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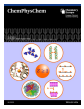

Multivalent ions and biomolecules: Attempting a comprehensive perspective

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Ions are ubiquitous in nature. They play a key role for many biological processes on the molecular scale, from molecular interactions, to mechanical properties, to folding, to self-organisation and assembly, to reaction equilibria, to signalling, to energy and material transport, to recognition etc. Going beyond monovalent ions to *multi*valent ions, the effects of the ions are frequently not only stronger (due to the obviously higher charge), but *qualitatively* different. A typical example is the process of binding of multivalent ions, such as Ca^{2+}, to a macromolecule and the consequences of this ion binding such as compaction, collapse, potential charge inversion and precipitation of the macromolecule. Here we review these effects and phenomena induced by multivalent ions for biological (macro) molecules, from the "atomistic/molecular" local picture of (potentially specific) interactions to the more global picture of phase behaviour including, e.g., crystallisation, phase separation, oligomerisation etc. Rather than attempting an encyclopedic list of systems, we rather aim for an embracing discussion using typical case studies. We try to cover predominantly three main classes: proteins, nucleic acids, and amphiphilic molecules including interface effects. We do not cover in detail, but make some comparisons to, ion channels, colloidal systems, and synthetic polymers.

While there are obvious differences in the behaviour of, and the relevance of multivalent ions for, the three main classes of systems, we also point out analogies. Our attempt of a comprehensive discussion is guided by the idea that there are not only important differences and specific phenomena with regard to the effects of multivalent ions on the main systems, but also important similarities. We hope to bridge physico-chemical mechanisms, concepts of soft matter, and biological observations and connect the different communities further.

1. Introduction, motivation and scope

In this review, we will focus on effects induced in biological and chemical systems by multivalent ions. While general overviews of the influences of electrostatics on soft matter are given, e.g., in Refs. [1–3], it is generally accepted that the effects of multivalent ions go beyond such considerations.[4–6] We will include different types of ions into our discussion: mono-atomic ions, such as Na^+ and Mg^{2+}; multi-atom ions (e.g., NH_4^+ or spermidine); nano-ions (such as polyoxometalates), and larger ions, e.g., oligo-arginine. We note that the classification of ions does not only depend on their net charge, but also on their other characteristics such as their (in)organic nature. The main aspects of this review are the charge-mediated effects of these ions whereas their other properties play a less important role. We note that specific interactions of monovalent ions with biomolecules, such as that between Ag^+ and DNA,[7] have also been shown, implying that the role of monovalent ions can go beyond that of purely inert electrolytes. However, such interactions are not the main focus of this review.

Generally, ions are present ubiquitously and are therefore of fundamental interest for a large variety of topics and research areas. Starting from biology and physiology, the importance of ions becomes apparent immediately. A typical animal or human cell contains approximately 130 mM K^+ and 10–20 mM Na^+ cations and relies on an active ion exchange with its surroundings to maintain its electrochemical potential.[8] Similarly, the signal transduction activity of neurons depends on charge exchange mechanisms.[9] Inside the nucleus, the highly negative charge of nucleic acids (DNA and RNA) implies that ions – most frequently, Mg^{2+} – are required to screen their charges, thus enabling, e.g., nucleic acid-protein interactions.[9]

Several other biological aspects depend on ions. For example, many enzymes host metal cations such as Ca^{2+} and Zn^{2+} in their catalytic centers; muscle contraction is made possible *via* myosin-Ca^{2+} interactions; and oxygen transport by hemoglobin is facilitated through Fe^{2+}/Fe^{3+} ions.[10] Generally, iron metabolism, storage and transport in mammals is a complex issue and involves, apart from hemoglobin, the proteins myoglobin, ferritin[10] and lactoferrin.[10,11] Divalent ions such as Ca^{2+} and Mg^{2+} have furthermore been shown to be present at the interfaces between virus particle subunits (see Ref. [12] and refs. therein), presumably fulfilling also structurally important roles in viruses. Investigations of silk feedstock

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indicated that the viscosity of the latter is strongly influenced by the type and valency of the cation present. Whereas the divalent Ca^{2+} increases the viscosity by bridging acidic amino acids, the monovalent K^+ reduces viscosity due to competition for binding to these types of residues.[13]

Given the prominent role of ions in physiology, it is obvious that biological and biotechnological experiments need to consider the native environment of the biomolecules they investigate. Thus, the success of *ex vivo* and *in vitro* experiments with, e.g. enzymes, strongly depends on the (ionic) composition of buffer solutions used.

The strong dependence of biological processes on ions can lead to peculiar evolution processes. As an example, due to low Zn^{2+} levels in the deep sea, a certain diatom species relies on Cd^{2+} instead[14] – a heavy metal cation known to be toxic for many land-borne creatures. Another dependence on seemingly less physiologically relevant ions has been observed in *M. fumariolicum* SolV, an extremophilic microbe native to Italian volcanic mudpots. This bacterium utilises lanthanide cations to catalyse methane-based metabolic pathways.[15]

From ecologic and ecotoxicologic points of view, ions play significant roles as fertilisers. A large amount of fertilisers is phosphate-based,[16] but lanthanides are also known to be used in agriculture.[17] In industrial settings, polyanions such as polyacrylate can be used to prevent $CaCO_3$ precipitation (i.e., as scale inhibitors) due to their specific interactions with Ca^{2+} in pipelines.[18]

At the same time, ions can be successfully employed to manipulate macromolecules in biotechnology, e.g. as crystallisation agents. This can be demonstrated in the case of negatively charged proteins where multivalent cations have been shown to promote crystallisation.[19–27] In addition, a purification protocol using Zn^{2+} ions has recently been established for recombinant antibodies.[28]

Here, we first introduce the theoretical aspects of charge-mediated interactions. We then summarise the current knowledge regarding the role of charges in (bio)(macro)molecules including DNA, surfactants, interfaces and proteins. We focus especially on charge-mediated modifications of static, dynamic or thermodynamic properties of the macromolecules in questions, including the intriguing possibilities to rationally tune (bio)molecular interactions. Finally, we briefly cover other systems such as ion channels and synthetic polymers. We mention experimental methods where necessary, but do not consider them the main subject of this review.

Through this review, we aim to provide a comprehensive overview of the manifold ways in which charges can influence the behaviour of macromolecules. By emphasising how physical concepts can be used to understand biological and soft matter systems, our goal is to enhance mutual understanding and communication between different scientific communities tackling manifold questions. We hope to stimulate further discussion and inspire both experimental and theoretical investigations of these complex aspects. We will mostly focus on the static/equilibrium behaviour, but we note that there are of course also interesting multivalent ion-mediated effects on the dynamics, kinetics, and viscosity of (biological) soft matter.[29,30]

2. Background and theoretical concepts

In this section (partly based on Ref. [31] and Ref. 39), we provide an overview of the theoretical concepts behind ion-related interactions of soft matter. In particular, we focus on charge effects as accounted for in mean-field, Poisson-Boltzmann and Derjaguin-Landau, Verwey-Overbeek (DLVO)[32,33] theories, outlining their strengths and shortcomings especially in the context of ion-specific effects and multivalent ions. The two latter aspects are then outlined in more detail using the

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Hofmeister series as an example. We note that for a detailed review on polyelectrolytes and, *inter alia*, their interactions with counterions, the interested reader is referred to Ref. [2]. In the following, we will describe mean-field and beyond-mean-field approaches to ion condensation. As a very general principle, we shall briefly mention here that the two main parameters governing the interaction of ions and (macro)molecules are the enthalpic contributions of their electrostatic interactions and the loss of the ions' conformational entropy upon binding to the molecules, the former compensating the latter.[3,34]

2.1. Mean-field theory of charged matter: Poisson-Boltzmann theory

Ion Distribution and Charge Screening: Poisson-Boltzmann and DLVO Theory

Considering a charged object in a solution with ions, the Poisson-Boltzmann (PB) theory provides a basic mean-field approach to describe the ion distribution. This approach combines the exact Poisson equation with a mean-field relation between the electrostatic potential and the potential of mean force on the ions.[1,35,36] The resulting ion distribution around charged objects forms the so-called electrostatic double-layer that causes a screening of charges in electrolyte solutions, over the Debye screening length

$$\lambda_D = \left(\frac{\varepsilon_0 \varepsilon_r k_B T}{2000 e^2 I}\right)^{-1/2} = \left(\frac{1}{8000\pi l \lambda_B}\right)^{-1/2} \quad (1)$$

where ε_0 is the vacuum permittivity, ε_r is the relative permittivity of the sample, k_B is the Boltzmann constant, and T the temperature. The Bjerrum length, λ_B, quantifies the distance on which the interaction between two elementary charges equals $k_B T$:[1,3,37]

$$\lambda_B = \frac{e^2}{4\pi\varepsilon_0 \varepsilon_r k_B T} \quad (2)$$

which, in water at room temperature, is approximately equal to 0.72 nm.[37] Given a solution of several ions with molar concentration n_i and valency Z_i, the ionic strength

$$I = \frac{1}{2}\sum_i n_i Z_i^2 \quad (3)$$

provides a valency-squared-weighted concentration of ions, implying that even in the mean-field approach multivalent ions have a stronger effect compared to monovalent ions.

The decay of the double-layer potential described by the Poisson equation has been historically rationalised by different approaches. The Helmholtz theory disregards thermal motion of the counterions, assuming an unrealistic rigidity of the counterion layer.[38] This drawback is addressed in the Gouy-Chapman model which pictures the counterion layer as diffuse, but has the shortcoming of assuming that the charges in question are point-like. Rigid and diffuse models are combined in the Stern model (Figure 1), resulting in a more comprehensive and realistic description of the interactions between charged surfaces and counterions.[38]

The PB theory and its linearised version, the Debye-Hückel theory,[40] provide a very useful and important framework for the understanding of electrostatic phenomena in soft matter. PB theory has been fairly successful in describing, e.g., distributions of mono- and divalent ions around DNA,[41–43] although it is well-known that PB theory cannot fully describe the effects of multivalent ions.

One very important consequence of the PB approach is the DLVO theory. In the DLVO potential, screened Coulomb interaction and van der Waals attraction are combined to explain the charge stabilisation of solutions with charged solutes (Figure 2). With increasing ionic strength, the charge

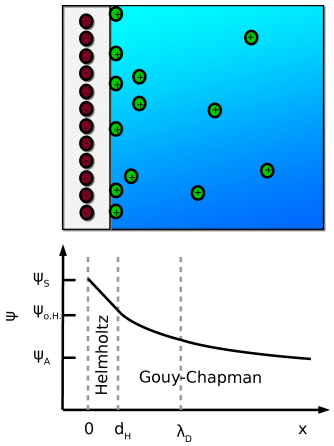


Figure 1. Stern model combining the rigid (Helmholtz) and diffuse (Gouy-Chapman) double layer models. The grey shaded area represents a surface immersed into bulk liquid (blue continuum). The red circles on the shaded area represent negatively charged particles, the green circles illustrate positively charged ones. The potential ψ decays linearly between the surface (ψ_S) and the outer Helmholtz layer ($\psi_{o.H.}$ at a distance d_H). At d_H, the diffuse double-layer begins and ψ decays exponentially, asymptotically approaching a value ψ_A at long distances from the charged surface. The thickness of the diffuse double-layer corresponds to the Debye screening length (eqn. 1). Figure reproduced and adapted from Refs. [38] and [39].

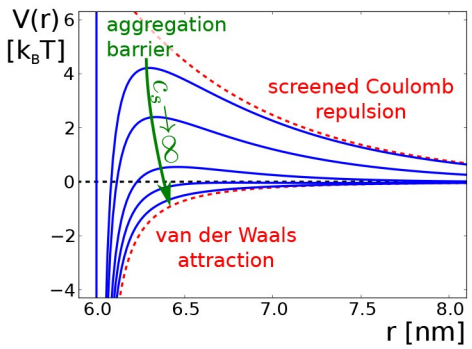


Figure 2. DLVO potential for varying salt concentration c_s. With increasing c_s, the potential changes from repulsive to attractive. The aggregation barrier reflects the charge stabilisation behaviour that becomes weaker due to charge screening.

screening decreases the electrostatic repulsion more efficiently and on shorter ranges, and finally the van der Waals attraction causes aggregation and precipitation.

Van der Waals forces (for a detailed description, the reader is referred to Ref. [44]) account for attractive interactions arising from interactions between permanent and induced dipoles, and their azimuthal orientation and range depend on the macromolecular structure. An essential aspect is the fact that the attraction decays on shorter length scales than electrostatics.[44] While not a part of the initial DLVO theory, we remark that in practice an attraction induced, e.g., by depletion or hydration can produce qualitatively similar effects, so that experimental interpretations using DLVO theory should not solely be attributed to van der Waals interactions .

The PB theory is based on strong assumptions. In particular it ignores ion–ion correlations, arising e.g. due to excluded volume and electrostatics. Furthermore, other ion properties such as polarisability and hydration effects are not included, but can actually cause significant effects. On the one hand, it is interesting that PB and DLVO theories perform so well in many cases, providing approximate theoretical predictions when a full description of the system is out of reach even with elaborate and costly computer simulations. On the other hand, it is not surprising that various ion effects have been observed that cannot be explained by PB DLVO theories.[1,45,46]

2.2. Charge effects beyond mean field: counterion condensation and ion-ion correlations

Very importantly, while in principle PB theory allows for $Z>1$, there are effects not contained in the PB picture. The most obvious of these is probably the possibility of charge inversion and like-charge attraction. We will briefly elaborate on these phenomena in the following.

