielectric and low dielectric media, since the solute behavior is dictated by local interactions between ions and charged or polar amino acid groups.

Conclusions

The following conclusions can be drawn from the present simulations of BPTI and HRP proteins in selected salt solutions:

- sulfate is always strongly attracted to the protein surface. With sodium and even more so with choline as counter ion there is a relatively high sulfate concentration close to the protein surface,
- chloride is never strongly attracted by the protein, despite the fact that the investigated proteins are positively charged,
- both with chloride and sulfate as counter ions, choline and sodium cations are attracted to the protein surface. Surface binding of choline is in general stronger but less specific than that of sodium,
- a mixture of the four ions around the proteins behaves more or less as a superposition of the sodium sulfate and choline chloride solutions, with the exception that choline is partly replaced by Na^+,
- at least for the two proteins and the selected salt solutions considered here, the

ion-protein interactions can be better described as those of ions with individual charged or polar groups of the amino acids in aqueous bulk, rather than interactions of ions within a smooth interface between two phases.

- The affinity of ions to the protein surface is very different from their propensity to the solution/vapor interface. Moreover, the increase in surface tension of aqueous alkali sulfate solutions is linear over a wide concentration range, whereas the interaction of sulfate with proteins can depend on the salt concentration in a strongly non-linear fashion. Therefore, care must be taken when relating surface tension data to ion properties near proteins.
- It is hard to conclude about ion-protein interactions from enzymatic activities. For instance, it is remarkable that sulfate and choline have antagonistic roles on the enzymatic activity of HRP, but the reasons are very different for the two ions:[18] sulfate binds strongly to the protein surface, but its influence on the activity comes mainly from a change in the buffer pH. Choline binds weakly, but it may bind near to the active site so that its influence on the enzymatic activity is as strong as that of sulfate, however, acting in the opposite way.

It is likely that the phenomenon summarized under the term "specific ion effects" is in fact a multitude of different subtle effects,[19] and we are probably still far from the point where we can make generalizations in order to become predictive in a quantitative way. In our future work, a whole series of protein-salt solutions will be investigated by MD simulations in order to create a much broader database, allowing hopefully for further generalizations.

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Figure captions

Figure 1: Distribution functions and cumulative sums for a) choline chloride – BPTI surface and b) choline chloride – HRP surface.

Figure 2: Distribution functions and cumulative sums for a) sodium chloride – BPTI surface and b) sodium chloride – HRP surface.

Figure 3: Distribution functions and cumulative sums for a) choline sulfate – BPTI surface and b) choline sulfate – HRP surface.

Figure 4: Distribution functions and cumulative sums for a) sodium sulfate – BPTI surface and b) sodium sulfate – HRP surface.

Figure 5: Distribution functions and cumulative sums for choline chloride and sodium sulfate – BPTI surface (a) 1ns and c) 10ns trajectories), and choline chloride and sodium sulfate – HRP surface (b) 1ns and d) 10ns trajectories).

Figure 6: Sulfate binding pockets. a) (ASN 231, LYS232, SER269, THR270, and GLN271) and b) (ASN198, THR200, and LYS241).

Figure 7: Snapshots from MD simulations of the proteins in the mixture of aqueous choline chloride and sodium sulfate showing ions at the protein surface. a) BPTI and b) HRP (green: Na^+, orange: Cl^-).

Figure 8: Distribution functions and cumulative sums for choline chloride & sodium sulfate – BPTI surface in a simulation employing a polarizable force field.

.

