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# Patchy proteins, anions and the Hofmeister series

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**Abstract**
We investigate specific anion binding to a range of patchy protein models and use our results to probe protein–protein interactions for aqueous lysozyme solutions. Our molecular simulation studies show that the ion–protein interaction mechanism and strength largely depend on the nature of the interfacial amino acid residues. Via direct ion pairing, small anions interact with charged side-chains while larger anions are attracted to non-polar residues due to several solvent assisted mechanisms. Incorporating ion and surface specificity into a mesoscopic model for protein–protein interactions we calculate the free energy of interaction between lysozyme molecules in aqueous solutions of sodium chloride and sodium iodide. In agreement with experiment, our finding is that 'salting out' follows the reverse Hofmeister series for pH below the iso-electric point and the direct series for pH above pI.

(Some figures in this article are in colour only in the electronic version)

## 1. Introduction

The stability of protein solutions is governed not only by the macromolecular net charge, salt concentration and valency, but also the salt *type* [1]. Traditionally, the latter falls into the category of Hofmeister or ion specific effects which in recent years has seen an appreciable renaissance—not the least from a theoretical perspective. In Hofmeister's original studies [2] ions were arranged according to their ability to precipitate or 'salt-out' proteins and for the anions it was found that $F^- > CH_3COO^- > Cl^- > NO_3^- > Br^- > I^- > SCN^-$. The effects of cations are usually less pronounced and will not be discussed these here. The situation is, however, slightly more complicated in that the Hofmeister order for proteins is in fact dependent on the solution pH and the protein iso-electric point. For example, second virial coefficient measurements on lysozyme [3] (pI = 10–11) showed that this protein follows the *reverse* Hofmeister series at low pH and anions such as iodide and thiocyanate very effectively induce protein association. In a systematic study using small angle x-ray scattering (SAXS), Finet *et al* [4] demonstrated that a Hofmeister reversal for pH < pI is observed not only for lysozyme but also for a range of small proteins including $\alpha$-crystallins, $\gamma$-crystallins, ATCase and BMV.

Let us for a moment assume that the ion *specificity* of protein association is governed by interactions between salt ions and the macromolecular surface [5]. We can now argue that for pH < pI any absorption of anions will effectively reduce the repulsion between the cationic molecules and thus assist complexation. The reverse is true for negatively charged proteins (pH > pI) where binding of anions will increase the repulsion and thus stabilize the solution. From the above mentioned experiments we thus conclude that anion binding to proteins follows the reverse Hofmeister series—i.e. thiocyanate binds more strongly than fluoride irrespective of the net charge. Following this line, Boström *et al* [6] showed that combining Poisson–Boltzmann electrostatics with ion specific dispersion forces between salt ions and spherical macroions, an essentially correct picture of the Hofmeister reversal is obtained. In the dispersion framework the larger ions such as iodide and thiocyanate interact more strongly with the (averaged) macromolecular surface than do for example chloride ones.

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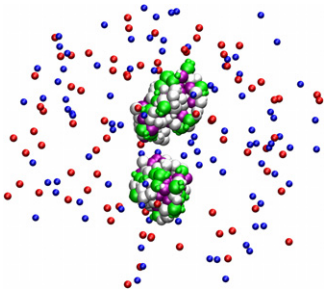

**Figure 1.** MC simulation snapshot of two coarse grained lysozyme molecules in a dielectric solvent with explicit salt particles.

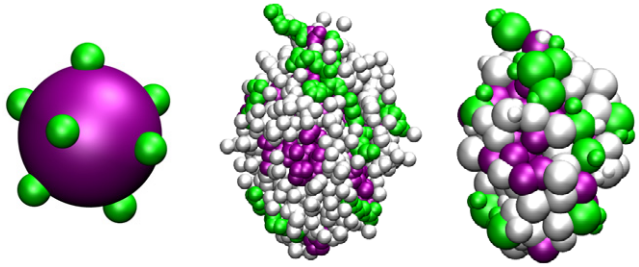

**Figure 2.** Three models for macromolecules with hydrophobic (purple/dark grey) and cationic surface groups (green/light grey). Left: a non-polar sphere with positively charged patches (section 3.1). Middle: the lysozyme in atomistic detail (section 3.2). Right: the lysozyme coarse grained to the amino acid level (section 3.3).