If the charge density in the system is strong, significantly modified ion distributions are obtained compared to the mean-field PB approach. Manning et al.[47] found a condensation of counterions on surfaces as long as the surface charge density is higher than a critical value which depends on the surface geometry and counterion valence. Generally, the Manning condensation model was introduced to obtain an estimate of the number of counterions condensing onto polyelectrolytes. The model assumes an idealised polyelectrolyte *via* an infinitely long, charged line. For simplification, interactions between these idealised polyelectrolytes are ignored and the dielectric constant of the surrounding medium is assumed to be that of the bulk solvent.[3,47] In addition, Olvera de la Cruz[48] observed a precipitation of polyelectrolytes induced by tri- and tetravalent salts in a computational study, accounting for the probability of the binding of a condensed ion layer by PB theory with cylindrical geometry.

A general overview on ion-ion correlations has been given by Jönsson and Wennerström.[4] More recently, statistically advanced approaches accounting for ion–ion correlations due to strong Coulomb coupling have found counterion condensation at strongly charged surfaces as well as ion distributions that depend on the valence and size of the counterions.[1,49–52] In this case, ion–ion correlations cause an inversion of the surface charge.[51,53–55] Theoretical approaches even predict a so-called "giant overcharging"[56] due to increasing monovalent salt content, while simulations suggest a lower reversed charge for these conditions.[57]

The effects of ion–ion correlations are, in general, expected to be small compared to specific interactions between ions and surfaces.[54] While ion–ion correlations might add a finite contribution to the protein–ion interaction, other more specific effects appear to be more relevant.[58]

An interesting point concerns competing-ion and co-ion effects, both for different multivalent ions as well as for a given species of multivalent ions[59] in the presence of a monovalent ion.[60] We shall mention that ion effects and in particular multivalent effects are also connected with the pH of the system, but unless explicitly stated otherwise, the effects we are discussing are dominated by the charge itself, and the pH is a secondary (although quantitatively important) effect. The effects of both ionic strength and pH (pD) has been studied for lysozyme by Kundu et al.[61] For information on the quantitative modelling of the coupling of charge state and pH in the context of multivalent ions we refer to Ref. [62].

2.3. Ion-specific effects: hydration, Hofmeister, coordinative binding

While DLVO theory performs well for dilute monovalent ionic systems, a classical DLVO approach to biological systems fails due to the fact that it is no longer applicable at physiologically relevant ionic strengths above 0.1 M, as elaborated by Boström et al.[46] In addition, Boström et al. point out that in order to allow for an appropriate comparison between theory and experiments, dispersion forces strongly depending on ion-specific effects need to be taken into account.[46] Such effects include ion size, electronic structure, charge density and the resulting polarisability. Moreover, given that an overwhelming majority of biological and physiological processes take place in aqueous environments, the hydration properties of ions are of particular importance.

Systematic reviews of ion-specific effects and properties have been published, e.g., by Kunz.[5,6] Detailed theoretical and simulation studies have been described, e.g., by Lund et al., Jungwirth et al., Kalcher et al., Moreira et al., Schwierz et al., Lenz et al., Kunz et al., Smiatek et al., Lesch et al. and Kalayan et al.[49,57, 63–70] We will not review the results here, but instead refer the interested reader to the corresponding publications.

In the following, we will briefly indicate the current state of rationalisation of the Hofmeister series. We will then focus our discussion on ion-specific effects and those mediated by multivalent ions in protein systems.

The study of salt-induced phase behaviour in protein solutions ranges back to the Hofmeister series on protein solubility in the presence of different salts[71] and the related salting-in and salting-out behaviour.[72] Figure 3 shows part of the Hofmeister series for anions and cations. The combination

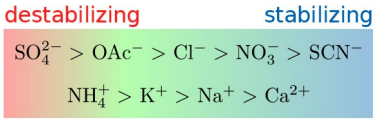


Figure 3. Part of the Hofmeister series for anions and cations. Ions on the left hand side of the series destabilise solutions and "salt out" solutes, whereas ions on the right stabilise ("salt in") solutions.

of cations and anions strongly affects the ability of the salt to precipitate ("salt out") or stabilise ("salt in") colloidal solutions.[73] These variations of the phase behaviour cannot be explained by the DLVO theory and imply that the protein–ion interactions vary considerably beyond Poisson-Boltzmann theory due to ion-specific effects.

A comprehensive molecular understanding of ion-specific effects is a challenge for theory,[5] although it is clear that water-mediated effects are a key ingredient. Baldwin[74] argues that the Hofmeister effect can be understood considering the two competing abilities of ions to "salt out" nonpolar functional groups and "salt in" the polar peptide group.

A prominent theme for the molecular origin of the Hofmeister effect is the propensity of ions to change the water structure, i.e. the ion hydration.[75–77] If certain ions – so-called "kosmotropes" – interact with water strongly, the surrounding water is aligned relative to the ion, and thus additional water structure is formed. Ions with weak interaction and inappropriate size – so-called "chaotropes" – are not able to induce any water structure and even distort the bulk structure. Kosmotropic cations are also referred to as "soft" and large with a low charge density and weak hydration while the opposite is true for kosmotropic anions which are considered "hard", strongly hydrated and assumed to have a high charge density. In turn, chaotropic cations are considered "hard" and are strongly hydrated and chaotropic anions are "soft" and weakly hydrated.[5,6] This concept of "hard" and "soft" ions has important implications for the formation of ion pairs in aqueous solutions. One possible interpretation of this phenomenon has been given by Collins[76,78] who formulated the "law of matching water affinities". This law approximates ions as spheres with point charges. In the case of small, "hard" ions, their hydration shell is strongly bound; the hydration shells of large, "soft" ions, however, are only loosely associated to the ions. Collins assumes that two "hard" ions of opposite charge experience a strong mutual attraction and, upon approaching each other, their strong attraction will allow them to shed their hydration shells and form an ion pair. Two "soft" ions with opposite charges will experience a much weaker mutual attraction than two "hard" ions, but their weakly associated hydration shells are readily shed, allowing them to pair up with each other easily.[5,78] Another theme involves the change of the dielectric constant at the protein–water interface, which allows non-localised adsorption of polarisable ions at non-polar, hydrophobic areas of the protein surface,[64,79,80] representing another possible mechanism for the Hofmeister effect *via* dispersion forces.[81–83]

Finally, the interfacial tension of the protein–water interface has been linked to the Hofmeister series.[84] Interestingly, Okur et al.[85] emphasise that Hofmeister cations and anions may follow different trends depending on the part of the protein they interact with. Potentially, the typical Hofmeister trend can even be reversed, an observation corroborated by Schwierz et al.[66,82] Thus, although of practical importance and known for over a century, the Hofmeister effect, and salt effects on protein solutions in general, remain an interesting and challenging field of research. While the multivalent ion effects discussed in the remainder of this review go beyond the Hofmeister effects, some of the ingredients at least for ion-specific effects (ionic radius, polarisability etc.) are similar.

In Sec. 2.4, we will provide a brief summary of selected physiochemical aspects of ions in solution before focusing on specific types of (biological) (macro)molecules and describing their interactions with multivalent cations in Sec. 3.

2.4. Physicochemical aspects of ions in solution

In the following, we will summarise selected physico-chemical properties of some ions of particular relevance in biological and soft matter systems. Table 1 provides an overview on the ionic radii, hydration numbers and electron configurations. We note that in the case of the binding of transition metals (especially, but not only lanthanides) to biomolecules, their electronic configurations and particularly the presence of f-orbitals, is likely to play a highly complex role at the quantum chemical level. Non-trivial trends in the protein binding behaviour of lanthanide and yttrium cations have been observed, e.g., by Gomez et al.,[86] Mulqueen et al.[87] and the authors of this review.[59] These effects can be tentatively attributed to the particular electron configurations of these ions and other highly complex properties of transition elements (polarisability, relativistic effects, anisotropic binding to biomolecules). A detailed discussion of these effects is beyond the scope of this review.

In the context of the hydration numbers given in Table 1, it is of interest to briefly mention the general effect that solutes

Table 1. Selected ion properties. Unless indicated otherwise, the ionic radii are taken from Table 2.2 in Ref. 88. [a] indicates an approximate value,[88] [b] from Ref. 89; [c] from Ref. 90; [d] from Ref. 91 [e] from Ref. 92.

Ion	Radius in solution (nm)	Hydration number	Electron configuration
Na^+	0.102	3.5	[Ne] $3s^0$
K^+	0.138	2.6	[Ar] $4s^0$
Mg^{2+}	0.072	10.0	[Ne] $3s^0$
Ca^{2+}	0.100	7.2	[Ar] $4s^0$
Fe^{3+}	0.065	6.0[e]	[Ar] $3d^5$
Y^{3+}	0.102[b]	8.0[d]	[Kr] $4p^0$ $5s^0$
La^{3+}	0.125[c]	10.3	[Xe] $4f^0$
$(CH_3)_4N^+$	0.280	1.3	–
Cl^-	0.181	2.0	[Ne] $3s^2$ $3p^6$
F^-	0.133	2.7	[He] $2s^2$ $2p^6$
CO_3^{2-}	0.178[a]	4.0	
SO_4^{2-}	0.230	3.1	
PO_4^{3-}	0.238	4.5	

such as ions can have on water structure. According to Marcus,[88] a highly suitable strategy to quantify ion-mediated effects on water structure is to determine the change of the average number of hydrogen bonds of the ion-water structures. This parameter can be quantified by exploiting the fact that hydrogen bonds in heavy water (D_2O) are stronger than those in light water (H_2O). The experimental determination of D_2O- and H_2O-specific parameters thus helps quantify the effect of solute molecules such as ions on water.[88,93]

When considering the binding of (multivalent) ions to macromolecules, an obvious question concerns the influence of the co-ions associated to the ions in question. While we note that a detailed discussion of this question is beyond the scope of this review, it is important to keep in mind that co-ions can have dramatic effects on ion adsorption to macromolecules. This has been shown for colloidal solutions, e.g., by Alfridsson et al.[94] and Karaman et al.[95] and for protein phase behaviour.[96] Importantly, in addition to unexpectedly specific co-ion adsorption, Karaman et al. found that gas dissolved in the solutions played an important role in the context of emulsion stabilities, with implications even for highly complex phenomena such as enzymatic catalysis.[95]

It is furthermore important to mention that, in solution, different ion hydroxide and oxide complexes can be formed. This is especially well-known in the case of iron[97] and implies that the ions can no longer be considered as single-atom ions. Iron is generally known to induce strong water protolysis, thereby acidifying aqueous iron solutions. In biological systems where pH regulation is essential for the functionality of biomolecules, the intricate interplay between the formation of such hydroxide complexes and pH effects[62] needs to be considered on a case-to-case basis for different ions.

3. Proteins

The interior of living cells features a high ionic strength with typical intracellular concentrations of Na^+ and K^+ being 12 and 140 mM, respectively.[9] These ions are of vital importance for, *inter alia*, maintaining a physiological osmotic pressure in living tissues and signal transduction in neurons. In addition to the ubiquitously present monovalent ions mentioned above, multivalent ions play equally crucial roles in ensuring the stability and functionality of different proteins. In this section, we focus on the interactions of multivalent cations with proteins as a specific example of biological soft matter. We describe the molecular mechanisms by which cations bind to proteins as well as the physiological effects these cation-protein interactions have. Finally, we give an overview on the physicochemical effects of cation-protein associations by describing phase diagrams of protein-multivalent cation systems. Remarks on ion channels, a very specific subtype of proteins interacting with cations, will be provided in Section 3.2.2. Parts of this section are based on Refs. [31] and [39].

As an example, calcium (Ca^{2+}) can be mentioned as a multivalent cation responsible for several phenomena related to the cytoskeleton[98] and muscle cells,[99] including the contraction of heart muscle cells.[100] In addition, Ca^{2+} is involved in the formation of protein aggregates of relevance for the food industry.[101–103] Crucially, the (dis)assembly of viruses features a pronounced dependence on Ca^{2+}.[104–108]

Further multivalent ions of physiological relevance for proteins include Zn^{2+}, which is a cofactor of several enzymes[9,109], as well as iron (Fe^{2+} and Fe^{3+}) being an integral part of a variety of so-called heme proteins.[110] These and other multivalent ions playing important roles in the context of physiology are discussed in more detail in Sec. 3.1.

There are some interesting aspects of physiological relevance of elements not occuring as natural constituents of living cells. Artificially introducing such elementary metals or their ions or their complexes into the human body is common practice in the field of medical imaging. For example, tumours and abscesses can be imaged by intravenously administering gallium ^{67}Ga-citrate.[111] The gamma radiation energies emitted by ^{67}Ga and suitable for imaging are 93, 184, 296 and 388 keV.[112] Animal studies showed ^{67}Ga-citrate to bind exclusively to transferrin and to be transported inside the body by the latter.[111] Another radioactive element, ^{166}Ho, has been shown to efficiently label chelate-conjugated antibodies,[113] which offers a valuable method to trace the uptake and distribution of antibody-based therapeutics.

Barium (Ba) and gadolinium (Gd) are often used as contrast agents for X-ray scans and for magnetic resonance imaging (MRI). The respective advantageous properties for the techniques in question are a high atomic number and therefore X-ray contrast enhancement (Ba) as well as an increase of the local longitudinal and transverse water proton relaxation rates.[114] Similarly, neodymium cations (Nd^{3+}) have been used for an investigation of histidine in aqueous solution[115] using nuclear magnetic resonance (NMR). Further information on lanthanides in structural biology can be found, e.g., in Refs. [116–118]. Metals and metal cations can also find applications in, e.g., cancer therapy. For example, attempts have been made to selectively target cancerous liver tissue by microspheres containing ^{90}Y.[119,120]

Apart from these obviously beneficial, albeit not side effect- or risk-free, interactions between proteins and ions, attention needs to be drawn to the potential toxicity of ions. Amongst other pathways, the latter can be mediated by ion-protein interactions. Poisoning due to an ingestion of, or exposure to, pathologic concentrations or levels of mercury (Hg), lead (Pb) or cadmium (Cd) are a well-known danger. Further metals with potentially toxic properties are copper (Cu) and aluminium (Al). Copper poisoning primarily affects the liver[121] with possible damage being inflicted to the kidneys and the brain as well.[122] Aluminium (Al) concentrations >0.1 mg/ml in drinking water have been associated with a potentially elevated risk of developing dementia and Alzheimer's disease.[123,124] In addition, Mn^{2+} has recently been proposed to be involved in neurotoxic effects in the context of parkinsonianism.[125]

3.1. Local picture of cation binding sites of proteins

Given the obvious importance of protein-ion interactions under both physiological and pathological conditions in medical and biological contexts, much research effort has been invested into studying these interactions from the chemical and physical points of view. A necessary step to investigate proteins the functionality of which depends on ions was to determine and characterise their ion binding sites.