Fig. 1

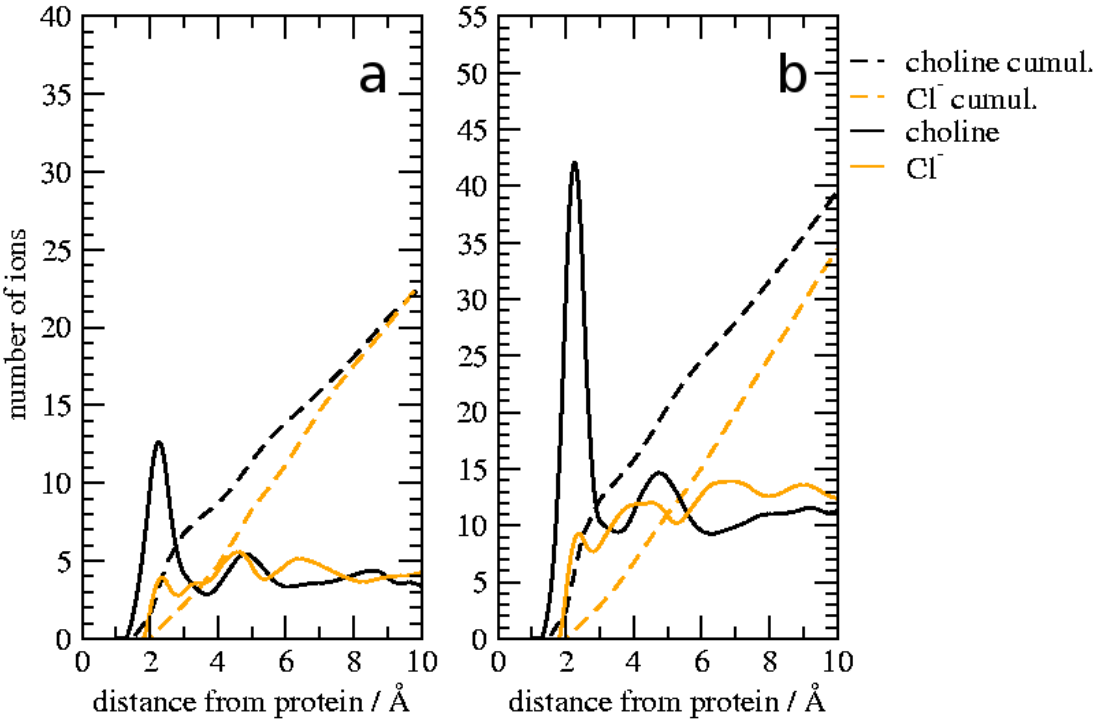
a
b
number of ions
distance from protein / Å
distance from protein / Å
choline cumul.
Cl⁻ cumul.
choline
Cl⁻

Fig. 2

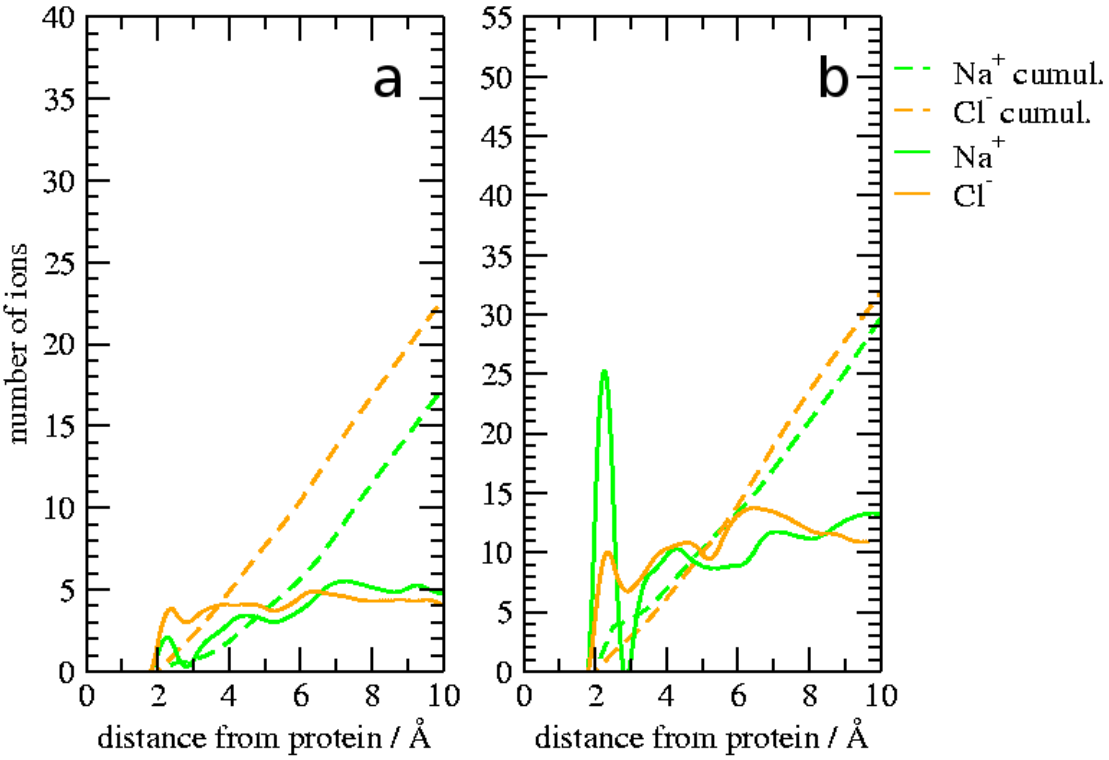
a
b
number of ions
distance from protein / Å
distance from protein / Å
Na+ cumul.
Cl- cumul.
Na+
Cl-

