CHEMICAL REVIEWS

REVIEW

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Hofmeister Phenomena: An Update on Ion Specificity in Biology

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CONTENTS

1. INTRODUCTION—THE BACKGROUND TO HOFMEISTER EFFECTS

Specific ion effects occur everywhere. They occur in bulk solutions and at interfaces (air/water, oil/water, solid/water, macromolecule/water, etc.). They are even found in nonaqueous polar organic solvents, indicating that water may not be necessary for their occurrence. They emerge typically as differences in behavior of salts in solution, usually above 100 mM, that are not explained by classical theory.[1] Although such "specific ion" or Hofmeister effects have been known since the end of the 19th century, physical chemists have been frustrated in the search for a predictive theoretical framework. Biologists and biochemists have worked more or less in a conceptual vacuum whenever specific ion effects are involved. Why, for example, among the alkali metal ions should lithium (acetate and chloride) alone be effective in treating bipolar disorder when no obvious biochemistry is involved?

Specific ion effects occur in simple aqueous solutions of electrolytes: properties such as viscosity, density, refractive index, heat capacity, activity coefficient, freezing point depression and boiling point elevation, and osmotic pressure all show significant changes from theory depending on the composition and concentrations of the added salt(s). Although adjustable parameters involving, say, "effective" ionic radii or hydration can be introduced to force-fit experimental data, the parameters—even for a single ion pair—change from one situation to another. They also appear in interfacial phenomena, for example, in surface or interfacial tension measurements. In passing, we observe that the most important ions that regulate life are Na^+, K^+, Mg^{2+}, Ca^{2+}, CO_3^{2-}, HCO_3^-, SO_4^{2-}, Cl^-, $H_2PO_4^-$, HPO_4^{2-}, and a separate group of others like Zn^{2+}, Mn^{2+}, Cu^{2+}, but significantly at very small concentrations.

At a symposium celebrating the 100th anniversary of the Nobel prizes for chemistry, Aaron Klug, Nobel Laureate of 1988, made the remark that it was well-known to biologists that the Debye−Hückel theory was limited to slightly contaminated water.[2] Klug received the prize "for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid−protein complexes". The work involved a role for and specificity of zinc ions. Such specificity is universal, and no systematics exist from physical chemistry to explain why.

Another biochemist participant at the Symposium remarked that mechanisms of enzyme action in biology were all understood. There was no need for further work. These two remarks encapsulate the confidence of the new discipline of molecular biology in the first flush of success. It was news to physical chemists.

Hofmeister's work on the relative effectiveness of different salts on the precipitation of proteins and some other colloids dates back to the 1870s.[3] Over the past half century, through a period of intense activity in sophisticated theories of electrolytes and in simulation, Hofmeister effects have often been ignored.

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The effects are not accommodated by the standard classical theories of colloid and physical chemistry. Workers in the biological sciences have always been aware of specific ion effects—obviously all biology is highly ion-specific. But they have lacked an overarching framework that ought to provide a useful unified intuition. Up until now the theories of physical chemistry that have difficulties with specific ion effects have been irrelevant to biologists. Hofmeister was a pharmacologist, and the biologists can rightly claim him as their own.

Our goal in this review goes beyond the outlining of more phenomena. It will be to explain how, following very recent progress, specific ion effects are becoming better understood, as well as how the specific forces that drive Hofmeister effects can be used as tools to probe structure and function.

The article is organized as follows: we first rehearse briefly the standard ideas on the different kinds of interactions that involve ions in simple solutions and other situations and the most relevant physicochemical parameters that characterize ion specificity. "Hydration", an intimately related problem that dominates interactions at short range, is determined by ionic interactions with the chimera that is "water" and with each other. We will rehearse the phenomenological correlations that reflect ion specificity in different phenomena. A detailed report of papers published between 2005 and 2011 on Hofmeister phenomena in bulk phases, in surfactant-based systems and at interfaces, in gels, in proteins and enzymes, and in real biological systems and medicine is our editorial charter.

1.1. A Caveat on Language: Progress in Theory

Before going further, we have to make two remarks. We say again, by specific ion effects we mean effects not accommodated by classical theories of electrolytes. A systematic explanation has eluded physical chemists. This is frustrating as many would agree that Hofmeister effects may be as important in the scheme of things as Mendel's work was for genetics. Although this review deals with papers published on the topic in the past few years, a considerable amount of relevant work occurred in the previous few decades during which considerable progress was made, experimentally and theoretically.

Classical theory, and the interpretation of measurements of pH, buffers, ion binding, and surface potentials, has drawn on an intuition built mainly on electrostatic forces and difficult-to-quantify concepts like ion radii, hydration, and hydrophobicity. Missing from our theoretical edifice has been the dispersion (many-body quantum mechanical forces) between ions and between ions and surfaces. These forces are ion-specific. It is as if one were working with Lewis's concepts of ion pairing and valence before quantum mechanics gave an account of molecular bonds. This is fine up to a point but lacks in predictability. Hydration, and hydration forces between molecules, gives rise to associations somewhere between real covalent bonds and unbound states. Once the quantum contributions are taken into account in an extension of classical theory, some of the mystery surrounding specific ion effects disappears. Hydration, electrostatic, and quantum mechanical forces are all coupled and cannot be separated.

There is emerging a reconciliation of new and old theory. These matters are highly technical, and we refer to refs 1, 4, and 5 for an account of the state of affairs. These developments are very new and suggest that our intuition on specific effects is finally coming into a form, quantitative even, that makes sense and provides at least a guide to predictability in biological phenomena.[5] In this revew we have retained the language and concepts of the older theory.

1.2. Why Hofmeister Matters in Biology

Our title refers to biology. By "biological" system we mean any molecule, macromolecule, or multimolecular self-assembled structure that plays a role in the hierarchy of biochemical events. These include proteins, polysaccharides, nucleic acids, and phospholipids that constitute the building blocks for biomembranes and self-assembled structures and whose functionalities regulate the vital processes of living organisms. That the characteristics of ionic interactions are so specific in biological systems is evident.

Salts and, crucially, trace amounts of specific ions determine the structure and function of the hierarchically lower structures that support life. They participate in the osmotic regulation of cells and in the main living processes. Any biological system will suffer a significant stress when specific salt concentrations are varied or one is replaced with another. An example is the acidic rain-induced mobilization of aluminum and manganese, which are toxic to plants and animals, in water basins.[6,7] Moreover, traces of other elements, like Mn, Ni, V, Cr, Zn, and Se, are necessary in plant and animal health for structure and function of enzymes.[8,9] These have whole-animal effects, as is clear from the classic example of cretinism due to iodine deficiency.[10]

The concentration at which specific ion effects occur is typically (but not always) around or above that of "physiological" solutions, ∼100 mM. That total concentration, and ratios of constituent ions, is about the same as that of "physiological" solutions, dominated by Na^+ and Cl^-, K^+, and Ca^{2+}, the same as that estimated for the Permian Era ocean when land animals and plants first emerged. However, in biology, overall concentration is misleading. Trace elements like Zn^{2+} and iodide already mentioned partition into and drive self-assembly, structure, and function of selective organelles and compartmentalized macromolecules.

We can expect that specific ion effects in simple physical chemistry experiments do not immediately or easily map over to biological systems. They are obviously quite complicated with a compartmentalized and segregated complex hierarchical set of interactions, chemical species, interfaces, and parameters/properties that are involved. The deceptively simple question of pH or buffers in such systems becomes complicated even in bulk systems, let alone in the presence of multiple components, salts, and macromolecular species such as proteins, polysaccharides, nucleic acids, and colloids as in seawater.[1,11]

The arguments for the Kluggian view, that in such a scenario physical chemistry is irrelevant, seem overwhelming. Indeed, to the contrary, before any global systemization in biology is possible, the problem of specific ion effects in physical chemistry has to be understood and brought to order. Here we come to a key issue. Ion specificity is not accessible to models based on electrostatic forces alone. In fact, classical electrostatic theories underpin the entire intuition of physical chemists regarding ionic interactions.

The classical theories include those for the Born energies of solution and transfer, the Debye−Hückel model of activities and its extensions of zeta and membrane surface potentials, and the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory of colloidal interactions. They do not account for specific ion effects. Attempts can be made to accommodate them through extensions in statistical mechanics via inclusion of hard-core interactions or "dressed" ions (hydration). However, these extensions fail in the sense that the fitting parameters that have to be invoked vary, even for the same electrolyte, depending on the system (e.g., the

particular amino acid or colloidal surface, or mixed electrolyte in a buffer).

The disjunction between biology and physical chemistry is doubly confounded. A specific example:

Measurements based on the techniques of physical chemistry, like pH or ion binding via NMR, do not account for Hofmeister effects, which affect the pH of the media used in biological studies.[11] The apparent pH is dominated by the presence of colloidal proteins or, in seawater, colloidal limestone. For the same reasons, the setting of a pH with a buffer to study a complex solution is not as simple as it is thought to be; indeed it is an open question.

To put the problem into a simpler context: sodium chloride or lithium thiocyanate should in principle work in the same manner, as they are both 1:1 electrolytes containing monovalent ions. Instead,

(1) Ion activity, or buffer pH in a background electrolyte solution, depends on the specific composition of the electrolyte (a cation−anion pair) and on its interactions with the solvent molecules and with the substrate.
(2) "Local" interactions between an ion and a solute (or portion of it) or with an interface mean that the ion is adsorbed specifically. This is accounted for in new theories by inclusion of many-body quantum mechanical dispersion forces missing from standard theory. These, and specific hydration determined by both dispersion and electrostatic forces, depend on the dielectric properties (at the entire gamut of electromagnetic frequencies) of the ion and of the substrate and solvent as we will see later.
(3) A further consequence of this is that—especially with systems such as cells, membranes, and supramolecular structures—the overall effect is the expression of several different single processes taking place at the same time. So the occurrence of anomalies, reordering in the classical Hofmeister series of ionic efficacy, and even inversions are often observed.

1.3. Hofmeister Series

Franz Hofmeister (1850−1922) first reported specific ion effects systematically in a series of papers. He studied the precipitation of egg yolk protein and of some other colloids in the presence of salts. There emerged an ordering of the ions depending on their effectiveness, measured by concentration, required to precipitate the protein. For the anions, the trend is[12]

$$CO_3^{2-} > SO_4^{2-} > S_2O_3^{2-} > H_2PO_4^- > F^- > Cl^- > Br^- \approx NO_3^- > I^- > ClO_4^- > SCN^-$$

Ref 3 reports the translation of Hofmeister's original works of the 1870s.

With the preceding outline as background, we now move to the next section. This deals with the different types of ionic interactions that are at play in Hofmeister phenomena.

2. MOLECULAR INTERACTIONS

The necessity to build in intermolecular interactions, beyond the familiar electrostatic forces and covalent bonds, is required because of[13]

(1) The existence of condensed phases (e.g., liquified gases), which occurs because of attractive van der Waals interactions.
(2) On the other hand, all matter in a condensed state strongly resists compression, reflected in the existence of an isothermal compressibility coefficient $\kappa = -(1/V)(\partial V/\partial P)_T$. It measures the strong and short-range repulsions that prevent coalescence when the molecules come into contact.

Grover and Ryall[14] wrote an interesting review and discussion on the process of salting-in/out—precipitation of colloidal suspensions in general. The *salting effect* is characterized by the Setschenow equation $\log (s_0/s) = kc_s$, where s_0 and s are the solubilities of a nonelectrolyte in pure water and in the electrolyte solution, respectively, c_s is the salt concentration, and k is the salting constant. Positive and negative values of k indicate salting-out and salting-in, respectively. Because different cations and anions have different ionic salting constants, their different combinations differ in their propensity to cause salting-out. This has been variously ascribed to the different nature, structure, size, charge density, hydration, and ion polarizability, as well as to the polarizability of the solvent. The existence of such concepts was real; their quantification was fuzzy. A long time ago, Randall and Failey[15] realized that the efficiency of some common cations as salting-out agents follows the order

For cations:

$$Na^+ > K^+ > Li^+ > Ba^{2+} > Rb^+ > Ca^{2+} > Ni^{2+} > Co^{2+} > Mg^{2+} > Fe^{2+} > Zn^{2+} > Cs^+ > Mn^{2+} > Al^{3+} > Fe^{3+}, Cr^{3+} > NH_4^+ > H^+$$

For anions:

$$OH^- > SO_4^{2-}, CO_3^{2-} > ClO_4^- > BrO_3^- > Cl^- > CH_3COO^- > IO_3^-, IO_4^- > Br^-, I^- > NO_3^-$$

The effect of the addition of salts on solutions of nonelectrolytes is very complex, due to the different types of intermolecular interactions that involve the ions, the solvent, and the solute molecules. The authors discuss them in terms of the different theories then extant. We give here a short summary.

2.1. Backdrop of Classical Theoretical Ideas

In *hydration theories*, ions attract and order surrounding solvent molecules forming hydration shells. In this process ions and nonelectrolyte molecules, including dissolved gas molecules, compete for the same solvent molecules. The hydration and solubility of the nonelectrolyte decrease, which brings about precipitation of the compound from the solution. Because in general cations are more hydrated than anions (at least in standard terms of hydration numbers),[16] then the cations are assigned responsibility for salting-out and salting-in, respectively, and the net salting effect of an electrolyte depends on the balance of these two opposing hydration forces. The water molecules confined in the hydration shell are strongly influenced by the ionic field and fluctuate continuously. The hydration number, i.e., the number of solvating immobilized water molecules per single ion, depends on the type of hydration (hydrophobic, hydrophilic, structure breaking, or polarization) and may change depending on the specific experimental property that is used for its evaluation. (This reflects the fact that the concepts are unquantified.) The major limits of the hydration theories are the assumptions that each ion ties up a finite number of water molecules, that no effect on the bulk solvent properties can be predicted, and—above all—that salting-in is not explained!

Water dipole theories consider that the solvent dipolar molecules in the hydration shell around an ion are oriented: cations attract the partially negative oxygen atom, whereas anions attract

the partially positive hydrogen side. Therefore, ions may play a significant role in either enhancing or disfavoring the orientation of the water molecules toward the nonelectrolyte solute (salting-in and salting-out, respectively), depending on the ionic charge. An insuperable difficulty is that the notion of an individual dipole moment for a water molecule in solution or at a surface is inadmissible if the interactions are many-body interactions, as they are. In reality hydration and dipolar ordering are coupled.

Electrostatic theories consider the difference in the work necessary to discharge the ions dissolved in the pure solvent from that required when the salt is dissolved in a solution containing the nonelectrolyte, due to a change in the (static-zero frequency) dielectric constant produced by the presence of the polar solute. A lowering in the dielectric constant induced by the solute should result in salting-out and vice versa. However, electrostatic theories do not explain why different electrolytes made up of ions with the same charge (for example, 1:1 salts) produce different effects, why the salting-in can turn into salting-out when the nonelectrolyte is changed, or why a postulated fitted (Born) radius of an ion changes with temperature.

Internal pressure theories are based on the experimental evidence that the dissolution of a neutral solute in water produces a decrement in the volume of water. The theory introduces the concept of internal pressure (P_{int}, defined as $P_{int} = (\partial U/\partial V)_T = (T\alpha/\kappa) - P$, where T, α, κ, and P are the absolute temperature, the isobaric expansion coefficient $\alpha = (1/V)\ (\partial V/\partial T)_P$, the isothermal compressibility $\kappa = -(1/V)\ (\partial V/\partial P)_T$, and the pressure, respectively) exerted by the solute on the solvent molecules. This modifies the ion−solvent interactions and may lead to the precipitation of the polar solute.

Theories based on *van der Waals forces* take into account the short-range interactions, which can be either attractive or repulsive, that involve ions, neutral molecules, and the solvent. Besides the Keesom (orientation) and Debye (induction) interactions, which derive from the interaction between permanent dipoles, ions, and induced dipoles, the London (or dispersion) forces are of quantum mechanical nature and describe the interaction between two instantaneous dipoles. They depend on properties such as polarizability and ionization potential that reflect the specificity of the ions. The conventional description of these interactions based on two-body perturbation theory is invalid in condensed media for which a many-body description like that of Lifshitz theory is required (ref 1 and references therein). We will come back to this issue later in this section. A brief dissection of the different electrostatic and nonelectrostatic forces operating in solution follows.

The problem is manyfold, due to the solvent (water) liquid structure. Ion-induced local water structure (hydration) depends on size and electrostatic and quantum interactions. The short-range interactions that involve the ionic species depend on the interactions between hydrated or "dressed" ions and surfaces due to overlap of hydration layers. Longer-range electrostatic interactions depend on the nature of solvent molecules in solution and are affected by "third-party" entities that may be present, for instance, proteins, polymers, and impurities. These entities affect the Debye length due to competitive adsorption and the nonlinear electrostatic effects.[1] Dissolved atmospheric gases have other major effects.[1] Relatively simple treatments based on electrostatics (Debye−Hückel theory and its extensions, DLVO treatment) and on a simple model for water (the *continuum* model), even with the inclusion of many-body attractive van der Waals forces, do not explain specific ion effects in terms of interactions.[1]

2.2. Interplay between Different Forces

Nonetheless, we persist in outlining how these ideas on molecular forces in vacuum, carried over to interaction in a solvent, have formed our intuition. The forces in reality are neither additive nor separable. However, it is important to understand the limitations of trying to employ them in interpreting specific ion effects.

A systematic explanation of the standard ideas on different electrostatic, dipolar, and dispersion forces that ions and molecules experience in different environments is beyond our brief. They can be easily traced in physical chemistry textbooks, as, for example, ref 13. We just summarize here the fundamental concepts.

In the expression of the electrostatic potential Φ_{el} for the Coulomb law,

$$\Phi_{el}(R) = \pm \frac{z_1 z_2 e^2}{4\pi\varepsilon_0\varepsilon_r R} \tag{1}$$

only the charges of the ions ($z_1 e$ and $z_2 e$) and the interionic distance (R) are considered, under the assumptions that ions are point charges, that R is far beyond larger than their actual physical size, and that the static dielectric constant of the solvent (ε_r) is taken as the average value for the pure solvent. In an electrolyte solution, the nonadditive effects of neighboring ions screen the electrostatic interaction, and the potential can be approximated as

$$\Phi(R)_{eff} \propto \Phi_{el}(R)\ \exp(-\kappa_D R) \tag{2}$$

where κ_D is the inverse of the Debye screening length:

$$\kappa_D = \left[\frac{e^2\rho(\nu_1 z_1^2 + \nu_2 z_2^2)}{\varepsilon_0\varepsilon_r k_B T}\right]^{1/2} \tag{3}$$

T, k_B, e, and ρ are the absolute temperature, Boltzmann constant, unit charge, and number density of ions, while ν_1 and ν_2 are the stoichiometric coefficients for the specific electrolyte $M_{\nu_1}^{z_1}X_{\nu_2}^{z_2}$, respectively.

In physiological media the Debye length, which measures the range of the electrostatic interactions, is ~0.8 nm, that is, about 3 water molecules! In biology proper, it is generally even much smaller. Even micromolar concentrations of highly charged electrolytes in the biological soup surrounding cells, polymers, or RNA have a very large effect on the screening length, which can be lower than expected from the salt concentration alone. The notion of a *continuum* solvent description of water and the dominance of electrostatic interactions becomes at least dubious.[1]

The ion−dipole interactions produce a potential given by

$$\Phi_{ion-dip}(R) = -\frac{ze\mu\cos\theta}{4\pi\varepsilon_0\varepsilon_r R^2} \tag{4}$$

where R is the distance between the ion and the center of the dipole (assuming R is much larger than the charge displacement in the dipolar molecule) and θ is the angle between the dipole vector and the ion−dipole axis.

Integrating over all orientations and using the Boltzmann distribution, we obtain the average value:

$$\langle\Phi_{ion-dip}(R)\rangle = -\frac{1}{6k_B T}\left(\frac{ze\mu}{4\pi\varepsilon_0\varepsilon_r R^2}\right)^2 \tag{5}$$

The main implication drawn from this formula is that the solvating water molecules that surround an ion are strictly forced in a "frozen" condition, with a significant loss of entropic freedom,

with respect to the bulk water molecules. The distance at which the hydrating molecules are strongly oriented by the ion electric field is approximately given by

$$R^* \approx \left(\frac{ze\mu}{4\pi\varepsilon_0\varepsilon_r k_B T}\right)^{1/2} \quad (6)$$

and for a generic ion in water, R^* is ~0.2 nm. This result suggests that only those solvent molecules that are located in the first hydration shell are restricted in their freedom of motion. This inference will be recalled later when we discuss the "water structure" issue. Again, the standard intuition drawn from this is misleading. Such dipolar interactions, valid for two molecular dipoles in a vacuum, have a different form in a condensed medium. In fact, the interaction is then *proportional* to temperature, not to its inverse.[1,17]

For two permanent dipoles μ_A and μ_B interacting over a distance R (much larger than the charge displacements in the molecules A and B), the thermal average over all angles of rotation for the Keesom or orientation potential is

$$\langle \Phi_{orient}(R) \rangle = -\frac{2}{3k_B T}\left(\frac{\mu_A\mu_B}{4\pi\varepsilon_0}\right)^2 R^{-6} \quad (7)$$

The induction or Debye term takes into account the mutual polarization between the dipoles A and B due to the induction effect of a permanent dipole of one molecule on the electronic cloud of the other through its static polarizability $\alpha(0)$:

$$\langle \Phi_{ind}(R) \rangle = -\frac{\alpha_A(0)\mu_B{}^2 + \alpha_B(0)\mu_A{}^2}{(4\pi\varepsilon_0\varepsilon_r)^2} R^{-6} \quad (8)$$

Another net attractive, London, or dispersion interaction derives from the formation of instantaneous dipoles in the two interacting species. This kind of potential, which exists in all species—polar and apolar, depends on the polarizabilities α and ionic potentials I of the intervening molecules as

$$\Phi_{disp}(R) = -\frac{3\alpha_A\alpha_B I_A I_B}{2(4\pi\varepsilon_0\varepsilon_r)^4 (I_A + I_B)} R^{-6} \quad (9)$$

(Such formulas used in simulation studies and extracted for bulk data are usually even wrong by factors of two and more, when ab initio calculations of polarizabilities are brought to bear and when many-body effects are properly described.)

It is clear then that, while Φ_{el} does not reflect any specific electronic property of the molecules/ions but only their net charges and stoichiometric coefficients (i.e., $NaNO_3$ and $LiClO_4$ should in principle behave in the same manner), all the other nonelectrostatic potentials do depend on the distribution and properties of the electronic clouds surrounding the nuclei through the values of μ, α, and I. The different "softness" of the electron cloud—quantified by the polarizability—will produce a different response of the molecule/ion to an external oscillating electric field, a field that includes electromagnetic fluctuations due to its neighbors. This means, e.g., that F^- ions are very hard, with the highest electronegativity and lowest polarizability, while I^- ions are very soft due to the presence of several internal electrons that shield the nuclear attractive force from the outer valence shell. Therefore, we can expect that hard ions such as fluoride will be less sensitive than soft ions such as iodide to other ions and dipolar molecules. As a direct consequence, ions can also interact with each other because of a mutual polarization effect. If $\alpha_A(0)$ and $\alpha_B(0)$ are the static polarizabilities of the two ions, the net attractive potential is

$$\Phi_{ind,ion}(R) = -\frac{\alpha_A(0) + \alpha_B(0)}{2}\left(\frac{ze}{4\pi\varepsilon_0\varepsilon_r R^2}\right)^2 \exp(-2\kappa_D R) \quad (10)$$

The presence of an interface that separates two media with different dielectric properties (air and water, or water and oil) will add an image potential.[1] Other interactions such as hydrogen bonding, π—ion interactions, acceptor—donor coupling, van der Waals forces, and steric and hydration interactions all participate in the specific ion effects.

Although the brief listing above forms the backdrop for traditional intuition, it was the best we had to inform that intuition. The assumption of additivity of, and division into, separate forces is often quite wrong and misleading.[18]

The physical chemistry of solutions of electrolytes drew heavily on electrostatic forces and hydration. The interpretation of experimental measurements, from neutron scattering to ion binding by NMR to pH to force measurements, all assume this is so. It has not involved quantum mechanics at the same level that we know is essential to explain the chemistry of gases and molecules, or of solids. Furthermore, in a real system many-body interactions change matters again.

Even when these matters are rectified, this is not the whole story. Shape is another crucial issue. Some ions, e.g., halides, calcogenides, and most of the cations, are spherical, and therefore the interactions they produce are isotropic. This is not true for ions such as nitrate, thiocyanate, dihydrogen phosphate, acetate, and hydroxide, which possess a nonspherical structure. Because of the existence of symmetry axes, their polarizability depends on the direction in which it is calculated. Moreover, the interaction between them and another molecule or interface will change, depending on the direction of approach. The shape issue shows up strongly in specific ion effects.