On the protein surface, numerous side chains with different physico-chemical properties are exposed to the solvent. As a first consequence, in aqueous solutions charge regulation of the protein surface occurs. Functional surface groups – basic (Lys, Arg) and acidic (Glu, Asp, His) amino acid side chains and the carboxy and amino termini of the protein – are (de)protonated depending on the pH and the charges in the environment,[126,127] thus coupling pH to protein surface charge.

As a second and indeed key effect in the context of this review, also ions other than the hydronium ion, in particular multivalent ones, interact with functional groups. Numerous studies report equilibrium constants for the binding of multivalent counterions to proteins specialised in metal storage and transport.[128–132]

Moreover, models for ion binding have been developed in order to understand the interactions of proteins with ions and ligands.[133–136] On the molecular level, amino acids with carboxylate, hydroxyl, thiol, thioether, and imidazole side chain groups bind transition metal ions coordinatively.[19,137–143] In fact, the binding of the potentially toxic heavy metal ions Cd^{2+} and Cr^{3+} to the cysteine-rich protein Cry has been suggested to be an environmentally friendly method of eliminating said cations from water.[144]

The binding of ions is enhanced at hydrophilic sites surrounded by hydrophobic surface areas.[145] The overall ubiquity of these surface groups suggests that the association of salt counterions with side chains of the opposite charge at the protein surface is at the heart of the model for the interaction of ions and proteins.[64] This notion has been explicitly supported for a study on the oligopeptide tetra-aspartate. Kubíčková et al.[146] observed a charge inversion both experimentally and by molecular dynamics simulations for tetra-aspartate with trivalent cations. Mono- and divalent ions also decreased the overall charge, but did not overcome the initial negative protein charge. As the basic mechanism, the ion binding to carboxylic acids is evidenced by radial distribution functions that also show the different behaviour of multi- and monovalent cations.[146]

As will be discussed in the following, this type of multidentate coordination of multivalent cations by negatively charged or polar residues is observed in many proteins. Nevertheless, other cation-binding mechanisms shall also be briefly mentioned here. As an example, the side chains of aromatic amino acids such as tyrosine, phenylalanine or tryptophan feature π electron systems which have been shown to undergo so-called cation-π interactions.[147,148] According to Dougherty,[148] all kinds of cations can be part of this type of interaction. Given the hydrophobic nature of π-electron systems, however, they may be more likely to occur between π systems and hydrophobic cations such as quaternary ammonium ions or even the protonated guanidino group of the amino acid arginine.[148]

We shall briefly note here that binding of (monovalent) anions to nonpolar surface patches has been observed in molecular dynamics simulations.[149] This phenomenon has been traced back to solvent-assisted attraction of the ion to the protein surface.

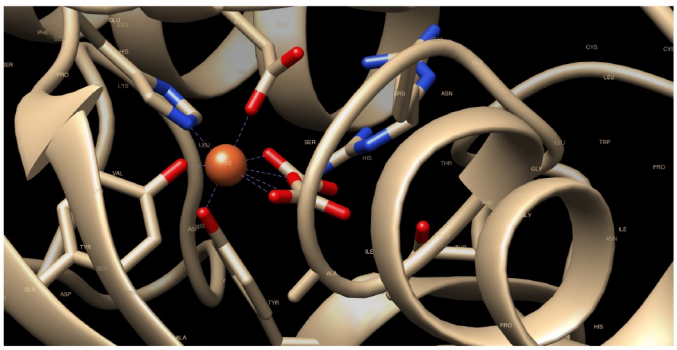
Having outlined general characteristics of cation binding sites on the protein surface, we will discuss specific examples of protein-cation systems in the following.

Calcium. In the human body, calcium is involved in a variety of processes in living cells, including cytoskeleton mobility, muscle contraction, bone formation, blood coagulation and hormone-mediated metabolism regulation[9,10, 109] (for a detailed description, see also the review by Kretsinger[99]). In fact, Ca^{2+} is often referred to as a so-called "second messenger" due to its ubiquity in physiological processes.[10,99] Thus, it is of particular physiological relevance to consider different Ca^{2+}-binding proteins. Amongst these, a specific helix-loop-helix motif referred to as the "EF-hand" is a commonly shared feature.[150–153] Examples of proteins containing an EF-hand motif are calbindin, myosin, troponin, calmodulin and parvalbumin (see Ref. [150] and refs. therein). The Ca^{2+} ion is usually coordinated by aspartic acid, asparagine or serine[150] (see visualisation in Figure 4). Experimental studies have shown that the affinity of the EF-hand motif for Mg^{2+} can be increased while decreasing that for Ca^{2+} by residue-specific mutations,[154] implying that subtle effects are important in determining the cation specificity of EF-hands.

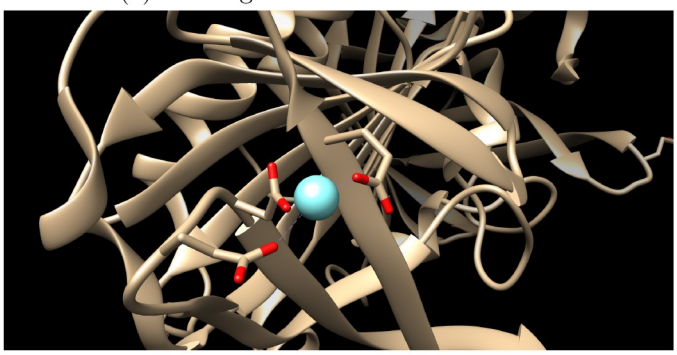
In addition to the EF-hand protein family, Ca^{2+} interacts with actin (see, e.g., the review by Janmey[155]) and gelsolin[156,157] or both actin and gelsolin simultaneously.[157] These Ca^{2+}-protein interactions are involved in the regulation of cytoskeletal motility. According to Robinson et al.,[157] both actin and gelsolin bind Ca^{2+} ions *via* aspartate and glutamate residues. Osteopontin, a protein abundantly present in the bone and teeth matrices, binds calcium in an inorganic form (hydroxyapatite) through phosphorylated serine and threonine residues as well as polyaspartate sequences.[158] The physiological role of osteopontin is briefly discussed in Sec. 3.2.1. Interestingly, in osteocalcin (another important protein in bone tissue), Ca^{2+} cations are bound by γ-carboxyglutamic acid residues,[159] a rarely occuring version of glutamic acid.[9] The tripeptide peptide Tyr-Asp-Thr with a very high Ca^{2+} chelating propensity has been isolated from whey protein,[160] evidencing the cooperative effect of several amino acids for binding of Ca^{2+}.

For more detailed and elaborate discussions on the binding of calcium ions to proteins, we refer the reader to Ref. [99]. Protein self-assembly in the presence of Ca^{2+} is discussed in Sec. 3.2.1.

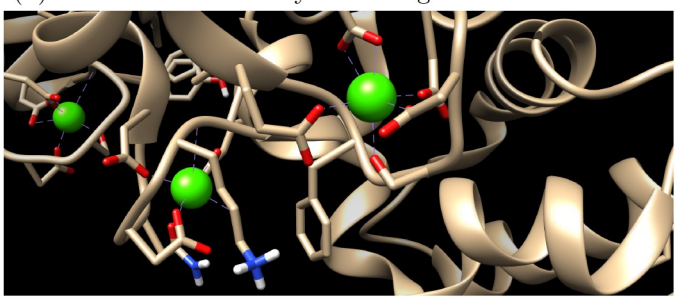
Iron. In mammal physiology, iron is known particularly well for its role in protein-mediated oxygen homeostasis and is often found in a coordination complex with porphyrin structures. This iron-porphyrin complex is referred to as a "heme group" and is



(a) Binding site of Fe^{3+} in transferrin



(b) Multidentate carboxylic binding site of Y^{3+} to BLG



(c) EF-hand binding site for Ca^{2+} ions in calbindin

Figure 4. Binding sites of multivalent ions in proteins (see text for details). The image illustrates the pivotal roles of negatively charged amino acid residues in coordinating the respective ions. As opposed to the Y^{3+} cations bound by BLG (b) and the Ca^{2+} ions bound by calbindin (c), binding of Fe^{3+} requires carbonate ions in addition to the protein residues coordinating the ion (seen on the right side of the orange sphere representing Fe^{3+} in (a)). The structures were visualised using UCSF Chimera[164] based on PDB IDs 1SUV, 3PH5 and 6FIE.

present as a prosthetic group in a variety of proteins such as myoglobin, hemoglobin, plant leghemoglobin and cytochromes (proteins involved in electron transport processes of the cellular respiratory chain)[109] as well as catalases, peroxidases, and mono- and dioxygenases[110] (enzymes catalysing redox reactions). For an overview of iron binding, the reader is also referred to Ref. [161].

The structure of myoglobin has been extensively studied, providing detailed insights into the local environment of the Fe^{2+} cation bound to the heme. The latter is located in a pocket-like structure of myoglobin, thus being protected from the surrounding solvent. Importantly, this steric protection prevents the Fe^{2+} ions from being oxidised to Fe^{3+}, which is not able to bind oxygen.[9]

Iron metabolism in mammals furthermore involves the non-heme proteins ferritin,[10] lactoferrin[10,11] and transferrin with its corresponding receptor protein.[162] As an example, one of the two iron-binding sites of transferrin consists of two tyrosines, a histidine and an aspartate residue and involves a carbonate anion[162] (Figure 4). Several so-called iron-sulfur proteins can furthermore bind Fe ions *via* cysteine side chains.[9] Moreover, while the ionic form of iron is clearly an important factor in the physiological context, it can also interact with proteins in other forms. Prominent examples are other types of iron-sulfur proteins, namely those hosting inorganic iron-sulfur (FeS) clusters. Those are, for example, found in the protein ferredoxin of the bacterium *Anabaena*. A particularly curious use of inorganic iron structures are so-called magnetosomes (inorganic, iron-containing crystals such as Fe_3O_4 crystals) providing bacteria with the ability to orient themselves along magnetic fields (for an overview on this interesting phenomenon, see Ref. [163] and refs. therein).

The iron- and calcium-binding sites mentioned above share a common feature – the multivalent cations bound to the respective proteins are complexed by charged and/or polar amino acid residues such as aspartate and tyrosine. Some examples of such binding sites are shown in Figure 4.

Magnesium. Mg^{2+} cations are known to play an important role in enzymatic reactions catalysing the cleavage of phosphate bonds. This can be especially relevant in sugar metabolism and nucleic acid (RNA and DNA) degradation. The latter is often catalysed by so-called nucleases such as ribonuclease H. Generally, magnesium ions are coordinated octahedrally.[165] In the active centre of the ribonuclease RNase H, Mg^{2+} ions are also surrounded by hydration shells (see Ref. [166] and refs. therein as well as Ref. [167]). As opposed to other alkaline metals, Mg^{2+} appears to have a particularly strong affinity to water molecules in its inner coordination shell,[166] making water an important constituent of catalytically active Mg^{2+}-enzyme complexes. In addition to these *in vivo* roles of Mg^{2+}, a very interesting magnesium-mediated transition from binary to unitary protein structures has been demonstrated by Künzle et al.[168]

Zinc. A crucial physiological role of zinc is its stabilisation of insulin microcrystals in the pancreas,[169,170] with obvious implications for diabetes. Zinc is furthermore known to be involved as a cofactor in several enzymes. Examples include carboanhydrase,[109] several proteases[10] and alcohol dehydrogenase.[9] In numerous proteins, Zn^{2+} ions are coordinated by a so-called zinc finger motif consisting of cysteine and histidine residues ("Cys_2His_2").[171]

Copper. In physiology, the most prominent role of copper is the electron transport in the respiratory chain. Here, it is bound to sulfhydryl groups of the protein cytochrome oxidase.[9] It is important to note that the oxidation state of the copper ions involved in the reduction of oxygen to water changes throughout the catalysis.[172] Interestingly, the enzyme copper-zinc superoxide dismutase – which catalyses the reaction of superoxide radicals to hydrogen peroxide and molecular oxygen – uses both copper and zinc for said catalysis.[173] Structural

studies reveal that the ions are coordinated by histidine and aspartate (Zn) and histidine and arginine residues (Cu).[173]

Molybdenum and vanadium. In plant metabolism, molybdenum (Mo), together with iron, plays an important role as a cofactor in the enzyme dinitrogenase. In addition, Mo is part of the active centre of the enzyme nitrogenase from *Azotobacter vinelandii*.[174] Interestingly, some dinitrogenase versions contain vanadium (V) instead of Mo.[9] Furthermore, vanadium is of great importance for the biosynthesis of halogenated products by marine organisms.[175]

Cobalt. A Co^{3+} ion is complexed by the corrin ring of coenzyme B12, a slightly modified form of vitamin B12. The latter is, *inter alia*, a cofactor of the enzyme methyl malonyl-CoA mutase which catalyses a step of a complex metabolic process referred to as β-oxidation of fatty acids. Importantly, the cobalt ion allows the reaction to proceed *via* an extremely unusual intermediate step involving a hydrogen radical[9] by undergoing a change in oxidation state from $+2$ to $+3$.

