Beyond Hofmeister

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Research efforts related to the Hofmeister series of salt ions have waxed and waned during its long and storied history. The past few decades have, however, witnessed a renaissance in its study, and the importance of the related solvation science is becoming ever more apparent.

Last year saw the 125th anniversary of the publication by Franz Hofmeister from the German University in Prague of a seminal paper[1] entitled 'Concerning regularities in the protein-precipitating effects of salts and the relationship of these effects to the physiological behaviour of salts'. This was the second and arguably most important article in a series of seven papers with the running title 'Lessons on the effects of salts' published between 1887 and 1898. This particular article presented a set of experiments ordering various salts according to their ability to precipitate egg-white proteins from aqueous solutions. By using salts with a common cation or anion, Hofmeister ingeniously separated cationic and anionic effects, thereby establishing what later became known as the lyotropic or Hofmeister series for ions. The original anionic series is cast in bronze on the building where Hofmeister carried out his experiments (Fig. 1).

As early as the third paper of the series[2], entitled 'On the water withdrawing effect of salts', Hofmeister made a heuristic attempt at interpreting his observations, based on the theory of electrolytic dissociation developed previously by Arrhenius and Ostwald. In this article, Hofmeister extended his studies to additional proteins and colloidal particles, and tried to connect the observed ordering of ions with their strength of hydration, denoted in his time as the water-absorbing effects of salts[2]. Hofmeister's explanation for the ionic ordering was eventually framed into the 1930s–1950s theory of structure-making and structure-breaking ions[3,4]. Within this model, ions are divided into structure makers (kosmotropes) and structure breakers (chaotropes), with the former having and the latter lacking the ability to order water molecules beyond their immediate solvation shells (for a recent review see ref. 5). According to this picture, strongly hydrated kosmotropes effectively 'steal' water from the protein, leading to a salting-out effect, whereas weakly hydrated chaotropes do not possess this ability[6,7].


Figure 1 | A commemorative plaque at the Medical Faculty of the Charles University in Prague reading (in Czech and German): "Professor Franz Hofmeister (1850–1922), who carried out research in this building, predicted that amino acids in proteins are connected by a peptide bond and, in 1888, derived the lyotropic (Hofmeister) series of ions." Photo courtesy of Pavel Jungwirth.

There are, however, at least two serious problems with the kosmotropes/chaotropes explanation of the Hofmeister ordering of ions. First, there is mounting experimental and computational evidence that the effect of ions (monovalent ones in particular) does not extend far beyond their immediate hydration layers, that is, there is no long-range water ordering by ions[8,9]. Second, this explanation leaves the chemical details of the surfaces of proteins or other hydrated solutes out of the picture. Without considering the nature of the protein itself, which indeed is the 'elephant in the room', it is impossible to properly rationalize not only the regular ordering of ions, but also the well-documented and long-known exceptions to Hofmeister behaviour[10]. As the most prominent example, precipitation of lysozyme follows the Hofmeister series only at high pH or high ionic strength. Under neutral and acidic conditions, and up to moderate concentrations, weakly hydrated ions salt out lysozyme better than strongly hydrated ones in an apparently reversed Hofmeister ordering[11,12].

Since the late 1950s there has been explicit interest in understanding how both cations and anions interact with proteins. These studies, which focused mainly on thermodynamic aspects of ion–protein interactions, were typically carried out using small model systems. For example, calorimetry and viscosity measurements were used to infer the interaction of Li+ with model amides such as N-methyl acetamide[13,14]. These efforts were soon followed by other studies looking systematically at solubilities of short polyglycine oligomers or even whole proteins to tease out the salting-in
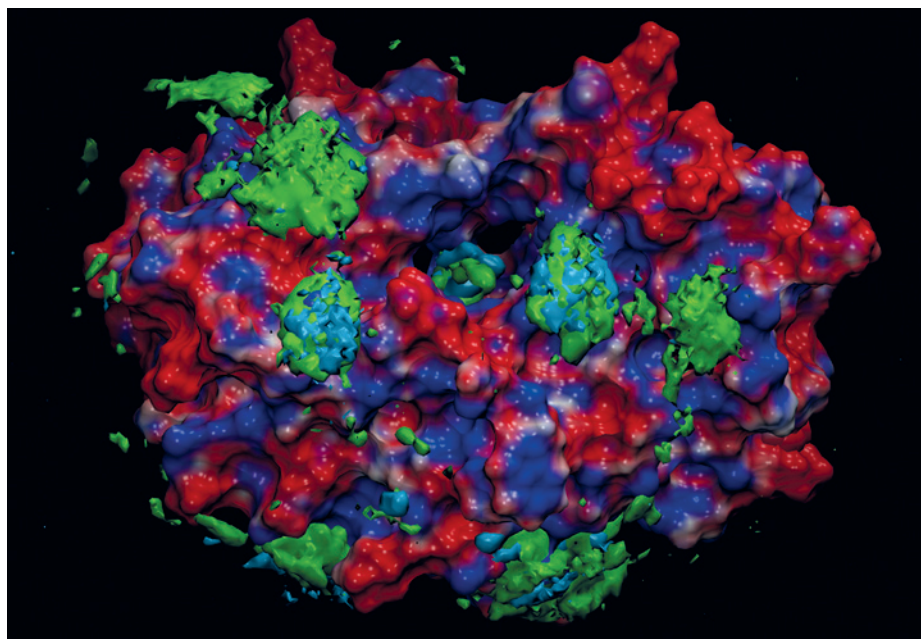
Figure 2 | Distribution of sodium (green) and potassium (cyan) cations around aqueous HIV-protease displayed with a colour-coded electrostatic surface (blue, negative; red, positive). The analysis is based on molecular dynamics simulations from ref. 45.