While dispersion may indeed be a significant driving force for ion specific effects, other important mechanisms are at play. Protein surfaces are far from uniform and consist rather of an intricate network of polar and non-polar groups to which salt ions have widely different affinities. For example, large anions are attracted to hydrophobic interfaces via surface modified solvation and polarization [7–9]. Direct ion pairing [10, 11] between salt particles and charged surface groups also gives rise to ion specific phenomena [12]. In this work we will pursue the contributions from these alternative mechanisms using a combination of atomistic and mesoscopic simulation techniques. The discussion will be rooted in our three recent studies [9, 13, 14], here expanded with additional theoretical predictions.

## 2. Computational methods

### 2.1. Monte Carlo simulations

For studying the free energy of interaction between *two* large macromolecules (section 3.3) a molecular level solvent description is hardly tractable. Instead, we treat the solvent as a structureless dielectric continuum and scale all electrostatic interactions with the relative dielectric constant of the medium. Ions and particles in the system are treated as (charged) Lennard-Jones particles. Furthermore, we coarse grain the experimental protein structure to the *amino acid level* [15]. That is, each residue in the protein is mimicked by a sphere located in the mass center of the amino acid. Two such proteins as well as salt particles and counter-ions are immersed in a spherical simulation cell; see figure 1. Configurations in the canonical ensemble (298 K) are sampled by the Metropolis Monte Carlo (MC) algorithm [16] via random displacements of mobile ions and proteins, with the latter also allowed to rotate around their center of mass. Ion specific interactions are incorporated as described in section 3.3. To obtain the free energy of interaction we sample the protein–protein mass center separation, $R$, and use the resulting distribution function, $g(R)$, to evaluate the potential of mean force via $\beta w(R) = -\ln g(R)$ where $\beta$ is the inverse thermal energy.

All MC simulations are performed using the Faunus coding framework [17].

### 2.2. Molecular dynamics simulations

Ion binding to *single* macromolecules is studied using molecular dynamics (MD) simulations. The aqueous solvent is treated in atomistic detail and for this purpose we use the simulation packages Gromacs 3.3.1 [18] (section 3.1) and Amber 9 [19] (section 3.2). All simulations are performed in the $NPT$ ensemble (1 bar, 298 K) using a cubic box with periodic boundaries; long ranged electrostatic corrections are accounted for by the particle mesh Ewald summation method. Our simulations contain a single macromolecule and >4000 water molecules. For a detailed account of the simulation parameters, refer to [9] and [14].

## 3. Results and discussion

Ultimately, our goal is to obtain the ion specific free energy of interaction between *two* proteins described in reasonable molecular detail, which allows us to validate our molecular theory against macroscopic measurements. Before reaching this point we first look at ion binding to *single* macromolecules—in particular a model nanosphere and a real protein in atomistic detail. The models that we will employ are shown in figure 2 and they all capture the hydrophilic and hydrophobic nature of protein molecules by including a patchy surface with alternating polar and non-polar groups.

### 3.1. Ion binding to a nanosphere

Binding of small and large anions to a patchy nanosphere has been studied in detail [9]. In figure 3 we show the simulated potential of mean force between a neutral, non-polar sphere and iodide and fluoride, respectively. Fluoride, being small and well hydrated, is repelled, as expected from classical reaction field continuum arguments. Iodide on the other hand is large, poorly hydrated and is attracted to the non-polar interface. The same behavior is observed at the vapor–water interface [7] and the affinity of iodide towards the hydrophobic region is comprised of a range of intermolecular interactions.