Lanthanides. Complexes of lanthanide ions and some organic ligands possess several favourable fluorescence properties such as long fluorescence lifetimes, strong Stokes shifts and distinct emission peaks;[176] depending on the choice of the ligand, the fluorescence can be enhanced.[177] Thus, lanthanides proved valuable structural probes to tackle questions related to cation binding sites of proteins. As an example, Harris and co-workers[178–182] examined the binding of various non-ferrous cations to human transferrin and lactoferrin, notably including the lanthanides Lu^{3+}, Er^{3+}, Ho^{3+}, Tb^{3+}, Gd^{3+}, Sm^{3+}, Nd^{3+} and Pr^{3+}. The authors report two lanthanide binding sites involving tyrosine residues[183] and a decrease in the number of cations bound with increasing cation radius.[180] Here, we remind the reader of the phenomenon of lanthanide contraction,[184] another important property of lanthanides in addition to those mentioned above. Lanthanide contraction describes the continuous decrease of the ionic radii from lanthanum (La) to hafnium (Hf) due to the successive increase in the occupation of the 4f orbitals and the simultaneous increase of the nuclear charge.[184]

Apart from purely structural studies, the influence of lanthanides on biological protein activities has been investigated as well. Smolka et al.[185] analysed the consequences of replacing calcium by trivalent lanthanides and Y^{3+} in the calcium-dependent enzyme α-amylase. This study suggests an inverse linear proportionality of the enzyme efficiency on cation radius, underlining cation-specific effects. No strong structural changes of the protein were observed. A similar study replacing Ca^{2+} by lanthanides in trypsinogen and evaluating the respective efficiencies of the cations in catalysing the conversion to trypsin was conducted by Gomez et al.[86] The dependence of the conversion rate efficiency is non-linear, but also inversely proportional to the cation radius. Interestingly, Nd^{3+} and Pr^{3+} were shown to be even better trypsinogen-trypsin conversion activators than Ca^{2+}, which was ascribed to their higher charge.[86]

The study conducted by Gomez et al. highlights an important property of lanthanides. With their radii being similar to that of Ca^{2+}, they can replace Ca^{2+} not only *in vitro*, but also *in vivo*, being of toxicological relevance.[128,186]

Lanthanides as well as yttrium are usually found in the form of trivalent cations that strongly interact with binding sites formed by carboxylic residues. This coordinative binding of Y^{3+} is apparent from protein crystals, where the cations bridge different protein molecules.[19] Importantly, the driving force for this binding is not enthalpy alone, but hydration entropy. In particular, a physicochemical characterisation of the binding reveals a lower critical solution temperature,[187] and the water coordination around Y^{3+} is reduced upon binding to the protein,[188] both of which evidence the release of hydration water molecules with substantial related entropy gains. Finally, we refer the interested reader to a recent overview on the roles of lanthanides in biochemistry by Daumann.[189]

Other cations. There are, of course, many other cations that could be mentioned here, but a full list would be beyond the scope of this review. We therefore refer the reader to the detailed works by Lipfert,[190] Permyakov,[128] Frausto da Silva and Williams[191] and Evans.[192] The above should suffice to indicate the main phenomena and concepts.

3.2. Physico-chemical and global effects of protein-cation interactions

In this section, we will discuss the global, physico-chemical behaviour of several systems composed of proteins and multivalent cations. We will explain the phase behaviour of several selected systems and discuss their respective origins. A particular focus will be on the role of the multivalent cations and their interactions with the proteins in question. We remark that we intentionally limit ourselves to a few examples of systems, and related implications, as a complete review of protein-cation interactions would be too voluminous for this review. In this context, we aim for a balanced account of basic references and new studies evidencing ongoing work.

3.2.1. Calcium-induced effects on protein assembly

Calcium represents one of the most common multivalent cations, which is why we dedicate an extra paragraph to it. It has effects on protein systems exploited both in nature (e.g. in blood coagulation, for viral assembly, and bone formation), as well as in nano- and biotechnological contexts such as food engineering. In the following paragraphs, we will outline a few examples to show the various functions and structures that are controlled by calcium ions.

Milk proteins. As one well-studied example in food science, milk proteins represent calcium-controlled molecules which significantly contribute to the calcium intake into the human body. The presence of calcium strongly affects the aggregation of whey protein,[193] and the resulting gel structure.[194,195] As a particular example, calcium has a dramatic effect on the speed of the gelation of whey aggregates, and mildly strengthens the resulting gels.[196,197] These structural variations have been shown e.g., to regulate the release of drugs from whey hydrogels.[198]

The second protein source in milk, casein, is also strongly affected by Ca^{2+} and calcium-phosphate clusters. The calcium effects range from changing the micellar structure of casein,[199–201] over varied aggregates after thermal denaturation,[202] to macroscopic effects such as the texture of milk-derived products, e.g. yoghurt.[203,204] For a detailed overview of the functionality, association and aggregation of caseins, we refer to Ref. [205].

Fibrin clot formation. Calcium is an essential cofactor in the initial step of blood coagulation, i.e., fibrin clot formation. Fibrin clots are the organism's immediate response to injury in order to prevent excessive blood loss, and fibrin assembly thus has fundamental as well as applied relevance e.g. for drug carriers and fibrin sealants. Ca^{2+} controls the cross-linking of fibrin protofibrils into fibers and hydrogel structures.[206] In particular, Ca^{2+} tunes the fibrin cross-linking rate,[207] resulting in values ranging from seconds to tens of minutes depending on the overall conditions of the fibrin solutions.[208] In this context, Ca^{2+} also affects the resulting gel structure, thereby generally enhancing the elasticity[209] and non-monotonically adapting the gel permeability.[210] Consequently, calcium is a very common additive in fibrin sealants, as well as in applications for drug delivery and bone tissue engineering.[211–213]

Inhibition of calcium crystal growth by osteopontin. Osteopontin is known to be an important constituent of body fluids with a high calcium content, such as milk and urine. It is therefore assumed that it is involved in the prevention of calcium salt precipitation (reviewed in Ref. [158]). Indeed, amongst its other roles (reviewed in Ref. [214]), e.g., in bone tissue homeostasis, osteopontin has been shown to inhibit the nucleation and growth of calcium oxalate.[215–217] A study combining molecular modelling and atomic force microscopy (AFM)[218] revealed that osteopontin strongly changed the morphology and growth of calcium oxalate crystals. Interestingly, the strength of these effects were pronounced to different degrees for different crystal faces of calcium oxalate crystals, indicating a strong interaction specificity between osteopontin and oxalate.[218]

Virus assembly. A crucial role of Ca^{2+} has been found for a large range of viruses. Early findings reported already that most plant viruses rely on correct Ca^{2+} binding for controlled structural assembly.[219] Similar indications were found for the bacteriophage PM2 and papillomaviruses, where Ca^{2+} was found to be essential for viral reassembly in vitro and during infection.[106,220] In the case of PM2, Ca^{2+} was hypothesised to stabilise the lipid bilayer of the virus before the protein outer layer is deposited on top of the lipid structure.[106] For bovine papillomavirus, the role of Ca^{2+} appeared to be to stabilise the protein capsid.[220] A recent study reports the requirement of Ca^{2+} for Rubella virus infections, as well as viral fusion and liposome insertion,[221] evidencing that Ca^{2+} enables virus function *via* structural adaption of the virus.

For simian virus 40 (SV40), Ca^{2+}, along with pH effects,[222] was found to be important for the accuracy of the assembled structure, and appropriate affinities of the viral protein capsid to Ca^{2+} regulate assembly and disassembly of the virus.[104] The presence of Ca^{2+} also proved relevant for the cell and nuclear entry during infection with simian virus 40, and Ca^{2+} was proposed to not only change the assembly state, but also the flexibility of the capsid.[105] Related to this, SAXS investigations showed that chelating Ca^{2+} caused a uniform swelling of SV40,[223] stressing the role of Ca^{2+} in regulating the virus structure.

A comparable picture is found for the hepatitis B virus (HBV), where calcium signalling plays an important role for DNA replication.[224] Again, Ca^{2+} was found to be important for the HBV core assembly.[108] Importantly, the knowledge on the Ca^{2+} effects for virus assembly even translates into nanotechnology. As an example besides the more general establishment of purification schemes,[219] an encapsulation system based on the hepatitis B virus allows to adapt the affinity to the cargo molecule via the Ca^{2+} concentration.[225]

Lipoprotein metabolism. Calcium has been found to be effective in regulating the low-density lipoprotein receptor (LDLR), which controls the body's cholesterol homeostasis. Indeed, a relation between calcium intake and the lipoprotein metabolism has been suggested.[226] On a molecular level, a recent study suggests that LDLR senses Ca^{2+} and unfolds partially,[227] thereby providing an alternative route for triggering of LDL release apart from the acidic-induced release.[228] Similar strong binding affinity of Ca^{2+} is found in a LDLR related protein abundant in the liver.[229] Furthermore, calcium also acts on the lipoprotein metabolism by assembling the lipoprotein lipase into its functional dimeric structure.[230]

3.2.2. Ion Channels

While ion channels are a specific type of proteins, they represent a slightly different topic in the context of this review, one particular characteristic being the fact that they are transmembrane proteins. We shall therefore limit ourselves to a few comments here.

The main functions of ion channels include the maintenance of physiological ionic strengths inside cells and the transduction of electrochemical signals along neurons. Prominent examples of ion channels include the Na^+ K^+ ATPase antiporter and the SERCA pumps (sarco/endoplasmic reticulum Ca^{2+} ATPase).[231,232] The function of the latter is to transport Ca^{2+} ions across the membrane of the sarco/endoplasmic reticulum. Thereby, SERCA pumps maintain an intracellular Ca^{2+} storage and also terminate Ca^{2+}-mediated signalling. Just as is the case for the EF-hand motif in proteins, SERCA proteins coordinate the Ca^{2+} ions *via* glutamate residues. In addition, glutamine and asparagine residues are involved (reviewed in Ref. [233]).

The functionality of ion channels relies on their selective permittivity with respect to different ion types. Indeed, the uptake of the "wrong" type of ions such as La^{3+} instead of Ca^{2+} can have drastic toxicological consequences.[128,186] Similar effects have been demonstrated for Gd^{3+}.[234]

On the other hand, ion selectivity can also be used to prevent cell death caused by toxic ion channels. This has been shown by Menestrina[235] in a study of the α-toxin of *S. aureus*. This toxin binds to the cell membrane, inserts itself into the membrane and forms ion channels, thus causing K^+ leakage,

which results in an osmotic shock and, ultimately, cell death.[236] Menestrina demonstrated that the channels formed by *S. aureus* α-toxin, which are open in a KCl solution, can be closed - and their function thus inhibited - by multivalent cations. The inhibition efficiency was shown to be

$$Zn^{2+} > Tb^{3+} > Ca^{2+} > Mg^{2+} > Ba^{2+}. \quad (4)$$

Menestrina provides a mathematical model to quantify the multivalent cation-mediated inhibition of the channel in which it is assumed that one multivalent cation binds to the channel in its open and one in its closed state. In addition, Menestrina suggests that a carboxyl group is involved in the binding of the cations,[235] which is consistent with the mechanisms described in Sec. 3.1. Similarly, Döbereiner et al.[237] observed an inhibition of the conductance of ion channels formed by α-hemolysin (HlyA) from *E. coli*[238] upon addition of multivalent cations. Here, the divalent cations Sr^{2+} and Ba^{2+} were able to induce HlyA-mediated erythrocyte hemolysis, albeit less efficiently than Ca^{2+}. Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} and Pb^{2+} did not lead to hemolysis; neither did the trivalent cations Fe^{3+} and La^{3+}. In addition, Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} inhibited HlyA conductance; Fe^{3+} and La^{3+} did so with greater efficiency. Döbereiner et al.[237] suggest that the cation radius plays an important role in cation recognition by HlyA.[237]

In order to better understand the selectivity of ion channels, Kumpf and Dougherty[239] performed computational studies on the affinity of Li^+, Na^+, K^+ and Rb^+ to benzene. The latter was chosen as a model of the hydrophobic core of a specific type of K^+ channel. Their results demonstrate a preference of benzene for K^+ and indicate that so-called cation-π interactions – that is, interactions of cations with delocalised π electron ring systems, of which benzene is representative – appear to occur in hydrophobic regions of ion channels. In particular, these interactions could give a hint towards the ion selectivity of ion channels. More information on this intriguing subject is found in Ref. [240].

3.2.3. Lanthanide-induced phase behaviour in protein solutions

Apart from their roles as structural probes and in medical imaging, lanthanide ions can be used to tune the phase behaviour of protein solutions, including the rational induction of protein crystal growth.

The local interactions of multivalent ions with proteins have profound consequences for the global behaviour, qualitatively different from, say, Na^+. Here, we discuss the phase behaviour and related collective phenomena of protein systems. Special attention will be paid to those types of phase behaviour induced by multivalent ions in negatively charged proteins.

Generally, a rich phase behaviour has been found in protein solutions, including liquid–liquid phase separation (LLPS), the formation of protein clusters, and crystallisation as well as other aggregates such as fibers. The nucleation kinetics differ considerably for different phases, which allows for metastable phases such as LLPS or clusters as precursor structures during crystallisation, as well as arrested phases such as gels to occur.[241,242] In this section, we provide an overview of the different phenomena that also play an important role in the present context.

Protein Surface Charge and Ion-Induced Charge Inversion. Charges on the protein surface are an important feature ensuring stability and functionality of proteins.[243–245] Charge patterns lead to anisotropic interaction patches that affect the phase behaviour of protein solutions[246–249] as well as pathways for aggregation and crystallisation.[250–252]

Protein–protein interactions are linked to charge regulation, which is, in turn, a complex process depending on system geometry and ion specific effects like binding or condensation. A comprehensive understanding of charge regulation at the protein surface is also needed in order to account for ion-specific effects such as binding and condensation as well as for the system geometry, e.g. the proximity of a wall.[245,253]

A special case is charge *inversion*, i.e., overcompensation, of surfaces in the presence of counterions. A comprehensive understanding of charge inversion has to account for both local ion binding and non-local contributions such as ion–ion correlations and hydrophobic effects.[254,255] Charge inversion has been observed for a broad range of systems such as silica spheres,[54] insoluble oxides[254] and also biological systems such as DNA.[56] The latter is discussed in more detail in Sec. 4.