and salting-out properties of Hofmeister salts[15–17]. Additionally, chromatography was used to elucidate the behaviour of salt ions at the model polyacrylamide/water interface[18]. Although some of the measurements described above were associated with certain experimental difficulties[19], the results correctly indicated that weakly hydrated anions (like I^{−}, ClO$_4^−$ or SCN[−]) and strongly hydrated cations (for example, Mg^{2+}, Ca^{2+} or Li^{+}) interacted with peptide backbones, whereas strongly hydrated anions (such as F^{−} or SO$_4^{2−}$) and weakly hydrated cations (such as Cs^{+} or NH$_4^+$) did not. The end of this period of primarily thermodynamic studies is associated with early attempts to use structural methods such as X-ray crystallography and NMR spectroscopy to put this work on a molecular-level footing[20,21].

After renewed interest in the Hofmeister series in the 1960s and 1970s, the topic fell out of favour for the next decade or two. Although the thermodynamic and statistical mechanics foundations of the phenomenon had been firmly laid out by earlier studies, the appropriate technologies, in particular spectroscopic techniques and molecular simulations, were generally not yet sophisticated enough to address ion-specific Hofmeister effects at a detailed atomistic level. The situation, however, changed gradually for the better, as was reflected in the organization of the first modern Hofmeister conference in 2004 and the subsequent special issue[22] of the journal *Current Opinion in Colloid and Interface Science*, which also provided an English translation of the two crucial Hofmeister papers[23]. This special issue provided invaluable testimony of the state of affairs at the dawn of the modern 'Rennaisance for Hofmeister', a term coined[24] by *Chemical & Engineering News* in 2007. It also demonstrated the breadth of phenomena and systems where Hofmeister ordering was investigated, ranging from processes involving proteins and DNA, to biomembranes and even non-biological problems, such as electrolyte activities, ion exchange, zeta potentials, surface tension and bubble coalescence[22]. Most importantly, it provided the impetus for subsequent attempts to properly understand the Hofmeister issue in molecular-level detail.

The claim at the 2004 conference that 'Hofmeister effects are ubiquitous…[and] as important as Mendel's were for genetics'[22] may have been in accordance with the original views of Franz Hofmeister, but it could have also raised false hopes that a simple and universal rationalization of all the observed phenomena actually exists (for an extensive recent review see ref. 25). Several more-or-less loosely defined explanations, such as dispersion interactions, hydration forces, or even dissolved gas, were suggested[22,26] as 'silver bullets', but as one of us said in the most recent *Chemical & Engineering News* article[27] on the topic: 'Those who hope

for a unifying theory of Hofmeister may ultimately be disappointed…[because] Hofmeister has no single Holy Grail.' On a positive note, it should be stressed that the role of the interface between the solute and its aqueous environment has been firmly established[28]. For proteins in particular, the focus on the interface goes nicely with the reductionist approach of dividing polypeptide effects into backbone and side-chain contributions, as originally advocated by Robinson and co-workers[29] and later adopted in other studies[17,30–33].

In terms of the protein backbone, the most significant affinities are observed for weakly hydrated, soft (polarizable) anions such as I^{−}, SCN[−] or ClO$_4^−$, giving rise to apparent equilibrium dissociation constants on the order of $K_D = \sim$200 mM (ref. 34). Interestingly, the binding site turns out to be a non-reducible combination of both the amide moiety itself and the adjacent α-carbon[34]. In polypeptides, these α-carbons bear a small positive charge because of the electron-withdrawing properties of the N–H and C=O groups on either side of it. Water molecules do not hydrogen bond with the α-carbon and as a result they are favourably displaced by larger and more weakly hydrated anions. Alkyl groups that are not adjacent to heteroatoms are less positively charged and so anion binding occurs less favourably at these sites.