Therefore a consistent and exhaustive theory needs to take into account the effect of nonelectrostatic forces, many-body interactions, and structural features such as anisotropy. For the long-standing fundamental problem of activity coefficients, proper prediction requires further the inclusion of quadrupolar and higher-order polarizability contributions. These matters are still works in progress, but happily in encouraging progress.[19,20]

Faced with this problem, researchers have devoted a great deal of energy to computer simulation. Sometimes it invokes water, the heart of the matter, and sometimes not. Simulation uses, again, electrostatic potentials with fitting parameters, molecular hard-core sizes, and temperature-dependent two-body solvent model water potentials that have difficulty in handling stark Hofmeister phenomena. The result is a proliferation of parameters that lack predictability. Very recently Parsons and coworkers have calculated ab initio frequency-dependent excess polarizabilities of ions in solution and shown that, once these properties are properly determined and included in the calculation of the interaction potential, the average ionic activity coefficient approximates the experimental value in a very satisfactory way.[4,6,19−24]

3. SPECIFIC ION EFFECTS IN THE PHYSICAL CHEMISTRY OF AQUEOUS SOLUTIONS

In the presence of water, the ion electric field orients the permanent dipoles of water around the ion, within one or two

hydration shells. This process is accompanied by a negative change of entropy ΔS because the solvent molecules have reduced dynamical properties (freedom of motion). They become part and parcel of the now "dressed" ions. That conventional picture has to be augmented: specific dispersion interactions between the ion and the solvent play an equally important role in the orientation, even a dominating effect in the process. They can oppose or enhance the electrostatic effects. Further, the degree to which water molecules are bound to the "dressed" ion varies enormously, giving rise to the idea of soft and hard ions (Gurney potentials).

The ion−solvent interaction affects not only the mobility of the water molecules in the hydration shell but also the effective size and properties of the ion itself. Thus, different ions (fluoride and iodide) with the same net charge (−1) but with different size and polarizability will be differently hydrated. Moreover, the amount of water available as a "true" solvent depends on the kind of ions it interacts with and ultimately on the hydration number of the ionic species. This point has been extensively treated by Zavitsas.[16,25]

To formalize this picture, we recall the standard theory of ions in solution in the *continuum* solvent model. The different interactions that involve the ions and the solvent in a solution determine the electrostatic self-energy of an ion. This is the energy involved in the dissolution of an ion from the gaseous phase. Assuming that the ion is a sphere with radius a and that the charge e is spread on its surface, the self-energy is given by[1]

$$E = \frac{e^2}{16\pi\varepsilon_0\varepsilon_r\kappa_D a^2}[1 - \exp(-2\kappa_D a)] \tag{11}$$

If the electrolyte concentration is so small that $\kappa_D a \ll 1$, then

$$E = \frac{e^2}{8\pi\varepsilon_0\varepsilon_r}\left\{\frac{1}{a} - \kappa_D[1 + O(\kappa_D a)^2]\right\} \tag{12}$$

The first term gives the Born self-energy of an ion immersed in a dielectric medium. The second term gives the correction to the self-energy due to the presence of the electrolyte. This can be used to provide some estimate of the free energy of transfer of an ion from water to another medium, say oil or the interior of a membrane with a different dielectric constant. It can be used to get some estimates of solubility by comparing the free energies of a solid ionic crystal with the free energies of transfer of ions to the solute, and to get some insights on hydration reflected in measured partial molal volumes, entropies, and enthalpies of solution.[26−28]

Besides the electrostatic part, there are other contributions such as that due to the temperature-dependent dispersion potential. This depends on the static polarization of the ion. In some circumstances, for example, in linear macroentities such as conducting polymers and DNA, it can lead to very long-ranged and nonadditive forces.[1,17,29] Such strong, very-long-range attractive interactions peculiar to linear molecules may be a key issue for molecular recognition. The contribution of dispersion forces to the Born free energy has a static contribution of[1]

$$E_{ind} = -\frac{2k_B T}{\sqrt{\pi}a}\kappa_D{}^2\alpha(0) \tag{13}$$

Another contribution derives from considering the frequency-dependent dispersion forces. In this case the corresponding

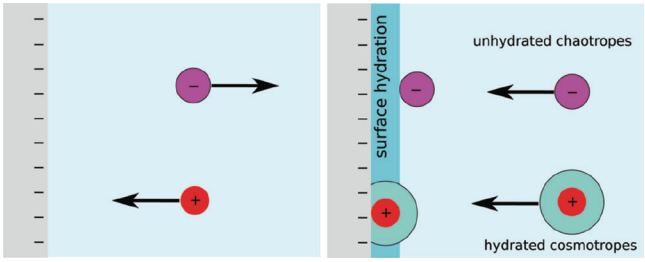

Figure 1. Effects of forces on ions near a surface. Electrostatic forces (left) and dispersion forces (right) act on cations and anions. The dispersion forces can be net either attractive or repulsive, depending on ion and distance, and are made up of a complicated sum from different electromagnetic frequencies. Reproduced with permission from ref 5. Copyright 2011 PCCP Owner Societies.

dispersion self-energy at finite temperature T is a sum over frequencies $\omega_n = 2\pi n k_B T/\hbar$:

$$E_{self-disp} = \frac{4k_B T}{\sqrt{\pi}a^3}\sum_{n=0}{}' \frac{\alpha^*(i\omega_n)}{\varepsilon(i\omega_n)} \tag{14}$$

with $\alpha^*(i\omega_n) = \alpha(0)/[1 + (\omega_n/\omega_1)^2]$ and $\varepsilon(i\omega_n) = 1 + (n_w{}^2 - 1)/[1 + (\omega_n/\omega_2)^2]$. Here, n_w is the refractive index of water and $\hbar\omega_1$ and $\hbar\omega_2$ are the electron affinity of the ion and the typical ultraviolet relaxation potential, respectively.[30] (In fact, whether an ion is considered to include a hydration shell or not, such estimates can be wrong by an order of magnitude once correct ab initio dynamic polarizabilities are used. See chapter 7 in ref 1 and ref 5.) More importantly, the Born electrostatic free energy seems to be wrong by a considerable margin and is highly specific once dispersion forces are correctly taken into account.

Figure 1 illustrates schematically the effect of forces on ions near a surface. The left panel represents the situation depicted by a classical model, where electrostatic forces act and determine the accumulation and repulsion of ions from a surface, depending on the ion charge. The right panel illustrates the effect of dispersion forces, which—in competition with repulsive electrostatic interactions—may produce an attraction on a soft polarizable ion of the same charge of the surface.[5]

The classical properties of aqueous salt solutions, such as osmotic pressure, lowering of the vapor pressure of water, freezing-point depression, boiling-point elevation, electrical conductivity, viscosity, etc., depend on the number density of the species in the system.[16] Ion pairing, charge transfer, and other specific interactions between the ions with the assistance of the solvent contribute to modify their physicochemical behavior in solution. However, these properties depend not only on the concentration and on the valence of the ions, but also on their nature.

In the following three subsections, we will present briefly the effects on the viscosity, activity coefficients, and pH of water solutions of salts. Our aim is to show how essential Hofmeister specific ion effects are in the physical chemistry of solutions and a fortiori when physical chemistry impinges on biology.

3.1. Viscosity

Jones and Dole[31] realized that some ions make water more viscous (kosmotropes) and some others make it more fluid (chaotropes) when compared to the viscosity of pure water at the same temperature. We will come back to discuss the terms kosmotropes and chaotropes later. For a wide range of concentrations

(between 5×10^{-3} and 1 M), the specific viscosity of a water solution can be fitted to the form

$$\frac{\eta}{\eta_0} - 1 = Ac^{1/2} + Bc \quad (15)$$

where c is the concentration of the salt and η_0 is the viscosity of pure water at the same temperature. For dilute solutions (and therefore in the electrostatic regime), the term in c vanishes and that in $c^{1/2}$ dominates. The parameter A reflects the viscous drag due to the ionic atmosphere, which should delay the motion of an ion and make the solution more viscous. But the term in c dominates in more concentrated solutions and carries the specificity of the added salt. The ion-specific Jones–Dole coefficient B can be negative (for chaotropes, $\eta < \eta_0$) or positive (for kosmotropes, $\eta > \eta_0$).[32,33]

At constant salt concentration (0.4 m), the ranking for sodium electrolytes is

$$PO_4^{3-} \geq \text{citrate}^{3-} > HPO_4^{2-} > CO_3^{2-} > SO_4^{2-} > H_2PO_4^- > OAc^- > OH^- \geq HCO_3^- > HCOO^- > BrO_3^- > Cl^- > NO_3^- \approx Br^- > ClO_4^- > I^-$$

and for potassium salts at the same concentration it is

$$HPO_4^{2-} > CO_3^{2-} > H_2PO_4^- > SO_4^{2-} > HCO_3^- > OH^- > Cl^- > NO_3^- \approx Br^- > I^-$$

For much higher concentrations, up to 5 M and above, more complicated formulas are necessary to fit the data.[34] For example,

$$\frac{\eta}{\eta_0} = \exp(Ac^{1/2} + Bc + Dc^2) \quad (16)$$

Two extensive reviews that discuss the viscosity of electrolyte solutions and the specificity of the B coefficients were published by Marcus.[32,33]

3.2. Activity Coefficients

The extended Debye–Hückel theory describes the average ionic activity coefficient $\gamma_\pm$ in terms of the ionic strength I of the salt solution:[35]

$$\log \gamma_\pm = -\frac{A|z_+z_-|\sqrt{I}}{1 + Ba\sqrt{I}} + bI \approx -A|z_+z_-|\sqrt{I} + (A|z_+z_-|Ba + b)I \quad (17)$$

As in the case of viscosity, the formula shows that the activity coefficients can be fitted with an electrostatic term ($I^{1/2}$) and a nonelectrostatic term that depends on the specific salt. The coefficient of the former ($A|z_+z_-|$) is purely electrostatic, whereas the coefficient of the latter contains the parameters (a, b, B) that reflect ion specificity, here via assumed hard-core radii or in extensions via hydrated ion interactions. The second term rapidly goes to zero at very low concentrations, and the Debye–Hückel limiting law model applies when electrostatics dominates the behavior in the solution. For moderately and highly concentrated salt solutions (for example, at 0.5 M), the second term prevails, the range of effectiveness of electrostatic interactions drops to ~0.6 nm, and salt specificity emerges. Matters are not significantly improved when more sophisticated statistical mechanical theories are brought to bear, and mixed electrolytes are impossible within the theory. However, when dispersion forces are included consistently in the theory, with polarizabilities calculated, along with ionic radii, and hydration from ab initio quantum mechanics, the agreement with experimental activity coefficients improves substantially.[19,20] (It becomes even better when quadrupole and higher-order multipole contributions are included, necessary for a consistent theory. Cavity contributions also missing from theories are necessary for even better results and are currently being investigated).

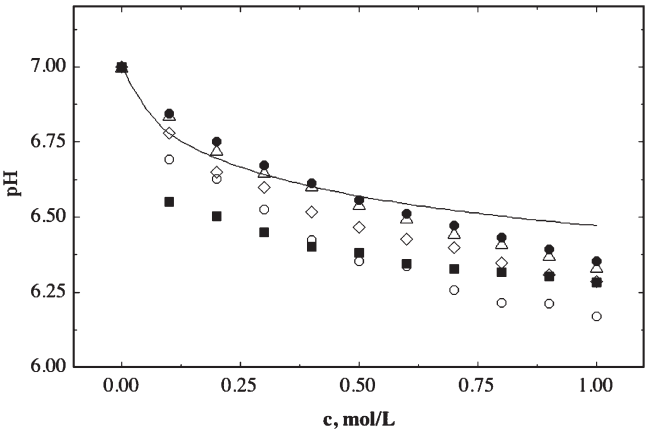

Figure 2. Measured pH values for a sodium phosphate buffer (5 mM) in the presence of NaCl (●), NaBr (△), NaI (■), $NaClO_4$ (○), and $NaNO_3$ (◇). Full line: pH calculated according to the Debye–Hückel equation for monovalent salts. Reprinted with permission from ref 36. Copyright 2006 American Chemical Society.

3.3. pH and Buffers

More important to biologists and biochemists are specific ion effects for pH measurements and buffers.[36] In fact pH measurements via glass electrodes (the standard procedure) or otherwise show that the value indicated by the pH meter depends on the specific background electrolyte.[11] Figure 2 shows the results of the experiments performed with phosphate buffers (5 mM), at pH 7.5, in the presence of some sodium salts.[36]

The experiments were conducted in buffered solutions, to avoid the effect of the electrolytes on the solubility of CO_2, which would affect the final pH reading. The pH decreases, as much as a pH unit, and the sequence reverses with a change of buffer anion and reverses again with a different cation (e.g., potassium instead of sodium).[36] Similar Hofmeister effects were seen with other buffers, like those based on citrate and triethanolamine, by Voinescu et al.[37] The former gives a decrement of the apparent pH in the presence of salts, and the latter gives an increment (see Figure 3).

A measurement of pH ultimately relies on two models: (i) the extended Debye–Hückel theory and (ii) the double-layer theory involving the Poisson–Boltmann distribution (at the glass interface). Both are based on electrostatic forces only. However, the adsorption of buffer ions at the electrode surface is driven by dispersion forces that introduce ion specificity but are neglected in the classical electrostatic theories. The matter is still open. Its interpretation relies on more reliable dynamic polarizabilities of ions obtained from quantum chemistry ab initio studies.[38] There are several other physicochemical properties that do depend on specific ion effects: optical activity,[39,40] conductivity, heat capacity, self-diffusion coefficients, refractive index, and freezing point.[1]

4. CORRELATIONS WITH PHYSICO-CHEMICAL PARAMETERS: THE LAW OF MATCHING WATER AFFINITIES

The lack of a consistent theoretical model that includes all ion interactions to explain and predict the behavior of a specific

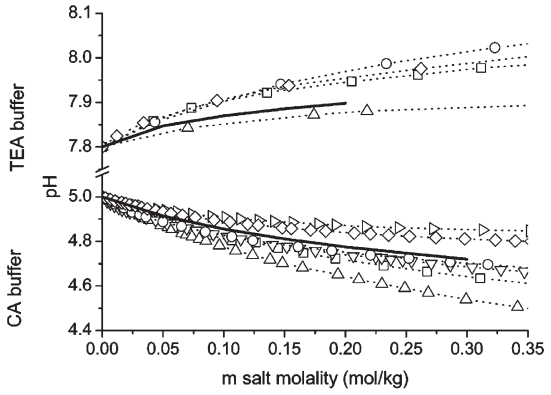

Figure 3. pH of the citric buffer (0.025 M) and of the triethanolamine buffer (0.025 M) versus chloride salt molal: $N(CH_3)_4Cl$ (triangle facing right), choline chloride (◇), CsCl (○), KCl (▽), NaCl (□), and LiCl (△). The full curve represents the pH calculated for both buffers from the extended Debye–Hückel equation for an ionic size parameter of $a_i = 4 \times 10^{-10}$ m. Reprinted with permission from ref 37. Copyright 2006 American Chemical Society.

electrolyte in a given situation led to extensive analyses of correlations between different experimental results in a search for some physicochemical properties that are specific "fingerprints" for different ions.[41] This approach is quite common now in the literature reports, and the correlations can be used to predict the behavior of other electrolytes in particular circumstances. The most common ion properties that are considered in establishing correlations include the static ion polarizability (α), the surface tension molar increment at the air–water (σ) or protein–water (σ_{pw}) interfaces, the Gibbs free energy and entropy of hydration (ΔG_{hydr} and ΔS_{hydr}), the ion radius and charge density, the lyotropic number (N),[42,43] the partial molar volume (v_s),[44] the molar refractivity (R_s),[45] the Setschenow constant (obtained from the solubility of hydrophobic molecules),[46,47] the viscosity B coefficient,[25,32] the entropy change of water (ΔS_{II}),[38,48−50] etc.

For example, Collins's *law of matching water affinities* is based on the observation that, for single valence ions, there is a stronger attraction of similarly sized ions than dissimilar sized ions in water.[51] This law relates the capacity of ions of opposite charge to form inner-sphere ion pairs to the difference in the Gibbs free energy of hydration of the constituting ions, the dependence of the salt solubility on the ion size, and other experimental evidence.[52] According to this law, ions that possess similar calculated hydration energies are deemed to have matching water affinities (how strongly/weakly they bind to water.) It is argued that this property correlates with the formation of close-contact ion pairs in water. The rough distinction of cations and anions into kosmotropes (k) or chaotropes (c) leads to four possible combinations: cation(k)–anion(c) like LiI, cation(c)–anion(k) like RbF, cation(k)–anion(k) like LiF, and cation(c)–anion(c) like CsI. Plotting the ΔH of solution for different alkali halides at infinite dilution versus the total calculated ΔG of hydration (for the pair cation + anion), one obtains a "volcano" graph depicted in Figure 4.

When the two ions have similar free energies of hydration (kosmo–kosmo or chao–chao), the solubilization in water is an endothermic process ($\Delta H_{sol} > 0$); it is argued that the uptake of heat corresponds to the formation of ion pairs being energetically favorable as heat is used up in separating them. In the kosmo–kosmo case, the favorability of ion–ion contact pairs is attributed

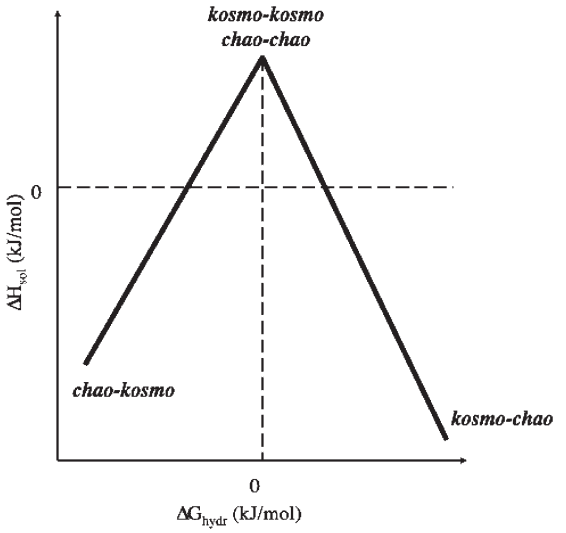

Figure 4. Volcano plot according to Collins. Standard heat of solution of crystalline alkali halides at infinite dilution versus the difference in the Gibbs free energy of hydration of the constituting ions, G(anion) − G(cation). Adapted with permission from ref 51. Copyright 2004 Elsevier.

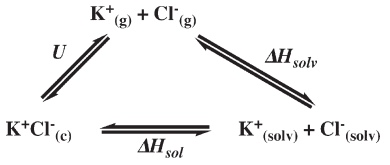

Figure 5. Thermodynamic cycle for the dissolution of potassium chloride in water. $\Delta H_{sol} = U + \Delta H_{solv}$.

to the large electrostatic interaction between the two small ions, which can overcome the strong water bonding to the ions. In the chao–chao case, this effect is attributed to the ions being squeezed together by the water so that the water can undertake more energetically favorable bonds with other water molecules in the bulk phase, rather than the weak water–chaotrope bonds. On the other hand, when the electrolyte is made up of a chaotropic and a kosmotropic ion, i.e., the global ΔG_{hydr} in absolute value is large (either foot of the volcano), the process is exothermic ($\Delta H_{sol} < 0$); it is argued that this means ion–ion contact pairs are energetically unfavorable as there is heat released when the ions pairs are separated. The explanation is that the energy gained by breaking the weak chao–water bond is not enough to balance the energy cost of breaking the strong kosmo–water bond, which is necessary to form an ion pair. In summary, ion pairs are formed at the top of the volcano, where the cation and the anion have similar ΔG_{hydr}. However, a detailed analysis of the model arises some relevant questions.

The heat of solution is determined solely by the ions' solvation energy (ΔH_{solv}) and their lattice energy (U), as depicted in Figure 5. At infinite dilution the ion will effectively never see its counterion so their interaction energy in water (and hence their propensity to form ion pairs) can not play a significant role in the heat of solution. Also, the calculated Gibbs hydration energy is not really a measure of the ion–water affinity, as it is due to the average electrostatic interaction of the ion with the bulk water not just an individual water molecule with which it is in contact.

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Another issue is the explanation for the attraction between the ions, as there is always a balance of energy loss and gain as the two ions approach each other and it is not clear which one will be more significant without quantitative modeling. For example, for the chaotrope−chaotrope case under the Collins model, two water−ion bonds are replaced with one stronger water−water and one weaker cation−anion bond, so it is not clear whether the final energy will be higher or lower. There are also other plausible explanations for the large−large ion attraction such as the strong dispersion interaction between two large soft ions. Also, there is an entropic contribution, which will act in an opposite way. In particular, the removal of water molecules from a large ion will bring about an entropy decrease, which will lead to a free energy cost of this change opposite to the electrostatic energy gain.[53]

If we now consider water and its orientations in the presence of an ion, Collins notes that there are two possibilities, due to the partial displacement of charges between hydrogens and oxygen, so that a water molecule will approach a cation with the oxygen and an anion with its hydrogen atoms.[53] This asymmetry in the water−ion interactions results in a change of water polarizability, as discussed by Leberman and Soper.[54]

The entropy ΔS_{II} was introduced by Samoilov and Krestov to discuss the Hofmeister series in different systems.[48−50] ΔS_{II} is given as the difference between the partial molar entropy of the ion and that of a water molecule surrounded by the other solvent molecules, and can be viewed as the local entropy change for turning a water molecule into an ion.[55] ΔS_{II} strongly depends on the specific ions and reflects two opposing factors:

(1) the disorder induced by the ion in the structure of the solvent molecules and
(2) the reordering effect due to the electric field of the ion that interacts with the permanent dipole of water.

A negative value for ΔS_{II} (positive hydration) reflects a strong ordering of water around the ion, e.g., with hard ions such as Li^+, Mg^{2+}, Al^{3+}, F^-, SO_4^{2-}, and HPO_4^{2-} (kosmotropes). Instead the existence of negative hydration ($\Delta S_{II} > 0$), as for soft species (chaotropes) such as Cs^+ or SCN^-, means that the disordering effect prevails. Collins noted that the variation of ΔS_{II} with the anion is an evidence of a charge-transfer process between the anion and the water molecule. This hypothesis is supported by the fact that often specific ion effects are more pronounced with anions (richer in electrons) than with cations.[56]

The correlations reported in several recent papers provide a link between the measured quantities and the properties that are specific for a given ion. The following sections address in detail the topics that we have been discussing so far, through the presentation of research articles published after 2005 on Hofmeister studies that deal mainly with biosystems and surfactants.

5. HOFMEISTER EFFECTS IN THE BULK PHASE

In this section we discuss some relevant reports dealing with Hofmeister phenomena in the bulk (aqueous) phase, particularly in the period between 2005 and 2010. Before so doing we cannot avoid comment on two major and still-debated issues that are intimately related to Hofmeister phenomena: the structure of water and the terms "kosmotropic" and "chaotropic".

The classification of molecules and ions into the familiar categories "kosmotropic" and "chaotropic" is based on the effect that different salts have on the structure of water. The specific effect of electrolytes on the viscosity of water dates back to Poiseuille's time (first half of the 19th century).[57] Kosmotropes increase the viscosity, chaotropes reduce it. The strength of water−water interactions in bulk solution acts as a critical reference energy level in classifying kosmotropic and chaotropic ions.[58] The explanation of such a phenomenon is usually assigned to the perturbation that ions induce in the local water structure.[26−28,33,59] The strong electrostatic field around the small and strongly hydrated (hard) ionic kosmotropes interacts with the permanent dipole moments of the surrounding water molecules and imparts a higher order on local water molecules via charge−dipole interactions. Instead, the big and scarcely hydrated (soft) chaotropes, which produce a weaker electrostatic field, perturb the dynamic quasi-ordered (hydrogen-bonded) array in bulk water and make the surrounding water molecules more disordered. Something like the effects embodied in such a description, with the corresponding words electrostriction and hydration often invoked interchangeably, probably captures some essentials of what occurs.

A general agreement still does not exist on how the water structure is to be defined and on how the extent of hydrogen bonding should be measured or computed. As Marcus pointed out,[33] the structure of water and the ion effects should be described by the extent of the hydrogen-bonding network, not by its strength nor its dynamics.

The electrostrictive effect of the ionic field (i.e., the pressure exerted by the ion) increases the mean density of the water in the hydration shells with respect to that of the neat bulk solvent. This is a clear effect of the ions on the structure of water. However, when the water structural effects of the ions are discussed, effects beyond their hydration shells are generally meant. The appellations "structure making" and "structure breaking", ascribed to Gurney,[60] have been applied to various ions. Whether and how much influence an ion has beyond the hydration shell(s) is still an open question.

In the attempt to quantify the concepts behind hydration, water structure, and its modifications, different parameters have been introduced.[33] We briefly summarize some of these.