Fig. 3

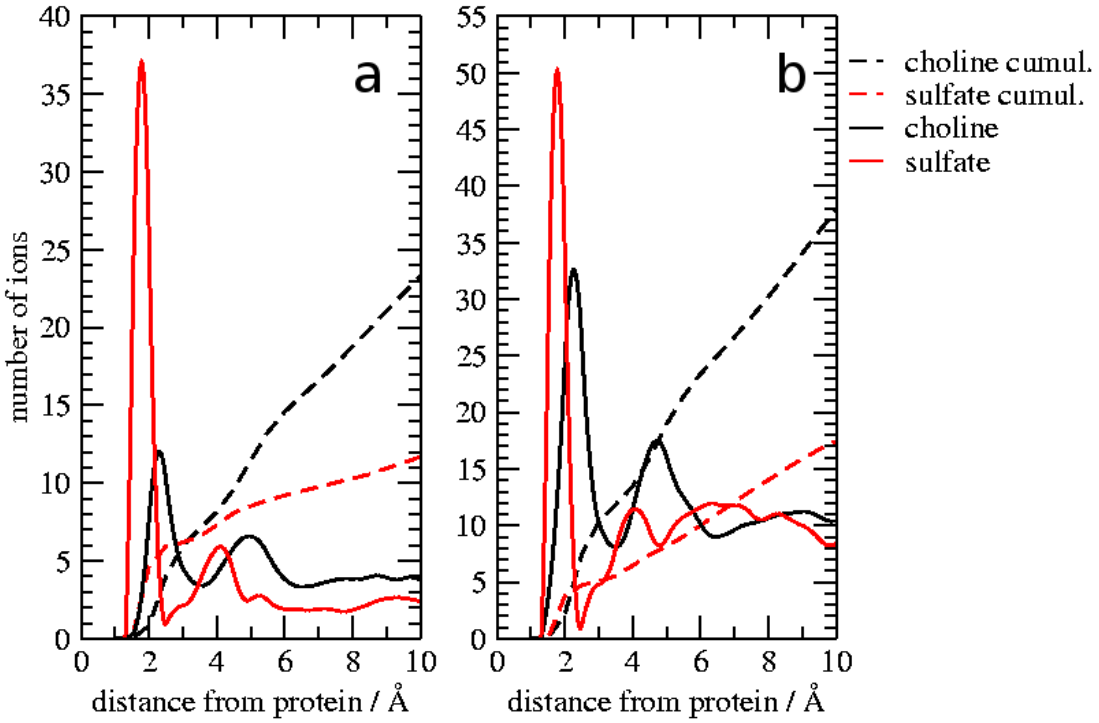
a
b
number of ions
distance from protein / Å
distance from protein / Å
choline cumul.
sulfate cumul.
choline
sulfate

