General discussion

Dr Goodman introduced the session: What information does a picture contain? A postcard of Cambridge University might be described as "Cambridge", but it is only a tiny part of the city. It might be labelled "King's College", if attention is focused on the highest peaks in the figure, ignoring the bulk of the data. It might have many other labels. Is the timescale hundreds of years (for the buildings) or seconds (for the tourists)? We need to know what phenomena we are investigating in order to interpret the data. Photographs are simple compared with the data in molecular dynamics simulations or DFT calculations. Why is an organic chemist like me interested in Hofmeister? The effects of ions are important in many areas of organic chemistry. For example, solubility; an important and familiar property that must be controlled to make useful pharmaceuticals and to get chemical processes to work effectively. However, we cannot calculate it accurately, and it is hard to measure it precisely (A. Llinas, R. C. Glen and J. M. Goodman, Solubility Challenge: Can You predict solubilities of thirty-two molecules using a database of one hundred reliable measurements?, *J. Chem. Inf. Model.*, 2008, **48**, 1289–1303.). Ions in the solution can have an important influence on solubility properties, and it is becoming possible to calculate the interactions between ions and biomolecular structures sufficiently accurately for the calculations to be related to readily interpretable experimental properties such as the extent to which oligopeptides form helices (for example, M. V. Fedorov, J. M. Goodman and S. Schumm, The effect of sodium chloride on poly-L-glutamate conformation, *Chem. Commun.*, 2009, 896–898). So we might hope that we can look at some molecular properties, such as size, charge, polarisibility, *etc.*, and go from these to the properties of solutions. But can all molecular properties be reduced to dials? Do we need a Hofmeister dial? Do we need to adjust size of the Hofmeister effect for different situations or even exchange it with a coulombic effect or other property? Are molecular structures much too complicated for this type of simplified analysis, and cannot be understood except by treating each molecule as an individual? In this session we will hear a series of presentations which describe the effects of ions on a wide range of other molecular systems.

Professor Levin added: I think it is difficult to decouple Hofmeister from Coulomb. The two are intimately tied together.

The underlying equations that describe the electrostatics in these systems are highly non-linear; ionic size, polarizability, and electrostatic potential are all mixed up together. For some problems, such as surface and interfacial tensions, we can have a very accurate theory that is highly predictive.

For hydrophobic colloidal suspensions we can calculate critical coagulation concentrations, see: A. P. dos Santos and Y. Levin, Ion specificity and the theory of stability of colloidal suspensions, *Phys. Rev. Lett.*, 2011, **106**, 167801. I am not sure if it will be possible to do something with proteins, which are much more complex than simple colloidal particles. Nevertheless, I think the theory can lead us to better water and ion models, which can then be used to simulate protein–electrolyte solutions.

Professor Record responded: Hofmeister effects on processes that expose molecular surface to water result from specific short-range preferential interactions of ions with the surface that is exposed in the process. We find that, to a good approximation, Hofmeister effects on protein unfolding and DNA helix melting can be separated from coulombic effects at high salt concentration, where coulombic effects are minimized (L. M. Pegram *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**(17), 7716–7721). For such situations the significance of the coulombic effect can be

predicted computationally, (though the adequacy of the Poisson–Boltzmann equation at molar salt concentrations may be questioned), or can be determined experimentally if a salt without a significant Hofmeister preferential interaction is available. Hofmeister salt effects which apparently are relatively free of coulombic effects are observable for preferential interactions of salt ions with uncharged surfaces, though of course the accumulation or exclusion of the salt ion in the vicinity of that surface has some coulombic consequences. Contributions of cation and anion to the Hofmeister effect of a salt appear independent and additive, at least up to 1 molal (L. M. Pegram and M. T. Record, *Chem. Phys. Lett.*, 2008, **467**, 1–8; *J Phys Chem B.*, 2008, **112**, 9428–36). For processes involving polyelectrolytes at low to moderate salt concentration, Hofmeister and coulombic effects can be tightly coupled because the coulombic accumulation of counterions to high local concentrations in the vicinity of the polyion drives specific interactions of interactive counterions with the polyion surface, which can result in "inverse" Hofmeister effects where interactive counterions are stabilizing at low salt concentration but destabilizing at high salt concentration (see for example Y. Zhang and P. S. Cremer, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**(36), 15249–15253.

Mr Marslaek opened the discussion of the paper by D. T. Bowron: You are showing the pair correlation function of SPC/E water before any EPSR. Your result shows no structure beyond the first peak, which is inconsistent with the result one gets from the same model sampled using molecular dynamics. I am curious about the source of this discrepancy.