The "stiffness" of a liquid is measured by the work necessary to create a cavity in the liquid (to accommodate a solute particle or a particle of the liquid itself condensing into it from the vapor). It can be expressed in terms of the difference between the cohesive energy density (the square of the Hildebrand solubility parameter, δ^2) and the internal pressure P_{int} as

$$\delta^2 - P_{int} = \frac{\Delta H_{vap} - RT}{V} - \frac{T\alpha}{\kappa} + P \qquad (18)$$

Here ΔH_{vap}, R, T, V, α, κ, and P are the molar enthalpy change of evaporation, the gas constant, the absolute temperature, the molar volume, the isobaric expansion coefficient, the isotherm compressibility coefficient, and the pressure, respectively.[61]

The order existing in a liquid can also be expressed in terms of the deficit of its molar entropy with respect to the same substance in the ideal gas phase. An approximate measure of this deficit is Trouton's constant, $\Delta S_{vap}(T_b) = (\Delta H_{vap})/(T_b)$, where T_b is the normal boiling point at atmospheric pressure, and $\Delta S_{vap}(T_b)$ is the molar entropy change of vaporization at T_b. Typically, an ordered liquid has $\Delta S_{vap}(T_b) > 12R$. Another measure for the order in a polar liquid is the Kirkwood dipole orientation correlation parameter, g:[62]

$$g = \frac{9k_B\varepsilon_0 VT}{N_A\mu^2}\,\frac{(\varepsilon_r - 1.1n_D^2)(2\varepsilon_r + 1.1n_D^2)}{\varepsilon_r(2 + 1.1n_D^2)} \qquad (19)$$

where k_B, ε_0, N_A, μ, n_D, and ε_r are the Boltzmann constant, the vacuum permittivity, the Avogadro number, the liquid dipole moment, the refractive index of the liquid at the sodium D-line, and the dielectric constant of the liquid, respectively. For ordered liquids g is >1.7, while structureless liquids have $g \approx 1.0$.

Another parameter that quantifies the structuredness of a liquid is the heat capacity density, defined as[63]

$$\frac{\Delta C_P}{V} = \frac{C_p(\text{liq}) - C_p(\text{ideal gas})}{V(\text{liq})} \quad (20)$$

Structured liquids have $\Delta C_P/V$ values larger than 0.6 J/K·cm^3, and water has a value of 2.32. The large value for water is due to the small volume of liquid water and mainly to its extensive network of hydrogen bonds.

The viscosity B coefficients are often used to classify the lyotropic behavior of ions, as we have seen before. They are obtained as the limiting slopes of plots. Hence, they pertain to infinite dilution and are additive in terms of the individual ionic contributions. However, there is no satisfactory theory for the ionic B coefficients that relates them to the ionic effects on the structure of water.[33]

The entropy of hydration of ions provides an approach for deciding on their structure-making or -breaking properties. Krestov considered that, to yield the water structure modifying entropic effect, the contributions of the ionic hydrate shell formation and, for multiatomic ions, also the limitation of the ionic rotational entropy should be deducted. In this way Krestov obtained ΔS_{II} that accounts for the changes in the structure of the water beyond the hydration shell.[50]

More recently the use of the terms "kosmotrope" and "chaotrope" has been questioned, not only because they refer directly to the vexed and undefined issue of water structure[58] but also because, in some cases under certain circumstances, the behavior of a specific ion does not match with this terminology, especially when it belongs to the central part of the Hofmeister ranking, e.g., sodium and chloride.[64]

In particular, Zangi discusses the results of some molecular dynamics simulations in terms of the ion-induced changes of structural and dynamical properties of water and the capability of the ions to modify the magnitude of "hydrophobic" interactions.[58] The results show that most of the properties of water in salt solutions change monotonically with the ability of the salt to increase/decrease the hydrophobic interaction. The ability of the ions to reduce the hydrophobic interaction (a property attributed to chaotropes) correlates with their ability to increase (and not decrease as the word "chaotrope" would imply) the structure between the water molecules, including the number and strength of hydrogen bonds, and, as a consequence, the water−water interaction energy. Moreover, Zangi correlated the change in the strength of the hydrophobic interaction induced by salts to changes in the structure and dynamics of the water molecules. A monotonic change in these properties is found with an increase in the salting-in/salting-out ability of the ions. However, he was not able to identify a single property that could predict the change in the strength of the hydrophobic interactions. He observed both salting-in and salting-out effects. Therefore, the salting effect must depend also on the properties of the solute or of the interfaces, in addition to the properties of the salt and water. This conclusion is quite general, as we will see when we discuss Hofmeister phenomena in proteins and at interfaces.

Moreover, recent spectroscopic investigations have cast serious doubts on the ion-induced perturbation of the bulk water structure. Apparently, only the first (or the first two) water solvation layer is really affected by the short-range electrical field produced by the ion, with no other long-range effect.[65,66] However, it should be kept in mind that impurities due to dissolved atmospheric gases, or other hydrophobic solutes in water, may impart a long-range water structure that does affect the tensile strength of water by 2 orders of magnitude, just as do defects in solids. This is not an insignificant problem. Such dissolved gas effects (50 mM at 1 atm) seem to be responsible for at least some long-range hydrophobic interactions.[1] In any case, this question is still far from being definitely set once for all. It looms large like an impending spectacular volcanic explosion. Having pointed out these caveats about water structure and kosmo- versus chaotropicity, we report now on the most recent papers dealing with Hofmeister effects in the bulk aqueous phase.

Zavitsas[25] evaluated the water activity in electrolyte solution from vapor pressure data using the Raoult law, in order to compute the thermodynamic hydration number (H_T), defined as the number of water molecules that are strongly bound to a solute molecule. H_T cannot distinguish between the water molecules that belong to different hydration layers. The small deviations found in the slopes of the colligative properties of strong electrolytes with respect to the theoretical expected values are related to the formation of ion pairs that occurs at high salt concentration. The relevance of ion-pair formation emerges in hard anions such as OH^- and F^- (with high charge density) or in chelating anions such as CO_3^{2-} and SO_4^{2-}. These species attract cations with low charge density and little hydration, with which they form stable ion pairs.

Craig and co-workers have been reporting on the very interesting effect of electrolytes on bubble−bubble coalescence, since 1993.[67] In their last contribution,[68] they interpreted the bubble−bubble coalescence results in term of ion partitioning at interfaces according to the Pegram−Record model (see refs 69−73). However, the unexplained bubble−bubble interaction problem and specific ion effects associated remain as *terra incognita* of obvious importance to the entire field.

Ruiz-Agudo et al.[74] studied the effect of electrolytes on the kinetics of dissolution of dolomite, $CaMg(CO_3)_2$. Gorrepati et al.[75] investigated the precipitation and flocculation of SiO_2 nanoparticles in HCl in the presence of salts. Salts accelerate the consumption of monosilicic acid and the flocculation of the primary particles (diameter 5 nm) with the trend: $AlCl_3$ > $CaCl_2$ > $MgCl_2$ > NaCl > CsCl. The salt concentration has an exponential effect on silica polymerization rates.

Rogers and Beck[76] used the quasichemical theory (QCT) approach to describe the salt hydration free energy as composed of three contributions: inner shell, outer-shell packing, and outer-shell long-ranged contributions. The long-ranged component of the hydration free energy in turn is divided into a first-order electrostatic, an induction, and a van der Waals part. The main contributions to the hydration free energy come from the first-order electrostatic and induction terms, with the latter comprising ~25% of the electrostatic part of the free energy. The first-order electrostatic part becomes slightly less favorable with increasing ion polarizability, while the induction one becomes more favorable. Because of the induction contribution, the total free energy becomes slightly more favorable with increasing ion polarizability. Contrary to the hydration free energies, the local solvation structure is affected more by ion polarizability than by

size: increasing the ion polarizability leads to more asymmetric solvation environments. Changes in size for a given polarizability lead to quite small changes in the solvation anisotropy. The average dipole moment magnitudes for water molecules in the first solvation shell are slightly suppressed from the bulk value. This highlights the importance of many-body water−water interactions in responding to the strong field of the nearby ion.

The competition between the enthalpic ordering of water molecules around an ion, due to the strong ion−dipole interactions, and the entropic lowering in the local structure of water, due to the disruption of the hydrogen-bond network, is usually considered as the reason that the entropic contribution of the ionic free energy of hydration is smaller than the enthalpic term. In a more recent contribution, Beck addresses this importat issue and shows that the entropy of hydration of hard-sphere solutes is negative, and that it is the dominant contribution to the van der Waals entropy of the Lennard-Jones particles.[55] The inner-shell contribution, which models the QCT form of the system free energy, is positive, with a magnitude that increases with the size and partly compensates the packing term. The long-ranged component of the entropy is negative and small, and it does not depend on the nature of the ion. Further, Beck discussed the far-field electrostatic contribution to the entropy, showing that it is negative and slightly dependent on the ion size. Instead, the local electrostatic contribution to the entropy ($S^{ex}_{es,loc}$) depends on the ion radius more significantly (small/large ions show negative/positive values, respectively), and the author concludes that the specificity of each ion emerges in the hydration entropy as $S^{ex}_{es,loc}$. This conclusion underpins the idea that small ions with a high charge density induce a massive ordering of the nearby water molecules and that this phenomenon overcomes the partial disruption of the hydrogen-bonding network. If the quantity ΔS_{II} can be viewed as the local entropy change for turning a water molecule into an ion (see section 4), $S^{ex}_{es,loc}$ can be considered as the entropy change for turning a Lennard-Jones particle into an ion that interacts with water through local electrostatic interactions. In particular, $S^{ex}_{es,loc}$ is negative for kosmotropes and positive for chaotropes: even if K^+ and F^- have similar sizes, potassium has a more positive value (1.5 cal/mol·K) than fluoride (−36.6 cal/mol·K), suggesting that cations are more chaotropic than anions of comparable size. This part of the entropy clearly reflects ion specificity and can actually be used to quantify the kosmo-/chaotropic behavior of an ion. The van der Waals contribution to the hydration entropy is more negative with increasing ion size. Instead, the far-field electrostatic component of the entropy does not reflect any specificity.

Thomas and Elcock[77] performed molecular dynamics simulations to investigate the thermodynamics of hydrophobic interactions in salt solutions that show weak or moderate Hofmeister effects. The calculations confirm the existence of anomalous behaviors, suggesting that the ion's charge density is not a useful parameter to explain Hofmeister phenomena. Instead, they can be explained by considering the hydrogen bonding in simple water solutions of salts without hydrophobic solutes. This result indicates that the change in water structure due to the addition of salt may be more fundamental to the Hofmeister effects of simple salts than preferential interactions between salt and hydrophobic solutes. In the case of lithium salts, the molecular dynamics (MD) simulations suggest the formation of linear strings (clusters) of ions that appear to be the cause of their observed anomalous behavior.

Heyda et al.[78] performed an MD simulation to study the ion pairing between halides and R_4N^+, R_3NH^+ and NH_4^+ in water. For ammonium, the pairing increases from I^- to F^-; for tetraalkylammonium, the series reverses, and R_3NH^+ shows an intermediate behavior (the proton prefers the small halides, and the alkyl chains have an affinity for the large halides).

Salts modify the mutual solubility of water and ionic liquids. Tomé et al.[79] studied the system composed of 1-butyl-3-methylimidazolium tricyanomethane and water and found that the mechanisms for salting-in and salting-out are different. The extent of hydration of the added ions is not sufficient to give an adequate description of the mechanisms operating in these systems. Nor is it sufficient to explain that salting-in and salting-out result from the interplay of different types of interactions between the ions, the ionic liquid itself, and the solvent. The two mechanisms involve (i) a direct binding between the ions of low charge density and the hydrophobic moiety of the ionic liquid, promoting salting-in, and (ii) an entropic effect that promotes the salting-out, related to the formation of hydration complexes away from the solute hydrophobic moieties and to an increase on the surface tension of water and thus on the energy of cavity formation, due to the presence of high charge density ions. High charge density ions are capable of weakening water−ionic liquid interactions, causing the dehydration of the solute by forming water−ion hydration complexes. The solubility of ionic liquids in water is essentially controlled by the specific interactions of the salt ions with the ionic liquid's hydrophobic moiety. The microscopic nature of the salting-out and salting-in phenomena is essentially different, with the former being the result of the tendency of high charge density ions to form water−ion hydration complexes that cause the dehydration of the solute and the increase of the surface tension of the cavity (an entropically driven effect), and the latter being a consequence of the direct binding of the anion to the hydrophobic moiety of the solute. This does not occur for salting-out-inducing ions because their binding with water molecules is preferential.

Salt effects in hydrophilic ionic liquids are relevant also for applications purposes. Bridges et al.[80] studied the extraction capacity of some ionic liquids (imidazolium-, pyridinium-, quaternary ammonium-, and phosphonium-based chlorides) salted-out by K_3PO_4, K_2HPO_4, K_2CO_3, KOH, and $(NH_4)_2SO_4$, and evaluated the salt concentration in the two separated phases at the equilibrium. The results are interpreted in terms of the Gibbs free energy of transfer per methylene group, which measures the energetic cost necessary to create a cavity for an additional CH_2 residue.

O'Brien et al.[81] studied the structure of water near ions through infrared photodissociation (IRPD) spectroscopy of gas-phase hydrated ions. The results indicate that ions do not substantially affect the reorientation time of water molecules beyond the first solvation shell and that ion-induced solvent structure effects are not the dominant factor behind the Hofmeister series. However, it appears unlikely that rotational dynamics of bulk water molecules reflects the presence or absence of large-scale patterning of the hydrogen-bond network.

Salts can be used to control the phase transitions that occur in aqueous dispersion of nonionic polymers at constant temperature. This topic was addressed by Magnusson et al.,[82] who studied the behavior of poly(ethylene glycol) (PEG) and poly(ethylene glycol) methacrylate (PEGMA) dispersions in the presence of CH_3COO^-, SO_4^{2-}, ClO_4^-, SCN^-, Cl^-, and I^-.

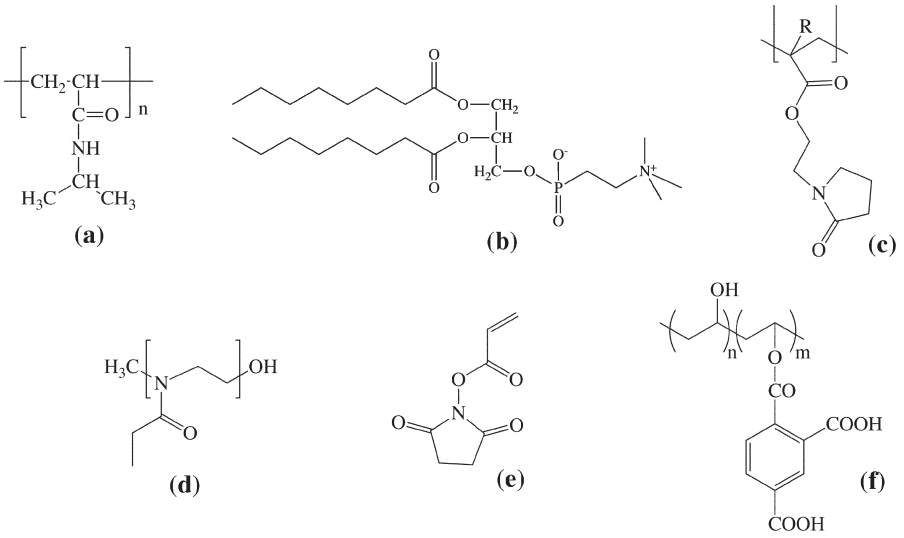

Figure 6. Chemical structures of (a) poly(*N*-isopropylacrylamide) (PNIPAM); (b) dioctanoylphosphatidylcholine (diC_8PC); (c) poly[*N*-(2-methacryloyloxyethyl)pyrrolidone] (PNMEP, R = CH_3); (d) poly(2-oxazoline); (e) *N*-acryloxysuccinimide (NASI); and (f) poly(vinylalcohol)trimellitate (PVA-T).

The effect of salts on the solubility of inulin dispersions in isopropanol/water mixtures was investigated by Naskar et al.[83] The salting-out effect to the addition of electrolytes follows the Hofmeister series only partially, with a partial correlation with the ion's radius and the lyotropic number.

Another interesting bulk effect is the variation of the optical rotation of chiral molecules due to the addition of salts. We and co-workers[39,40] studied the effect of some sodium salts on the optical rotation of D- and L-glucose and some D- and L-α-amino acids (Ala, Asp, Gln, Glu, Ser, Pro, Thr, and Trp) in aqueous solution at room temperature. The variation of the specific optical rotation with the salt concentration (c_s) can be accurately fitted with the equation

$$\frac{[\alpha]_{salt}}{[\alpha]_{water}} - 1 = A\sqrt{c_s} + Bc_s \qquad (21)$$

where $[\alpha]_{salt}$ and $[\alpha]_{water}$ are the specific optical rotation of the chiral molecules in the presence of the salt and in pure water, respectively. The coefficients *A* and *B* reflect the electrostatic and the ion-specific nonelectrostatic force contributions that dominate at low and high salt concentrations, respectively. Similar dependencies are found in other specific ion phenomena, such as, for example, the critical micelle concentration of a short-chain lecithin (see ref 84 and references therein), the formation of pseudopolyrotaxanes, and the viscosity of aqueous solutions (see eq 15).[1] The ion effect on $[\alpha]_{salt}$ was discussed in terms of the specific interactions (namely, hydrogen bonding and hydration), ion-pair formation, and dimerization of the amino acid molecules. Apparently, ions perturb the solute−solvent interactions and produce conformational changes in the chiral solute, and in turn these modify its optical activity. These conclusions match with the results obtained by Scolnik et al.,[85] who showed that the mechanism is presumably connected to the direct and specific interactions that the ions establish with the chiral solute, as well as to their different hydration properties, which perturb the solute−water interactions and therefore lead to a modification of their conformation in solution.

6. HOFMEISTER EFFECTS IN SURFACTANT AND POLYMER SYSTEMS AND AT INTERFACES

Surfactants, polymers and interfaces are some of the most studied systems that exhibit Hofmeister studies. Most such quite dramatic effects have been hidden because it requires hard work to prepare systems with uncommon ions, which is abhorred by most. Salts affect the critical micelle concentration (CMC), solubility, thermal phase behavior of amphiphiles, and the shape, size, and dynamic properties of the self-assembled nanostructures. They also control the phase behavior and swelling of responsive gels (see, for example, ref 86 on poly(styrene sulfonic acid)) and DNA, and can finely tune the properties of oil/water, air/water, and macromolecule/water interfaces by modifying the charge distribution, pK_a, hydration, and ion adsorption.

Specific ion effects have been studied on aqueous dispersions of nonionic, cationic,[87] and anionic surfactants, as well as on hydrophilic/lipophilic polymers and especially in ionic microemulsions and vesicles formed from membrane mimetic double-chained surfactants, for decades. Hofmeister effects here change headgroup forces and can be used to design microstructures and templates for nanostructures at will. Progress in that field has been inhibited because of lack of awareness of Hofmeister effects.[1]

Du et al.[88] performed MD simulations on poly(*N*-isopropylacrylamide) (PNIPAM, Figure 6a), a very well-known and studied polymer as a model of protein behavior.

PNIPAM is a thermoresponsive hydrated polymer that adopts an extended coil conformation and shows an LCST (lower critical solution temperature). Above its LCST, it becomes dehydrated and adopts a folded structure.

At each concentration, a lowering of the LCST (305 K is the value in pure water) was found.[88] It follows the sequence: Na_2CO_3 > Na_2SO_4 > $Na_2S_2O_3$ > NaH_2PO_4 > NaF > NaCl > $NaClO_4$ > NaBr > $NaNO_3$ > NaI > NaSCN, indicating a remarkable anion effect. However, while cations interact directly with the polymer through the amide oxygen, anions do not interact with the polymer. Apparently, the interaction of the cation with the polymer is related inversely to the formation of

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the anion/cation ion pair. Therefore, it seems that the anion effect is mediated by the cation/anion interaction.

Heyda et al. performed MD simulations on alkali halides and *N*-methylacetamide.[89] They confirmed that cations possess an affinity for the amide bond whereas the halide anions do not. Only the larger halides establish an appreciable interaction with the hydrophobic methyl groups. Na^+ appears to be stronger than K^+ in interacting with the C═O oxygen. These results suggest that the destabilization of proteins induced by weakly hydrated anions is at least partly due to the exposure of the hydrophobic groups to the solvent, which is directly originated from their interactions with the larger anions.

MD simulations were performed by Vácha et al. to investigate the interactions between alkali cations or halide anions with a dioleoylphosphatidylcholine (DOPC) bilayer in an aqueous dispersion.[90] Among the alkali cations, only Na^+ shows an enhanced concentration around the headgroups, while K^+ and Cs^+ do not adsorb preferentially at the interface. The anions weakly accumulate near the choline residues, partly compensating the sodium binding. Moreover, large anions penetrate deeper into the hydrophobic layer of the membrane, in a way that recalls their behavior at the air/water interface.

Nelson and Rothberg studied the adsorption of single-stranded DNA onto citrate-coated gold nanoparticles (GNPs) in the presence of different salts, and propose that the process depends on ion solvation.[91] In fact, the effect correlates with the viscosity *B* coefficient and suggests that chaotropes accelerate the adsorption of ssDNA onto GNPs. In general, salts strongly influence the binding rate of ssDNA onto GNPs. Unexpectedly, however, chaotropic anions resulted in faster binding whereas strongly hydrated kosmotropic anions slowed the reaction down. The authors speculate that strongly hydrated anions bind to hydrophobic residues on the DNA, increasing the coiling of the helix and consequently the solubility.

Lee et al.[92] investigated the destabilizing effect of salts on monolayers of 1-octadecylamine (ODA). In particular, they checked the effect of potassium salts of different anions on the crystal nucleation of K_2SO_4 as interfacially templated by a monolayer of ODA, in an aqueous microdroplet system bounded by a liquid−liquid interface with 1-decanol. The experiments indicated Hofmeister trends for anion-specific changes in monolayer ordering. The monolayer order was disrupted most significantly by thiocyanate and perchlorate, and this was attributed to the ability of these ions to penetrate into the monolayer's hydrophobic regions. An electrostatic template mechanism involves the attraction of sulfate anions of the crystallizable solute to the cationic headgroups of the ODA monolayer. As a result, the interfacial concentration of sulfate would be significantly higher than in the bulk. A concentration increment of sulfate ions would then provide a driving force for overcoming the barrier in crystal nucleation. Chaotropic anions are expected to preferentially reside near the charged headgroups of the surfactant at the air−water interface,[93] and are expected to form such ion pairs, thus screening the positive charges of ODA in the monolayer from the crystal nucleus and mitigating the promoting effect of the monolayer on nucleation. In this way, the authors explain the behavior of thiocyanate as having a disordering effect on the ODA monolayer, leading to the introduction of channeling or porosity, which then facilitates water transport through the monolayer. Figure 7 shows the calculated concentration of K_2SO_4 in the microdroplet at the onset of crystallization (C_{onset}) and the corresponding frequency for different potassium salts.

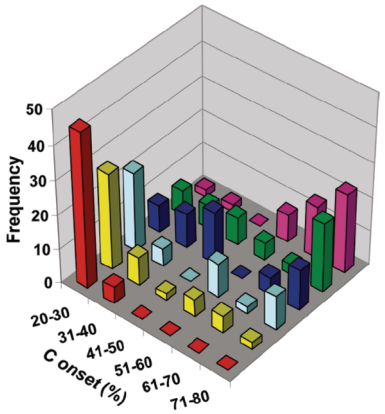

Figure 7. Distribution of C_{onset} values for ODA-templated crystal nucleation of K_2SO_4 in the presence of 10 mM solutions of K_2HPO_4 (red), KBr (yellow), KCl (light blue), KI (deep blue), KNO_3 (green), and KSCN (plum). Reprinted with permission from ref 92. Copyright 2010 American Chemical Society.

The results seem to confirm that the Hofmeister effect observed in crystallization studies has an interfacial origin.

Spreading films of 1-octadecylamine were also used to investigate the ion's ability to penetrate the interfacial thickness of a monolayer and perturb its chain packing. Gurau et al. performed vibrational sum frequency spectroscopy (VSFS) and found that SO_4^{2-}, Cl^-, and NO_3^- have a small effect, while I^-, ClO_4^- and SCN^- penetrate more deeply into the interfacial thickness.[94] The surface potential measured on the same systems confirmed the different levels of penetration of the different anions.

Another interfacial effect was investigated by Jungwirth and Tobias in a work that addresses the interactions between the first solvation shell and long-range effects at the air/solution interface. The polarization of the ions and of water molecules, as well as solvent exclusion and hydrogen-bonding rearrangements, contribute to determine the affinity of an ion for the aqueous surface. The case of H_3O^+ is particularly interesting as it deals with the phenomenon of bubble−bubble coalescence. In fact, while alkali halides inhibit bubble coalescence in water, their corresponding acids do not. The authors suggest that this effect is related to the weaker surface segregation of cations and anions in the acidic solutions compared to the neutral salts.[95] This subject will be expanded later (see section 7.1).

The variation of the cloud point of lipids and surfactants induced by the addition of different electrolytes to their aqueous dispersions is probably the most studied topic in this field. The cloud point, that is, the phase separation that leads to the formation of two coexisting liquid phases with different lipid/water ratios, mainly depends on the hydration shell that surrounds the amphiphile's polar headgroups. The addition of salts may strongly perturb the hydration layers at the aggregate−water interfaces and produce ion binding, depending on the composition of the electrolyte.