(i) Loss of ion–dipole energy occurs, as described in classical electrostatics with a reaction field.
(ii) Association of poorly solvated species is induced due to a reduction of the ordered water network surrounding these.

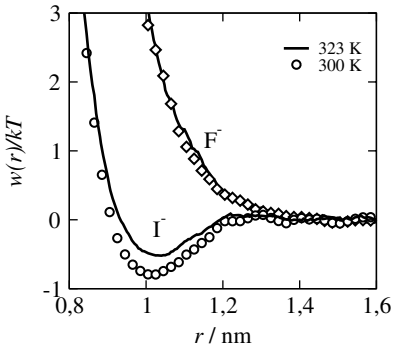


**Figure 3.** Simulated potential of mean force, $w(r)$, for fluoride and iodide with a neutral, nanosized sphere in SPC/$E$ water at 300 K (symbols) and 323 K (lines).

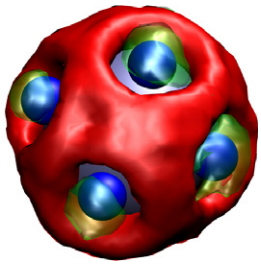

**Figure 4.** Iso-density surfaces of iodide (red) and fluoride (green) around a nanosphere with positively charged surface patches (blue).

(iii) Aligned water molecules near the non-polar interface set up an electric field that leads to induced dipole interactions with polarizable ions. (These are neglected in the calculations shown in figure 3.)
(iv) Solvent–solute, solute–solute and solvent–solvent dispersion interactions can lead to both an attraction and a repulsion of ions [6, 20, 8].

It is noteworthy that the attraction of iodide to the non-polar sphere increases with decreasing temperature; see figure 3. This is compatible with temperature dependent experiments performed by Finet *et al* [4] and indicates that non-negligible entropic components are at play. Attaching charged patches on the surface of the nanosphere (figure 2, left) we observe an increased binding of both iodide and fluoride. The latter binds very specifically, via ion pairing, to the charged surface groups, while iodide binding is more diffuse. We can illustrate this by plotting the spatial densities of the two ions around the nanosphere as shown in figure 4.

### 3.2. Ion binding to a protein

From the above study of ion binding to a significantly coarse grained model protein we now proceed to a 'real' fluctuating protein treated in atomistic detail; see figure 2 (middle). In a recent simulation study [14] of chloride and fluoride binding to the protein lysozyme, we analyzed the ion distributions around the non-spherical protein surface. Qualitatively we obtained the same picture as for the model nanosphere: the larger iodide anion prefers non-polar surface groups while the smaller chloride ion shows an increased affinity for charged groups. In figure 5 we present additional results for iodide and fluoride. Compared to our previous study [14], a non-polarizable force field was employed. The charged/non-polar ion segregation is largely retained even though we have neglected induced dipole contributions as mentioned under interaction type (iii) in section 3.1.

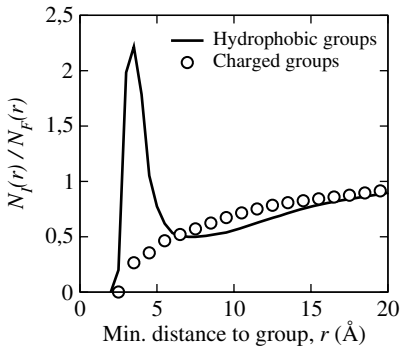


**Figure 5.** Relative cumulative sums, $N(r)$, of fluoride and iodide for charged and non-polar patches in lysozyme, simulated in atomistic detail at pH 4.5, 150 mM salt in SPC/$E$ water and no atomic polarizabilities.