Fig. 4

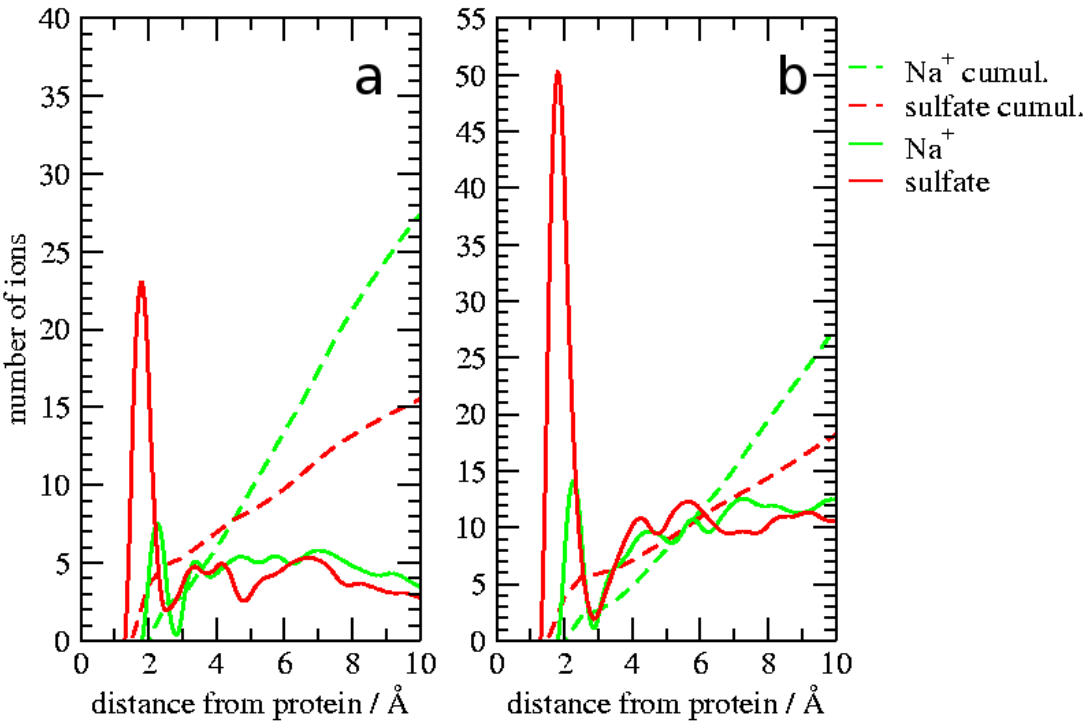
a
b
number of ions
distance from protein / Å
distance from protein / Å
Na+ cumul.
sulfate cumul.
Na+
sulfate

Fig. 5

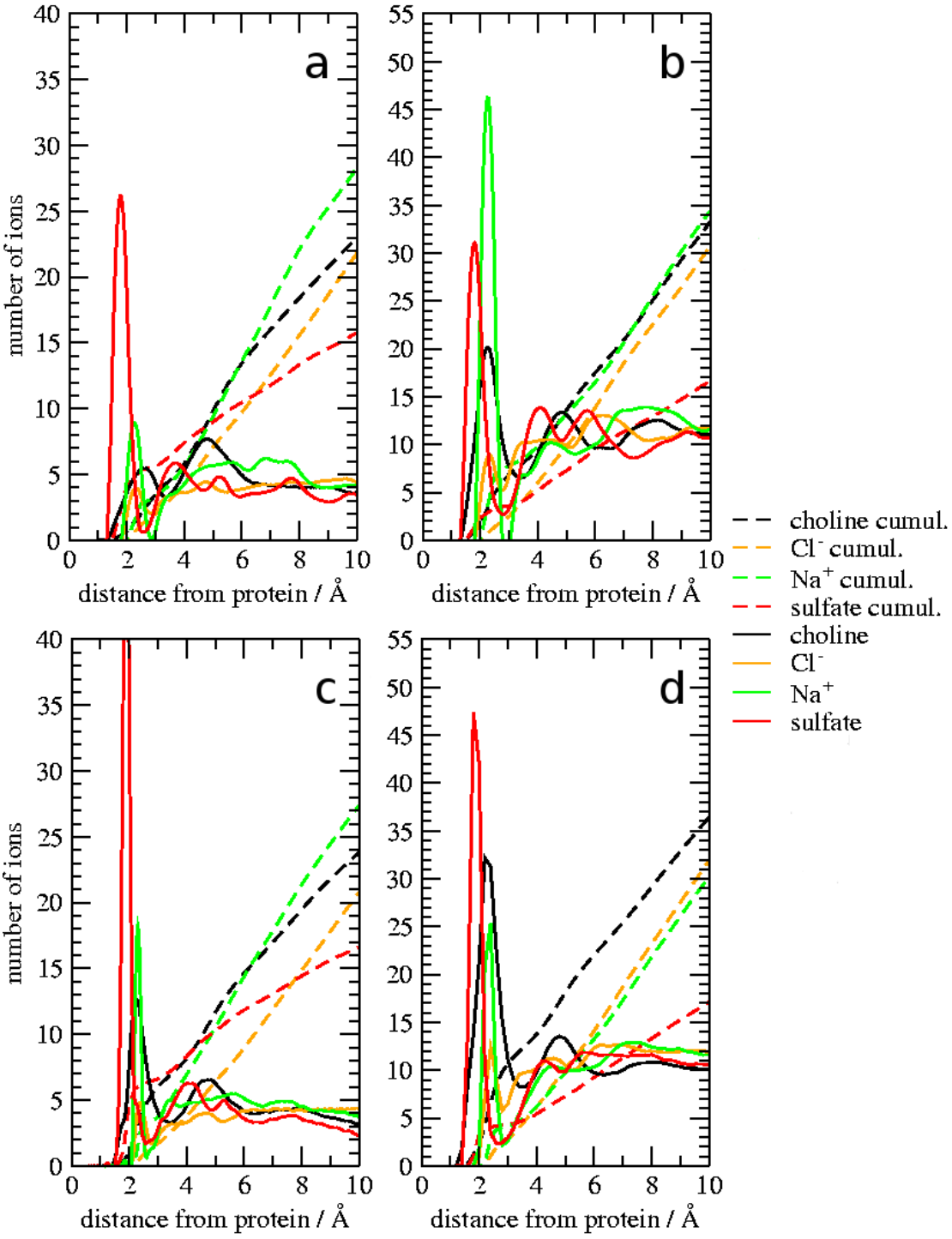
a
b
c
d
number of ions
distance from protein / Å
choline cumul.
Cl⁻ cumul.
Na⁺ cumul.
sulfate cumul.
choline
Cl⁻
Na⁺
sulfate