Kadam et al.[96] studied the effect of pH and salts (Na_3PO_4, Na_2SO_4, and NaCl) on the cloud point of Tetronics in water. Tetronics with hydrophilic terminal blocks are octablock, star copolymers with four poly(ethylene oxide)/poly(propylene oxide) (PEO/PPO) arms attached to an ethylenediamine core. In particular, T904, $(HO-(CH_2-CH_2-O)_{15}-(CHMe-CH_2-O)_{17})_2N-CH_2-CH_2-N(O-CH_2-CHMe)_{17}-(O-CH_2-CH_2)_{15}-OH)_2$, forms spherical micelles with an aggregation

number of ~10 at 30 °C; the aggregation number increases with increasing temperature and upon addition of Na_2SO_4. The mechanism involves the hydration layer around the surfactant; in fact, an increase in temperature or addition of salt dehydrates the PEO shell, particularly the EO units close to the PO core, leading to the observed effects.

The effect of salts on the upper consolute curves of a short-chain lipid, namely, dioctanoylphosphatidylcholine (diC_8PC, Figure 6b), in water was studied in the presence of different salts and in deaerated dispersions by Lagi et al.[84] The coexistence curves (Figure 8) were fitted according to the Blankschtein–Thurston–Benedek model, and the fitting parameters ($\Delta\mu/k_B$ and C/k_B) were obtained. The former reflects the chemical potential gain related to the growth of the rodlike micelle along the main axis, and the latter quantifies the intermicellar interactions. The addition of chaotropic anions shifts the coexistence curves to lower temperature with respect to the pure water case. Instead, the presence of kosmotropic ions increases the cloud point. The cloud-point temperature, the lipid mole fraction corresponding to the upper limit, and the parameters $\Delta\mu/k_B$ and C/k_B show monotonic trends with the polarizability, the free energy of hydration, and the surface tension increments of the anions. After equilibration of the phase-separated system upon addition of salts, the concentration of the anion was assessed through ion chromatography and showed that they partition asymmetrically, with a partition coefficient that depends on the nature of the salt.

In summary, the results show that (i) specific ion effects on the cloud point can be interpreted in terms of ion adsorption at the micellar interface, which alters the free energy gain related to micellar growth ($\Delta\mu/k_B$) and the intermicellar interaction parameter (C/k_B). (ii) After phase separation, the partition of anions between the upper and lower phases is asymmetric (depending on the specific anion), due to adsorption at the micellar interface. (iii) Degassing the solvent produces an increment in the cloud-point temperature. Instead, the solution of gases induces a lowering in the cloud point, which depends on the gas polarizability. This is an example of a phenomenon not widely recognized: hydrophobic interactions are mediated and caused by the presence of dissolved gas.[1]

Sun et al. investigated the Hofmeister effect on the cloud point of pyrrolidone-based polymers (Figure 6c) in water.[97] The cloud points of these polymers are correlated to Hofmeister series. The addition of Na_2CO_3 or NaCl leads to a salting-out effect, lowering the cloud point. The salt effect is more pronounced in Na_2CO_3 solution, leading to a dramatic decrease of the cloud point. Iodide anions bind specifically to the amide nitrogen of the pyrrolidone groups; therefore, the addition of NaI leads to a salting-in effect and significantly increases the cloud point, up to full solubilization at 95 °C. The solvent isotopic effect in NaCl or Na_2CO_3 solution is the same as in the salt-free solution. The results are discussed in terms of a dehydration of the polymer that eventually leads to phase separation.

Pegram and Record[69,70] addressed the issue of ionic partitioning between the bulk phase and the air–water interface. Partitioning is ion-specific, almost independent of concentration, and basically controlled by the ionic hydration. A careful analysis of surface tension increments provides a quantitative model of ion partitioning that is largely consistent with the results of recent surface-sensitive spectroscopy experiments and MD simulations.[69] Additionally, the authors determined a minimum estimate for the thickness of the surface region and deduced that concentrations

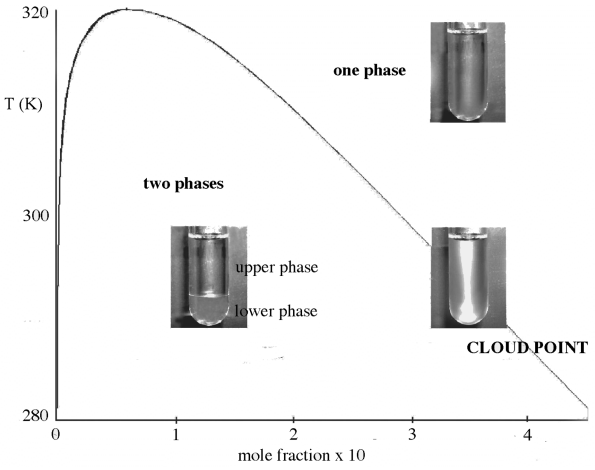

Figure 8. Coexistence curve for aqueous dispersion of diC_8PC. The picture shows the monophasic sample, the onset of turbidity across the cloud-point curve, and the biphasic sample where an upper and a lower phase are in equilibrium. Adapted with permission from ref 84. Copyright 2007 American Chemical Society.

of ions in this region are proportional to their bulk concentrations with proportionality constants (partition coefficients) that are ion-specific and relatively concentration-independent. Patel et al.[98] observed that a triblock copolymer PEO_{103}–PPO_{39}–PEO_{103} (TBP) does not form micelles and does not show a cloud point in water, unless an electrolyte is added, which modifies the hydration of the polar groups.

Yan et al.[99] studied the phase-separation and cloud-point curves of sodium dodecyl sulfate (SDS) + poly(vinyl alcohol) (CPVA) mixtures. They found that chaotropic ions bind to the cationic polymer, decrease its interactions with SDS, and increase the micellar charge density. Instead, kosmotropic anions lower the cloud point of nonionic surfactants, and in most cases the cloud point in the mixed systems of ionic surfactant + nonionic polymer mixtures decreases with increasing salt concentration. In the present study, Na_2SO_4, NaCl, and NaBr increase the cloud point, and the increment is proportional to the salt concentration (c) for $c < 100$ mM. The polarization capacity of anions increases in the order of the Hofmeister series, which naturally leads to stronger binding in the same order. It can be expected that, due to the binding of anions to the CPVA molecules, the adsorption layer of CPVA that wraps up the surface of SDS micelles will be depleted, reducing the interaction between the copolymer and SDS micelles.

Bloksma et al.[100] studied the effect of salts on the cloud point of poly(2-oxazoline) (Figure 6d) with different hydrophobic chain lengths and different side groups. Basically, the salt effect depends on the hydrophilicity of the polymer. Chaotropes produce salting-in and increase the cloud-point temperature (T_{cp}) with the salt concentration. Kosmotropes produce a salting-out, and T_{cp} decreases with the salt concentration. The polar residue can shield the polymer skeleton and therefore determine the salt effect. As already suggested by Cremer and co-workers,[101] the kosmotropes cause a salting-out effect by destabilizing the H-bonds between water and the polar groups of the polymer and by increasing the cost of hydration, resulting in a linear decrease of the LCST with increasing salt concentration. In addition, chaotropes can also cause a slight salting-out effect by increasing the hydrophobic hydration. Nonetheless, the stronger salting-in

effect of chaotropes is caused by direct interactions of the salts with the polymer, resulting in an overall salting-in behavior. In fact, binding of the anion to the amide group of the polymer increases the cloud point with increasing salt concentration until a saturation effect takes place. The response of poly(2-oxazoline)s to the addition of salts from the Hofmeister series depends on the hydrophilicity of the polymer. The cloud point of the most hydrophilic polymer can be tuned over almost the whole temperature range of liquid water under atmospheric pressure.

Zhang et al.[102] investigated the effect of salts on gold nanoparticles (GNPs) coated with polymeric (oligoethylene glycol, OEG) thiols. They found that sulfate destabilizes the colloidal dispersion, whereas chloride, thiocyanate, nitrate, and perchlorate stabilize the aggregates for months even when the salt concentration is as high as 4 M. The study was extended to clusters formed by GNPs with a protein (BSA). Here, chaotropes stabilize the GNP + protein dispersion. Anions located at the left-hand side of the Hofmeister series typically have a salting-out effect. Sodium sulfate destabilizes the OEG self-assembled monolayer (SAM)-coated colloidal gold in solution above a critical salt concentration. Therefore, it is reasonable to expect that adding this salt to the protein−colloid mixture will dramatically speed up the aggregation process. The added sodium sulfate, especially at high concentration, plays a similar role on the colloid in both pure colloid solution and the mixtures with proteins. Protein (BSA) solutions with the addition of any salt used in this work (sodium chloride, sodium nitrate, sodium thiocyanate, ammonium chloride, and magnesium chloride) up to 2.0 M are always stable. For OEG SAM-coated colloidal gold, they are stable in aqueous solutions with salt up to 1.0 M, except for Na_2SO_4; that is, the colloid starts to aggregate with Na_2SO_4 above a critical concentration. This observation indicates that the nature of added salts (i.e., the Hofmeister effect) does not change the stability of either the OEG SAM-decorated colloidal gold or BSA in solution. On the other hand, there is a strong dependence of the stability of colloid−protein mixtures on the nature of salts. Anions and cations on the same side of the Hofmeister series give a similar effect: thiocyanate, nitrate, and magnesium enhance the stability of the mixture with increasing salt concentration, whereas sulfate and ammonium lead to the aggregation of colloids. In summary, compared to the effects on the one-component systems (protein or colloid solution), an enhanced Hofmeister effect is observed in two-component systems.

Efrat et al.[103] studied the effect of ions on the phase behavior of monolein (GMO) and oleyl lactate (OL). In these systems, the different cubic mesophases can transform one into the other. The transitions are dependent on the temperature, component ratios, and coingredients. It has been shown that the cubic bicontinuous mesophase composed of GMO/water and ethanol may also be formed if part of the GMO is replaced by an anionic surfactant. The cubic bicontinuous areas in the phase diagram are enlarged in the parameters A_T (the total area in percentage of the isotropic cubic phase), ΔW_{max} (the maximum water solubilized in percentage at a selected GMO/OL ratio that enables the solubilization), and $EtOH_{max}$ (the maximum required percentage of ethanol to achieve the minimum viscosity at any given GMO/OL ratio). The size of the cubic bicontinuous region depends on the counterion (cation). NaOL and HOL had a relatively minor effects whereas the OL has a relatively large effect on the swelling of the cubic region as seen from the pseudoternary phase diagram. The presence of both Na^+ and H^+ ions yielded a synergistic effect on the cubic region as a result of the mixed chaotropic and kosmotropic character that they impart to the system.

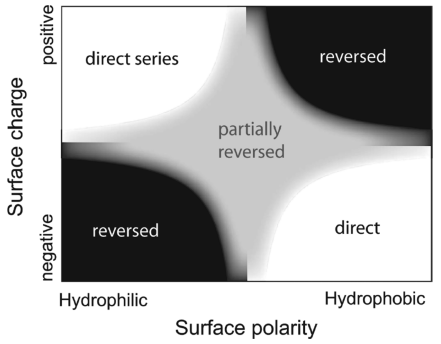

Figure 9. Hofmeister phase diagram showing the different direct or reversed Hofmeister sequences depending on the surface polarity and charge. Reprinted with permission from ref 106. Copyright 2010 American Chemical Society.

Ericsson et al.[104] studied the effect of salts on the size of micelles produced by alkylglycosides. The size change follows the Hofmeister series: the micelle size decreases according to the sequence $SO_4^{2-} > Cl^- > NO_3^- > I^- > SCN^-$. Here, I^- and SCN^- act as salting-in anions that give rise to a decrease of the micellar size as compared with neat water. Although the salt effect on micelle size is quite substantial, the results illustrate the high salt tolerance of alkylglycosides in the sense that up to 1.5 M salt could be added without inducing any phase separation.

Peula-García et al.[105] addressed the water structure near a hydrophilic interface to get insights into short-range repulsive forces. Independently of the property studied, positive surfaces usually order ions in an inverse Hofmeister sequence in comparison to that produced by negative surfaces. The authors found that, when the sign of the surface charge is kept constant, the sequence in which the ions are ordered according to colloidal stability with hydrophilic surfaces is reversed in comparison to hydrophobic surfaces. Moreover, in intermediate situations of hydrophilicity, partial reversals were observed. The most accepted mechanism to explain these results considers that chaotropic anions with high polarizability adsorb at the hydrophobic regions of the surface. The current mechanisms used to explain Hofmeister effects consider that chaotropic anions accumulate at interfaces whereas kosmotropic ones are excluded from them. In this interesting paper, the authors demonstrate that this accumulation or exclusion takes place only when the interface is hydrophobic. An inversion in this behavior is observed when the surface is hydrophilic. In this case, chaotropic anions are excluded from the hydrophilic interface while kosmotropic anions accumulate on it. Hofmeister effects in colloidal systems are strongly governed by the microscopic water structure around both the particle surface and the solvated ions. From these findings, it is inferred that the mechanism that can explain the accumulation and exclusion of ions at interfaces is related to the entropic forces originating in the attraction between ions and surfaces when they have similar water arrangements or the repulsion between them when they have dissimilar water arrangements.

The Hofmeister phase diagram in Figure 9 shows the different Hofmeister regimes, that is, direct and reversed Hofmeister sequences, in terms of surface polarity (from hydrophilic to hydrophobic) and of surface charge (from negative to positive).[106] The central partial reversal region is quite extended, particularly when

the polarity and the charge of the surface are intermediate, which is a typical result of biology-related Hofmeister studies. This is a very important feature: a partial reversal of the series is not an indication of the absence of a clear correlation in Hofmeister phenomena, but rather a direct consequence of the effects due to surface charge and solvation.

An interesting application of this scheme is the patchy surface of a protein (see also section 7.1), where hydrophilic and hydrophobic regions are intermingled. Can we consider the effects of these two regions as being additive? On the basis of the results proposed in Schwierz et al.'s paper and other contributions in this review, the adsorption of ions to polar and nonpolar surfaces can be assumed to be additive, at least to first approximation.

On the same track, López-Léon et al. discuss the reversal of Hofmeister series when the charge and/or the polarity of the surface are modified, in terms of the structure of the hydration shell around a hydrophilic surface.[107] In summary, they propose that the mechanisms of stabilization/restabilization processes that are relevant to hydrophilic systems depend on the degree of hydrophilicity (surface polarity), on the strength of the ionic hydration, on the different density of water molecules, and on the accumulation or exclusion of ions from the surface. The mechanisms are illustrated in Figure 10.

Panel a shows the distribution of chaotropic ions (green circles) and kosmotropic ions (red spheres) at an interface between a hydrophobic surface and water. In the presence of a hydrophilic surface (panel b), the interfacial water has a higher density than in the bulk (dark blue area), kosmotropic ions accumulate near the surface, and chaotropes (surrounded by a layer of low-density water) are excluded. In panel c the ion distribution between two hydrophilic surfaces is depicted. Chaotropic ions (green spheres) are excluded from the high-density water areas (dark blue), whereas kosmotropic ions (red spheres) accumulate near the interfaces. This spatial distribution of ions and high-density water near two facing hydrophilic surfaces hinders the direct contact between the surfaces. This steric factor does not appear in hydrophobic surfaces (panel d). As a matter of fact, the absence of high-density water layers near the hydrophobic surfaces allows a closer approach of the two surfaces.

The interaction between hydrophobic surfaces at the nanoscale level is an important issue for understanding the intermolecular interactions between, e.g., two protein units. Zangi et al. performed some MD simulations to investigate the interactions between two hydrophobic surfaces in salt solutions and discussed the results in terms of charge density and binding of the ions onto the surfaces.[108] In another contribution, Zangi and Berne evaluated through MD simulations the tendency of Lennard-Jones particles to aggregate in the presence of salt solutions at different ionic strengths. They found that ions with high charge density promote particle aggregation. They speculate that this effect may play a significant role in the melting of the hydrophobic tails of lipid bilayers, depending on the charge density of the lipid headgroups. Low charge density species preferentially bind to the particle surface reminiscent of counterion binding at a micellar interface. However, an increment in the salt concetration causes a separation of the particles.[109]

Klasczyk et al.[110] studied the interaction of alkali metal chlorides with lipid vesicles made of palmitoyl oleoyl phosphatidylcholine (POPC). This investigation was undertaken due to the fact that in the extracellular media of mammalians sodium is present at a high concentration, while potassium is located in comparable concentrations in the cytoplasm (~0.1−0.15 M).

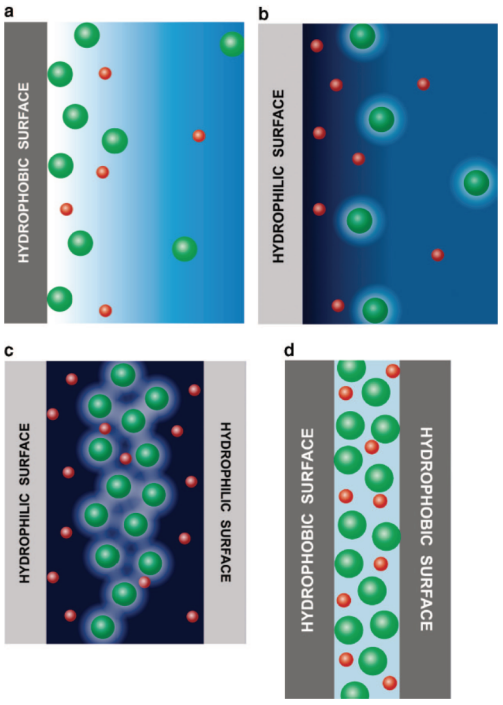

Figure 10. Distribution of chaotropic ions (green circles) and kosmotropic ions (red circles) at a hydrophobic surface/water and at a hydrophilic surface/water interface. The light halo around the chaotropic ions represents low density water, whereas the dark blue color near the hydrophilic surface represents high density water molecules. Reprinted with permission from ref 107. Copyright 2008 American Chemical Society.

This means that the interaction of potassium rather than sodium with proteins and lipids is more important for the cellular functionalities. Moreover, although sodium is balanced by extracellular chloride, intracellular cations like K^+ are not balanced equivalently by free anions. This phenomenon is known as the "anionic gap". The intracellular electroneutrality is warranted by the negatively charged proteins and lipids located in the inner leaflet of the membranes.

Lithium, rubidium, and cesium are present only at micromolar concentrations in the human body but are of medical importance as they can be curative or toxic depending on concentration.[111,112] The medical applications relate to antidepressive, psychotherapeutical indication, whereas a small variation from the curative concentration causes cardiac defects or even death. X-ray scattering techniques and infrared spectroscopy showed that alkali chlorides influence the phase behavior of concentrated lipid bilayer systems. At concentrations in the molar range, alkali ions were found to shift the main phase-transition temperature of lipids by a few degrees. The effect of the different ions follows the order in the Hofmeister series. Monovalent cations were found to decrease the dipole potential of the polar headgroups in phosphatidylcholine vesicles, and the interaction was discussed in terms of free energies of hydration. In particular, the bilayer bending rigidity decreases at high salt concentrations, whereas the force needed to puncture supported bilayers increases with salt concentration. Lithium induces the strongest decrease in the area per lipid, and the maximal number of ions bound per lipid was estimated to be 0.28. Sodium and potassium showed weaker effects following the Hofmeister series. Sodium was also observed to bind more strongly to membranes than potassium; the

chloride anions were found to mostly stay in the water phase. The negative zeta potential has been interpreted in terms of the orientation of the hydration layers and lipid headgroups, water polarization, and impurities. Above a concentration of ~150 mM, the effective charge exhibits a plateau, suggesting saturation of the ion−membrane interactions. Note that this occurs at ratios of lipid to ion bulk concentrations smaller than 1:50. The molar enthalpies measured for all alkali metal cations adsorbing to the membrane are positive (endothermic), indicating that this interaction is entropically driven. A naive estimate for the number of water molecules released can be made from the change in the internal energy. The latter is approximately equal to the enthalpy change ΔH if we assume that both the density and the pair distribution functions of the bound water molecules are roughly equal to those of the liberated water molecules. Then, from the equipartition theorem, it follows that $\Delta H = n(f-3)k_BT/2$. Here, n is the number of released water molecules and f is the number of classical degrees of freedom. When released, a single water molecule gains three translational and up to three rotational degrees of freedom, i.e., $3 < f < 6$. In salt-free solution, the vesicle suspension shows an endothermic phase transition. The occurrence of the ripples has been considered as formation of defects of fluid molecules. Apparently, the results suggest that these defects are less expressed in the presence of lithium and sodium chlorides, supporting furthermore the stabilizing effect of these salts. The isothermal titration calorimetry (ITC) measurements show that binding of ions to phosphocholine bilayers occurs spontaneously but is an endothermic process and, therefore, entropy-driven. The gain in entropy presumably arises from the release of several water molecules from the hydration shell of the ion as well as dehydration of the lipid membrane. This behavior is easily explained by considering the ion−membrane interaction as a "binding-by-dehydration" process.

Ryhänen et al.[113] investigated the effect of anions on the self-assembly of a cationic gemini surfactant, (2*R*,3*R*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)butane dibromide, through differential scanning calorimetry (DSC) and Langmuir spreading isotherms. They found that F^- does not adsorb but is rather depleted from the monolayer interface. Instead I^- penetrates between the surfactant's heagroups. Because the amphiphile is a cationic gemini, anions occupy the first layer, while cations remain in the second layer. Cl^-, Br^-, and I^- show an anomalous behavior and stabilize the monolayer. Moreover, chloride interacts strongly with the polar heads and forms a pseudocrystalline lattice of salt on the surface of the aggregate.

Giner et al.[114] investigated the effect of ions on the behavior of spreading monolayers of a cationic amphiphile (viologen) at the air−water interface. In particular, chaotropes produce more compact films and an overshoot in the isotherm, while kosmotropes produce a kink. Anions on the right side of the Hofmeister series can easily penetrate into the positively charged viologen monolayer and thus better compensate the positive charges of the amphiphile. There are differences relative to the surface pressure at which the liquid expanded-liquid condensed transition occurs: perchlorate (right side) shows the transition at 20 mN/m, and the other anions shift it to 30. Monovalent anions produce an overshoot in the isotherm; the multivalent anions produce a kink.

Hennig et al.[115] studied the transport of anions across lipid membranes and interpreted their results in terms of anion−macrodipole (protein) interactions. This study is relevant also for ion pumps. Oligourea/amide macrocycles self-assemble into parallel nanotubes and operate with macrodipole−potential and anion−macrodipole interactions. The results indicate that the key factor is the dehydration of the anion before it penetrates the bilayer rather than its selectivity in binding the carrier.

Jäger et al.[116] investigated the effect of sodium and potassium on persistent T-shaped dendrimer-based micelles. Grijalba et al.[117] investigated the effect of electrolytes on amphotericin and some of its derivatives. This study addresses an important topic, i.e., the effect of salts on the pharmacological and toxic properties of the polyene antibiotic amphotericin (AMB) that are related to its self-association and aggregation properties. The understanding of these processes at the physicochemical and molecular levels should contribute to elucidation of the mechanisms of action and toxicity of this widely used antibiotic and to develop more efficient and less toxic formulations. Aqueous AMB dispersions contain polydisperse aggregates whose properties depend on concentration, ionic strength, and time. The monomer aggregation was evaluated spectrophotometrically by the ratio between the absorbances at 409−414 nm (attributed to the monomer, A_M) and at 320−335 nm (attributed to self-associated forms or aggregates, A_A), A_M/A_A. The vibronic peak at 409 nm in AMB's optical absorption spectra is due to the monomer, whereas the broad band in the 325−360 nm region is ascribed to small and large aggregates. Kosmotropic anions promote the association of the polyenes, causing a drastic increase of polydisperse aggregates in a concentration-dependent manner. Sulfate-induced aggregation was observed for a wide range of antibiotic concentrations, from 0.1 to 50 μM. Sulfate also promotes the self-association of AMB derivatives. On the other hand, monomerization increases in the order perchlorate < urea < thiocyanate < trichloroacetate. When comparing the various polyenes, the effect is less strong for the charged derivatives. This is due to the fact that they are more soluble, even in the absence of salt. This observation is corroborated by the analysis of the dependence of A_M/A_A on the trichloroacetate concentration. The less soluble the macrolide, the more pronounced is the monomerization effect.

Manet et al.[118] investigated the self-assembly of 14−2−14 gemini surfactants $C_nH_{2n+1}-N^+Me_2-CH_2-CH_2-N^+Me_2-C_nH_{2n+1}$ paired to small counterions. As expected, the CMC of the gemini 14−2−14X followed the direct Hofmeister series: $I^- < NO_3^- \approx Br^- < Cl^- < F^- \approx CH_3COO^- < H_2PO_4^-$. The micellization free energy ranges from about −18 kJ/mol for the least favorable micellization with kosmotropic ions ($H_2PO_4^-$) to about −27 kJ/mol for the most favorable micellization with chaotropic ions (Br^-) following the order: $Br^- \approx NO_3^- < Cl^- < CH_3COO^- < F^- < H_2PO_4^-$. The same order was observed for the evolution of the CMC values as well as ionization degree: a higher Gibbs free energy of micellization ($\Delta G_{M.25}$) correlates with a higher CMC and ionization degree with the presence of more ionized micelles, and the micellization process is less endothermic compared to gemini with chaotropic counterions. An important and general finding is that the effect of halide ions follows the predicted trend, whereas the variation of the parameters for polyatomic anions such as NO_3^-, CH_3COO^-, and $H_2PO_4^-$ is not systematic and deviates from the expected behavior. In particular, all the physical properties of the ions (see Table 1) correlate to their CMCs as long as *the ions preserve the same electronic configuration and the same spherical morphology*, such as the halides. This is a very general and important conclusion, which can often be traced in Hofmeister studies. The authors observe also that hydrated and charged headgroups and counterions in solution can associate reversibly to form ion pairs.