Dr Bowron answered: The damping of the structure beyond the first neighbour peak in Fig. 5 from the paper relates to the requirement for flexible water molecules if experimental data is to be correctly modeled. Standard Monte Carlo and Molecular Dynamics engines are generally focused on inter-molecular correlations, and consequently treat the water molecules as identical rigid structures. This prevents their models from generating accurately the intra-molecular terms that are unavoidably captured in experimental scattering data. EPSR restores the structure in the intermolecular correlations through the perturbation potential that it generates.

Professor Mason commented: I think what you have done in Fig. 5 is simply take TIP3P water which has the experimental geometry of water (104 degrees), and applied to it the SPC/E charges? It is documented that this will just look like TIP3P water, whereas if you take the SPC/E geometry (109 degrees) and apply the TIP3P charges, this looks like SPC/E water (P. E. Mason and J. W. Brady, *J. Phys. Chem. B*, 2007, **111**(20), 5669–5679, Fig. 2). Basically geometry plays a larger role than charge in determining the presence of the second peak in gOO(r). Further the presence of the second peak at 4.5 Å in gOO(r) is actually a relatively poor indicator of 'tetrahedral' structure, in that it can be shown that TIP3P water, despite having virtually no second peak in gOO(r) has a very similar three dimensional structure to SPC/E water. In essence all that Fig. 3 in the paper has shown is that EPSR can change a bond angle from 104 degrees to nearer 109 degrees.

Dr Bowron responded: The fundamental requirement of EPSR is to reproduce experimental total scattering data, which would not be possible if the simulation were performed using rigid molecules in the normal fashion of classical methods. All the molecules in an EPSR model are flexible and their flexibility is optimized to match the intrinsic level of quantum zero-point disorder present in the nuclear positions. As a result, it is not useful to obsess about fixed molecule geometries that are essential components of conventional classical simulations. EPSR is effectively a hybrid method, incorporating elements of classical, quantum and effective many-body interactions into the structures it generates. The method certainly does not change the H–O–H angle of the water molecules in its ensembles to 109 degrees,

the mean water molecule geometry is specified based on intramolecular O–H and H–H distances of 0.98 Å and 1.55 Å respectively.

Mr Marslaek asked: Is the main reason for the introduction of flexibility the approximation of zero point disorder?

Dr Bowron responded: Yes. As the structure refinement method aims to reproduce experimental data, it must take into account the finite widths of the intra-molecular correlation features in the total radial distribution functions that are derived from the structure factors measured in the scattering experiments. Fixed geometry molecular configurations would result in unphysical delta functions in the radial distribution function at the intra-molecular correlation distances.

Mr Marslaek remarked: Is then perhaps the primary message that changing the geometry of the SPC/E model and introducing flexibility makes for a poor water model?

Dr Bowron answered: The structural disorder introduced into the molecular geometries to reflect the quantum zero point effects, leads to a distribution of dipole moments for local interactions. This is because we do not vary the partial charges assigned to the nuclear sites on each molecule and this results in the near neighbour inter-molecular correlations being disordered. In EPSR this problem for the inter-molecular interactions is then compensated for, by the empirical perturbation potential derived from the experimental data.

Professor Bain opened the discussion of the paper by Dor Ben-Amotz: Could you explain what assumptions you make *a priori* in your MCR approach about the nature of the component spectra? Do you assume that the spectra are a linear combination of two or three concentration-invariant spectra? Would you, for example, distinguish between the cases where the TBA molecules were in two states with and without a bound I^- ion, as opposed to a continuous shift in the C–H stretching frequency with increasing I^- concentration?

Professor Ben-Amotz answered: That is an excellent question. I believe that in these particular experiments the answer is that we could not tell the difference between the two cases you described, for the following reasons. Our experimental results suggest that the TBA molecules that do contain one I^- anion in their first hydration-shell produce a red-shift of about 5 cm^{-1} in the CH frequency of TBA (and the latter shift is consistent with our theoretically predicted shift of about 4 cm^{-1}). However, since this shift is significantly smaller than the width of the CH band (as well as the sub-peaks in the CH band), I would not expect to be able to distinguish whether our measured difference between the CH frequency of TBA in a particular NaI solution and that of TBA in pure water is due to a mixture of bound and unbound I^- ions or due to a continuous distribution of TBA–ion distances. More specifically, the results shown in Fig. 5 of our paper indicate that our measured TBA CH stretch frequency in 1 M NaI is red-shifted with respect to that of TBA in pure water by about 1 cm^{-1}. The latter red-shift may be due entirely to a two-state linear combination of TBA molecules which do or do not contain I^- in their first hydration-shell, or it could equally well be due to the average shift arising from a continuous distribution of I^- ions about TBA. Having said that, I also want to stress that the two-component SMCR analysis procedure that we have used in this work is appropriate, independent of the above two-state *vs.* continuous distribution issue. In other words, our two-component SMCR analysis only assumes that when TBA is added to a given salt solution, then the spectrum of the resulting solution is expressible as a linear combination of the spectra of the pure salt solution (with no TBA) and the TBA solute-correlated spectrum which contains features that reflect