In particular, charge inversion has been observed in solutions of globular, negatively charged proteins with multivalent cations.[58,256, 257] The lower charge density of the protein surface speak against ion–ion correlations being the main cause of charge inversion. Instead, Zhang et al.[19] support the notion of a charge inversion due to ion binding to acidic residues on the protein surface, based on information from crystal structures. Note that not only cations, but also *negatively* charged molecular complexes have been shown to interact specifically with net negatively charged proteins, as demonstrated for human and bovine serum albumin.[258] Remarkably, a protein crystallisation strategy similar to the one demonstrated by Zhang et al.[19] has been pursued using negative multivalent ion complexes.[24–26, 259]

Reentrant Condensation. The inversion of the surface charge is related to a specific phase behaviour called reentrant condensation known from polyelectrolytes (see, e.g., Ref.[260]), which has been observed in aqueous solutions of negatively charged proteins with trivalent[58,256,261] and tetravalent cations,[262] as illustrated schematically in Figure 5. At a given protein concentration c_p and a low salt concentration c_s, the system is a homogeneous liquid (Regime I), charge-stabilised by the initially net protein charge. A continuous increase in c_s, while keeping c_p constant, decreases the negative surface charge of the protein and eventually condenses the protein molecules in solution into cluster-like structures. This condensed state is referred to as Regime II, the entrance into which is marked by a critical salt concentration, c^*. A further increase of salt concentration leads to overcharging of the protein, and the clusters redissolve upon surpassing a second critical salt concentration, c^{**} (Regime III), stabilised by the reversed charge of the protein-cation complex. Computer simulations confirmed

the reentrant behavior in the protein-protein potential of mean force,[263] and support a picture of very directional interaction due to binding of multivalent ions.[264,265]

Although induced by salts, this phenomenon is clearly beyond the usual salting-in and salting-out behaviour of proteins and needs an individual explanation linked to the ion binding to proteins. We remark that a macroscopically similar phenomenon is observed for DNA, but arises from a different microscopic driving force (see Sec. 4.3).

Liquid–Liquid Phase Separation (LLPS). Under certain conditions within the condensed regime II of the reentrant condensation (see paragraph above and Figure 5), a liquid-liquid phase separation (LLPS) into a protein-rich and a protein-poor liquid phase is observed.[257,266]

In general, LLPS was found in several protein systems, first in mammalian eye lenses[267,268] with implications for cataract[269,270] and exemplifying critical phenomena in a biological model system.[271–275] A metastable LLPS in hemoglobin solutions has been found to be the primary event of sickle cell anemia.[276]

In addition to the above examples, LLPS is a process which is often invoked to explain how living cells regulate signal transduction pathways and organise their interior. This organisation often occurs *via* the formation of membraneless organelles, e.g., so-called P granules, which feature liquid-like properties.[277] Such organelles can consist of different types of proteins and RNA molecules. The proteins driving phase separation in cells are in many cases so-called intrinsically disordered proteins (IDPs).[278–281] The propensity of IDPs to undergo LLPS is strongly influenced by their molecular interactions, which are, in turn, determined by properties such as net charge and hydrophobicity. It is known that IDPs are often enriched in aromatic, polar, and positively and negatively charged amino acids.[282–286] This implies that salt/ion effects are an important factor capable of influencing the behaviour of IDPs.[280] Indeed, theoretical studies of block polyampholytes containing positive and negative charges as IDP models[287] have shown that divalent ions decrease the width of the coexistence region of high- and low-polymer density phases of the symmetric block polyampholytes, but only have a weak influence on the coexisting concentrations. Trivalent ions have a stronger effect, significantly shifting the dense branch of the binodal to lower concentrations.[287] This study thus demonstrates how ions can influence the phase behaviour of IDPs.

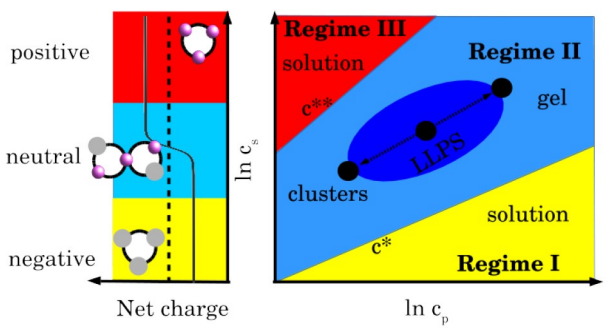


Figure 5. Phase diagram showing regimes I, II and III, reentrant condensation and LLPS. See text for details.

While condensed structures formed by IDPs are often functional and physiologically relevant, they can also play a role in several pathologies such as Alzheimer's and Huntington's diseases.[278,288] This can be due to the formation of aggregates which perturb the physiological processes of cells. Interestingly, this aggregation process can be enhanced by metal ions, e.g. if these allow IDPs to populate certain conformations prone to aggregation (see Ref. [289] for a detailed overview). As an example, the fibrillation kinetics of α-synuclein, an IDP involved in the pathogenesis of Parkinson's disease, has been shown to be strongly enhanced in the presence of Cu^{2+}, Fe^{3+}, Co^{3+} and Mn^{2+}.[290]

Experimental results confirmed the metastability of the LLPS with respect to the crystal phase[291] as theoretically expected for an attraction that is short-ranged compared to the protein size.[292–294] Interestingly, such a short-ranged attraction can be introduced into protein solutions by multivalent cations that induce cation-activated attractive patches.[265] Multivalent cations such as Y^{3+} are able to form cation bridges between negatively charged areas on protein molecules,[19] thus introducing an effective short-ranged attraction between the proteins. Interestingly, under appropriate experimental conditions, LLPS occurs[257,266] with a lower critical solution temperature (LCST-LLPS), *i.e.*, representing an entropy-driven transition,[187] most likely related to the release of hydration water around the multivalent cation. Given that entropic considerations usually favour phase separation for globular, folded proteins upon a temperature *decrease*,[295] this behaviour is rather unusual and most likely linked to release of hydration water.[187] It also provides clues regarding the water-ion interactions and entropic contributions (cf. Ref. [188])

The width of the reentrant regimes, the lower critical transition temperature as well as the overall strength of the interaction can be strongly influenced by the type of multivalent cation.[59] Pronounced anion as well as solvent isotope effects on the phase behaviour of protein-multivalent salt systems have also been shown.[96,296]

Generally, metastable LLPS in protein solutions is of specific interest due to its connection to protein crystallisation. In this context, control of the phase behaviour is essential to optimise nucleation conditions for high-quality protein crystals (see below).

Additives in protein solutions such as PEG, glycerol, monovalent salts or a second protein species have been found to shift the coexistence curve of protein solutions in temperature.[297–305] Theoretical studies have reproduced these shifts based on colloidal models and nonspecific interactions between proteins and additives.[246–248, 305–308] More information on how multivalent cations can be used to induce protein crystallisation can be found in Section 3.2.3.5.

Cluster Formation. The formation of equilibrium clusters in solutions of charged particles has been predicted by a simple argument:[309–311] if particles exhibit a short-ranged attraction and a long-ranged (Coulomb) repulsion, monomers attach due to the attraction until the repulsion of the entire cluster grows strong

enough to destabilise further attachment. Indeed, transient clusters, potentially of this type, were observed e.g. for solutions of lysozyme,[312–315] β-lactoglobulin,[316,317] hemoglobin,[318] and lumazine synthase.[319] Reversible cluster formation in protein solutions is not only of fundamental, but also of practical interest, since it would be promising for drug delivery at high antibody volume fractions and moderate viscosity.[320]

An ion-induced short-range attraction via bridges of multivalent cation is also expected to stabilise protein clusters.[265] Soraruf *et al.*[321] studied the formation of multivalent cation-induced cluster formation in bovine serum albumin, as evidenced by an increased structural correlation length and significantly slowed down diffusion. In addition, the slowing down of the diffusion with increasing salt concentration was found to depend mainly of the ratio of cations per protein, consistent with the ion-bridge picture.[322]

The presence of flexible clusters might affect pathways of protein crystallisation, as will be discussed in the following.

Protein Crystallisation and Nucleation Pathways. The lack of a systematic and general procedure to obtain high-quality protein crystals has inspired numerous studies on the connection of phase behaviour and crystal nucleation as well as the control of optimum conditions for protein crystallisation.

George and Wilson[323] suggested the so-called crystallisation slot for the protein attraction. While for too weak attractive interaction the nucleation is very slow, too strong attraction causes multiple nucleation events and irregular and arrested assembly of proteins. Vliegenthart and Lekkerkerker[324] explain optimum crystallisation conditions by two effects: nucleation rates can be enhanced by the proximity to the critical point of a metastable LLPS, or the presence of small dense droplets[325,326] or clusters,[327] both of which have indeed been found in experiments.[298,328]

Both conditions proposed represent multi-step nucleation pathways in the sense that the two order parameters density and structure, which are coupled in classical nucleation theory, are separated and develop independently. In a first step, the solution forms a dense precursor which then reorders to a structured crystal nucleus.[329] The exact nature of the precursor in protein solutions remains unclear and is, most likely, not a general feature.[330]

Notably, cation-mediated bridging of negatively charged protein molecules can promote the nucleation and growth of protein crystals,[19,331,332] thus allowing for a controlled growth of crystals which is considered a major obstacle in protein crystallography. Intriguingly, the location of the samples in the protein-salt phase diagram, i.e., their composition, determines their crystallisation pathways. Using β-lactoglobulin (BLG) and YCl_3 as an example, classical nucleation dominates at low salt concentrations,[21] while a two-step mechanism can be observed at high salt concentrations.[22] For an overview of non-classical protein crystallisation, we refer to Ref. [332]. The ion-mediated approach has also been successfully applied to positively charged proteins using polyoxometallates (POMs), a specific type of anions.[24–26, 333]

Arrested Phases: Gels and Amorphous Aggregates. Besides the equilibrium properties, also kinetic pathways matter for the observed phase behaviour. In systems with short-ranged attractions, arrested phases such as gels and glasses[311,334, 335] are observed in colloidal systems. For the case of proteins, the gel formation has been related to an arrested metastable LLPS[242] and the formation of clusters.[336–338] Poon[339] argues that the arrested LLPS might be the reason that crystals cannot grow at high attraction strength.

In the case of cation-induced LLPS, arrested states are also possible. The LCST-LLPS of BSA-YCl_3 systems has been shown to occur *via* spinodal decomposition and the kinetics of the latter have been studied using ultra small-angle scattering.[340] It was found that the characteristic length ξ of the respective systems grows as a function of time t as $\xi \sim t^{1/3}$ for $T < 45\,^{\circ}C$. For $T < 45\,^{\circ}C$ and at $t > 30$ s, the growth of ξ slows down. At even higher temperatures, arrest is observed as indicated by constant values of ξ until protein denaturing interferes with further investigations above 55 °C. Interestingly, the kinetics of LCST-LLPS samples as well as the onset of arrest can be strongly influenced by the choice of multivalent cations used. Matsarskaia et al.[341] studied arrested states in systems consisting of BSA and varying mixtures of $HoCl_3$ and $LaCl_3$, finding that higher $HoCl_3$ concentrations progressively lower the temperature of the onset of arrest. These results indicate that Ho^{3+} induces stronger interprotein attractions than La^{3+}.

4. Nucleic Acids (DNA and RNA)

DNA and RNA are the two most commonly known dominant types of nucleic acids and play pivotal roles in cell division, protein biosynthesis and the regulation of various cell signaling pathways.[9] Similarly to the primary structures of proteins, they are also chain-like, but do not undergo an equally elaborate folding process, although especially RNA is known to form different secondary and tertiary structures.[10,342] Nucleic acids also exhibit a charge pattern on their "surface" and are therefore also classified as polyelectrolytes.[343] However, as opposed to proteins, the net charge of nucleic acids is typically dominated by the negative charge of their phosphate backbones. Not surprisingly, charge-driven or charge-mediated interactions therefore play a key role in DNA/RNA research and several strategies have been employed to control and manipulate their interactions with charges. The vital involvement of DNA in cell proliferation implies its role as a target in chemotherapy *via* different, specific types of interactions, which will be briefly explained.

In the following, we will first discuss the local picture of the ion distribution around DNA and RNA, and then focus on the resulting more macroscopic ion effects in these systems.

4.1. Local picture: ion distribution and binding

Given the high charge density of DNA and RNA, the ion distribution around the nucleic acids is dominated by electrostatic interactions. The regularity of DNA furthermore provides

an interesting experimental test case for validation of theoretical assumptions.

In particular, several experimental studies contributed to a detailed characterisation of ion clouds around DNA by exploiting the anomalous X-ray scattering around the absorption edges of the counterions Rb^+, Sr^{2+} and $(Co(NH_3)_6)^{3+}$. Interestingly, both monovalent and divalent salts show quantitative agreement with predictions based on atomic scale non-linear Poisson–Boltzmann theory (NLPB).[42] ASAXS difference spectra characterising the DNA-ion spatial correlations show ion distributions which are more extended for monovalent ions (decay length 4.2 Å) than divalent ions (2.9 Å), as expected theoretically.[42] Furthermore, the number of ions per base pair was obtained, yielding 1.36 monovalent and 0.76 divalent cations per base pair, in good agreement with NLPB predictions for excess ions of 1.43 and 0.85, respectively.[344]

As another test of NLPB, Andresen et al.[41] performed an experiment on mixed cation solutions where monovalent and divalent cation compete. As predicted, the shape of the ionic clouds remained invariant, and the ratio of surface-close monovalent to divalent cations follows a simple Boltzmann relation,[41] overall preferring divalent ions to be bound.