Table 1. Partial Molar Volume (ν_s in cm^3/mol), Polarizability (α in Å^3), Hydration Number (n_H), Free Energy of Hydration ($-\Delta G_{hydr}$ in kJ/mol), Calculated Partition Coefficient (as logP_{calc}), Free Energy of Transfer from Aqueous to Organic Phase (ΔG_{HB} in kJ/mol), Liotropic Number (N), and Acidity Constant (as pK_a) at 25° C (Reprinted with Permission from Ref 118; Copyright 2010 American Chemical Society)

ion	ν_s	α	n_H	$-\Delta G_{hydr}$	log P_{calc}	ΔG_{HB}	N	pK_a
I^-	41.7	7.51	1.6	283	1.04	−1.09	12.5	−11
NO_3^-	34.5	4.13	2.0	306	0.21	−0.68	11.6	−1.3
Br^-	30.2	4.85	1.8	321	0.63	−0.80	11.3	−9
Cl^-	23.3	3.42	2.0	347	0.54	−0.61	10.0	−7
CH_3COO^-	46.2	5.50	2.2	373	0.09	0.12	4.8	
$H_2PO_4^-$	34.6	5.79	1.8	473	−0.77	−0.10	8.2	2.2
F^-	4.3	0.88	2.7	472	0.23	0.08	4.8	3.2

In this process some hydration water molecules are released into the bulk with a resulting entropy increment. Hydrophobic counterions interact more strongly with an amphiphilic micellar interface, which results in stronger ion-pair formation, favors micelle formation, and decreases the CMC. However, if two ions with similar hydrophilicity are compared, other parameters that are directly linked to the entropy gain upon ion-pair formation must be considered.

For example, the size (expressed as partial molar volume ν_s), the polarizability (α), and the hydration number (n_H) are those which clearly differentiate $H_2PO_4^-$ from F^-; all are related to the large size of the dihydrogen phosphate ion. Indeed, for these parameters, $H_2PO_4^-$ ions are more similar to ions such as Br^- or I^-. These differences in ion properties are presumably the origin for the very high CMC value induced by $H_2PO_4^-$. Briefly, ions with high hydration numbers result in the high entropic gain for surfactant aggregation.

Although the hydrophilicity of an ion does not alone favor micellization, the increased hydration of the counterion does promote micellization (thus lowering the CMC) for entropic reasons. $H_2PO_4^-$ ions are large, hydrophilic, highly polarizable and weakly hydrated, and thus more unfavorable toward micellization, leading to higher CMC than the highly hydrated fluoride. Acetate ions, which are relatively hydrophilic like Cl^- but larger (similar partial molar volume as I^-), have more modest but similar properties: high polarizability (similar to that of Br^-) and intermediate hydration ($n_H \approx n_H$ of Cl^-) with a high CMC (about the same CMC of F^-). Conversely, the combination of relatively high hydration and strong hydrophobicity (ΔG_{hydr} = −306 kJ/mol, an intermediate value between those of Br^- and I^-) of NO_3^- does not lower the CMC of the surfactant (similar to the CMC in the presence of Br^-). Here, it seems that other parameters such as its relatively low polarizability and high pK_a disfavor ion-pair formation and decrease the entropy gain upon micellization, leading to higher-than-expected CMC values for NO_3^-. The ensemble of these observations clearly demonstrates that these opposing effects (e.g., ion hydration, ion polarizability, intra/intermolecular hydrogen bonds, and steric hindrance) all affect the entropy gain upon micellization and the propensity of the counterions to form ion pairs with the amphiphile headgroups. In other words, ion-pairing cannot be accurately described using one single parameter but requires an ensemble of several parameters. This is why, for example, two ions with similar hydrophilicity but with different hydration and/or with different steric effects may lead to very different CMCs. Indeed, by using different families of anions and varying independently the effect of various ion properties (position in the Hofmeister series, hydrophobicity, hydrogen bonding, and substitution), we could unambiguously demonstrate how such effects can cooperatively affect the propensity of counterions to form ion pairs with surfactant headgroups and alter the entropic energy gain upon micellization. These results provide new insight into understanding the effect of ions on the delicate balance of forces controlling aggregate morphology and solution properties of charged amphiphilic molecules. The investigation of the aggregation number and the concentration of micelles revealed intriguing micellar properties. Regardless of the absolute CMC values and the ionic properties that show an important variation with the counterions, the aggregation number of micelles just above the CMC depends only on the normalized concentration D_t/CMC (D_t is the total surfactant concentration) as long as the molecular structure of the cationic moieties of gemini surfactant is fixed and spherical micelles are considered. On the other hand, the concentration of the micelles depends on the absolute concentration of the surfactant, that is, at a given concentration above the CMC, all the gemini 14−2−14 studied have the same number of micelles per unit volume, which increases with the surfactant concentration, indicating that this process is primarily controlled by entropy.

Minnes et al.[119] investigated the effect of different salts on the partitioning of hematoporphyrin IX (HP) and hypericin (Hy) into liposomal membranes. HP is one of the most and best-studied sensitizers in biological photosensitization and photodynamic therapy (PDT) treatment. In all cases, increasing the concentration of electrolytes strongly enhances the partitioning, even by more than a factor of 4 as in the case of $MgSO_4$. The authors found that the effect of the ionic components of the salts exhibit some, although not complete, correspondence with the Hofmeister order of chaotropic ions. This indicates that the partitioning of these amphiphilic sensitizers between the aqueous and lipid phases is strongly affected by their solvation in water. This, in turn, is influenced by the effect of ions that are known to have a chaotropic effect. The authors extract the following consequences from the observed salt effects on the partitioning constants of photosensitizers to liposomes: (i) Mg^{2+} does indeed have a stronger effect than K^+ and Na^+ ions, in accordance with the Hofmeister series. (ii) SO_4^{2-} does not seem to affect the partitioning more strongly than Cl^-. This is not in accord with the accepted ranking of the series. (iii) NO_3^- has a weaker effect than that of Cl^- ions, and of course SO_4^{2-}. (iv) The effects of Na^+ and K^+ are almost identical, as expected by the regular series. (v) It appears that the cation is responsible for most of the effect of HP partitioning. This arises from conclusion (ii) and from the similar effects of KCl and KF, whereas $MgCl_2$ exhibits a markedly different influence from KCl or NaCl. These results indicate a clear salting-out process of the photosensitizer molecules from the aqueous phase into the lipid environment. These results further imply that for each sensitizer−lipid pair we might find an optimal type and concentration of salt, at which the partitioning would be optimally amplified and possible unwanted side effects of high electrolyte concentration would be minimized. The strong enhancement of passive uptake of these sensitizers by lipid membranes by electrolytes could be employed for photosensitization in liposomes as models of biological membranes, as well as for their use in natural cellular membranes. This salt effect on passive

partitioning into membranes may be applicable for cancerous tissues in which the osmotic pressure can be controlled, such as kidney and melanoma. The salt effect may be important also for tissues where currently we cannot control the osmotic pressure. Also, changing electrolyte concentration may be relevant in topical photosensitization of bacterial infections. Liao et al.[120] studied the wettability changes of silica nanoparticles coated with a copolymer obtained from *N*-isopropylacrylamide (NIPAM) $H_2C{=}C{-}CO{-}NH{-}C(CH_3)_2$ and *N*-acryloxysuccinimide (NASI, Figure 6e).

The nanocomposite grafts were prepared by covalent layer-by-layer assembly and exhibit striking changes in surface wetting in response to changes in solute identity or concentration according to the Hofmeister series. Furthermore, the same surface was found to be reversibly either hydrophilic or hydrophobic, depending on the identity of the salt. The solute-induced wettability changes were found to be sensitive to the concentration of the given solute with significant differences in contact angle (Θ) for even dilute aqueous solutions of a solute such as sodium citrate. According to the results, the swelling of the polymer on the textured surface follows the expected Hofmeister series, which predictably alters the surface roughness and the associated wetting and dewetting of the surface.

A similar topic was addressed by Fu et al.,[43] who studied the effects of salts on the LCST of PNIPAM and the wettability of PNIPAM-coated SiO_2 nanoparticles. Below the LCST, these polymers are soluble and hydrophilic with an extended coil conformation. Above the LCST, these macromolecules undergo a sharp phase transition and assume a collapsed hydrophobic conformation. The LCST of a thermoresponsive polymer changes upon the addition of salts to an aqueous solution, and their effects on the LCST follow the Hofmeister series (see also refs 12 and 91). Although temperature-induced wettability changes of a thermoresponsive surface can be sufficient for a particular application, additional triggers such as changes in ionic strength sometimes can enhance the utility of a stimulus responsive surface. These studies showed that changing the anion identity and concentration of various sodium salts had a significant impact on water contact angles, an effect similar to that seen in studies of temperature effects on the wettability of PNIPAM grafts on nanostructured surfaces. These anion effects on the wettability of PNIPAM−silica nanocomposite grafts paralleled the Hofmeister-like effects of the same alkali metal salts on PNIPAM LCSTs, wettability changes that are larger than those seen on PNIPAM grafts that do not contain silica nanoparticles (see ref 101). In this study the effect of the cation nature and charge on the surface wettability of these nanocomposite PNIPAM grafts was investigated. Using activities instead of concentration, the strength of the cation effect on wettability follows the order trivalent > divalent > monovalent. These changes observed by atomic force microscopy (AFM) experiments correlate with the changes in wettability and are consistent with the notion that these solutes produce a solute-responsive swelling or chain collapse that alters the hydrophobicity of the PNIPAM component of the nanocomposite graft. These studies show that cations have large effects on the phase separation of PNIPAM solutions and surface wettability of PNIPAM nanocomposite grafts. In summary, these effects on surface wettability are correlated with a variation of solvation and swelling and depend on the surface roughness of the nanocomposite grafts.

Maiti et al.[121] investigated the effect of inorganic and organic salts on the micellization of SDS and tetradecyltrimethylammonium bromide (TTAB) at a relatively low ionic strength (10 mM). They found that micellization and viscosity depend on the interaction between the headgroups and the counterions. The low salt concentration is presumably the reason why they did not observe large Hofmeister effects.

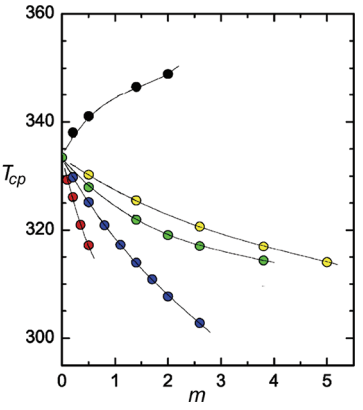

Figure 11. Cloud point (T_{cp}, in K) of a 2% v/v C_8E_5 aqueous dispersion as a function of the salt concentration (*m*, in mol/kg) in the presence of NaF (red), NaCl (blue), LiCl (green), NaBr (yellow), and NaI (black). Reprinted with permission from ref 125. Copyright 2007 Elsevier.

Abezgauz et al.[122] studied the addition of different salts to cetylpyridinium chloride. They found that typical chaotropes (Br^-, NO_3^-, ClO_3^-) induce a sphere → rodlike → wormlike transition, while kosmotropes (CO_3^{2-}, PO_4^{3-}, OH^-, and SO_4^{2-}) do not affect the shape (spherical) and viscosity of the micelles. The authors assume that the pyridinium headgroup behaves as a chaotropic species and interpret the results in terms of Collins' law of matching affinities. (Experiments on exchange in counterion in a Hofmeister series with the double-chained cationic surfactant didodecyldimethyl ammonium bromide to form vesicles and with corresponding microemulsions and their predictable microstructure long predated such work. This is similar for double-chained anionic surfactants.[1])

Jiang et al.[123] studied the self-assembly properties of C_{16}TABr when the counterion is exchanged to F^-, Cl^-, NO_3^-, or SO_4^{2-} through ion-exchange chromatography. The values of the CMC, micelle ionization, ΔH_{mic}, and ΔS_{mic} follow the Hofmeister series, and the results are interpreted in terms of counterion binding at the micellar interface. The interpretation is supported by electron paramagnetic resonance (EPR) measurements.

Onorato et al.[124] investigated the effect of the thiocyanate anion on a monolayer of dodecanol at the air−water interface, through UV second harmonic generation spectroscopy. As expected, due to its chaotropic nature, thiocyanate adsorbs at the film interface, whereas the effect of the cation seems to be irrelevant. The effect of salts on the cloud point and on the shape of the phase-separation curve was investigated by Weckström and Papageorgiou[125] up to large salt concentrations, in a very interesting paper.

The experiments show a significant effect of the anion nature on the position and shape of the cloud-point curve: NaF and NaCl produce a strong salting-out and a significant change of the boundary shape, NaI induces a strong salting-in, whereas intermediate species such as LiCl and NaBr produce intermediate changes and small changes in the shape. Figure 11 shows the cloud-point temperature as a function of the salt concentration for a 2% v/v aqueous dispersion of C_8E_5.

The experimental results are interpreted by means of an equation that relates the surface properties of the alkali halides to the cloud-point temperatures of C_8E_5 and of a PEO polymer in the presence of salts at different concentrations. The surface properties of the electrolytes are used to set a reference for their effects in solution. The authors propose a new concept, a hypothetical electrolyte that possesses "matching" (ideal) properties, based on the values of the surface excess of water Γ_w.

Kresheck[126] studied the effect of salts on the micellization of *n*-decyldimethylphosphine oxide. The results were analyzed in terms of the salt ion partitioning model[72] and related to the solute free energy increment (SFEI), which needs the calculation of the solvent accessible area (ASA). The latter parameter can be evaluated from the heat capacity change for micelle formation.

Lynch and Piculell[127] investigated the behavior of C16-, C14-, and C12-trimethylammonium surfactants with bromide, chloride, or acetate as counterions. The results indicate that the counterion binding is mainly determined by the surfactant hydrophobicity. Above a certain hydrophobicity threshold, which is specific for a given surfactant, the binding depends on the counterion nature and follows the expected Hofmeister trend ($Br^- < Cl^- < CH_3COO^-$). The presence of a second threshold indicates that the gel is hydrophobic enough to induce a saturation in the binding below the surfactant CMC. After that it does not depend further on the counterion. Because of the large charge density of the micellar surface, and the great amount of condensed counterions, the differences in the polarizability of the counterions are not relevant to explain the swelling and ion-binding processes. Therefore, according to the authors, the specific ion effects are relevant only for a narrow range of hydrophobicity, i.e., when the gel is sufficiently hydrophobic to induce counterion binding but not so hydrophobic as to reach a saturation level before attaining the CMC.

In another contribution by the same authors and Sjöström, the binding of a cationic amphiphile (C_{16}-trimethylammonium bromide, chloride, and acetate) on neutral polymeric hydrogels (from polyacrylamide) at different degrees of hydrophobicity was investigated.[128] In particular, they studied SDS, SD−EO_2−S, DAM (H_2C═CH−CO−NMe_2), NIPAM (H_2C═CH−CO−NH−$CHMe_2$), and BM (H_2C═CH−CO−NH−CMe_3). The results suggest that the binding depends on the counterion size. To have an interaction between the surfactant and the gel, it is necessary that the CAC_{gel} (CAC = critical aggregation concentration) is not greater than the CMC and that the gel-forming polymer has a minimum degree of hydrophobicity. The behavior of the mixed system can be various: (i) either the swelling increases above the surfactant CMC and is affected by the nature of the counterion, (ii) or the swelling increases up to the CMC before reaching a plateau, and the counterion does not affect the swelling, (iii) or the swelling reaches a stable level even before the CMC is reached.

Swann et al.[129] studied the swelling of poly(methyl methacrylate) (PMMA) polymers in water in the presence of salts. Salts modify the swelling behavior of the pH-responsive hydrogel, following the Hofmeister series. The response parallels parameters that describe the interactions of the ions with water such as surface charge density, viscosity coefficient, and entropy of hydration; therefore, the major role seems to be played by the ion−water interactions that affect the polymer behavior. Increasing the salt concentration leads to a reduction in swelling. At fixed electrolyte concentration, the reduction of swelling is the result of the partitioning of the specific anions between the gel and the solution. The more chaotropic the ions, the more they partition within the gel, increasing the local ionic strength and allowing a greater collapse in the gel structure.

The aggregation of NIPAM and methylbenzylamine (MBA) charged microgels was studied by Hou et al.[130] The results indicate that the aggregation is regulated by interfacial effects, and the aggregation temperature follows the direct Hofmeister series. The electric charge on the polymer or copolymer seems to be the leading factor in determining whether the salts induce aggregation or not.

The swelling of hydrogels produced by salt-resistive polymers was investigated by Wang and Satoh[131] on poly(vinyl alcohol)-trimellitate (PVA-T, Figure 6f), in the presence of different salts with a combination of different cations (Li^+, Na^+, K^+, and Cs^+) and anions (SO_4^{2-}, Cl^-, and SCN^-). Although the super salt-resistivity of poly(4-vinyl phenol) and poly(4-vinyl benzoic acid) was previously ascribed to the simultaneous presence of the π-electron conjugated system of the benzene ring and of the acid protons, here the authors demonstrate that poly(vinylalcohol), a common hydrophilic polymer, can be transformed into a salt-resistive polymer by introducing a trimellitic acid group, and the salt-resistivity may be modulated depending on the esterification degree of PVA with trimellitic anhydride. Regardless of the esterification degree, a salting-in effect was observed and the ion-specificity followed the trend $SO_4^{2-} \gg SCN^- > Cl^-$ for anions and $Li^+ > Na^+ > K^+ > Cs^+$ for cations. This means that even a relatively low level of substitution induces a significant variation in the response of the parent polymer (PVA) to ions, especially to anions.

A clear example of specific ion effects occurs in the case of the widely occurring natural polymer, chitosan, whose conformation changes upon replacement of chloride with acetate.[132] The polymer is collapsed and insoluble in chloride form, and it is highly soluble and extended in acetate form. This sensitivity of conformation to anions is exploited in snake, spider, and blue ring octopus venom and is well-known (see chapter 9 in ref 1). Most specific ion effects that occur in gels and cause changes in gel structure are exactly parallel and due to the same specific ion-induced molecular forces that occur in surfactant and lipid systems and dictate self-assembly.

7. HOFMEISTER EFFECTS IN PROTEINS AND ENZYMES

Hofmeister's studies were conducted on a protein, egg albumin (see ref 3 for an English translation). Earlier Wilhelm Ostwald, the father of the founder of German colloid science, Wolfgang Ostwald, had developed a major pioneering interest in enzymes. Specific ion effects on proteins predate all others. They have consequences and applications that are central to modern biotechnology. Specific salt−protein interactions determine phenomena like protein folding, association, stability, and precipitation. These can be controlled, prescribed, and applied to affect the stability, dosage, formulation, and administration of protein-based drugs for biomedical applications (see refs 72, 133, and 134). The onset of Alzheimer's, Huntington's, and Parkinson's diseases appears to be related to the aggregation of proteins.[134] These issues have been tackled by different authors in part in studies of effects of electrolytes on proteins and enzymes in vitro.

7.1. Proteins

In the description of the process that leads to protein association and eventually phase separation, Collins[51,52,56] addresses the issue of the different levels of hydration that occur at a

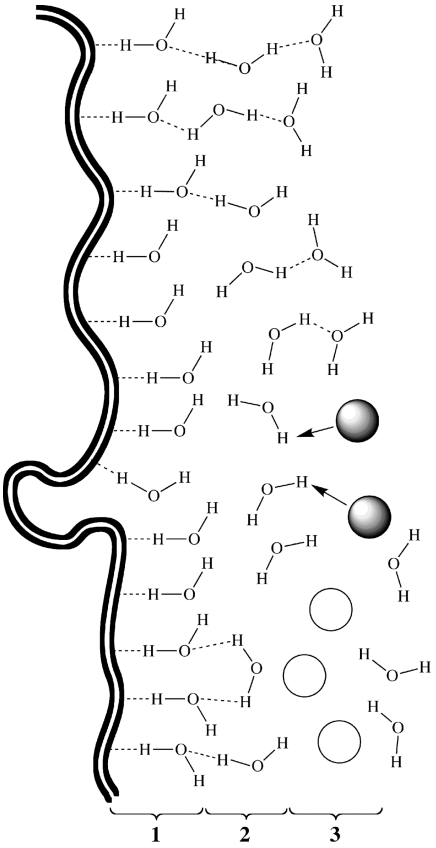

Figure 12. The three layers of hydration around a protein molecule (1, solvation layer, 2; transition layer; and 3, bulk layer). The dark and white circles represent a kosmotropic and a chaotropic ion, respectively. Adapted with permission from ref 51. Copyright 2004 Elsevier.

protein surface. The context is within the same schema as that of the law of matching water affinities described above. Briefly, the interfacial water that wraps up a protein macromolecule can be thought of as divided into three interdependent layers (see Figure 12). The first layer, immediately facing the protein surface, is the solvation layer (1), followed by a transition (2), and the bulk (3) layers. The specific structure, charge, and composition of the protein determine the solvation layer, whereas the composition and properties of the bulk solution determine the behavior of the bulk layer. The first and third layers compete for hydrogen bonding with the second layer.

If ions or neutral solutes are added to the bulk layer, they will affect the capability of the transition layer to solvate the protein surface. In particular, a kosmotrope in the bulk layer will "divert" the second-layer molecules from participating in the hydration of the protein, and the solution is a poorer solvent for the protein, which eventually minimizes the solvent exposed area by increasing its compactness (folding) and rigidity. On the other hand, the presence of chaotropic species in the third layer promotes the solvation of the protein surface by the transition layer; the solution becomes a better solvent for the protein, which unfolds, exposing more surface to the solvent.

Dér et al.[135] discuss the effect of interfacial water structure on protein conformation, a very interesting topic. Comparisons with the air/water surface tension increment do not correlate well with the effect of different salts on protein conformation. We remark that this popular correlation, with properties of ions at the

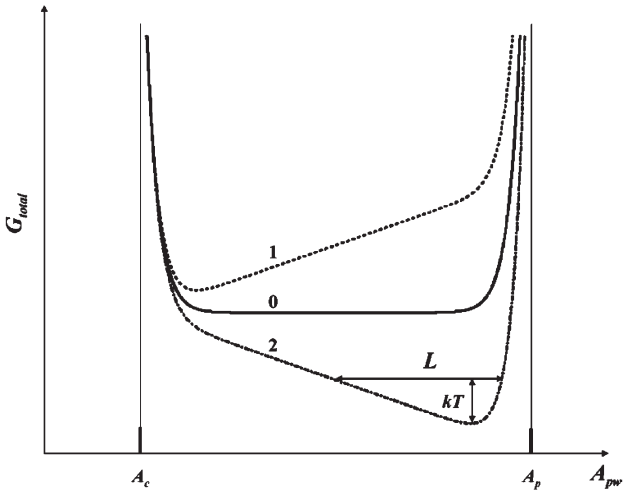

Figure 13. Sketch of the protein energy landscapes (total free energy G_{total} as a function of interfacial area A_{pw}) for the three protein families, defined as flat-bottomed (family 0, rare), closed conformation (family 1, the most represented), and open conformation (family 2, rare). L is the lability parameter, i.e., the allowed range of A_{pw} at a height of k_BT above the energy minimum (drawn for a natively open protein) is a measure of lability, or fluctuation amplitude. Reprinted with permission from ref 135. Copyright 2007 American Chemical Society.

air−water interface, is not very fruitful. Adsorption of ions at the oil−water or protein−water surface is driven by dispersion forces mainly, not electrostatic forces at physiological salt concentrations. Indeed, the dominating determinant of ion adsorption is due to the missing dispersion forces, which can change in sign from the air−water to the oil−water interface. The specific water/protein interface tension parameter ($\Delta\gamma_{pw}$) is a more appropriate entity. This parameter can be calculated from the change in the interfacial term of ΔG ($\Delta G_{interfacial}$) during the conformational change as $\Delta\Delta G_{interfacial} = \Delta\gamma_{pw}\Delta A_{pw}$, where A_{pw} is the protein−water interfacial area. $\Delta\gamma_{pw}$ depends on the nature of the solvent-exposed amino acid residues, on the strength of the hydrogen bonds in water, and on the excess surface concentration of salts.