In comparison with anions, the backbone interactions of cations are less pronounced. Strongly hydrated cations, such as Li^{+}, Mg^{2+} or Ca^{2+}, can interact with the carbonyl oxygen of the amide moiety. These interactions are, however, weak because the tight hydration shell of the cation must be disrupted along with the hydrogen bonding of the amide with water[35]. Less strongly hydrated cations, such as Na^{+} or K^{+}, could in principle more easily shed their hydration shells to interact with the amide carbonyl group[36,37], but interactions of such lower-charge-density cations with the amide are even weaker than those seen for strongly hydrated cations.

Although the backbone is clearly important when it comes to understanding the Hofmeister phenomenon, there are other sites on the protein surface that interact appreciably with salt ions from the surrounding aqueous solution. These are, in particular, the charged amino acid side-chains, which can get involved in pairing with salt ions. Specifically, cations from the solution tend to pair with the negatively charged carboxylate groups of the glutamate and aspartate residues, whereas anions pair with the positively charged ammonium group of lysine, the guanidinium of arginine, and the imidazolium of (doubly protonated)

histidine. Such simple electrostatic considerations do not, however, provide much guidance about the Hofmeister ordering for individual cations or anions in terms of interactions with these side-chain groups. To provide at least a qualitative rationale, an empirical 'Law of matching water affinities' was formulated in the 1990s, stating that oppositely charged ions of comparable hydration free energies tend to pair in water[38,39].

More recently, Hofmeister ordering of salt ions at charged amino acid side-chains has been investigated in detail by molecular dynamics simulations, spectroscopy and light-scattering methods[40–43]. The results clearly show that the negatively charged carboxylic acid side-chain groups prefer pairing with smaller, rather than larger, cations. This translates to protein surfaces being more easily 'poisoned' by sodium than by potassium[44–46] (see Fig. 2), which may be one of the clues why cytosol is rich in K^+ but poor in Na^+. Similarly, the positively charged side-chain groups of basic amino acids pair more efficiently with smaller, rather than with larger, anions, leading to reversed Hofmeister ordering at these sites[19,41]. Interactions with these charged groups can be comparable to — or even overwhelm — those with the backbone, which would then cause Hofmeister reversal for the whole peptide or protein[19,47].

Further progress in understanding ion-specific effects in biological systems will require researchers to go beyond the simplifying concept of separate anionic and cationic Hofmeister series. What matters is not only the behaviour of individual ions at the protein surface but, to varying extents, also interactions between the salt ions themselves, both near the protein and in the bulk aqueous solution[48]. Such effects become operational at relatively high salt concentrations and are distinct from non-specific electrostatic interactions, known for a long time to lead to salting-in at very low ionic strenghts[49]. As an example, concentrated guanidinium chloride acts as a strong denaturant of model peptides, whereas concentrated guanidinium sulfate does not; this difference in behaviour arises from the passivation of the denaturating guanidinium cations by appreciable ion-pairing with sulfate anions in the solution[50,51]. Finally, more specialized

ion-binding sites with tailored functional groups and geometries, such as active sites of enzymes or membrane ion channels, are usually considered to be beyond the purview of generic Hofmeister effects. Nevertheless, new insights may be gained by extending the realms of Hofmeister chemistry in this direction, because there is significant mechanistic overlap between the Hofmeister ion ordering and ion-specific interactions in these specialized sites.

We can conclude in the spirit of Hegel's dialectic triad of thesis, antithesis and synthesis. The original thesis that Hofmeister effects can be fully rationalized in terms of the hydration behaviour of ions in bulk solution has clearly been disproved. It has been replaced by a much more powerful antithesis that the anionic and cationic Hofmeister ordering results from local interactions of individual ions at the surfaces of hydrated proteins. Nevertheless, there is already an emerging synthesis that goes beyond the ordering of anions and cations into separate Hofmeister series. In what may be referred to as contemporary Hofmeister solvation science, it takes into account interfacial ion–protein interactions, as well as ion–ion interactions at the protein surface and in the surrounding aqueous medium. At the very recent Faraday Discussion on ion-specific Hofmeister effects, the closing speaker, Philip Ball, addressed the audience by stating[52] encouragingly: "Compared with genomics or dark energy, you might fear that you are working in a deeply unfashionable field. But on the contrary, this general area of solvation science should, and will be, a growth area." ❐

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