### 3.3. Protein–protein interactions

Now that we have established that anion binding to proteins is highly dependent on the surface residues, we proceed to examine how different ions influence *protein–protein* interactions. To achieve this we perform coarse grained Monte Carlo simulations of two lysozyme molecules (see figure 1) in aqueous solutions of sodium chloride and sodium iodide [13]. In our model, detailed in the methodology section, we invoke the dielectric continuum model for water and thereby average out all structural features of the solvent. It is, however, precisely water structuring that gives rise to ion specificity and we therefore need to include this *implicitly* [13]. Applying effective potentials, obtained from explicit solvent simulations, between hydrophobic surfaces and ions [8], we account for the solvent assisted interaction between ions and non-polar amino acid residues (defined as ALA, LEU, VAL, ILE, PRO, PHE, MET, TRP). In this framework, iodide is attracted to these groups while sodium and chloride are repelled.

As shown in figure 6 we see that at low pH, the inter-protein potential of mean force, $w(r)$, is more attractive in solutions of sodium iodide than in sodium chloride. This can be attributed to the fact the iodide absorbs on the non-polar patches, thus decreasing the effective charge on the cationic proteins (pH $<$ pI).

Changing the solution pH from 4.5 to '12.5' the isoelectric point of lysozyme is exceeded ($pI_{lys} \sim 11$) and the protein net charge goes from $+9$ to $-5.5$. The quotation marks are to signal that the authors, despite being simulators, are aware that strong alkaline conditions are hardly practical for protein solutions. Nevertheless, as shown in figure 6 our simulations predict—in accord with measurements [4]—that salting out of anionic proteins does indeed follow the direct Hofmeister series while the reverse is true for cationic proteins.

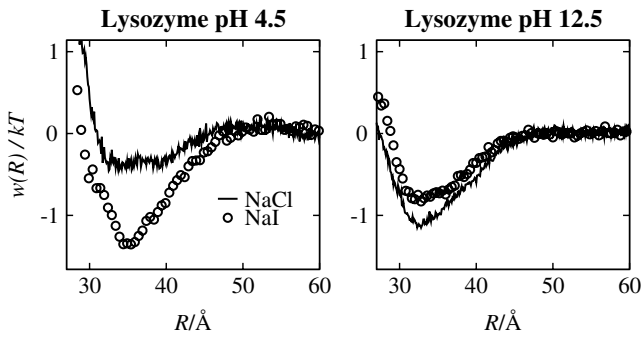


**Figure 6.** Free energy of interaction, $w(R)$, between two lysozyme molecules as a function of their mass center separation, $R$, calculated in 0.1 M sodium chloride (lines) and 0.1 M sodium iodide (symbols) at pH 4.5 (left) as well as pH 12.5 (right).

## 4. Conclusion

Using (1) a nanosphere with charged and non-polar patches as well as (2) a protein in atomistic detail, we have demonstrated that anion binding to molecular surfaces strongly depends on the detailed nature of the interface.

- Small, well hydrated ions bind to polar side-chains via ion pairing.
- Large, poorly hydrated ions are attracted to non-polar surface groups via solvent assisted interactions.

The affinity of large ions to hydrophobic regions is attributed to two main mechanisms caused by subtle properties of the interfacial water structure. Firstly, bringing together two poorly hydrated species, the ordered water network around these is reduced. Secondly, an electric field, set up by aligned interfacial water dipoles, induces a dipole moment in polarizable ions. Both of the these 'solvent assisted' mechanisms, like the dispersion contribution, increase the attraction of large ions to molecular surfaces. A pragmatic approach to ion specificity is therefore to include a *single*, attractive force between ions and the whole interface. Such a force, though, will be comprised of widely different physical mechanisms and also be oblivious to specific surface features.

Incorporating a surface and ion specific interaction between ions and surface patches on proteins we show, in agreement with experiments, that protein–protein association follow the reverse Hofmeister series for pH $<$ pI and the direct series for pH $>$ pI. While the same theoretical conclusion was obtained within the dispersion framework [6], the present results underpin that surface patchiness and solvent assisted ion interactions play an equally important role.

## Acknowledgments

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