Through Fourier transform infrared (FTIR) measurements, the strength of the hydrogen bonds in water is known to decrease in the series $F^- > CH_3COO^- > Cl^- > SCN^- > ClO_4^-$ (fluoride and acetate strengthen, the other ions weaken hydrogen bonds in water). (We remark here that bromide and chloride ions bind strongly to the dimethyl- or trimethylammonium cation, whereas acetate and nitrate and other anions interact in completely different ways.)[1] It is then not surprising that kosmotropes increase and chaotropes decrease $\Delta\gamma_{pw}$. Here the conformational changes are discussed in terms of *fluctuations* between "open" and "closed" conformations (see Figure 13).

These salt-induced changes imply different solvent exposed areas. If so, that modifies the functional efficacy of proteins.[135,136] In particular, Dér et al. tested myoglobin (Mb, the most abundant protein in animals) and the membrane protein bacteriorhodopsin.[135] In these cases, chaotropes stabilize the proteins, reduce the amplitude of the fluctuations, and increase the melting temperature of the protein if it has an open conformation in its native state. On the other hand, chaotropic ions destabilize those proteins that possess a closed conformation in the native state. The effect of salts is discussed in terms of amplitude changes in the

fluctuations in the interplay between conformations, and of hydration. In fact, water is essential to keep a sufficient structural flexibility that is necessary for the local motions.

Along the same lines, Varhač et al.[137] studied the stability and dynamic flexibility of cytochrome C. These depend on dynamic properties of the solvent because the protein−water interactions lower the energetic barriers between conformation states, and the salts can have a direct impact on these interactions. In particular, the binding of phosphate ions destabilizes the protein and increases the flexibility of the heme region. This issue is of significance for physiological reasons. Weak accessibility of the heme group to the solvent at physiological ionic strength conditions, and the ion-specific different mobilities of the heme region, affects the redox properties and the stability of this protein. Moreover, the binding of SCN^- ions to the protein proceeds at a kinetic rate that depends strongly on the background electrolyte anion. It follows a bell-shaped, nonmonotonic trend with increasing concentration (see Figure 14).[137]

Tadeo et al. reported on the stability changes in protein L from *Streptococcus magnus* induced by different salts and discuss the effect of anions in terms of the nonpolar solvent-accessible area and of the protein−solution interfacial tension.[138] In an interesting study on chymopapain, López-Arenas et al.[139] try to separate the effect due to electrostatic interactions from dispersion forces. A general "electrostatic screening" stabilizes the transition state over the native state of the macromolecule, while Hofmeister (nonelectrostatic) effects alter the unfolding rates according to the known structure "stabilizing" or "destabilizing" properties of the particular ions. The small ions affect the unfolding kinetics of chymopapain in two ways: (1) at low concentration, different electrolytes produce a similar increase of the reaction rate, a phenomenon that is assigned to "electrostatic screening" of the charged groups in the macromolecule, and (2) at high electrolyte concentration, ion-specific effects are clearly manifest in a way that agrees with the Hofmeister characteristics of each particular ion. Hofmeister effects were further used to estimate the amount of area exposed to solvent (ASA) on formation of the transition states. The rate constant of the unfolding process can be written as

$$\ln k = \ln E - \frac{\Delta G_0^{\ddagger}}{RT} - \frac{\Delta G_{el}^{\ddagger}}{RT} - \frac{c_i m_i^{\ddagger}}{RT} \tag{22}$$

where $\ln E - (\Delta G_0^{\ddagger})/(RT)$ is the value of $\ln k$ without salt. $\Delta G_{el}^{\ddagger}$ is the contribution of the electrostatic screening to the activation free energy, $m_i^{\ddagger}$ is a parameter specific for each electrolyte whose concentration is c_i. $m_i^{\ddagger}$ is given by the difference between the interaction parameters for the transition state and the native state: $m_i^{\ddagger} = \beta_{TS,i} - \beta_{N,i}$, where $\beta_{TS,i} = \partial\mu_{TS}/\partial c_i$ and $\beta_{N,i} = \partial\mu_N/\partial c_i$. Therefore,

$$\begin{aligned} m^{\ddagger} &= \beta_{TS} - \beta_N \\ &= \beta_{pol} \cdot \Delta ASA_{pol} + \beta_{npol} \cdot \Delta ASA_{npol} \end{aligned} \tag{23}$$

where ASA_{pol} and ASA_{npol} are the polar and nonpolar solvent-accessible surface areas, respectively. β_{pol} and β_{npol} are computed from the respective salting-out constants. In its native state, chymopapain possesses 14 acid side chains. However, 11 of these are located <5 Å away from one or more positively charged groups, so very short ion pairs are formed; all these groups are located close to the surface but with no, or little, area exposed to the solvent. Some of these acid groups may remain unprotonated even at pH 1.5 and might form strong ion pairs that strongly stabilize the native molecule.

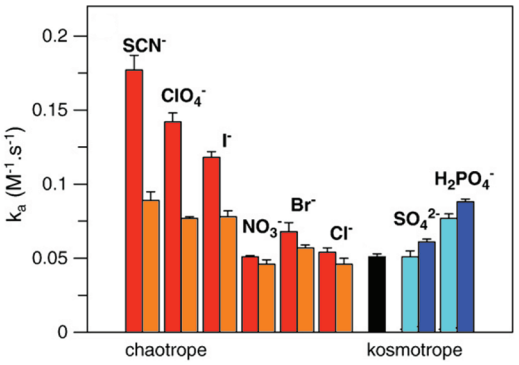

Figure 14. Association rate constants of cyanide binding into cytochrome C for different anions at 0.5 M (orange and light blue), 1.0 M (red and deep blue), and without salts (black box). Reprinted with permission from ref 137. Copyright 2009 Elsevier.

Pegram and Record[72] recently proposed a solute partitioning model (SPM) that, starting from the surface tension increment data, calculates the partition coefficient K_p for a single ion between the bulk and an interface. The issue of ion partitioning is a very clear effect of specific dispersion forces that act in ion−substrate interactions.[84,140] Nonuniform distribution of small solutes, and of salt ions near surfaces, is responsible for the often large effects of concentration of these solutes on a very wide range of processes in water.[72] These include formation or disruption of biopolymer−water interfaces, molecular solute−water interfaces, and macroscopic air−water interfaces. Salt ions and uncharged solutes exert their effects depending on whether they are accumulated in, or excluded from, the water of hydration at the biopolymer surface exposed/buried in a particular process.[73] The partition coefficients K_p are assumed to be independent of the ion concentration and of the nature of the companion ion. The model considers the transfer of a model compound from a nonaqueous phase to water. It envisages a situation in which the salt affects the thermodynamics of transfer when the concentration of the ions (cation and anion) is different in the bulk phase from their concentration in the interfacial hydration layer of the model compound. The partition coefficient K_p is then considered as arising from different contributions due to the apolar regions, to the amide polar groups, and to other polar residues (such as the ester oxygen atoms in a polypeptide). In general, anions accumulate indifferently in proximity of the amide groups, and chaotropic species generally induce an increment in the solvent-exposed surface of the macromolecule. Such modeling may also be useful in other kinds of association processes, such as those that involve the formation of an α-helix in a peptide, or in DNA−protein complexes.[72]

The partition coefficient ($K_{p,3}$) is defined as the ratio of molal concentrations of solute in the local and bulk domains:[73]

$$-\frac{\partial \Delta G_{obs}^0}{\partial m_3} = RT\frac{\partial \ln K_{obs}}{\partial m_3} = RT(1 + \varepsilon_3)\frac{b_1 \Delta ASA(K_{p,3} - 1)}{m^*_1} \tag{24}$$

where b_1 is the number of water molecules per unit surface area in the local biopolymer domain defined by ΔASA and m_1^* is the solvent molality. The term $1 + \varepsilon_3$ accounts for nonideality when the solute activity is converted into molality. Solute effects on the solubility of sparingly soluble model compounds (such as hydrocarbons, nucleic acid bases, amino acids with nonpolar acid

chains, and end-blocked amino acids) are interpretable in terms of partitioning of solute molecules between the bulk solution and the local phase of water of hydration at the molecular surface of the model compounds. *Accumulated solutes increase the aqueous solubility; excluded solutes decrease the solubility.*

When hydrocarbon solubility data are analyzed via the SPM approach, similar trends to those seen for the air−water interface are observed. Alkali metal cations are all excluded, whereas the different anions exhibit a wide range of partitioning behavior (strong exclusion of sulfate and strong accumulation of thiocyanate), which follows the Hofmeister order, as depicted in Figure 15.[71]

The alkali metal cations would have to be accumulated moderately at some other region of the protein surface to compensate for their strong exclusion from nonpolar surfaces in order to explain their intermediate (relatively nonperturbing) position in the series. Preliminary analysis of solubility and distribution coefficient data for model peptides indicates that Hofmeister salts are accumulated at a polar amide surface and that differences between different salts are relatively small in comparison to the situation observed with a hydrocarbon surface.

Because protein unfolding and other protein processes involve mostly changes in exposure of nonpolar and amide protein surface, the above interpretation of model compound data quantitatively confirms the previous proposal that the Hofmeister salt series observed for biopolymer processes results from the compensation between highly salt-specific net exclusion from nonpolar surfaces and net accumulation at amide surfaces.[73] However, the model does not discuss the different, specific interactions that involve the ions and the different regions of the protein interface.

An extensive report on different model systems has been recently published by Zhang and Cremer.[12] In particular, they considered the thermal behavior of uncharged models (hydrophobic collapse of thermoresponsive macromolecules) and of cationic proteins (liquid−liquid phase separation). In fact, proteins can undergo both cold and thermal denaturation. When the temperature of the solution is raised, a protein will denature as thermal energy starts to break hydrogen bonds, activate low-lying vibrational modes, and unravel the macromolecule. On the other hand, cold denaturation occurs because of the enthalpically favorable interactions between the more hydrophobic interior of the protein and solvent water molecules. To mimic the cold denaturation of proteins, the authors studied poly(*N*-isopropylacrylamide) (PNIPAM, Figure 6a) as a model macromolecule. The effect of Hofmeister anions on the hydrophobic collapse of PNIPAM can be explained on the basis of direct interactions of anions with the macromolecule and its first hydration shell. An anion, X^-, can polarize a water molecule that is directly involved in hydrogen bonding with the amide. The ability of an anion to polarize the first hydration shell of the polymer is manifest quantitatively in the hydration entropy, S_{hydr}, of each anion. Second, anions can interfere with the hydrophobic hydration of PNIPAM by increasing the surface tension at the hydrophobic−aqueous interface. As the salt concentration increases, the surface tension is increased at the aqueous/polymer interface. Moreover, the energy for cavity formation at the interface will be raised. The surface tension increase is measured quantitatively by the surface tension increment, σ. Both the water polarization and the surface tension effect cause a depression in the lower critical solution temperature (LCST) of PNIPAM as the salt concentration is increased. These effects should vary roughly linearly with salt concentration at least up to moderate concentrations. By contrast, anions can also bind to the amide moieties in PNIPAM.

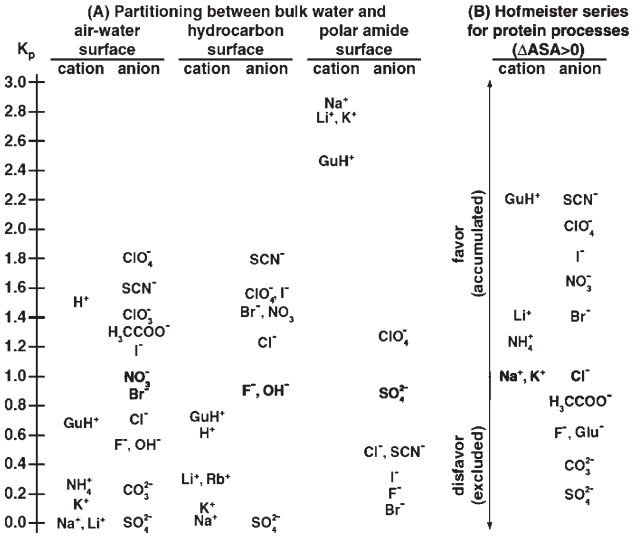

Figure 15. (A) Comparison of single-ion partition coefficients K_p for partitioning between bulk water and the air−water, hydrocarbon, and amide surfaces. (B) Relative Hofmeister rankings of anions and cations for processes that expose the protein surface to water (e.g., unfolding). K^+, Na^+, and Cl^- divide the cations and anions into those that are accumulated (and thus drive unfolding) and the excluded protein stabilizers. Reprinted with permission from ref 71. Copyright 2008 Elsevier.

This causes an increase in the LCST because it charges the polymer surface. Moreover, it is a saturation phenomenon. The results indicate that strongly and weakly hydrated anions affect the LCST of PNIPAM by different mechanisms. The chaotropes decrease the LCST via a surface tension effect, which causes hydrophobic collapse. For kosmotropic ions, the polarization of hydration shell water molecules and surface tension effects are both at work. Elastin-like polypeptides (ELPs) have an advantage over PNIPAM as a model system, in that their primary sequence is identical to that of protein systems. Additionally, their sequence and chain length can be precisely controlled by genetic expression in bacteria using recombinant DNA technology.

In the case of charged proteins, the relative efficacy of anions in influencing the physical properties of proteins follows distinct Hofmeister series depending on whether the macromolecules bear a net negative or net positive charge. For pH > pI of the protein, the direct series is followed. Conversely, an inverse Hofmeister series is typically observed when the solution pH < pI. As such, negatively charged proteins are thought to obey a direct Hofmeister series, and positively charged proteins are thought to follow an inverse Hofmeister series. This central issue has been addressed in a recent paper by Boström et al.[141]

Liquid−liquid phase-transition behavior can be found for numerous concentrated protein systems in water, especially if poly(ethylene glycol) is also added to the system. When these systems are cooled below the phase-transition temperature, they form micrometer-sized droplets of aggregated proteins. More polarizable anions have a stronger ability to partition to the protein/aqueous interface and decrease the interfacial tension. The behavior of these anions at the protein/water interface stands in stark contrast to their behavior at the air/water interface, where they all are known to increase the surface tension. The difference in behavior at the protein/water interface stems from the macromolecules having a much higher dielectric

constant than air. Indeed, it is well-known that the most chaotropic anions decrease the surface tension at oil/water interfaces. Their ability to do this correlates to their octanol−water partition coefficient, which is also dependent on the polarizability of the anion. Definitely, Hofmeister effects depend on the protein charge.[142]

Sedlák et al. have reported that ions interact with the peptide bond, which is considered as composed of a chaotropic dehydrated residue (the amide) and a kosmotropic hydrated group (the carbonyl moiety).[143] They studied the thermal stability of proteins through circular dichroism experiments on dispersions of apoflavodoxine (which carries −19 charges at pH 7) or cytochrome C (bearing +17 charges at pH 4.5), in the presence of different electrolytes. They found a correlation between the ion effect on the thermal stability and the surface tension increment σ, which suggests a direct interaction with the first hydration shell of the proteins. Apparently the ion charge is the dominant factor that describes the ion effectiveness, whereas there is no correlation with the ion polarizability. The results are interpreted in terms of a two-state reversible process that depends linearly on the ion concentration for values larger than 0.3 M, and anions are more efficient than cations. In conclusion, hydration—which depends on all of ionic size, electrostatics, and dispersion interactions with the solvent—determines the interactions between ions and peptide bonds when Hofmeister effects dominate.

Schwartz et al.[144] studied triglycine as a model peptide via near-edge X-ray absorption fine structure (NEXAFS) experiments. They modeled the results theoretically, seeking to further characterize the interactions of proteins with Na_2SO_3 and NaBr. Chaotropes (such as Br^-) were believed to interact directly with the protein analogue near the nitrogen sites, as well as with the exposed hydrophobic surfaces. Kosmotropes, on the other hand, were believed to polarize the water interacting with the nitrogen- and oxygen-containing groups and interact with the hydrophobic groups. The results then, based on a molecular probe, suggest that kosmotropes interact directly with the nitrogen backbone of peptides. This conclusion is apparently controversial when compared to previous reports.[12] Here the more kosmotropic anions are supposed to interact with the protein via mediating water molecules, whereas chaotropic anions should interact with a protein directly. However, the two studies were conducted at different ionic strengths, which affects specific ionic adsorption so that there is not necessarily a conflict. The matter deserves more detailed analysis in the light of recent theoretical progress. Interestingly, Schwartz et al. do point out the difficulty of trying to infer molecular phenomena from measurements that are inherently macroscopic, thus casting uncertainty on their molecular-level interpretation.

Mesophilic proteins are usually stabilized by the presence of salt, whereas thermophilic and hyperthermophilic proteins are destabilized.[136] Anions and cations produce different effects on the activity and thermal stability of enzymes by controlling their flexibility. Unexpectedly, Tóth et al. found that the thermophilic enzyme is stabilized by high ionic strength and shows a bell-shaped dependence of the protein activity on the position of the anions in the series (see Figure 16).[136]

The active site of the enzyme is too flexible with chaotropic anions and too rigid with kosmotropes: both situations may lead to a decrease in enzyme activity. Kosmotropic cations (Li^+) have a tendency to bind to peptide bonds and stabilize the open conformation of the active site (water molecules present in the active site prevent binding of the substrate).

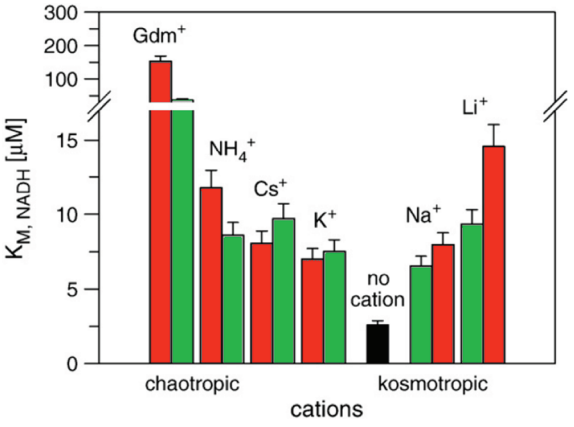

Figure 16. Michaelis−Menten constants ($K_{M,NADH}$) for NADH in the presence of 1 M (green) and 2 M (red) salt concentrations. Black box represents $K_{M,NADH}$ in the absence of salts. Reprinted from with permission ref 136. Copyright 2008 Elsevier.

Usually formulations contain peptides at high concentration (typically 100 mg/mL). In such conditions protein molecules aggregate and can undergo irreversible α-helix/β-sheet transitions that reduce the stability of the protein. The aggregation process depends on the surface charge and hydration of the protein.[133] Moreover, Le Brun et al. have shown that ions can strongly modify the protein−protein interactions by changing the second virial coefficient,[133] to the point that attractive forces can be converted into repulsive interactions. In our view this effect may depend on two things. An example is the salt effect on the cloud points of polyoxyethylene surfactants, where the forces go from repulsive to attractive, but simultaneously the micelles grow to cylinders, necessary to make the forces large enough for aggregation. In the protein case, unravelling above the cloud point is probably also involved along with the change in sign of the forces.

Besides its technological relevance, the aggregation process in protein dispersions is an obviously fundamental issue. Zhang and Cremer[145] investigated the behavior of lysozyme, the protein most commonly studied. Lysozyme undergoes a phase-separation process that can be investigated by measuring the cloud point. A cloud-point determination provides a simple physical measurement of the forces acting between biomacromolecules. Higher cloud-point temperatures mean stronger attractive forces between the protein molecules. Above the protein isoelectric point, the macromolecule bears a net negative charge and a direct Hofmeister series is normally observed. In this case, chaotropes such as I^-, ClO_4^-, and SCN^- help to unfold proteins and salt them into solution. By contrast, kosmotropes such as SO_4^{2-} and F^- lead to the stabilization of the folded state and cause a salting-out effect.

The aggregation behavior strongly depends on the nature and concentration of the background salt. This observation is explained by two simultaneous processes: (i) the attenuation of electrostatic repulsion through the specific association of chaotropic anions and (ii) the ability of the ions to alter the tension at the protein−aqueous interface. Qualitatively, the interplay of these two phenomena can be described as follows: lysozyme is a basic protein with a pI of ∼11.2, and hence, its surface is positively charged at pH 9.4. Repulsive electrostatic interactions between lysozyme molecules keep them from flocculating at low

salt concentrations. The presence of electrolyte ions in the solution (that modify the value of pI as well), however, changes this situation. When chaotropic anions associate with positively charged sites on the protein surface, the effective surface charge on the macromolecule will be reduced. This in turn diminishes the surface potential of the protein/water interface, causing the cloud-point temperature to rise. Ion partitioning to the protein/water interface due to ion adsorption will also modulate the interfacial tension. The results show that the larger (softer) anions are more effective at associating with the positively charged lysozyme surface. As a consequence, these anions are more efficient at screening the electrostatic repulsion between protein molecules and promoting salting-out behavior. The partial molar volume V_i^0 for a given anion in aqueous solution is directly proportional to its hydration free energy, ΔG_{hydr}. Bigger anions have a lower free energy cost for shedding their hydration shells and engaging in ion pairing relative to smaller anions. It should also be noted that large anions (e.g., SCN^- or ClO_4^-) are poorly hydrated.[146] The interfacial tension increments at the lysozyme/water interface (σ_{pw}) for the sodium salts of all of the chaotropic ions used in these studies are obtained by fitting the experimental data in the linear regime, using Cl^- as a calibrating ion, because it is known that its interfacial tension increment value is approximately the same at the air−water (σ) and oil−water interfaces. By extension, the authors assume this value also remains constant at the protein/water interface. The values of σ_{pw} for ClO_4^-, SCN^-, I^-, NO_3^-, Br^-, and Cl^- are reported in Table 1 of ref 135.

Tardieu and co-workers[147,148] studied the reversal of the Hofmeister series, depending on the pH with respect to the protein's isoelectric point, with different proteins such as lysozyme and γ- and α-crystallins. The size of the ion does not help in understanding the reason why the series reverses at different pH; instead the addition of nonelectrostatic dispersion forces can explain the onset of a short-range attractive potential, which depends on the ion nature and brings about precipitation of the protein in some circumstances. The short-range potential can change sign depending on whether the pH is lower or greater than pI. Therefore, the solubility of the protein depends on the specific electrolyte and on the particular protein under study.[141]

The effect of some salts on the liquid−liquid phase separation of a lysozyme−SDS complex as a function of the ionic strength was investigated by us and co-workers.[140] In that paper we showed that, at pH 10 and with a lysozyme/SDS mole ratio of 1:87, the cloud-point temperature (see Table 2) decreases upon the addition of chaotropic anions, as the plots of the cloud point versus the surface tension increment (σ) and versus the partial molar volume (ν_s) indicate. After, the lysozyme−SDS complex phase separates, with the formation of a protein-rich lower phase coexisting in equilibrium with a protein-depleted upper phase.

The concentration of the added anion was measured through ion chromatography essays, and the partition coefficient [down]/[up] was calculated (see Table 2). The results indicate that for anions smaller than bromide the partition coefficient increases with ν_s, whereas for bigger anions the coefficient decreases regularly. The data were interpreted in terms of the solvent accessible area (ASA), protein−water interfacial tension (σ_{pw}), excluded volume, ion pairs, and specific ion binding at the protein surface.[140]

As we have already anticipated, protein liquid−liquid phase separation (LLPS) is an intriguing thermodynamically driven event, in which a homogeneous protein solution separates into a protein-poor top layer and a protein-rich bottom layer as the temperature decreases.[140,149] Often this event is reversible simply by mixing the two phases and raising the temperature of the solution. Protein LLPS has wide implications in many biological processes. It has been postulated that the LLPS occurs in the cytoplasm, where the protein concentration may reach 350 mg/mL. Apparently an LLPS process is involved in mammalian cataracts (involving the lens γ-crystallins) and in sickle-cell disease.[150] Protein LLPS is also a prerequisite for one of the pathways in protein crystallization. The occurrence of protein LLPS has been attributed to short-range protein−protein interactions, most likely attractive in nature.