TBA-induced spectral changes. That assumption should be valid as long as there are no significant TBA–TBA interactions at the experimental TBA concentrations. The validity of the latter assumption is confirmed by the fact that our TBA solute-correlated spectra are found to scale linearly with TBA concentration. The same goes for the results in Fig. 7 in our paper, whose red curve is the solute-correlated spectrum obtained when NaI is added to an aqueous solution of TBA. Thus, the latter red curve reveals how adding NaI has changed the Raman spectrum of aqueous TBA. We have again confirmed that the latter change is a linear function of the NaI concentration, which implies that there are no significant ion–ion interaction contributions to the solute-correlated spectrum of NaI in aqueous TBA.

Dr Ottosson commented: In your paper you show that iodide directly interacts with the hydrophobes, whereas fluoride does not, but that the local iodide concentration at the hydrophobe interface is only 60% of that of the bulk concentration. Since your observable in the solvent-correlated spectra is the shift of the C–H stretch peaks I wonder how you can distinguish between a situation where a low local concentration of iodide is binding strongly (thus giving a large shift per ion) and that where the a larger local concentration (which could be larger than the bulk concentration) binds weakly and causes a small shift per ion? In general, isn't it so that solvent correlated spectra (without additional input from simulations) only can give an estimate of the lower bounds of the number of affected molecules and ions due to the difficulty to distinguish between these kinds of scenarios?

Professor Ben-Amotz replied: Our experimental estimate that only about 60% of the TBA molecules are significantly perturbed by their interaction with iodide is in fact a lower bound, as you have noted, and so it could be larger. However, our Raman-MCR results, shown in Fig. 7, imply that some of the TBA molecules in the system are not significantly perturbed even in a 3 M NaI solution. That would seem to require that the local concentration of iodide in the first hydration-shell of TBA is indeed lower than the bulk iodide concentration. On the other hand, I would agree that there is more to be done in order to address these important issues. So, at this point, I would say that our evidence suggests, but does not yet definitively prove, that the local iodide concentration around TBA is lower than the surrounding bulk iodide concentration. Note that I have provided some further comments related to your question in my reply to Colin Bain's question above.

Dr Ottosson asked: You compare your solvent correlated spectra with simulations and get a near quantitative match. However, the shift in the experiments still results from an ensemble average of the changes induced by the solute so I wonder how straight-forward it is to directly compare with simulations. For example, did you perform an analysis of how the calculated spectral shift depends on the distance from the hydrophobic group? Even if you have a completely accurate description of this dependency, I feel there must still be some uncertainty in how the density profile of the iodide ions with respect to the distance from the hydrophobes must look, even if your calculations match the boundary conditions posed by your experiments – would you agree with that? Reasonably, a large number of qualitatively different types of density profiles should be able to cause the same solvent correlated spectrum.

Professor Ben-Amotz responded: Our calculated CH frequency shifts of TBA (given in Table 1 in the paper) are average values obtained from all of the iodide ions within the first hydration shell of TBA. Further work would be required in order to obtain sufficient statistics to accurately map the dependence of the CH frequency shift on the angular and radial location of the iodide ions. At this point, all we can say is that the magnitude of the CH frequency shift that we have measured, when compared with our calculated shifts, is consistent with our conclusion that iodide ions do penetrate significantly into the first hydration shell of TBA.

Mr Sharma said: How much does it effect the experimental spectra due to presence of any possible triiodide (triple anion) complexation with increasing concentration of NaI in aqueous solutions? Have you ever obtained any such evidence in any other monovalent ionic solutions?

Professor Ben-Amotz answered: We have not seen any evidence of triiodide, but I imagine that we would have been able to see it if it were present, as our solute-correlated spectra contain features arising both from the solute itself and from any molecules in the solution whose vibrational spectrum is perturbed by the solute. Thus, for example, if triiodide were formed then we should see its vibrational spectrum in our NaI solute-correlated spectrum (either in pure water or in water plus some other solute such as TBA).