Fig. 6a

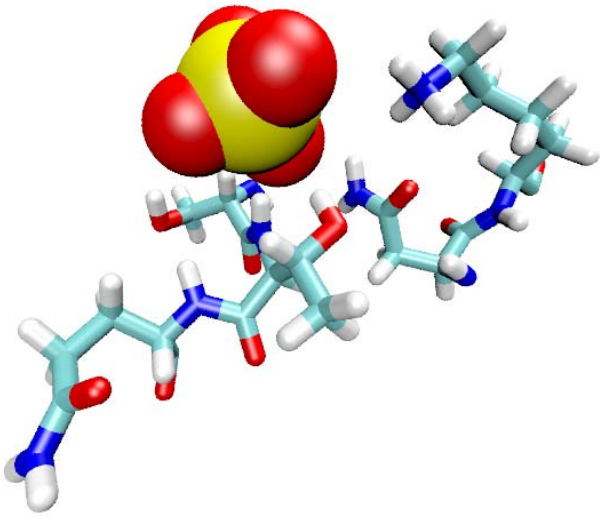

Fig. 6b

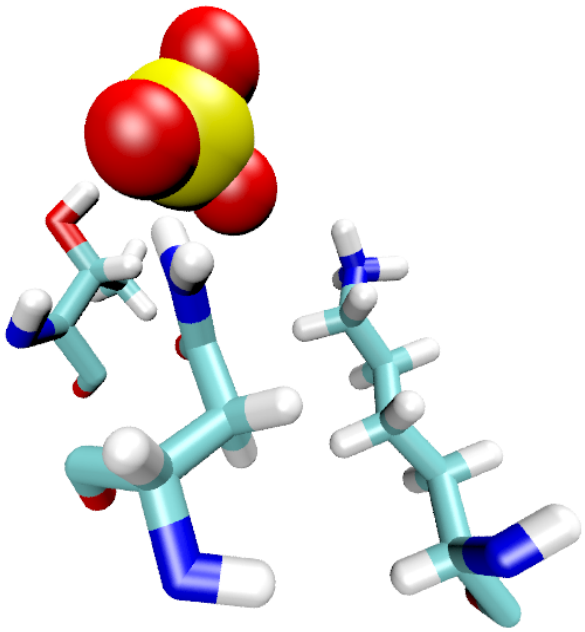

Fig. 7a

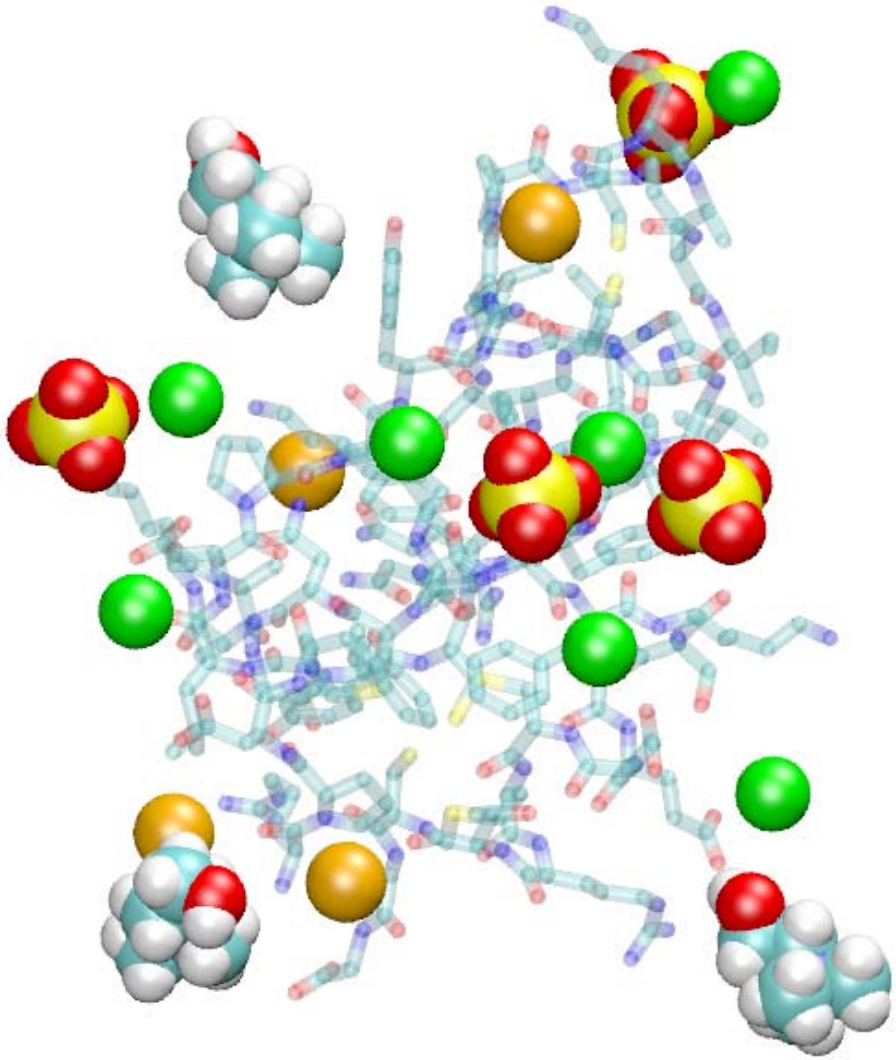

Fig. 7b

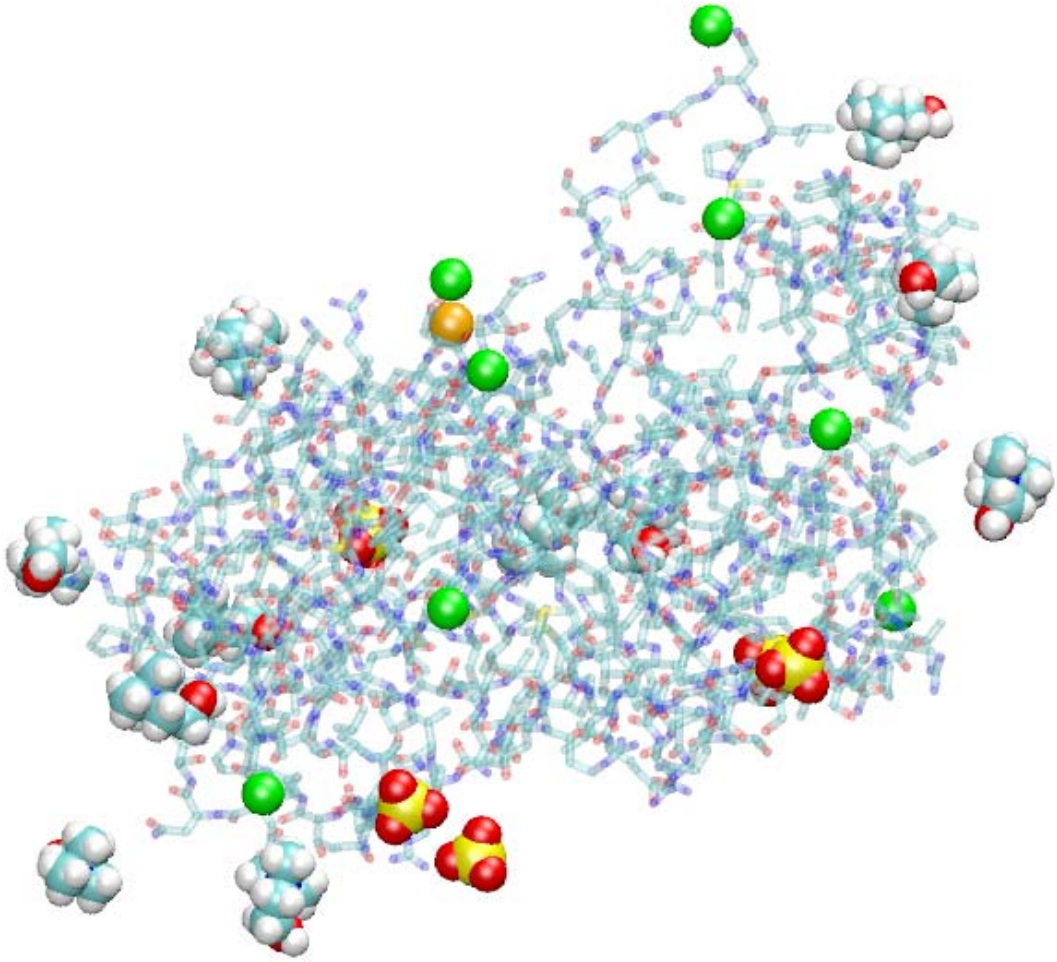

Fig. 8

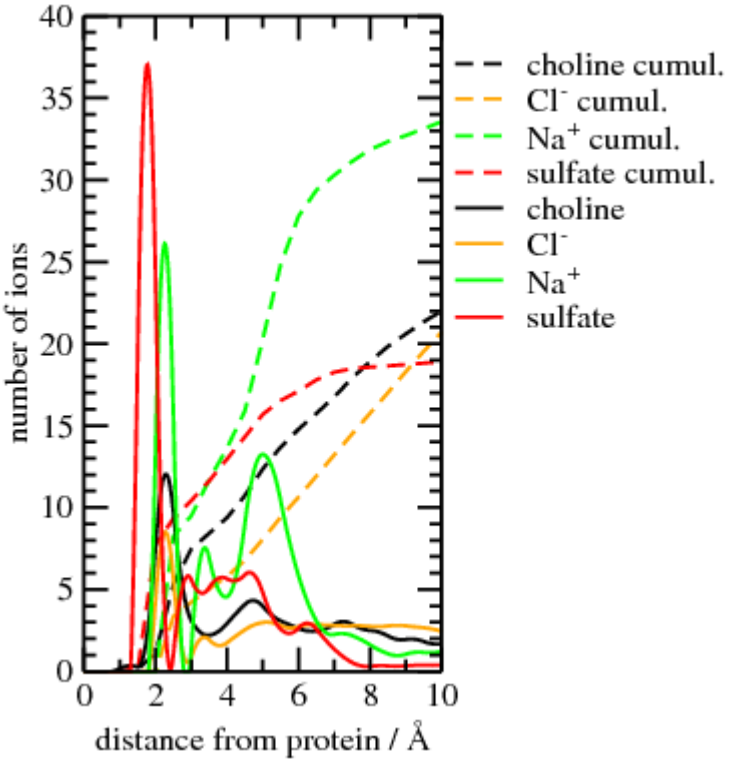
40
35
30
25
20
15
10
5
0
number of ions
0
2
4
6
8
10
distance from protein / Å
choline cumul.
Cl- cumul.
Na+ cumul.
sulfate cumul.
choline
Cl-
Na+
sulfate

Table 1: Amino acid content of the protein surfaces

amino acid	BPTI		HRP	
	number	content %	number	content %
ALA	2	6	3	3
ARG	5	16	18	16
ASN	0	0	19	17
ASP	2	6	7	6
CYS	0	0	0	0
GLN	1	3	7	6
GLU	2	6	3	3
GLY	1	3	2	2
HIS	0	0	1	1
ILE	2	6	1	1
LEU	2	6	11	10
LYS	4	13	2	2
MET	1	3	0	0
PHE	1	3	5	4
PRO	3	10	10	9
SER	0	0	8	7
THR	3	10	15	13
TRP	0	0	0	0
TYR	2	6	0	0
VAL	0	0	2	2