Table 2. Cloud-Point Temperature T_c (K), and Anion Partitioning Coefficient ln([down]/[up]), at 20° C for the Different Salts in 0.25 M Aqueous Solution (Reprinted with Permission from Ref 140; Copyright 2010 American Chemical Society)

anion	T_c	ln([down]/[up])
(water)	283.0	
NaF	283.8	0.26
CH_3COONa	286.8	
NaCl	284.4	0.37
NaBr	285.4	0.44
NaN_3	286.1	
$NaNO_3$	285.9	0.14
NaI	287.5	−0.02
Na_2HPO_4	283.0	
Na_2SO_4	284.7	
HCOONa	286.8	
Na_2SeO_4	286.5	
$NaClO_4$	288.3	−0.22
NaSCN	288.6	−0.20
NaOCN	287.6	0.27
KSeCN	307.3	
KCl	303.3	
KSCN	304.4	

Mason et al.[149] studied the LLPS of a recombinant monoclonal antibody, by constructing the liquid−liquid coexistence curves, in the presence of monovalent salt solutions (KF, KCl, and KSCN) at low ionic strength at different pHs. Fluoride was least effective for decreasing the attractive interactions among the three anions. It is proposed that, in a monovalent salt system, when the pH is below the pI at low ionic strength, the electric double-layer repulsion dominates the antibody−antibody interactions. Thus, an increase of the ionic strength begins to weaken the double-layer repulsion and the antibody−antibody interactions become more attractive. The anion with stronger binding to the positively charged antibody, possibly with an ion-correlation force, decreases the double-layer repulsion more effectively (inverse Hofmeister series). After neutralization, preferential interactions between the anions and antibody result in a decrease in the antibody's solvation free energy. The antibody−antibody interactions then become less attractive, following the direct Hofmeister sequence. A similar effect was observed under the pH condition close to the pI.[149]

Mason et al.[151] investigated through molecular dynamics simulations the interaction between a model peptide (a single melittin α-helix) and tetrapropylammonium (TPA) sulfate or guanidinium chloride (GdmCl). Clusters were already found

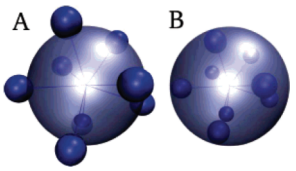

Figure 17. Models for a macromolecules with the charged groups exposed to the solvent (A) or buried under the surface (B). Reprinted with permission from ref 146. Copyright 2008 American Chemical Society.

with Cs_2CO_3 and Gdm_2CO_3. The results suggest that (1) ion pairing can be responsible for partially or totally reversing the denaturant potency of an ion. This fact would explain the occurrence of reversed Hofmeister series. (2) The effectiveness of an ion in denaturing a protein and the ion's position in the Hofmeister steries are a complex result of the ability of the ion to disrupt hydrogen bonding, nonpolar interactions, and electrostatic effect that contribute to protein stability. The position in the series can change depending on the extent to which each category of interactions stabilize a particular protein. Whereas some ions such as guanidinium have the ability to attenuate many or all of these interactions, the current study indicates that TPA competes almost exclusively for hydrophobic interactions and is likely to be ineffective at influencing hydrogen bonding. If confirmed experimentally, this finding suggests that the Hofmeister series should be assessed in more detail, as the order in which the ions appear in the series will depend on the type of interaction stabilizing the protein in question. (3) The specific locations at which the ions interact with the protein will affect the charge density profile of the protein. In summary, the study suggests that the Hofmeister series can be better understood by assessing the capability of ions to affect hydrogen bonding, salt bridges, and hydrophobic interactions in the protein and how these effects are altered by the counterion.[150]

Lund et al. investigated the distribution of fluoride and iodide around a spherical macromolecule, with and without discrete charged patches, through MD simulations.[146] Figure 17 shows two possible models for a macromolecule with a nonpolar core and charged groups that can be either exposed to the surface (A) or buried underneath the surface (B).

When the macromolecule is uncharged, the strongly hydrated F^- ions are repelled while I^- ions are weakly attracted (see Figure 18A). When the particle is charged, both anions are attracted, but with different mechanisms: (i) fluoride binds through cation−anion interactions that overcome the repulsion due to the hydrophobic core and (ii) iodide binds through ion pairing and is attracted by the hydrophobic pool. Burying the charged groups of the protein below the surface favors the binding of iodide (see parts B and C of Figure 18). On the other hand, if the surface charge density is increased, binding of F^- is favored. In any case, the binding of a specific ion is determined by local interactions.

In another contribution, Lund et al. observed that moving a large ion closer to a nonpolar region involves different contributions to the intermolecular interaction potential.[152] In particular, the ion−dipole energy is weakened, the unfavorable accommodation of water molecules around the large ion and the nonpolar surface is reduced, and the attractive induced-dipole interactions with the polarizable ion and the electrostatic potential are set up by the oriented water molecules at the interface. These topics

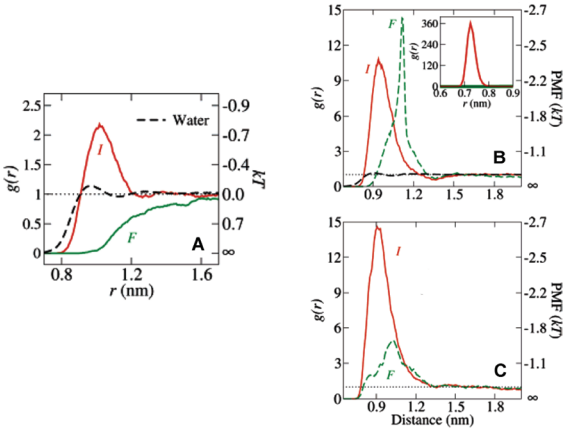

Figure 18. Radial distribution of iodide (red), fluoride (green), and water (black) in different cases: outside a neutral Lennard-Jones sphere of diameter 1.6 nm (A), outside a nanosphere with eight attached charges that are either 1 Å above the surface (B) or 1 Å below the surface (C). The inset shows the case where all charges are placed in the center of the sphere. Reprinted with permission from ref 146. Copyright 2008 American Chemical Society.

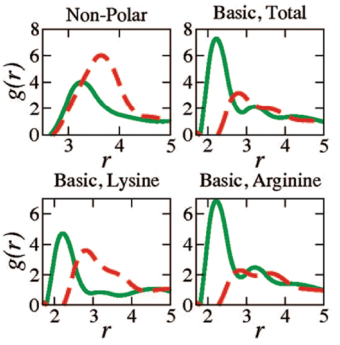

Figure 19. Distribution functions of chloride (green) and iodide (red, dashed) around various groups on lysozyme. The $g(r)$'s are normalized to unity at a 5 Å separation, approximately the Debye length of the system. Reprinted with permission from ref 152. Copyright 2008 American Chemical Society.

were addressed in a molecular dynamics simulation study on lysozyme in water dispersions. The results indicate that Cl^- is preferred by the cationic groups whereas I^- accumulates near the nonpolar moieties (~6 times compared to its bulk concentration), as illustrated in Figure 19.

In conclusion, the affinity of lysozyme for Cl^- and I^- and the salt effect on the protein association and salting-out derive from a balance between direct ion pairing (e.g., between Cl^- and Arg residues) and solvent-assisted attraction of large polarizable ioins to nonpolar surface patches. Similar conclusions were obtained in another paper by Lund et al. based on MD simulations on lysozyme dispersions in the presence of Cl^- and I^-.[153] In particular, when the salt concentration is 0.1 M, the density of I^- in the midplane between two protein molecules is about twice that of Cl^-. Besides neutralizing the protein's positive charges, iodide may also establish bridges between nonpolar surface groups, resulting in a net attractive protein−protein interaction. Mean field studies performed on planar hydrophobic surfaces indicate the opposite effect, that is, I^- charges the surfaces due to specific

adsorption and therefore promotes the repulsion between the surfaces more than Cl^-. In the case of a cationic protein, the preferential ion adsorption brings about a lowering of the net charge. The calculation of the second virial coefficient B_2 indicates that at very low salt concentration the specific ion effect is very small, because B_2 is dominated by the electrostatic repulsion between the two charged proteins. At higher salt concentrations the difference between Cl^- and I^- is larger and the calculations agree with the experimental findings. Again, the overall result derives from at least two effects: the direct interaction of the anion with the cationic amino acid residues at the protein's interface and the specific binding of the anion to the hydrophobic patches of the macromolecule. The former effect is stronger for Cl^-, and the second is more significant for I^-.

The comparison between air/water and protein/water interfaces is discussed in detail in an interesting review by Jungwirth and Winter.[154] First, the authors recall that some ions (e.g., alkali and fluoride) are repelled from the air/water interface whereas others (such as bromide and iodide) are significantly accumulated at an aqueous surface. Surprisingly, the hydronium ion (H_3O^+) belongs to the second group. Ion accumulation and exclusion are generally driven by the anion's size and the polarizability of the ion and of the solvent molecules. Hard nonpolarizable ions are repelled from the surface, whereas soft polarizable ions are strongly accumulated because they can overcome the repulsion due to electrostatic image force, produced by the surface. This behavior is shared by chaotropic ions like Br^-, I^-, SCN^-, and ClO_4^- and is not restricted to air/water interfaces. MD simulations and scattering experiments have proved that polarizable anions are accumulated at the surface of formamide, glycerol, ethylene glycol, and liquid ammonia as well. The behavior of H_3O^+ seems to be determined by its specific hydrogen-bonding features: it is a good donor but a poor acceptor, due to the reduced charge on the oxygen atom.[155]

On the other hand, classical and ab initio MD simulations show that the OH^- ion prefers to be solvated in the aqueous bulk phase (chapter 7 in ref 1). In fact, it is an excellent hydrogen-bonding acceptor and a poor donor, just the opposite of H_3O^+. This is why hydroxide is only occasionally found at the air/water interface with the oxygen involved in hydrogen bonds, and the hydrogen is confined in the gas phase. OH^- disrupts the hydrogen bonding in water at a lower extent than H_3O^+, and therefore it favors bulk solvation and does not accumulate at the interface. Regarding the analogy between air/water and protein/water interfaces, there is little correspondence in terms of ion segregation. Instead, local pairing between ions and the polar or charge amino acid residues at the protein surface offer a starting model for explaining ion−protein interactions, especially for small hard ions. In the case of large soft ions, the hydrophobic regions distributed on the macromolecule external surface come into play in determining the specific effects.

Rubin et al. have studied the aggregation of lysozyme and BSA in the presence of different salts. Repulsive forces and hydrodynamic drag inhibit aggregation. To quantify the aggregation, they evaluated a protein−protein interaction parameter (ν) from the self-diffusion coefficient measured by light scattering (see Figure 20):[134]

$$D(c) = D_0[1 + \nu c + O(c^2)] \quad (25)$$

Large ν values imply strong repulsions. For both proteins, chaotropic ions induce aggregation even at low ionic strengths.

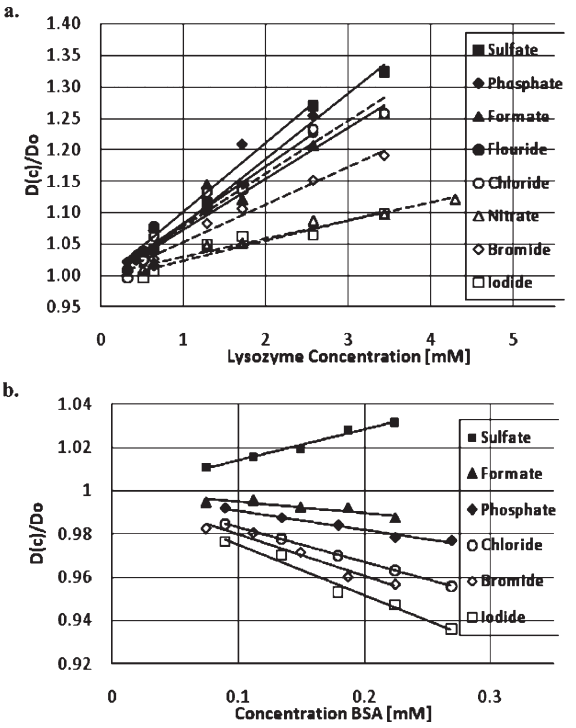

Figure 20. Plots of normalized relative mutual diffusion coefficient $D(c)/D_0$ as a function of the protein concentration for lysozyme (a) and BSA (b) at pH 4.25 and 25 °C. Reprinted with permission from ref 134. Copyright 2010 American Chemical Society.

For lysozyme, the aggregation kinetics is greatly affected by chaotropic ions such as I^- and Br^-, whereas kosmotropes produce similar effects. Such findings seems to suggest that kosmotropic ions are excluded from the protein surface. The kinetics of aggregation for BSA follows a direct Hofmeister series.[134]

According to Lodderstedt et al., the aggregation of fibrils of the nuclear polyalanine binding protein (PABPN1) is strongly affected by anions, which induce salting-out, while cations have no effect. The authors conclude that electrostatic forces are of subordinate importance with respect to salting-out specific ion effects.[156]

A similar subject was addressed by Jain and Udgaonkar,[157] who studied the salt effect on the fibrillar aggregation of β-amyloid. Prion diseases are a group of fatal neurodegenerative diseases that appear to originate from the misfolding of normal cellular prion protein, PrPC, into an alternative disease-related conformation, PrPSc. PrPC exists as a glycosylphosphatidylinositol-anchored, monomeric, protease-sensitive conformation and is rich in α-helix. Although its structure is not known, PrPSc is known to be oligomeric, protease-resistant, and rich in β-structure. Hence, understanding the process of amyloid formation by the prion protein becomes imperative. The experiments showed a relevant effect of the nature and concentration of anions. In the case of amyloid-β, both the Hofmeister effect and specific anion binding appear to be important. Figure 21 shows the different concentrations at which different anions promote the wormlike fibril formation by moPrP (mouse prion protein) at low pH, and Figure 22 shows the AFM images of the fibrils in the same sodium salts.

This effect is not electrostatic nor due to water structure, but depends on the anion/protein binding. Determining how a change in aggregation conditions, effected by the presence of

different salts, affects the kinetics of prion protein aggregation is important, because it might provide an insight into the mechanistic basis of the structural heterogeneity inherent in prion protein aggregation and would therefore help in the understanding of the phenomenon of prion strain diversity. This suggests that the salt effect is primarily an anion-induced effect. The apparent rate constant is seen to increase sharply in a sigmoidal manner with an increase in NaCl concentration, over a very narrow concentration range (100−150 mM), which suggests that NaCl may affect the kinetics of wormlike fibril formation by a direct interaction with the protein. The extent of fibril growth, as monitored by the amplitude of the change in the thioflavin-T (ThT) fluorescence signal intensity, is seen to be dependent on the nature as well as on the concentration of the anion. Hence, the order of the efficacies of the anions for promoting fibril growth was as follows: $SO_4^{2-} > I^- > NO_3^- > Cl^-$. Interestingly, the same order of efficacies is obtained when the minimum salt concentration required for maximum fibril formation is used as the criterion for efficacy, even though the dependence of the extent of fibril formation on salt concentration is different for each salt. The bell-shaped dependence has suggested that the effect of salts or organic cosolvents on amyloid formation reactions depends on how these additives affect the balance between hydrophobic and electrostatic interactions. The preferential interaction (or binding) of anions with the positive charges on the protein modulates amyloid fibril formation.[157]

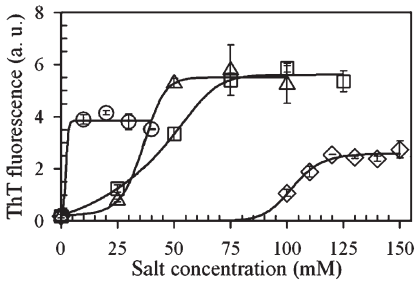

Figure 21. Effect of different anions on the growth of wormlike amyloid fibrils by 25 μM moPrP at pH 2 and 50 °C: Na_2SO_4 (○), NaI (△), $NaNO_3$ (□), and NaCl (◊). Reprinted with permission from ref 157. Copyright 2010 American Chemical Society.

Yeh et al.[158] investigated the effect of salts on the formation of amyloid starting from yeast prion protein, by measuring the binding of Congo Red dye to the β-sheet portions of the protein. The results indicate that the kosmotropic anions accelerate the polymerization reaction; the chaotropes inhibit the same process, according to the Hofmeister series, and their effectiveness correlates with the viscosity B coefficient and chaotrope concentration. The authors conclude that the interactions between the solvent and the protein induce the formation of amyloid structures.

An interesting study was conducted on the activity of the beetle antifreeze protein (AFP) in the presence of different salts by Wang et al.[159] Usually its activity implies the protein's

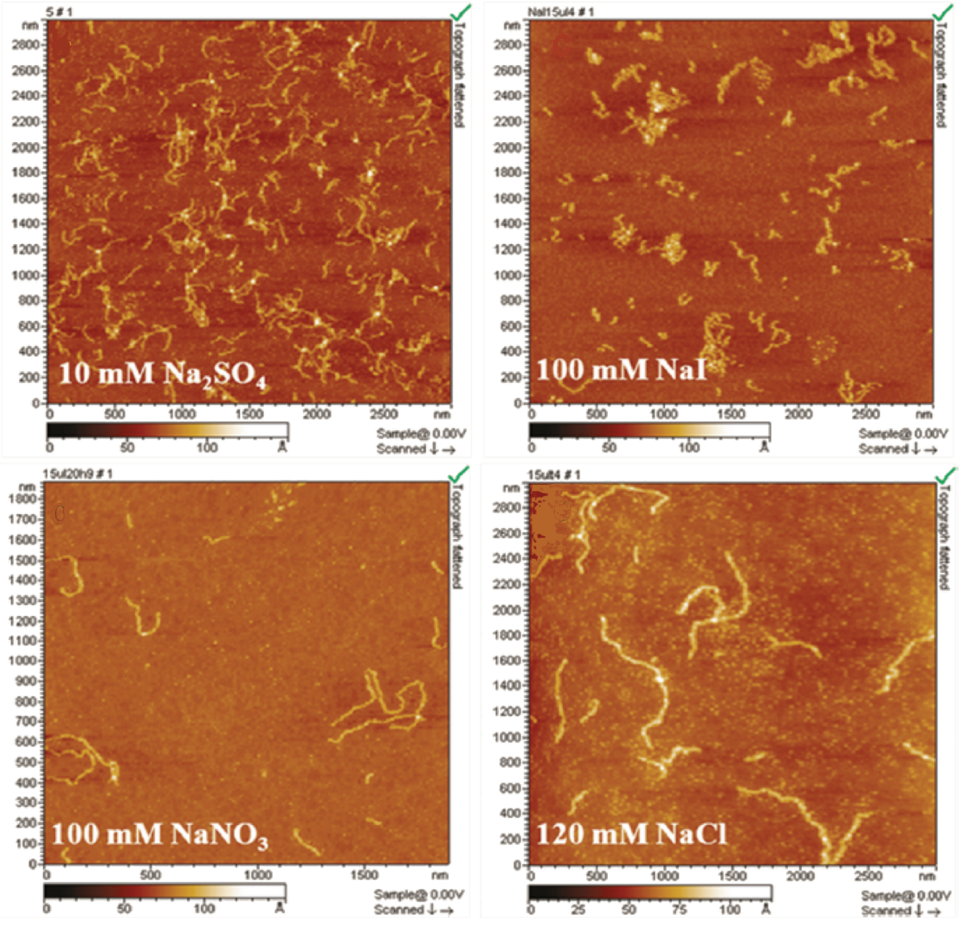

Figure 22. AFM image of wormlike amyloid fibrils formed in Na_2SO_4, NaI, $NaNO_3$, and NaCl. Reprinted with permission from ref 157. Copyright 2010 American Chemical Society.

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absorption onto the ice crystal surface at preferred growth sites, up to a maximum point where the ice crystal growth is further inhibited by the Kelvin effect. The antifreeze activity is assessed through the "thermal hysteresis" (TH), i.e., the temperature difference between the melting point and the nonequilibrium freezing point. The effect of salts on TH was determined, and the results suggest two possible mechanisms in terms of AFP and of modification of the protein−ice interactions.

A similar topic was addressed by Peltier et al.[160] in their study of the antifreeze glycoprotein (AFGP). Antifreeze (glyco)proteins are believed to operate by adsorbing onto specific faces of ice crystals, usually (but not exclusively) the prism faces, thereby inhibiting crystal growth. Once the crystal reaches this typical geometry, its growth is stopped until the temperature further decreases below a new depressed freezing point, also called the "hysteresis freezing point", at which crystal growth accelerates. Reports in the literature indicate that the presence of certain salts enhances the activity of native antifreeze (glyco)proteins. Increasing the size of the cation or the anion increases the thermal hysteresis value. Moreover, sodium citrate is the additive that induces the greatest increase in the thermal hysteresis activity of insect antifreeze proteins. In the present study, it is shown that kosmotropes favor the precipitation of proteins, promoting their adsorption on ice. Polar fishes that survive under these freezing temperatures have been found to produce a range of (glyco)proteins that have the capacity to lower the freezing point of their blood by inhibiting the growth of ice crystals that enter their bodies. One possible explanation is that added solution species could salt-out the proteins, promoting their adsorption onto ice. Two factors must be taken into account in predicting the salt-induced binding enhancement of a glycoprotein: the size of the ion and its affinity with water.

We note in passing that removal of dissolved gas from a hydrophobic protein suspension stabilizes it. Hydrophobic forces are turned off by removal of dissolved gas. They also are affected by specific ion effects, with the interplay between them being complex;[1] the forces between proteins vary by orders of magnitude depending on the specific counterion, and this provides a simple method to control protein crystal structure.

In protein-based formulations, handling procedures such as agitation and addition of other ingredients are important factors that determine the aggregation and immunogenic response in patients. Often the monoclonal antibodies (mAbs) are administered at high concentration, so that stability, solubility, and viscosity of the final formulation need to be controlled.[161] Salts are commonly used to adjust the pH (in buffers) and to set the isotonicity or to reduce the viscosity. Fesinmeyer et al. studied the turbidity of IgG_2 mAbs dispersions in the presence of different electrolytes and found that the cation does not affect the aggregation.[161] The onset of turbidity increases according to the Hofmeister trend $F^- < Cl^- < Br^- < I^- < ClO_4^- < SCN^-$; it increases with the ionic strength, and the aggregation accelerates at higher temperatures.

The effect of salts on BSA monolayers at the air−water interface was studied by Chen et al. by investigating the interfacial water structure through vibrational sum frequency spectroscopy (VSFS), at different pHs above and below the pI.[162] The same investigation was performed on monolayers of cationic, anionic, and zwitterionic surfactants. The results suggest that the interfacial water structure depends on the surface charge.[162]

Hess and van der Vegt performed simulations of ion−protein interactions, using the acetate ion as a model of carboxylate on the protein surface.[163] According to the results, sodium binds more than potassium, similarly to other recent studies, but it is not assured that protein carboxylates behave as do the free acetate ions in solution. The preference of sodium over potassium binding to such complex surfaces may therefore be not only ion-specific but also protein-specific. Model calculations by Dill and co-workers[164] have, on the other hand, shown that the nature of ion-pairing between a free ion from the solution and a surface-embedded ion may be quite different from the pairing of similar ions in bulk solution.

The colloidal stability, electrophoretic mobility, and restabilization phenomena of IgG-coated polystyene latex at pH 4 and 10 were studied by López-León et al.[165] The results clearly indicate that the DLVO theory does not work (see also refs 1, 5, and 18). The most effective ions in destabilizing the latex particles and promoting their aggregation are the chaotropic anion thiocyanate and the kosmotropic cation calcium. The efficacy of the ion depends on the sign of the surface during the destabilization process: the ions' ranking found for aggregation with positive surfaces reverses when the latex particles have a negative surface. Anions can restabilize the particles when they act as co-ions and as counterions.[165]

The relevance of dispersion (nonelectrostatic) forces in protein interactions is addressed by Moreira et al.[166] The authors investigated how the protein charges modify the local pH and vice versa. They found that even for physiological concentrations (∼0.1 M) the salt specificity may be important, especially close to the isoelectric pH. The main conclusion of this theoretical work is again that ionic dispersion forces acting between ion and protein play a very important role in interpreting properties of biological and colloidal systems, especially at high salt concentrations. The surface adsorption per headgroup is highly affected by the ion specificity of the counterion at a negatively charged surface.

7.2. Enzymes

The effect of salts on the stability and kinetic activity of enzymes is a crucial issue for formulation, bioprocessing applications, biocatalyst lifetimes, protein-based pharmaceutical shelf life, and active protein lifetimes in vivo. Following a previous and pioneering study on the Hofmeister effects, and their reversal with change in buffer, or with potassium rather than sodium, on the activity of a DNA nuclease enzyme,[167] Bauduin, Pinna, and co-workers studied the effect of buffers and salts on the activity of horseradish peroxidase (HRP).[168,169] This enzyme belongs to the class of peroxidases that help in removing H_2O_2. The authors found that the addition of salts to protein buffer solutions induces pH changes that depend on the nature and the concentration of the ions, and that ions produce a specific direct effect on the protein. All added salts lead to a decrease in measured pH values. The higher the salt concentration, the more pronounced is this effect. An interesting discussion on the effect of choline on the enzyme activity is also reported. In fact, the super activity induced by sulfate ions is counterbalanced by a stoichiometric amount of choline, $HOCH_2CH_2NMe_3^+$. However, because choline decreases the activity of HRP, it is not a good osmoprotectant for the cell.[169]

Other relevant studies deal with the hydrolytic activity of *Aspergillus niger* lipase[170] and *Candida rugosa* lipase.[171] Pinna et al.[170] give support to the hypothesis that Hofmeister effects are not due to water structure changes but rather to specific binding of ions at the enzyme surface that modifies its activity.

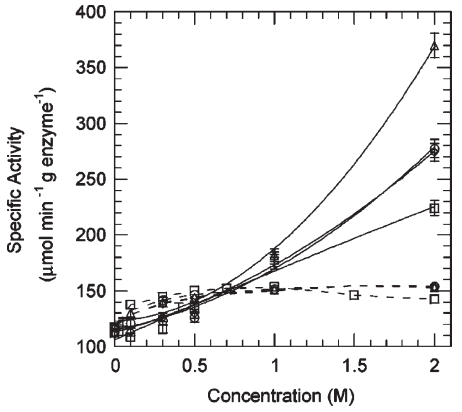

Figure 23. Effect of sodium chloride (◊), bromide (△), nitrate (○), and perchlorate (□) on the hydrolytic activity of lipase A in phosphate buffer (5 mM, pH ≈ 7) as a function of the salt concentration. The solid and dashed lines represent the experimental and the calculated activities, respectively. Reprinted with permission from ref 170. Copyright 2005 American Chemical Society.