Dr Gibb commented: From my perspective, as a flat surface is curved into a convex one, the number of non-covalent interactions that it can form with another entity decreases. Likewise, as a flat surface is made concave so the number of possible interactions that it can make increases (assuming that the other species can fit within the concavity). Isn't it the case that the interaction surface of *t*-butanol is simply too flat/small for any interaction with the iodide ion to be anything but transient? Replacing the *t*-butanol with a hydrophobe possessing a larger interface would lead to a stronger interaction that might be quantifiable using the C–H shift data.

Professor Ben-Amotz responded: Our experimental results suggest that iodide does penetrate into the first hydration shell of *t*-butanol, while fluoride does not. Moreover, our simulation results suggest that the iodide population is localized primarily around the higher curvature periphery of the three methyl groups, rather than near the flatter hydrophobic end of the molecule. It would be interesting to further explore the influence of surface curvature on the affinity of various ions for hydrophobic interfaces.

Dr Gibb asked: With the example of concave molecules we synthesize, we can measure – with excellent accuracy and precision – the association of chaotropes to the hydrophobic pocket. The values we measure are between 0.65 and 2.67 kcal mol^{-1} (iodide, 1.4 kcal mol^{-1}).

Professor Ben-Amotz answered: I would be interested in learning more about this and considering whether our experimental technique could be applied to such ion binding processes in hydrophobic pockets.

Professor Goodall said: Beyond simple ions to Raman active compounds, could you look at the Raman of more complex ions at the same time as your Raman-MCR measurements on water? Examples I would find particularly interesting are thiocyanate and nitrate.

Professor Ben-Amotz replied: Yes, we certainly can look at molecular ions, and we are currently doing just that. I expect that we will publish a follow-up paper describing the interactions between TBA and various ions.

Professor Record remarked: We've analyzed thermodynamic data for the effects of Hofmeister salts on hydrocarbon solubility to obtain a lower bound on the amount of "local" water at a molecular hydrocarbon surface (0.18 H_2O $Å^{-2}$) and local-bulk partition coefficients quantifying the individual distributions of the cation and the anion between this local water and bulk. How do your spectroscopically derived results for ion distributions in the vicinity of the *t*-butyl group compare with these thermodynamically derived results?

Professor Ben-Amotz answered: Thank you for this question. Your results are in fact more directly relevant to our measurements than I had realized. More specifically, your thermodynamic analysis[1] of hydrocarbon solubility in the presence of Hofmeister salts, as well as the partitioning of various ions between bulk water and either air–water or oil–water interfaces, are all generally consistent with our findings. For example, our spectroscopic measurements confirm that both sodium and fluoride ions are excluded from the hydrophobic hydration shell of TBA. Moreover, our additional unpublished measurements confirm that the same is true for sulfate ions, and that the affinity of halide ions for the first hydration shell of TBA increases with increasing size. The only apparent quantitative discrepancy between our conclusions and yours is that our results suggest that the concentration of iodide ions is comparable to, but slightly lower than, the concentration of the surrounding bulk aqueous salt solution. Although the latter conclusion is consistent with both our experimental and EFP simulation results, I would say that further work is required in order to verify this particular conclusion. I am more confident that our results do clearly show that I^- ions are present in the first hydration-shell of TBA, in contact with its hydrophobic groups, while F^- is not.

1. L. M. Pegram and M. T. Record, *J. Phys. Chem. B*, 2008, **112**, 9428–9436.

Dr Cremer asked: In direct relation to Tom Record's comments on the partitioning of iodide to *t*-butyl alcohol, would iodide partition better to a hydrophobic moiety that was slightly positively charged compared with a purely hydrophobic moiety such a *t*-butanol? Such hybrid hydrophobic/positively charged sites are present in the backbones of polypeptides.

Professor Ben-Amotz replied: Yes, one would clearly expect that a positively charged species would have a higher affinity for I^- than a neutral species. We have performed some measurements similar to those I have talked about, but using TMAO rather than TBA as the solute. As you know, TMAO looks quite similar to TBA but is zwitterionic, with a partially negative O atom and a partially positive N atom attached to three methyl groups. Thus, one might expect the methyl groups of TMAO to have a slightly positive net charge. Our preliminary measurements of TMAO in aqueous NaI suggest that the I^- ions do in fact interact more strongly with the methyl groups of TMAO than they do with the methyl groups of TBA. We plan to publish the results of these and other such measurements at some point.

Professor Bakker asked: For both I^- and F^- you observe a similar increase of the response of the OH dangling peak of the water molecules in the hydration shell of TBA, indicative of a slight distortion of the hydrophobic hydration shell by the ions. However, you also find that F^- is strongly excluded from the first hydration shell of TBA, and that the local concentration of I^- around TBA is nearly as large as in the surrounding bulk. Can you explain why nevertheless F^- and I^- lead to a similar increase of the response of the dangling OH?