Even for trivalent cations, NLPB predictions were valid up to a critical threshold beyond which DNA condensation sets in.[345] The authors speculated that, at these low ion concentrations, the ion-ion correlations might not be strong enough to significantly vary the profiles. For competition between mono- and trivalent ions, NLPB had to be corrected for effects of finite ion size.[345]

For RNA, a study combining anomalous small-angle X-ray scattering (ASAXS) with MD simulations provides a consistent picture on the ion distribution around a more flexible RNA segment.[346]

Recent computer simulations for cations around DNA and RNA suggest that tighly bound divalent Mg^{2+} ions can occur in two different surface areas with different binding distances, as opposed to tight binding of monovalent ions in one broad population.[347] Figure 6 shows a schematic representation of ion binding to a DNA molecule as well as condensation and charge inversion of DNA molecules induced by ion-ion correlation effects.

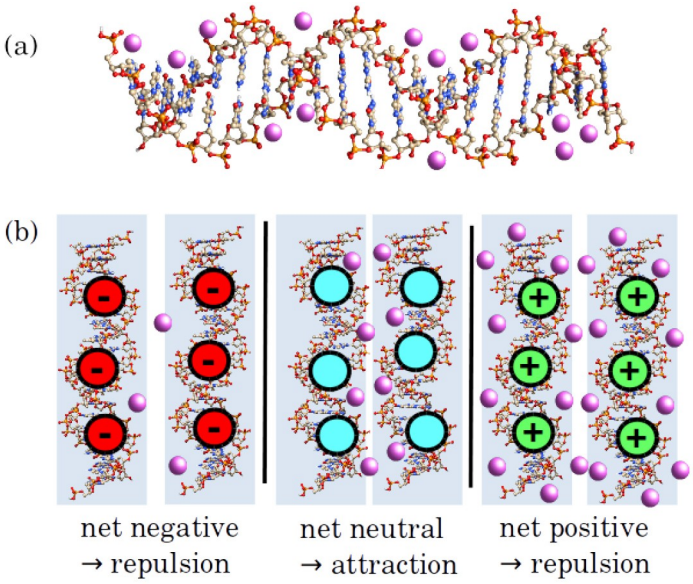


Figure 6. Schematic of DNA-ion correlations at different ion concentrations. (a): Cation binding to minor and major grooves (inspired by Refs. [347] and [348]). (b) Condensation and charge inversion of DNA molecules induced by ion-ion-correlation. The circles on the DNA molecules indicate the net charge of the latter (red: net negative; turquoise: net neutral; green: net positive). Figure was rendered using UCSF chimera[164] and Avogadro.[349]

4.2. Structural stabilisation by cations

DNA and RNA fulfil different functions in the cell,[9] but their chemical compositions are rather similar. Indeed, their interactions with cations also feature similarities,[350,351] including the fact that both types of nucleic acids can be stabilised by delocalised as well as site-specific cation interactions.[352]

In the case of RNA, it is interesting to note that this type of nucleic acid can, starting from an initially rather simple linear structure, proceed to form more complex structures. This phenomenon, also known as RNA folding, is particularly important in the case of transfer RNA (tRNA) which is involved in cellular protein synthesis.[10] Since the formation of such structures involves a compaction of the negatively charged RNA phosphate backbone, inorganic cations are required to screen these charges and facilitate the compaction process.[353,354] Both mono- and divalent cations can fulfil this role[355,356] and the melting temperature of tertiary RNA structures has been shown to have a non-monotonous dependence on dehydrated monovalent cation radii[352] with the effectiveness of stabilisation indicated as

$$Li^+ \approx Na^+ < K^+ \ll NH_4^+ \gg Rb^+ > Cs^+$$

In addition to inorganic ones, Heerschap et al.[357] tested organic cations and found the following order of stabilisation for a certain type of tRNA:

$$\text{spermine} > \text{spermidine} > \text{putrescine} > Na^+ \approx NH_4^+$$

Divalent cations are more efficient in stabilising RNA structures than monovalent ones,[356] as exemplified by the prominent role of Mg^{2+} in tRNA folding.[358] NLPB theory-based calculations show that, in an RNA solution with a constant monovalent ionic strength mediated by NaCl, the addition of $MgCl_2$ stabilises the folded RNA conformation.[359] This is traced back to the entropically favourable release of roughly 2 Na^+ ions per Mg^{2+} binding event.[359] In addition, due to their higher charge density, half as many divalent cations are needed to neutralise RNA phosphate backbone charges upon folding and subsequent compaction. Hence, the entropically unfavourable decrease in conformational space of the cations is less thermodynamically relevant than in the case of monovalent cations.[356]

Importantly, for Mg^{2+} ions bound to RNA, the cation-mediated effects go beyond pure electrostatics, and include polarisation and charge transfer.[353] Along these lines, the

intricate interplay between the hydration shell of the cation and its interaction with the phosphate residues of the RNA need to be considered.[353]

How strongly cations influence RNA structure depends on the type of their interaction with RNA. The latter can be classified on a scale from "chelated" to "free in bulk"[353] and those classified as "chelated" (i.e., coordinated and fully dehydrated) are the ones of greatest structural significance. Moreover, the more RNA ligands a cation coordinates in its first shell, the more it contributes to RNA folding.[353] A clear prediction for structural changes of RNA in the presence of cations is challenging, and clear differences exist between the structure of RNA in KCl and $MgCl_2$ solutions.[347] Interestingly, for both salts, the RNA structure deviates also significantly from the assumed canonical A-form of RNA.[347]

Interestingly and potentially counterintuitively, quasi-elastic neutron scattering (QENS) measurements have shown RNA folding to go along with an increased flexibility of its backbone as is reflected, *inter alia*, in an increased mean-squared displacement and mobile atom fraction.[360] The authors of Ref. [360] propose that the diffusion of hydrated cations close to the RNA molecules lead to stronger electrostatic fluctuations. Another possible explanation involves more strongly fluctuating hydrogen bonds between hydration shell water molecules and the RNA backbone screened by Mg^{2+} cations.[360] For a more comprehensive discussion of RNA folding in the presence of metal ions we refer to the review by Woodson.[361]

In addition to electrostatic interactions between the RNA phosphate backbone and cations, both mono- and divalent cations have also been shown to interact with DNA bases through cation-π interactions as well as through the first hydration shell water molecules of the cations.[362] These types of interactions can lead to an enhanced stabilisation of certain DNA binding motifs, thus playing a role in cellular processes relying on, e.g., protein-DNA binding.[362]

Because of its crucial role in cell proliferation, DNA is an obvious target for cancer treatment. In the context of this review, the chemotherapeutic cisplatin needs to be emphasised. Once inside a cell, cisplatin is hydrolysed, yielding the charged complex $[Pt(NH_3)_2ClH_2O]^+$. Importantly, this cation undergoes a very specific coordinative reaction to a guanine or adenine base, enabling an interaction between the cisplatin-DNA complex and DNA repair proteins.[363] A different approach to cancer treatment involves the direct suppression of the production of a cancer-promoting protein *in vivo*. Such an approach involved the targeted delivery of small interfering RNA (siRNA) molecules using the large organic cation oligoarginine has been described by Cantini et al.[364]

4.3. Reentrant condensation of DNA by multivalent ions

Reentrant condensation of DNA macromolecules by multivalent cations, particularly spermine, has been studied intensively. In this system, the stability of DNA solutions is determined by two transition concentrations of multivalent cations. At low cation concentrations, the DNA solution is stable. Crossing the condensation cation concentration, DNA condenses and precipitation sets in. Beyond the second reentrant transition concentration, the DNA solution is stable again.[365–367]

The phenomenon of reentrant condensation in DNA can be explained by a charge inversion[55] and like-charge attractions[368] of DNA molecules induced by ion–ion correlations of multivalent counterions.[51,53,369,370] Similarly to the question on ion distributions (see above), DNA again provided a well-characterised experimental system with a surface-charge density sufficiently high to allow the observation of clear effects beyond PB theory. These observations inspired the further development of theories and simulation approaches, also accounting for ion–ion correlation effects. Ion–ion correlations in the strong-coupling limit induce a rather ordered distribution of condensed ions over the surface, which leads to both overcharging and like-charge attraction without the need for specific interactions.[51]

The effect of competing monovalent salt represents a debated topic. Experimentally, monovalent cations induce an increase of the condensation concentration of multivalent cations, whereas the reentrant transition remains constant.[365,371] From analytical theory, a so-called giant charge inversion, i.e. a larger reverted positive charge than the initial negative charge, was predicted at large monovalent cation concentrations,[56] but not observed in simulations.[57] Burak et al.[43] concluded that the actual number of condensed multivalent ions depends on the choice of short-range interactions, and thus has to be salt and model-dependent.[43] Indeed, the structure of multivalent cations has been found to affect DNA condensation and condensates,[372] which was traced back to a non-specific recognition process of complex cations to DNA.[373] On a finer structural level, it was suggested that DNA condensation is connected to ion condensation in the major groove of DNA which depends on ionic properties and also allows for a temperature-driven transition due to entropic effects.[374] Recent simulations indeed found that aggregation of DNA and RNA induced by multivalent ions depends on the helical structure (A or B form), suggesting a critical role of the condensation area of the ions.[375]

In the context of the relevance of local structure, it is helpful to contrast the different pictures for reentrant condensation in DNA and proteins. Proteins provide a very irregular condensation pattern, as both charges are distributed irregularly on the surface, and local binding sites have different geometries, making solvation effects very dependent on the specific surface area. As a result, proteins typically have few strong interaction sites with multivalent ions, which dominate the binding and require a more local picture of cross-linking as the main cause of attraction. By contrast, DNA provides a fairly regular structure with repeating condensation areas, which allowed initial approaches based on uniformly charged cylinders to recapitulate the overall effects due to ion–ion correlations. Even when considering the atomic picture, ion distributions are more uniform along the full chain, and attraction due to ion–ion correlations can thus still be expected to play a major role.

4.4. DNA: stiffness, kinks, and persistence length

There have been very intense and detailed studies of the mechanical properties of DNA,[376] including in particular single-molecule DNA. A comprehensive feature and overview was written by Bustamante et al.[377] ds-DNA is 50 times harder to bend into a circle than ss-DNA,[377] although material-wise ds-DNA is only two strands of ss-DNA plus the twist. The stiffness is reflected in a rather large flexural persistence length, A, which in the worm-like chain (WLC) model is about 50 nm (the length of roughly 150 base pairs) for dsDNA in physiological buffer.[377] The phosphates in the DNA backbone make it one of the most highly charged polymers known.[377] As a result, its structure is "pre-stressed" by electrostatic self-repulsion.

Thus, we can expect that charges can provoke a strong response of DNA. Other charged macromolecules, such as DNA-binding proteins, can, provided a suitable charge pattern, effectively associate with DNA and even bend DNA.[377] Interestingly, even enantiospecific kinking of DNA by a partially intercalating metal complex was reported.[378]

A landmark study on "ionic effects on the elasticity of single DNA molecules"[379] reported several effects of ions, some of which were unexpected based on macroscopic elasticity theory. While details of the analysis of the elasticity of DNA (with in fact three different elastic regimes, and some effects opposite to what would be expected from macroscopic elasticity theory) are beyond the scope of this review, it was clearly shown that *multi*valent ions can have a much stronger effect on the persistence length than *mono*valent ions for the same nominal ionic strength[379] (cf. quadratic functionality of valency Z in Eq. 3). For further work in the context of mechanical properties of DNA and charges, see, e.g., Refs. [380–384].

Generally, we can expect that the mechanical properties of DNA impact biologically relevant reactions, and vice versa, i.e. the impact of other biomolecules attached to DNA have an impact on the mechanical properties, which means that the latter can be used as a sensor for the former.

We remark that in addition to its biological relevance, DNA is also employed in nanoscience and nanoengineering applications including, e.g., "DNA origami".[385] These are beyond our scope here, but we note that of course also for these systems charges typically play a key role for the interactions and the resulting behaviour.

5. Amphiphilic molecules and interfaces

Amphiphilic molecules assembled at interfaces are excellent model systems. They can be found as monolayers or bilayers, the latter serving, e.g., to mimic biomembranes. This is especially important given the crucial role of cations, including divalent ones, in different processes involving cellular membranes such as signal transduction.

Amphiphilic molecules in general typically exhibit a hydrophilic and, in some cases, charged head and a hydrophobic tail. They play a key role as constituents of bilayer structures and biological membranes such as those surrounding cells. Given that they can be used *in vitro* as model systems mimicking cell membranes, they are thus also of relevance for pharmaceutical research.

In addition, amphiphilic molecules are essential ingredients of everyday consumer products such as cosmetics or detergents. In this context, also terms such as "surfactants" and "tensides" are used.[386] These are not the main focus of this review, but we wish to point out some of their properties with special attention being paid to those involving multivalent cations. Importantly, there are ionic surfactants and especially these (but not only these) are subject to various ion-mediated effects. In this section, we shall organise the material according to their nature, e.g. interface layers and micelles or, more generally, interface and bulk behaviour, respectively. We shall also comment on ion effects at interfaces, including those occuring in the absence of amphiphilic molecules, as model studies. We note that the distinction between interfaces and bulk is not always sharp since the bulk can also involve internal interfaces that may be formed in solution.

5.1. Monolayers and remarks on the local picture

A general overview on phase behaviour of monolayers, including lipids, is found in Ref. [388]. A general schematic of ions and amphiphilic molecules in an aqueous solvent is shown in Figure 7. Here, we begin our discussion with lipid monolayers, which are also very suitable to study local ion-lipid interactions.