Figure 23 shows the effect of some anions on the hydrolytic activity of lipase A.

In ref 171, Salis et al. debate the role played by ions in regulating the enzyme activity. They assert that ions are not simple pH (such as in buffer solutions) or ionic strength regulators, two roles that invoke electrostatic interactions only. Rather, they induce specific effects related to their nature and to the interactions they establish. Bilaničová et al.[172] studied the enzymatic activity in nonaqueous media in the presence of different sodium salts and concluded that the activation or deactivation of the enzyme cannot be related to the water structure change induced by the ions, as they used lyophilized lipase with a very low water content.

Broering and Bommarius[173] studied the activity of horse liver alcohol dehydrogenase (HL-ADH), α-chymotrypsin, and monomeric red fluorescent protein (mRFP), in the presence of different sodium salts. The authors found that the surface tension increment σ is not a good parameter to describe the effect of the different anions on the kinetic activity of the proteins; instead a better correlation was obtained using the viscosity B coefficient, which is indicative of different ion hydration. The results indicate that the less hydrated chaotropes accelerate the protein deactivation, but the reverse is not true for kosmotropes, which probably do not slow protein deactivation past a certain threshold but only prevent it from occurring faster. The explanation invokes a mechanism where chaotropes interact with the protein molecules, and the differences in hydration of the various ions lead to a proportional effect on the protein stability by lessening the hydrophobic effect at the protein−water interface. On the other hand, kosmotropic ions are preferentially excluded from the protein−water interface; then in their presence the protein "sees" less ions in solution and reacts less to the differences in hydration among them. The protein unfolding rate or the rate of intrinsic irreversible deactivation can control the observed deactivation rates.

Yang et al. studied the activity and stability of alkaline phosphatase. The results indicate that effect of salts on buffer pH is ion-specific and presumably due to electrostastic and dispersion forces.[174] Ions affect the hydration water molecules around the enzyme and interact with both the enzyme surface and bulk. When the activity of the enzyme is plotted versus the viscosity B coefficient, the trend is bell-shaped, with a maximum for KNO_3.

The enzymatic activity of HIV-1 protease in water dispersions in the presence of NaCl and KCl was studied by Heyda et al. through kinetic measurements and MD simulations.[175] The results suggest that the enzymatic activity increases with the salt concentration, especially when the buffer contains K^+ rather than Na^+. Moreover, the MD simulations show that Na^+ is more attracted to the protein interface than K^+ due to the interactions with the COO^- groups of Asp and Glu. These results may explain why the presence of sodium at the active site can lead to a lower efficiency of the enzyme in binding the substrate in aqueous NaCl.

A reversal in the Hofmeister sequence was found by Salis et al.[176] for the adsorption of lysozyme on functionalized SBA-15 mesoporous silica when the concentration of salt is 0.2 M. For higher ionic strengths instead, the trend follows a direct series.

8. HOFMEISTER EFFECTS IN BIOLOGY AND MEDICINE

In this last short section, we review a few papers dealing with Hofmeister phenomena that show up with real biological systems, or in medicine and in other biorelated matters. The use of salts for applications such as conservation of foodstuffs and as biocides has been known forever and a day. However, in spite of the paucity of reports on specific ion effects in living organisms, this topic is of particular interest because it shows that physical chemistry is at work, in a more or less complicated manner, in determining functions and behavior of complex systems, through the interplay of different interactions that mainly involve ions, cell membranes, and, in general, biological matter. One historic example is preservation of cod by drying with lye, perhaps the major industry in Europe for centuries.

Biology probably offers the broadest set of examples where specific ion effects take place. Biological systems are always based on water and comprise a wide collection of ions. Typically these occupy the kosmotropic side or intermediate position in the direct Hofmeister ranking: phosphate, sulfate, carbonate, chloride, nitrate, sodium, potassium calcium, magnesium, and a few others. Instead, chaotropes are less present in biological samples or are even toxic (iodide, thiocyanate, cyanide, cesium, barium, and so forth). Interestingly, the inorganic world also is dominated by kosmotropic species: silicates, carbonates, oxides, aluminum, and the above-mentioned ions.

Salt tolerance is a key issue in the optimization of transgenic plants that have to be modified to improve their specific resistance to biotic (pathogens, wounding, etc.) and abiotic (light, ozone, temperature, drought, salinity, etc.) stresses.[169] Specificity here is usually at low salt. Trace elements like copper, vanadium, zinc, manganese, and selenium, missing from poor soils and added to superphosphate and other fertilizers, have vast effects on plant growth and yield.

Tanaka and Oka[177] studied the effect of cations and chaotropic anions on the mobility of spermatozoa of the fish *Poecilia reticulata*. The effect of cations and anions is additive. Interestingly, the authors conclude that the strong dependency on the ion valency and the small specificity among ionic species suggest that their effects are not biological but physicochemical.

A significant salt effect on living microorganisms was detected by us and co-workers in the case of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In that study we demonstrated that different anions have a different effect on the growth rates of the two bacterial strains.[178] Figure 24 shows the cell growth of the two bacterial strains in the presence of different electrolytes at

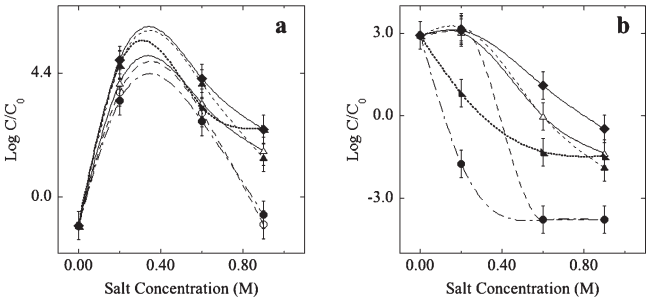

Figure 24. Cell growth for *Staphylococcus aureus* (a) and *Pseudomonas aeruginosa* (b) as a function of salt concentration of NaSCN (●), NaI (○), NaBr (▲), $NaNO_3$ (△), NaCl (♦), and CH_3COONa (left-facing triangle). Reproduced with permission from ref 178. Copyright 2005 IOP Publishing Ltd.

different concentrations. The effects of the electrolytes investigated depend on their nature and concentration and are presumably related to the interactions between ions and the bacterial enzymes.

Ions may have a significant impact on different biological systems. The relevance of the main ions (sodium, potassium, magnesium, calcium, chloride, fluoride, iodide, etc.) is well-known and will not be dealt with here. Instead, the less common ions may have important effects. One example is lithium and its therapeutic or toxic effects, and not only in psychiatry.[111,179,180] The data for rubidium are still scarce, and its biological effects unknown.[180,181] Cesium has been tested as a potential antitumoral agent, but the results are still controversial.[182] An interesting review of the therapeutic and toxic effects of nitrite and nitrate for the human body can be found in refs 183 and 184. Another interesting paper[185] reports the effect of ClO_4^-, SCN^-, and NO_3^- as inhibitors of iodide transport, with serious implications for thyroid-related functions. We suspect that here a Hofmeister phenomenon might be involved.

Between 2005 and 2011, there were only two contributions we could find that addressed the topic of Hofmeister series in medicine. One is due to Grover and Ryall and discusses the effects of salts on urinary stones.[14] Kosmotropes (pyrophosphate, citrate, and magnesium) inhibit the growth of calcium oxalate crystals (CaOx), and salting-out is responsible for the observation that dissolved urate promotes the crystallization of CaOx in undiluted human urine in vitro, a finding that has enormous implications for pathogenesis of urinary stones.

De Cristofaro et al.[186] investigated the effect of salts on the activity of the so-called von Willebrand factor (VWF) in hemostasis. It mediates platelet adhesion to the sites of vascular damage and acts as a carrier protein for coagulation factor VIII. There is a definite inverse relationship between the viscosity *B* coefficients, i.e., Hofmeister effects are implicated. This corroborates the concept that the inhibitory effect of anions results from specific binding regulated by the physicochemical nature of the anion involved. We can surmise that the major effect of anions in modulating the rate of the interaction is not due to Debye—Hückel screening phenomena. The potency of the inhibitory effects of anions followed the Hofmeister series, that is, $ClO_4^- > Cl^- > F^-$, and was inversely related to the relative Jones—Dole viscosity *B* coefficients. Specific binding of different anions must play a key role in the regulation of VWF functions if one considers that a gradient of ion concentration exists between the intra- and extracellular compartments. In particular, it is known that intracellular anions are represented mainly by phosphates, sulfates, and protein carboxylates, whereas the chloride ion concentration is low, amounting to 1—3 mM. On the other hand, the extracellular chloride concentration is much higher (~100 mM) and may easily form inner-sphere ion pairs with the chaotropic cation groups on proteins (protonated imidazolium and amino group of lysine). The net effect on VWF structure of anions present in the intracellular compartment is not known. However, once secreted into the extracellular compartment, VWF is readily bound by chloride ions, which are abundantly present in that compartment and have a high affinity for the protein. These findings open up a way to investigate whether and how this regulatory mechanism also could be involved in the pathogenesis of some thrombotic microangiopathies.

An application of specific ion effects in foodstuff is offered by Calligaris and Nicoli.[187] They reported that acetate and carbonate slow down the lipid oxidation rate in foodstuffs, showing an antioxidant activity.

9. THE OTHER SIDE OF THE COIN. FUTURE PERSPECTIVES: INTERPLAY BETWEEN ION SPECIFICITY, MEMBRANE ORGANIZATION, PROTEIN STRUCTURE AND ACTIVITY

Hofmeister effects occur in the biological sciences at several levels. At an overall level there is very little room for maneuver. For example, the composition of ions in the blood is fixed. A systematic study of whole-animal effects of changes in composition, say, by replacing the chloride anion in blood in a Hofmeister series, is obviously a forlorn proposition. This, with our lack of understanding of the source of specific ion effects, has led to a situation where very fragmentary evidence of any systematics exists. All ions are specific. One question is why?

The other, perhaps more interesting, question is what indirect physicochemical consequences might follow from their existence. Put more brutally, is there any useful global role for physical chemistry in the biosciences beyond the technical support provided by instrumentation? To a reductionist, the answer is obviously yes. But how is an open question. We here try to dissect the problem at different levels in an attempt to gain some insights into the proposition.

9.1. Specific Ion Effects at the Cellular Level

Usually questions like why the concentration of Na^+ outside a red cell is 4 times higher than outside a cell and why K^+ is much higher inside than outside are resolved by appeal to active ion pumps. The mechanism that maintains the required partitioning is assigned entirely to a biochemical pump. Half a century ago, Ling and colleagues attempted to explain this phenomenon by calling on a Donnan equilibrium-specific ion partitioning in the finite volume of a red cell. The attempt failed and led to the necessary postulate of an ion pump, yet the energetics of the process pose a question not entirely resolved. The (electrostatic) theory used to seek a physicochemical, as opposed to a biochemical, origin for the partitioning was based on then extent notions that excluded the very ion-specific quantum mechanical forces that it was supposed to explain. For the red cell problem, the interior of the cell comprises almost close-packed hemoglobin molecules to which the potassium ion binds preferentially—presumably through specific dispersion forces and associated compatible hydration. The cell sits within, and has available to it, a surrounding medium of size not too much larger than its own volume. For that reason parameters like activities relevant to bulk

electrolytes have to be reformulated. This is on top of major effects on activities due to the presence of extracellular proteins and other macromolecules. So there is at least a possibility that these Hofmeister, physico-chemical effects may ultimately be seen to play a role in establishing the balance. A hint at such a possibility can be seen in the experiments on dioctanoyl phosphatidylcholine[84,188] and lysozyme.[140]

Here substantial anion-specific partitioning occurs in a finite volume that contains phase-separated, self-assembled lipids. For that system, analogous to a red cell in the finite volume of its accessible physiological medium, there is a "pump" but no pump, due solely to specific ion effects that we understand. However, analogies are dangerous, so let us move to a finer level.

9.2. Enzymes

Restriction enzyme activities as a function of the Hofmeister series of both cations and anions have been explored in ref 167. A first question occurs in why a particular ion like Mg^{2+} (which can be replaced by Mn^{2+} or Ni^{2+} in that case) is necessary to set enzyme structure and create a hydrophobic pocket in which the action takes place, as well as how that binding occurs. It is impossible to answer such a question and that of the energetics of selective binding of cations to calixarenes and cryptates within the framework of standard electrostatic plus "hydration" theories. The same applies to hydrated methane and other inert gases such as in clathrates. However, once the absence of dispersion self-energies from standard estimates of Born free energies of transfer is noted, the problem is not so incomprehensible—the real free energies of transfer require these additional dispersion energies. Their inclusion, with a consistent definition of ion size obtained from ab initio studies, can change the Born estimates by more than 100%. The missing forces in turn dictate specific hydration and compatibility of ionic hydration shells with the hydration of the amino acid backbone.[1,5]

Some light on a second question—what is the source of the energetics of the enzymatic catalysis?—seems to follow from a study of the enzyme activity as a function of the Hofmeister series. What emerges is a testable, more complex consequent mechanism, beyond thermodynamics, that provides the energetics of substrate−enzyme interactions. (This involves hydrophobic cavitation and probably depends on dissolved gas; see chapter 6 in ref 1.) The inference is that using Hofmeister effects as a probe of reactivity is indeed useful. The supra activity of the sulfate ion in plant enzymes is a further example of how Hofmeister series studies can throw light on a complex enzyme system.[169−172]

Such studies cannot be fully illuminating because of uncertainties in existing theories: the reversal of Hofmeister series for phosphate and cacodylate buffers, nominally at the same pH, and reversal again when Na^+ is replaced by K^+, point to deep problems still existing for the interpretation of measurements of pH and of buffers, and indeed of measurements of membrane potentials in physiology that ignore specific ion adsorption. (The source of the energy for the fundamental reaction ADP to ATP is a catalysis that occurs on a surface. It makes sense within the emerging theoretical paradigm: the many-body polarization modes of these complex molecules change on adsorption and give rise to lowered energy barriers.)

9.3. Bacterial Growth

As we have already discussed, bacterial growth as a function of ion type provides an example where, presumably, a rate-limiting step in metabolism is associated with specific ion effects like those for restriction enzyme activity.[178] A parallel alternative explanation assigns the changes in growth rates to bacterial surface adhesion or to bacterial association (and conjugation) affected by the specific ion-dependent forces between them. In any event, specific ion effects provide an as yet unexploited tool for pharmacological and biotechnology applications for biofouling through bacterial adhesion.

9.4. Inorganic Nanoparticle Effects

Another area where specific ion effects are at work in biology is illustrated by the problem of precipitation of nanoparticles of magnetite in bird brains, for migration, and in bacteria, for orientation. The precisely defined synthesis occurs in a region containing a complex mixture of sugars among other reagents impossible to mimic.

However, it is significant that laboratory studies of nanoparticle synthesis in different background Hofmeister salts and sugar isomers show that particle size and nanostructure can be varied more or less at will. For example, Baglioni and co-workers studied the formation of $Mg(OH)_2$ nanoparticles starting from different magnesium salts (see Figure 25).[189]

The possibilities of making amorphous apatite to understand bone growth are open because no systematic studies have been made of effects induced by a combination of sugars and Hofmeister sequences that undoubtably affect nucleation and growth and size of nanoparticles. Dissolved atmospheric gas affects matters too. In any event we can expect useful developments in this field by using Hofmeister effects as an exploratory tool.

The effect of NaCl and $CaCl_2$ has been investigated in lung surfactant monolayers.[190] Worthy of investigation is the claimed effect of calcium on the formation of amyloid fibrils, which is presumed to be the origin of Alzheimer's disease.[191]

Further unexplained ion-specific effects occur in physiology with secretion of salt by seagulls, as well as in coastal plants. Perhaps the most challenging is how dugongs, which live entirely on salt-saturated sea grass, eliminate salts with mysterious super kidneys.

9.5. Membrane Structural Changes

Physico-chemically induced membrane structural changes can be used as probes of drug and toxicity mechanisms. A vast amount of work in the last four decades has gone into effects of specific ions on forces between self-assembled aggregates of surfactants and lipids in electrolytes, on microemulsions formed from surfactants, oil, and electrolytes, and on their microstructure.

Hofmeister effects on bilayer interactions, charged or uncharged, are enormous. Replacement of one counterion by another in a lamellar phase can cause swelling, change in headgroup curvature,[1] and formation of spontaneous stable vesicles. Or it can cause phase transitions from lamellar phase to bicontinuous cubic or hexagonal and micellar phases. Microstructure is determined by local headgroup curvature, which changes with specific counterions, and global packing constraints, which include interaggregate forces. On the other side of the coin, curvature is set by oils that penetrate into the hydrophobic head of the surfactant water interface. These matters in model membrane mimetic systems are very well understood and are rediscovered in every decade.[1,18]

What is perhaps not so well known is that, due to the specificity of ionic adsorption forces, missing from standard theory, the addition of a small amount of one ion to a system of self assembled aggregates formed with a much larger concentration of counterion may produce very large effects. An example is a cationic didodecyldimethyl ammonium sulfate/water/decane microemulsion. It forms a normal oil-in-water microemulsion made up of droplets. The addition of trace amounts of Br^- ion displaces the divalent sulfate counterion, and the system becomes a reverse curvature bicontinuous water-in-oil microemulsion.

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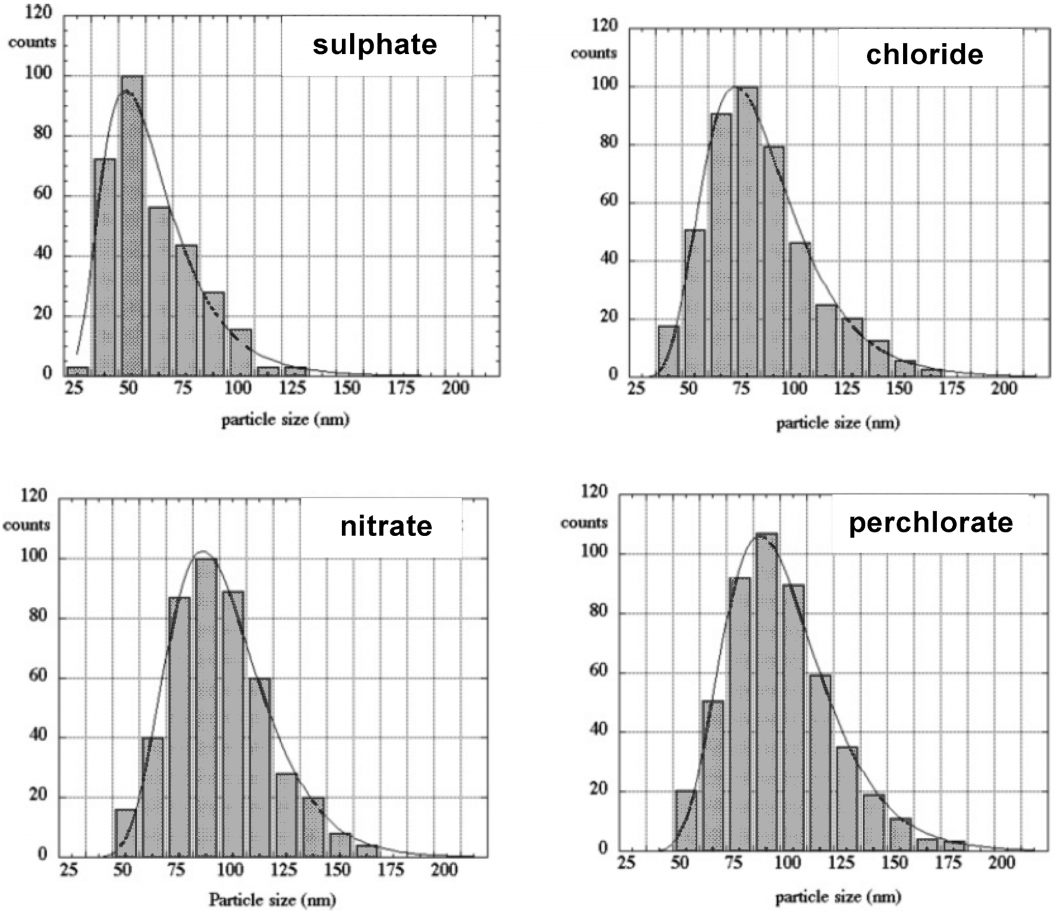

Figure 25. Size distribution of $Mg(OH)_2$ nanoparticles obtained from $MgSO_4$, $MgCl_2$, $Mg(NO_3)_2$, and $Mg(ClO_4)_2$ through transmission electron microscopy. Reprinted with permission from ref 189. Copyright 2005 American Chemical Society.

This is incomprehensible if one thinks of electrostatic forces alone.[192] It is a universal phenomenon understood in the new emerging theories. Similar specific ion effects startling within the classical paradigm occur in other systems such as vitamin K in monoolein/water dispersions.[193]

Another important area where Hofmeister effects can and have been used to probe biochemical processes is that which involves interactions and structural changes that occur with light. A nice example is the extensive work of Vogel and co-workers on rhodopsin action in membranes (see refs 194−196 and references therein).

Playing with curvature induced by specific ion effects, with a balance imposed by specific oil adsorption, allows one to identify and explore the consequences of drug and toxic chemical agent uptake in biological membranes. The physical-chemical, not specific biochemical, effects can be explored. Changes of membrane structure induced by apparently nonspecific agents may provide insights into mechanisms not previously explored. Progress in understanding the subtleties of membrane structure has been considerable. However, the part played by the surface chemistry of membranes, especially the role of lipids and their interplay with ions in influencing drug action, represents *terra incognita*. It is open for exploitation.

10. CONCLUSIONS

The intuition we have used in analyzing Hofmeister effects draws on a theory based on electrostatic forces and associated hydration alone. But the classical theories that capture some essentials of specific ion effects ignore, at least explicitly, the quantum dispersion forces that are ultimately responsible for the specificity.[1,5] There is still a long way to go before matters like theories behind specific ion effects as they affect pH, buffers, colloidal dispersions, and membrane potentials are modified. The edifice we rely on is not based on straw but would be decidedly more useful were the foundations more secure. We are confident that this is something that we can look forward to, soon. It is a work in progress. Apart from that, if we had to sum up in a line or two what we have learned over the past decade, we could say this: Previously biologists and physical chemists lived in parallel universes, almost disjunct. What has happened and is encouragingly revealed by recent papers we have reviewed is an awareness now of specific ion effects, their affect on forces, and their interplay with lipids in setting self-assembled microstructures.

The number of papers dealing with Hofmeister effects related to biology and medicine is already large and keeps growing every year. The effect of background electrolytes and buffer ions, pH, and dissolved gases on the structure and functionality of proteins, enzymes, biomacromolecules, nucleic acids, cytoplasmatic organelles, and other supramolecular self-assemblies is certainly a key area for development in science. The further refinement of physicochemical concepts such as nonelectrostatic interactions and hydration can help to unravel the complex mechanisms through which ionic species participate in the processes that belong to the realm of life science.

The area is replete with all kinds of claims, some patently unquestionable, e.g., the bubble−bubble interaction problem of Henry and Craig[68] and the effect of removal of gas on colloid interactions, and some outrageous. We refer the interested reader to ref 1 for effects of dissolved gas and to the book of Pollack.[197]

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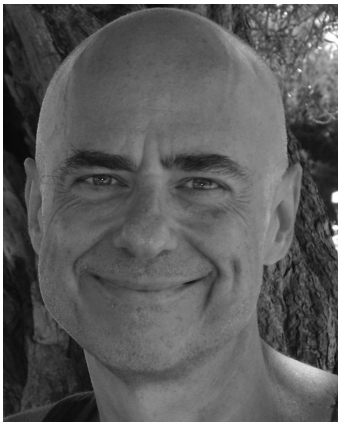
BIOGRAPHIES



Pierandrea Lo Nostro is associate professor at the University of Florence (Department of Chemistry) and CSGI. He received his Ph.D. in Chemistry (Physical Chemistry of Interfaces) in 1992 from the University of Florence. Since 1989, he has been carrying out experimental studies at MIT (on liquid−liquid phase separation, self-assembly of bolaamphiphiles, and semifluorinated alkanes), at the University of South Florida (self-assembly of biocompatible surfactants), and at the Australian National University (specific ion effects). Currently he teaches classes in Physical Chemistry, Environmental Physical Chemistry, and Physical Chemistry of Formulations. His main research interests are Hofmeister phenomena in bulk, interfacial, and living systems, nanoparticles, supramolecular chemistry (pseudopolyrotaxanes), and self-assemblies from multifunctional surfactants.

Barry Ninham founded the Department of Applied Mathematics (Natural Sciences) at ANU in 1970. He and his colleagues pioneered, and continue to work extensively on, molecular forces, experiment and theory, as well as on putting forces to work in self-assembly of soft matter. Contributions are wider than that and embrace physical and inorganic chemistry, nanotechnology, and chemical engineering, especially in geometry and structure as well as in porous media, and a new language of shape.

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