Professor Ben-Amotz answered: The fact that both I^- and F^- produce a similar increase in intensity (and slight red-shift) of the dangling OH peak arising from the hydrophobic hydration-shell of TBA is surprising. Alhough we do not yet understand why the two ions produce such a similar effect on the dangling OH, we have speculated that the increase is related to the disruption of the tetrahedral water structure in the second hydration-shell of the anions.

Dr Tyrode asked: TBA tends to aggregate in aqueous solutions at concentration usually exceeding 2 M. These aggregates remain soluble in solution and may have long associated correlation lengths (see for example the paper presented in this *Faraday Discussion* by D. T. Bowron; or S. Paul and G. N. Patey, *J. Phys. Chem.*

B, 2006, **110**(21), 10514; or R. Gupta and G. N. Patey, *J. Chem. Phys.*, 2012, **137**(3), 034509). In the work presented here you carried out Raman-MCR measurements of varying concentrations of NaI and NaF at a fixed TBA concentration of 0.5 M. In contrast to NaF, at high NaI concentrations (3 M) you observed a 3 cm^{-1} red-shift in the CH stretching modes of TBA, which you interpreted as iodide ions present in the first hydration shell. Although you have confirmed in previous work (*e.g.* ref. 7 in your manuscript) that no TBA aggregates are formed at 0.5 M concentrations in PURE water, this may not necessarily be the case in the presence of NaI concentrations exceeding 1 M. I am concerned that red-shift detected as well as some of the other spectral changes, are a consequence of TBA aggregation triggered by an increase in the iodide concentration in solution (particularly above 1 M), rather than the anion adsorption to the first hydration shell, as described in the manuscript. In principle, this possibility could be discarded, collecting a series of spectra at different TBA concentrations. However, judging for what is described in the manuscript, it appears that this possibility was not explored.

Professor Ben-Amotz answered: This is a good question. Although we have not investigated this possibility in detail, we are quite convinced that TBA aggregation is not responsible for the salt-induced spectral changes that we have observed. The simplest evidence for that is that we do not observe any such spectral changes in NaF, which should have a greater "salting out" effect than NaI. We are currently pursuing additional studies aimed at extending our Raman-MCR methodology to the detection of hydrophobic aggregate formation. Those measurements indicate that there is essentially no direct contact TBA–TBA aggregate formation below a TBA concentration of 1 M.

Professor Harries added the general comment: I think that, as a community, our understanding of important biological processes that relate to proteins in solutions containing ions and many other cosolutes is becoming better over time. For example, over the past decade significant progress has been made in deciphering the way that the thermodynamic stability of small solute molecules, such as amino acids and peptides, is affected by salts and other cellular cosolutes such as osmolytes. The guiding idea is that understanding the effect on these smaller model compounds will afford a basis for learning about and predicting the effect of the same cosolutes on large molecules undergoing processes such as protein folding or self-assembly and aggregation (for example into amyloid fibers). This progressive, incremental strategy is helpful, as it allows a smoother transition between scales, is more easily accessible experimentally, and is easier to analyze. In addition, this approach has been used to successfully predict the changes in stability of several large proteins due to cosolutes.[1–3] Nevertheless, it is already clear that, in the process of going from small molecules to large, we will inevitably encounter considerations that apply to one scale but not to another. A full understanding of these factors is an essential part of our learning, and serves as another reason to improve our knowledge on all relevant length scales. In this respect, we are already making considerable progress in formulating predictive theories that will eventually allow quantification of the effect of cellular solutes on biologically relevant processes.

1. M. Auton and D. W. Bolen, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 15065.
2. L. M. Pegram, T. Wendorff, R. Erdmann, I. Shkel, D. Bellissimo, D. J. Felitsky and M. T. Record Jr, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 7716.
3. M. Auton, J. Rösgen, M. Sinev, L. M. F. Holthauzen and D. W. Bolen, *Biophys. Chem.*, 2011, **159**, 90.

Professor Mason continued the discussion of the paper by D. T. Bowron: In the second paragraph of your paper in the 'structure refinement' section you state:

"However it is important to recognize that any pair distribution functions not heavily constrained by the scattering data will primarily reflect the prior assumptions made for the underlying interatomic potential." And that "in spite of this, provided that these primary constraints are reasonable, the model can provide a useful guide for improving our understanding of how the structure of system gives rise to its observed physical and chemical properties". These two statements together suggest that if a structure factor of the solution is not heavily constrained by the neutron data, that the molecular mechanics will provide the insight. This is indistinguishable from the statement that molecular mechanics can provide insight into solution properties, and would render, in such a case the inclusion of neutron data in such a study irrelevant.