Bu et al.[389] used X-ray spectroscopy to investigate a Langmuir monolayer of dihexadecyl phosphate on a cesium iodide (CsI) solution. The experiments showed that the monovalent Cs^+ ions form a diffuse Gouy-Chapman layer on the surface of the monolayer. With 0.58 Cs^+ ions per lipid molecule, the cations were found to be surrounded by oxygens from the aqueous solution as well as from the dihexadecyl phosphate head groups. The counterions (I^-), on the other hand, appeared to be depleted from the monolayer-water interface.[389] Moreover, the pathologically relevant divalent cation Be^{2+} has been shown to cross-link and thereby compact phosphatidylserine (PS, an anionic lipid) monolayers.[390] The authors also showed that Be^{2+} was able to displace Ca^{2+} from PS, indicating a potential mechanism behind the pathological condition berylliosis.[390]

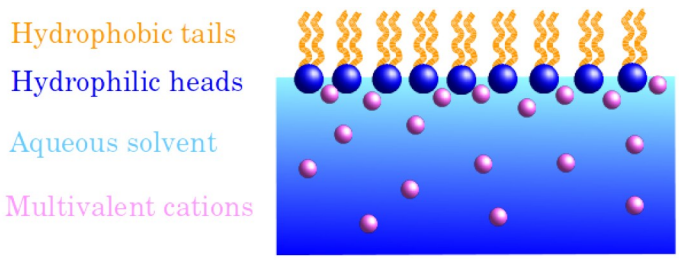


Figure 7. Schematic representation of the arrangement of cations in the bulk solution and near a monolayer of amphiphilic molecules. Image inspired by Ref. [387].

A powerful technique to study the local distribution of ions around amphiphilic molecules is X-ray reflectivity. In a very elegant XRR experiment exploiting anomalous scattering, Vaknin et al.[387] characterised in detail the ion distribution at biomimetic membranes, specifically Ba^{2+}, near DMPA (1,2-dimyristoyl-sn-glycero-3-phosphatidic acid). They found an unexpectedly large concentration of barium at the interface, 1.5 per DMPA, forming a Stern layer of bound ions and a cloud of less densely bound ions near the lipid headgroups.[387]

Interestingly, in the case of divalent cation mixtures (Ba^{2+} and Ca^{2+}), DMPA was found to exhibit a strong preference for Ca^{2+} with the ratio of Ca^{2+}:Ba^{2+} at the DMPA-water interface being roughly 4:1 even though theoretical predictions based on cation hydration behaviour suggest the opposite.[391] In addition, the fact that more than the one Ca^{2+} ion required for charge neutralisation is bound to the DMPA surface suggests that charge inversion of DMPA occurs upon Ca^{2+} binding. Similarly to the study by Bu et al.,[389] no accumulation of I^- ions at the interface was observed here and, also similarly, some hydroxide species ($Ca(OH)^{2+}$) are assumed to be present. The fact that this preferential binding of Ca^{2+} is in stark contrast with data obtained using a different surfactant[392] underlines the strong molecular specificity of cation-surfactant interactions.[391]

Pittler et al.[393] extended the studies performed on DMPA and monovalent and divalent ions to the trivalent ion La^{3+}. Their results showed a charge inversion of DMPA at very small $LaCl_3$ concentrations of 0.5 μM, much lower than those observed by other experiments investigating a silicon (Si) surface.[394] The charge inversion observed is believed to be mediated by La^{3+} intercalation between the negative charges of DMPA phosphates and/or hydrogen bonds between the phosphate oxygens and $La(OH)^{2+}$.[393]

5.2. Micelles and bulk behaviour

Amphiphilic molecules can, under suitable conditions, assemble into micelles,[395] which may then be considered (soft) nanoparticles in solution, essentially in a colloidal sense. If these are subject to the influence of multivalent ions, they can exhibit a variety of different behaviours which we will discuss in the following.[396]

An intriguing approach to tuning the phase behaviour of micelles using multivalent cations was demonstrated by Carl et al.[397,398] The authors synthesised block-copolymers consisting of polyacrylate (PA) and polystyrenesulfonate (PSS). Combining these two polyelectrolytes prevents the well-known Ca^{2+}-induced precipitation of PA. Instead, the diblock copolymers underwent reversible micellisation which could be tuned by varying the Ca^{2+} concentration and temperature.[397] In another study, the authors exploited thermodynamic differences in cation-polymer interactions of PSS and PA and demonstrated that Ca^{2+} triggers micellisation at high temperatures, while Ba^{2+}- and Sr^{2+}-induced micelles form at both high and low temperatures. At intermediate temperatures, single block copolymer chains are found. Importantly, the micelle structure can be inversed by changing the temperature. At low temperatures, PA forms the outer layer of the micelle; at high temperatures, the outer layer consists of PSS.[398]

In addition to micelle formation, the ion-dependent behaviour of amphiphiles and lipids in bulk is also of interest. An interesting example of ion specificity on the behaviour of a lipid derivative, lecithin, has been described by Lee et al.[399] The authors observed that, depending on the cation and anion added to their lecithin samples, the tendency of lecithin to form a gel can be strongly altered. Studying lecithin-Ca^{2+} as a reference system, they established that lecithin gelation goes along with the formation of cylinder-like structures and an increase in the viscosity of the sample. A possible application for this ion-induced gelation is a reversible, isothermal gelification of kerosene for transport purposes.[399]

Similarly, the cation-dependent bulk properties of water-in-oil microemulsions stabilised by the sodium salt of the surfactant bis-2-ethylhexylsulfosuccinate were investigated by Eastoe et al.[400] The authors demonstrated the formation of rod-shaped aggregates of the water-oil emulsion when Na^+ was exchanged for Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} . Interestingly, the aggregates formed in the presence of these cations assume a spherical shape upon water addition. Spherical structures were also observed in the case of Mg^{2+} and Ca^{2+}, indicating a pronounced sensitivity to ion-specific effects of this system.

For a system with an anionic surfactant and anionic surfaces (the isoelectric point for silica is approximately pH = 2) normally repulsive interactions would be expected, but divalent ions (Ca^{2+}) can form bridges between the negatively charged surfactant and surface, thus enabling their binding.[401] Furthermore, the authors of this study varied the pH and compared their results to Na^+, showing that the behaviour can be qualitatively different depending on the type and valency of the used cation. We note that Ref. [401] did *not* report *reentrant* effects, in contrast to the study of protein adsorption at solid-liquid interfaces using trivalent ions (Y^{3+}), where reentrant adsorption reflecting bulk reentrant behaviour could be observed.[402]

The adsorption of mono- and divalent anions to cetryltrimethyl ammonium (CTA) surfactant salts and the resulting effects on the surface tension of the latter was investigated by Para and Warszynski.[403] The authors found that the monovalent ions Br^-, Cl^- and HSO_4^- decrease the surface tension of CTA more efficiently than the divalent anion SO_4^{2-}. This result is traced back to a strong hydration of SO_4^{2-} anions, preventing them from penetrating the surfactant surface layer[403] and it is an interesting example of non-trivial effects as a function of charge.

An overview on the phase equilibria of selected ionic surfactants in the presence of mono- and divalent ions is given in Ref. [404], demonstrating ion-specific effects on the stability of liquid crystalline phases of sodium di-2-ethylhexylsulphosuccinate in D_2O. In the presence of divalent cations, the water uptake of the lamellar crystalline phase of an octylsulphate-decanol-D_2O is reduced. Furthermore, Mg^{2+} leads to the formation of two additional liquid crystalline phases of the system.[404]

Surfactants at the solid-liquid interface with divalent ions were studied in Ref. [401] using neutron reflectometry.

5.3. Bilayers: vesicles and membranes

In biology, one of the most important manifestation of lipid bilayers are membranes surrounding various cell types including cells of the human body, but also bacteria.[9] An interesting example is the outer membrane of Gram-negative bacteria. This membrane is strongly asymmetric with the inner side consisting of phospholipids, whereas the outer part contains a significant proportion of lipopolysaccharides (LPS).[405] Mg^{2+} and Ca^{2+} ions are known to bridge these molecules, thereby compensating their mutual electrostatic repulsion. Clifton et al.[406] demonstrated that removing these cations from a model closely mimicking the outer membrane of Gram-negative bacteria leads to a destabilisation of membrane asymmetry and intermixing of LPS from the inner and outer parts of the membrane. This study illustrates the key role divalent cations can have especially in a biological context.

In the context of physiological relevance, pulmonary surfactants need to be mentioned. Dipalmitoylphosphatidylcholine (DPPC), which is considered the main component of lung surfactants (see, e.g., Ref. [407]), was investigated with respect to the effect of divalent cations on its structure and activity by Efrati et al.[408] These authors established that, in the presence of the divalent ion-dependent surfactant proteins SP28-36 (which are part of the tubular myelin fraction of lung surfactants), the critical ion concentration inducing DPPC liposome aggregation decreased in the case of Ca^{2+}, Ba^{2+} and Sr^{2+}. Mg^{2+} and Mn^{2+}, on the contrary, did not show this effect. The formation of tubular myelin structures required the presence of Ca^{2+}. The authors suggest that the physiological role of Ca^{2+} is partly due to a neutralisation of the negatively charged carboxyl groups of the SP28-36 proteins.[408]

An interesting approach to estimate the affinity of (multivalent) cations to membranes is to use channel proteins inserted into reconstituted lipid systems. Gurnev et al.[409] employed a cation-selective channel to estimate the extent of charge inversion of lipid membranes of multivalent cations, revealing that La^{3+} cations were more efficient at inverting membrane surface charge than hexaamminecobalt and spermidine. The effect of another trivalent cation was demonstrated by Ermakov *et al.*[410] who showed a pronounced compaction of brain PS upon binding of Gd^{3+}. Interestingly, the authors observed Gd^{3+}-mediated blocking of the large mechanosensitive channel MscL from *E. coli* inserted into reconstituted lipid bilayers containing PS. The authors trace this effect back to Gd^{3+}-mediated compaction of anionic lipids, which in turn exhibits pressure on the channel, thus blocking it.[410] These studies thus evidence an intriguing correlation between local effects of multivalent cations translating into a large-scale, global influence on a biological system.

Generally, just as in the case of proteins, Ca^{2+} appears to play a particularly important role for lipids, their structures and their interactions (for a detailed account, see Ref. [411] and refs. therein). Amongst other aspects, the fusion of lipid membranes during, e.g., the uptake or release of vesicles by cells, is often mediated by Ca^{2+}. Churchward et al.[412] demonstrated the importance of cholesterol for such Ca^{2+}-induced membrane fusion processes: removing cholesterol from model membranes significantly reduced the Ca^{2+} sensitivity of the fusion.[412] A possible explanation for the importance of cholesterol is that it contributes negative curvature to the membranes (i.e., promotes the formation of concave surfaces at lipid-water interfaces) involved in the fusion process (see Ref. [413] and refs. therein). This effect can also be mediated by other lipids the structures of which are not necessarily similar to cholesterol, but need to have a certain threshold negative curvature.[414] One of the first biological systems shown to respond to Ca^{2+} were PS vesicles.[411] In particular, the presence of Ca^{2+} induces the formation of dehydrated, multilamellar $Ca(PS)_2$ complexes from PS bilayers.[415,416] This process is referred to as a gel-to-liquid crystal bilayer transition and the periodicity and order of the bilayer structures were shown to differ depending on the type of cation present (Li^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+} or Pr^{3+}).[416]

The effect of neutral lipids on this gel-to-liquid crystal bilayer transition was investigated by Coorssen et al.[417] The neutral lipid molecules were shown to be incorporated into the dehydrated multilamellar PS bilayers. At higher concentrations of the neutral lipids, the interactions between the PS bilayers became weaker, the bilayers showed larger separations and, under certain conditions, two structures were observed.[417]

A further aspect of strong physiological relevance of Ca^{2+}-lipid (and Ca^{2+}-protein) interactions involves vision.[418,419] In particular, the photoresponse of rod outer segment membranes is known to depend on Ca^{2+}.[418] Huster et al.[418] investigated the effect of unsaturated docosahexaenoic acid on the interaction between membranes and Ca^{2+}. Here, the fatty acid saturation degree of the lipid membranes under study affected the Ca^{2+} affinity of the membrane. The more important factor determining Ca^{2+} affinity, however, was the content of PS.

McLaughlin et al.[420] studied the effect that divalent ions (Ca^{2+}, Mg^{2+}, Sr^{2+}, and Ba^{2+}) have on the surface potential of phospholipid membranes. While no surface potential could be induced by cations in the case of an overall neutral surfactant (phosphatidylethanolamine, PE), the authors did observe a decrease of the surface potential in the case of the negatively charged surfactant phosphatidylserine (PS) upon addition of Sr^{2+} and Ba^{2+}, consistent with predictions based on double layer theory. The same phenomenon was observed in the case of Ca^{2+} and Mg^{2+}. Interestingly, however, smaller concentrations of the two latter cations were required in order to achieve the surface potential decrease mentioned above, implying that cation-specific binding parameters and properties need to be taken into account.[420]

Vanderkooi and Martonosi[421] used a dye molecule the fluorescence of which is strongly enhanced in a hydrophobic environment to investigate the influence of different parameters on lecithin microsomes (membranes). They observed that trivalent ions are more effective than mono- and divalent ones in enhancing the fluorescence signal. This observation was traced back to two possible reasons – a stronger hydrophobicity of the membranes induced by the cations or the cations affecting the binding of the dye to the membranes. The latter possibility was deemed more likely.[421]

Lis et al.[422] investigated the binding of Ca^{2+} to bilayers of dipalmitoylphosphatidylcholine (DPPC) by measuring the repulsive forces between the bilayers. They found that a low concentration of Ca^{2+} leads to a strong increase of the bilayer separation distance in aqueous solution, implying that Ca^{2+} increases these repulsive forces. High Ca^{2+} concentrations had a smaller effect on the interbilayer distance. The addition of a monovalent salt (NaCl) was found to weaken this effect of Ca^{2+}. Lis et al. found that pushing together the lipid monolayers forming the bilayer led to a desorption of Ca^{2+} and a decreasing surface potential. They tentatively attribute this phenomenon to the possibility of the double-layer electric fields deforming the arrangement of polar surface groups of the bilayer, thereby altering their Ca^{2+} binding pattern. They speculate that this change in arrangement might, in turn, be due to changes in the conformations of lecithin head groups.[423]

The same authors then extended their studies to other surfactant bilayer systems as well as to other divalent cations.[424] The order of preferential cation binding to DPPC was found to be

$$Ca^{2+} \approx Cd^{2+} \approx Mn^{2+} > Co^{2+} \approx Mg^{2+} > Ba^{2+}. \quad (5)$$

Moreover, a higher density of polar groups present on the surface of the bilayer as well as a higher concentration of divalent cations invokes a higher density of cations bound. Concerning the phase behaviour of the surfactants studied, the authors found that phase separation into two different lamellar phases occured in phosphatidylcholine (PC) mixtures differing only in their hydrocarbon chain residues in the presence of Ca^{2+}.[424] In the context of the cation-DPPC affinity study, it is interesting to note that McLaughlin et al.[425] found the following order for divalent cation affinity to PC:

$$Mn^{2+} > Mg^{2+} > Ca^{2+} > Co^{2+} > Ni^{2+} > Sr^{2+} > Ba^{2+} \quad (6)$$

These results emphasise the importance of cation-specific effects in the context of cation-lipid interactions. As a general conclusion regarding the binding of divalent cations to PC, Lis et al.[424] established it to be a highly sensitive and multifactorial phenomenon, depending not only on the length of the PC hydrocarbon chain, but also on the distance between the bilayers, and, importantly, the concentration and type of divalent cations used.

In addition to the extensive studies of cation-bilayer interactions described above, several studies were performed on the interactions between cations and vesicles. Ohki et al.[426] studied the propensity of both mono- and divalent cations to induce aggregation of phospholipid (phosphatidylserine (PS) and -choline (PC)) vesicles. The effectiveness of monovalent cations to induce PS aggregation was determined to be

$$H^{+} > Na^{+} > Li^{+} > K^{+} > TMA. \quad (7)$$

where TMA is short for tetramethylammonium. The threshold concentrations of divalent cations causing PS vesicle formation were ranked in the order

$$Mn^{2+} < Ba^{2+} < Ca^{2+} < Sr^{2+} < Mg^{2+}. \quad (8)$$

The explanation of these observations proposed by the authors involves the surface potential and surface charge densities of the vesicles as well as the repulsive interactions between vesicles.[426] We note that a detailed review on ion effects on amphiphilic molecules is provided in Ref. [427].

All of these studies underline that subtle, cation-specific effects should not be neglected when discussing cation-mediated phenomena.

5.4. Other interfaces

While this review is not specific for interface phenomena, they play, of course, a major role in soft and biological matter in general, not limited to surfactants. Therefore, we shall briefly comment on this issue, without claiming to be exhaustive. Obviously, simpler, non-biological interfaces can serve as model systems, e.g., to study the charging behaviour with better spatial resolution. Examples are found, *inter alia*, in Ref. [428] and references therein. Furthermore, interesting and remarkably strong ion-specific effects at interfaces were reported in Refs. [429] and [430].

An important area is that of protein adsorption at interfaces.[431–433] Since proteins frequently exhibit amphiphilic properties, there are even some analogies to the surfactant systems discussed above. Importantly, in the context of this review, they usually also have a (nontrivial) charge pattern on their surface. Multivalent ions can, of course, induce a richer phenomenology, including charge inversion.[402] This was demonstrated using BSA and Y^{3+} in water at SiO_2 surfaces. Depending on the salt concentration, "reentrant" effects in the adsorption were found, nicely mirroring the bulk reentrant behaviour.[58,256,257]

Other biomolecules such as DNA/RNA were, of course, also investigated with respect to their adsorption and interface behaviour.[434] Several of the studies discussed in Sec. 4 were, in fact, related to interfaces, meaning that we will not further elaborate on them here.

Furthermore, we wish to mention several other systems because of their model character. As an example, the adsorption of β-lactoglobulin to the air-water interface was studied by Richert et al.[435] in the presence of Y^{3+} and Nd^{3+} cations using a multi-method approach including sum frequency generation (SFG) spectroscopy. Binding of the cations resulted in a reduction of the net protein charge and subsequent aggregation. Ion binding to protein residues located at the air-water interface was concluded to mediate foam stability *via* structure-property relations.

An interesting system to investigate with respect to their sensitivity to charges are polyelectrolyte brushes due to their intriguing surface geometry. Yu et al.[30] studied the effect of multivalent ions on the lubricity of polystyrene sulfonate brushes. Even small concentrations of multivalent cations such as Y^{3+}, Ba^{2+} and Ca^{2+} were found to strongly increase the

friction between layers of the brush polyelectrolyte due to cation bridging effects. Yu et al.[30] underline the significant effect of multivalent counterions on the lubricating properties of polyelectrolyte brushes, stressing the importance of this effect for applications such as biomedical devices.

6. Remarks on other systems

In addition to those described above, there are other systems in the broader area of soft and biological systems which exhibit interesting charge effects, also and in particular with multivalent ions. While we cannot discuss them in detail, we nevertheless wish to give some examples below.

6.1. Synthetic polymers

While these are not the primary scope of this review, we feel that some comments on the ion-dependent properties of synthetic polymers are in order, since a lot can be learned from the comparison between synthetic polymers and, in particular, proteins and DNA/RNA.

It is interesting to note, for example, that the Hofmeister effect, initially described for proteins, has also been observed in systems of the polymer poly(N-isopropylacrylamide) (PNIPAM).[436] The authors of this study found that kosmotropes can polarise water molecules forming hydrogen bonds with the amide groups of the polymer and increase the surface tension of the backbone cavity, thus interfering with hydrophobic hydration of these moieties. Additionally, a direct binding of the anions to the polyamide groups of PNIPAM is possible. Those interacting directly and via the surface are classified as chaotropes; they decrease the lower critical solution temperature of the system. Those anions exhibiting mostly polarising effects represent kosmotropes.[436]

The multivalent ion-driven behaviour of polyelectrolytes is a complex area of research (see, e.g., Ref. [437]). In particular, the charge-driven interaction between polyelectrolytes (PE) and alkali cations is exploited in industrial processes where polyacrylates are frequently used as, *inter alia*, scale inhibitors.[18] This cation-PE interaction has been studied in detail using various scattering methods.[18,438–443] Using anomalous small-angle X-ray scattering (ASAXS) on a sodium polyacrylate (NaPA)-Sr^{2+} system, Goerigk *et al.*[441] demonstrated that the Sr^{2+} counterions reside in spherical structures on the NaPA chains.[441] Hansch *et al.*[443] investigated the temperature-dependent phase behaviour of sodium polysterene sulfonate (NaPSS) in the presence of Ba^{2+} and Al^{3+} cations. Interestingly, while in the presence of Al^{3+} NaPSS shrinks with increasing temperature, Ba^{2+} cations decrease the dimensions of NaPSS upon a temperature decrease. This strong difference is traced back to differences in cation binding thermodynamics and underlines the strong specificity of cation effects in macromolecular systems. As such, it is yet another example of non-monotonic cation-mediated effects.

In addition to inorganic cations, the influence of multivalent organic cations on PE molecules has also been investigated. Mechtaeva et al.[444] observed crosslinking of polyacrylic acid by oligoethylenimines, leading to the formation of ionic complexes. The shape of the complexes formed was investigated by atomic force microscopy (AFM) and scanning electron microscopy (SEM) and found to depend on the the polyelectrolyte concentration as well as on the ratio of amine and carboxylic groups of the oligoethylenimines.[444]

In another study performed by Buyukdagli and Podgornik,[445] it was found that PE adsorption to like-charged membranes immersed in a monovalent salt solution was facilitated by the addition of tri- and tetravalent cations. The PE adsorption is traced back to a condensation of the multivalent counterions at the membrane which strengthens the monovalent salt-mediated screening. The critical concentration of the multivalent counterions is found to decrease with increasing charge of the counterions. Additionally, an increase of monovalent salt concentration leads to polymer desorption from the membrane.[445]

The effect of trivalent ions on a biologically relevant PE (hyaluronic acid (HA), a charged polysaccharide) was studied by Innes-Gold et al.[446] by combining magnetic tweezers force spectroscopy as well as simulations and theory. The results indicate that in the presence of the trivalent cations hexammine cobalt (III) chloride and hexammine ruthenium (III) chloride, the system displays a remarkably decreased sensitivity to the ionic strength of its surroundings, reflected in a decreased elasticity of HA. Innes-Gold et al.[446] trace these observations back to the formation of a tight "jacket" of trivalent ions around the HA molecules.

Ion-specific effects on hydrogels were shown by Fullenkamp *et al.*[447] The authors synthesised these gels in order to perform model studies on the self-repairing thread structures used by marine mussels to attach to various surfaces. In the hydrogels studied, ion binding proceeds *via* chelation by histidine residues. Interestingly, the different ions used by Fullenkamp et al. (Zn^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+}) lead to differences in the brittleness, self-repair rates, gel relaxation times and colouring of the gels.[447]

6.2. Colloids and nanoparticles

The first experimental observations of certain phase behaviours closely reflecting those of atomic systems were made in colloidal systems.[448] In particular, a seminal publication by Pusey and van Megen[449] proved principles based on atomistic explanations to be able to account for phase behaviours observed experimentally for polymethylmethacrylate (PMMA) colloids. This study thus provided a strong indication that aspects of atomic systems can be applied to colloidal ones and vice versa. Colloids are hence sometimes referred to as "superatoms".[450] In fact, also the phenomenon of charge inversion has been discussed in terms of the colloidal picture.[254] Thus, while colloids are obviously not "molecules" in the true sense of the word, colloidal models and model systems are frequently very useful in particular in the interpretation of observations on globular proteins. In the context of multivalent

ions, the analysis of patchy colloid models provides a valuable link between simplified models and much more complex protein systems.[249,265,451–453] The different effects of mono-, di- and trivalent ions on colloidal particles has been described by Linse and Lobaskin[454] using canonical Monte Carlo. In particular, the authors report decreasing intercolloid distances and a stronger tendency to aggregate with increasing counterion valency, indicating the importance of theoretical approaches rationalising high charge densities of counterions.

In addition to purely charge-mediated effects, cation-induced pH changes need to be considered as well. Schubert et al.[455] studied the influence of metal ions and their hydroxides and the associated pH changes on NPs coated with BSA. This study reports both charge inversion of the NPs and a decrease of the solution pH upon addition of several trivalent cations, going along with the adsorption of metal hydroxide species on the NPs. Schubert et al.[455] trace the charge inversion observed to these hydroxides, thus connecting pH and pure charge effects.

7. Concluding Remarks

We have attempted to provide a comprehensive perspective on multivalent ions and biological (macro)molecules.

It is rather suggestive to expect similarities in the local picture (binding and bridging) and indeed these are found, since essentially the same functional groups are involved. While the local chemistry is not in all cases fully understood in terms of a quantitative quantum-chemical picture of the interactions of multivalent ions (in particular lanthanides) with, e.g., carboxylic acids in the presence of water, it is expected to be similar for different types of macromolecules containing similar functional groups. An interesting case in point is Ca^{2+}, which appears to play a very special role in many different systems. Importantly, for most of the systems described in this review, i.e., including proteins, nucleic acids, synthetic polyelectrolytes and amphiphilic molecules, non-trivial ion-specific effects were found. The latter can sometimes be traced back to "simple" features such as ionic radii, but this one parameter is not always sufficient to accout for ion specificity and it is thus clear that aspects such as ion hydration properties and possibly quantum chemical effects need to be taken into account.

In terms of the overall/global picture, there are also *some* similarities, certainly on a qualitative level. As an example, both proteins and DNA can bind multivalent ions and, as a consequence, undergo charge inversion. However, the strong differences in charge distribution – rather homogeneous, "polymer-like" in the case of DNA vs. typically highly inhomogeneous for proteins – will generally lead to different overall macroscopic behaviour, including the response to higher concentrations of multivalent ions. In addition, at east double-stranded DNA is somewhat special in terms of its high stiffness and large persistence length. This leads to a rather unique response to multivalent ions.

The comparison with soft-matter model systems, such as colloids, is in many cases fertile in terms of the overall phase diagram (most notably patchy colloid models), especially for the case of globular proteins. In particular, applying the concept of "colloids as superatoms" greatly helps transferring the knowledge obtained on the phase behaviour of well-controlled, colloidal systems to much more complex (bio)molecules.

For amphiphilic molecules, the presence of large, potentially bulky hydrophobic areas strongly distinguishes these from nucleic acids and proteins, as does the dominant role they play for interfaces (and vice versa). Thus, the similarities in the overall/global behaviour are limited, and usually a different approach is needed. Nevertheless, amphiphilic molecules actually offer unique opportunities to study ion effects in a very targeted way, such as exposing a specific group in a large area (at the surface of a liquid) and thus sufficient signal for a specific study of, e.g., ion association parameters. In addition, they exhibit their own very rich phase behaviour, which can be subjected to and influenced by multivalent ions.

We hope that the material compiled here and the perspective offered help to understand the effects of multivalent ions more comprehensively. We also hope that there will be some cross-fertilisation with the areas beyond the scope of this review, covered only in passing, such as ion channels in membranes, synthetic polymers, and colloids. It may also spark new studies and inspire new discoveries by translating ideas from one field to the other.

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Conflict of Interest

The authors declare no conflict of interest.

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