In the current case, if we take a look at Fig. 7 in the paper we find that all of these correlations contribute a very small amount to the total scattering. For instance, in the 1 molal TBA, 0.5 m AB electrolytes, each of the correlations shown in Fig. 7 contributes less than about one part in ten thousand to the total scattering data. To put this into perspective this is probably a couple of orders of magnitude smaller than the thickness of the line chosen to represent the residuals shown in Fig. 2. In this case it is probably reasonable to say that the empirical potential structural refinement technique is delivering the same as the molecular mechanics simulation. To me it looks like this point seems to have been all but lost in the conclusions of this paper. Do you think this is a reasonable criticism of the technique applied to this aspect of this system?

Dr Bowron answered: In an ideal world, where we have a perfect knowledge of the potentials of interaction between, atoms, ions and molecules, we should be able to rely on a molecular mechanics simulation to provide an accurate model of reality. Unfortunately we do not live in such a world, and we are dependent on experimental observations to establish the veracity of our knowledge. Empirical Potential Structure Refinement aims to generate configurations of atomic and molecular coordinates that are consistent with experimental scattering data and known physicochemical constraints such as density and molecular structure. The method starts from a baseline of our best guess at a set of interaction potentials, and then develops a set of perturbation potentials with a magnitude that scales in proportion to the structural contributions to the data that is provided for refinement. If the reference potentials were perfect and able to account for the provided data, the method would not change anything, but to date there are no perfect potentials and a perturbation function is always required to bring the model into good agreement with experiment. For example, existing classical potentials tend to overestimate the magnitude of polar group interactions between molecules.[1–2]

With regards to whether we learn anything about the dilute species interactions in an EPSR model, the answer is, yes we do. This is because in any condensed state system, atoms, ions and molecules do not live and act in isolation. The chemistry that takes place in a solvent does not occur on a structureless uniform background. What an EPSR model does, is optimize the nuclear positions in the structural ensembles it generates to be consistent with the best scattering information that we have about the *total* system, and this means that dilute species interactions that are to be accommodated in the model, have to do so against a background of the best solvent model that we can generate. In essence the method enforces rigorous, experimentally established, boundary conditions for the atomic and molecular interactions of interest. Although not presented in this work, chemically specific structural information from dilute species can be incorporated into the EPSR model through a requirement for the structural ensembles to reproduce X-ray absorption spectroscopy data, which can be sensitive to structural correlations at the parts-per-million level.

1. D. T. Bowron, J. L. Finney and A. K. Soper, *Mol. Phys.*, 1998, **93**, 531–543.
2. D. T. Bowron, J. L. Finney and A. K. Soper, *Mol. Phys.*, 1998, **94**, 249–251.

Dr Ball asked: I have a small question which is starting to seem like part of a bigger question. In your previous studies you reported finding salt bridges – halide ions – between the hydroxy groups of *t*-butyl alcohol. Is this something that survives your EPSR treatment? More generally, your results make me wonder how secure we can feel about using simple systems as analogues of more complex ones – tertiary alcohols as models of macromolecules with hydrophobic and hydrophilic regions, say. It seems that we might start to see qualitatively new phenomena appear (or vanish) as we alter the degree of complexity. We know that even for alcohols – even for methanol, in fact – there are effects such as clustering that might determine the hydration characteristics, which might not apply in the same way, or at all, to long-tail lipids or to protein side-chains. What would you say about that? How much continuity can we expect as we change the complexity of our solutes?

Dr Bowron replied: First, salt bridges do still survive in the EPSR simulations, but these studies performed with more powerful computers that allowed for better sampling of the experimentally consistent structural ensembles, suggest that they are more prevalent at higher alcohol concentrations. The earlier results in the most dilute system,[1–2] appear to have been given undue weight due to insufficient system equilibration.

The challenge of extending fundamental knowledge to the science of complex systems is extreme, but there is however the counter argument that without the fundamental understanding of the rich phenomena displayed by the toy models, we would not know where to begin to tackle the bigger problems. What is already known is that complexity can arise from very simple laws, so at the very least we need to study simple systems to establish these. Speaking as an experimental scientist, I actually think we should be very grateful for the fact that complexity can drive new phenomena as this means there is likely to be an almost endless supply of new and exciting things to discover, and good measurements will always have a role to play in their observation and characterization.

1. D. T. Bowron and J. L. Finney, *Phys. Rev. Lett.*, 2002, **89**, 215508.
2. D. T. Bowron and J. L. Finney, *J. Chem. Phys.*, 2003, **118**, 8357–8372.

Professor van der Vegt opened the discussion of the paper by Daniel Harries: Enthalpy and entropy variations contain contributions of changes in solvent–solvent interactions that do not affect the free energy. This is referred to as exact enthalpy–entropy compensation. Shouldn't we for this reason refrain from statements on enthalpy (or entropy) driven stabilisation?

Professor Harries replied: Regardless of the name we give these findings, the overall entropic and enthalpic parts of the free energy are important considerations or constraints on proposed molecular mechanisms for the action of cosolutes. It is true that the effect of cosolutes on the free energy of processes such as protein folding and aggregation can be much smaller than the underlying entropic and enthalpic terms, which often seem to compensate each other to a large extent. We simply call processes "enthalpically driven" if the net enthalpic contribution of cosolutes as measured in an experiment is the largest contribution to the relevant free energy. In addition, we find that at least for the peptide folding we have studied, the entropic and enthalpic contributions are in fact often of the same order of magnitude as the total change in folding free energy due to solute addition (for example, in the presence of PEG 2000 at 0.1 M, $\Delta\Delta G = -1.8$ kJ mol^{-1} while $\Delta\Delta H = -1.1$ kJ mol^{-1} and $T\Delta\Delta S = 0.7$ kJ mol^{-1}). Moreover, we know that these terms in the free energy can usually be further dissected into several contributions on the molecular scale. Take, for example, the process of protein folding in the presence of cosolutes. Sometimes the net effect is simply found to be dominated by entropy or enthalpy, while the other is smaller and acts to stabilize the protein (we and others

have found indirect or direct evidence for this[1–2]). Even more dramatically, other co-solutes, such as polyols and sugars, can show stabilizing enthalpic dominance, while the net entropic contribution is *destabilizing*.[3] It is difficult to explain this last experimental observation by mechanisms that invoke entropy, such as excluded volume effects, as they would result in an entropic contribution of the wrong sign. While excluded volume effects undoubtedly contribute to the overall entropy, in this case they do not dominate the net action of these cosolutes. Therefore, the molecular mechanism that is responsible for this stabilizing effect necessarily includes contributions that are overwhelmingly enthalpic. Calling such mechanisms "enthalpically driven" serves as a mnemonic reminding us of the necessity to find new mechanisms that can account for the dominating enthalpic contributions, as well as the possible unfavorable entropy.

1. D. B. Knowles, A. S. Lacroix, N. F. Deines, I. Shkel and M. T. Record Jr, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 12699–704.
2. Y. Wang, M. Sarkar, A. E. Smith, A. S. Krois and G. J. Pielak, *J. Am. Chem. Soc.*, 2012, **134**(40), 16614–16618.
3. R. Politi and D. Harries, *Chem. Commun.*, 2010, **46**, 6449–51.

Dr Canchi remarked: It is very interesting to see that certain cosolutes can stabilize proteins by an enthalpic mechanism, even though stabilizing cosolutes are excluded from the vicinity of proteins. What do you think is the molecular picture for this effect?

Professor Harries replied: In models that describe the action of "depletion forces" or "molecular crowding", the solvent itself is often ignored.[1–2] Scaled particle theory, often used to describe the action of excluded cosolutes, typically parametrizes only the shape and size of the cosolute and the protein, and ignores the interactions of the solvent with these species.[3] However, water has strong, direct interactions with both protein and cosolutes, particularly in biological systems.[4] What we know about the enthalpic mechanism you ask about is based mostly on molecular dynamics simulations.[5] It seems that even in the absence of peptides or proteins, polyol osmolytes modify the hydrogen bonding network around them, so that the hydrogen bonds between water molecules becomes shorter and more linear, and hence stronger. Since the cosolute molecules are excluded from the vicinity of the peptide, a layer of hydration is formed between cosolutes and the peptide. This first hydration layer, too, is interconnected with stronger hydrogen bonds than in pure water, and as a consequence waters in this layer are less available for hydrogen bond formation with the peptide, forcing the peptide to find alternative internal hydrogen bonds. This translates into a stabilization of the peptide in the folded state. Overall, water mediates the interaction of cosolute with the peptide, and the concomitant changes in water hydrogen bonding are responsible for the enthalpic contributions. Further evidence of this mechanism is necessary. Already, recent experiments[6] have shown that the water–water hydrogen bonding in glycerol aqueous solutions is indeed altered in the way predicted from molecular dynamics simulations.[7]

1. S. Asakura and F. Oosawa, *J. Chem. Phys.*, 1954, **22**, 1255–1256.
2. H.-X. Zhou, G. Rivas and A. P. Minton, *Annu. Rev. Biophys.*, 2008, **37**, 375–397.
3. D. M. Hatters, A. P. Minton and G. J. Howlett, *J. Biol. Chem.*, 2002, **277**, 7824–7830.
4. G. N. Somero, C. B. Osmond and C. L. Bolis, *Water and life: comparative analysis of water relationships at the organismic, cellular, and molecular levels*, Springer-Verlag, 1992.
5. R. Gilman-Politi and D. Harries, *J. Chem. Theory Comput.*, 2011, 3816–3828.
6. J. J. Towey and L. Dougan, *J. Phys. Chem. B*, 2012, **116**, 1633–1641.
7. R. Politi, L. Sapir and D. Harries, *J. Phys. Chem. A*, 2009, **113**, 7548–7555.

Dr Henchman commented: You state that the salts tend to have an overall destabilizing effect on the model peptide and yet you include supposedly stabilizing salts such as Na_2SO_4. Could you explain why this is?

Professor Harries replied: The effect of salt on protein folding depends not only on the salt used, but also on the protein itself. For the peptide we have been studying, electrostatic interactions seem to play an important part,[1] so that at low concentration the screening of these interactions dominate, and the folded state is destabilized by salts. As salt concentrations increase, we find a stabilizing effect (compared to the initial destabilization) that is ion-specific. For example, in sodium sulfate the effect is much stronger than in sodium chloride at the same concentration. For this peptide, increasing the concentration of salt even further resulted in a tendency to aggregate. However, the theoretical predictions shown in Fig. 4 indicate that had the experiment been possible, salts would have stabilized the peptide in the folded state, compared to its state in pure water.

1. M. S. Searle, S. R. Griffiths-Jones and H. Skinner-Smith, *J. Am. Chem. Soc.*, 1999, **121**, 11615–11620.

Dr Gibb commented: Going way back to Philip Ball's comment, it seems that a natural next step for the field is to fill in the gap in molecular complicatedness between simple molecules such as *t*-butanol and complicated ones such as peptides or proteins. In that regard it is worth noting that over the last decade or so the field of supramolecular chemistry has developed considerably into the aqueous realm. In other words, there are many classes of highly symmetrical, water-soluble hosts that have a lot to offer the Hofmeister field in regards to identifying how small-scale effects come together synergistically to engender larger-scale Hofmeister phenomena.

Professor Tiddy continued the discussion of the paper by D. T. Bowron: Can you relate your measurements of the species present in solution to thermodynamic data such as water and TBA activities?

Dr Bowron replied: The Empirical Potential Structure Refinement process is performed in the constant NVT (canonical) ensemble. In consequence the calculation of thermodynamic parameters such as solution component activities requires the evaluation of the system's chemical potential through a method such as test particle insertion. At the current time, the method is not optimized to do this reliably as the simulation is parameterized to reproduce structural data over any grand-canonical thermodynamic observable.

Professor Ben-Amotz said: Am I correct in understanding that the small magnitudes of the ion–TBA association equilibrium constants that you have obtained imply that the local concentration of the ions around TBA is smaller than the total concentration of the corresponding ion and that your results imply that the affinity F^- for TBA is greater than that of Br^-? In a related comment, you indicated that your results indicate that there is significant TBA–TBA hydrophobic association at a mole fraction of 0.04, which is equivalent to a TBA concentration of over 2 M. At such a high concentration one might expect there to be significant TBA–TBA contacts even in a random mixture. So, it is not clear to me how you can distinguish such random contacts from contacts induced by a true hydrophobic interaction.

Dr Bowron answered: At the lowest investigated alcohol concentrations there is sufficient water in the solutions to independently hydrate the alcohol molecules, the cations and the anions. The coordination histograms tell us that the affinity of the ions for direct interactions with the alcohol molecules occurs at the 5% to 10% level in these systems whilst the equilibrium constants allow us to compare the relative propensities. Our results do show that there is a preference for alcohol interactions with fluoride ions compared to bromide, but with the caveat that the fluoride

ions are always found to be hydrated and consequently the alcohol-ion interaction is solvent mediated. Solution crowding appears to become a significant factor as the concentration of the alcohol is increased. In the sodium chloride system, the apparent preference for alcohol–cation interactions at the lowest concentration is reduced and the anions start to play a more significant role. This correlates with the growth of a 9 Å alcohol–alcohol interaction feature (Fig. 9 in the paper), which had previously been assigned to salt bridged interactions.[1]

The concentration of the alcohol in the solutions investigated was deliberately chosen to bridge the 0.04 mole fraction composition as this was known to coincide with the maximum degree of hydrophobic effects displayed. The salt free systems have already been extensively characterized,[2–3] so this study was designed to allow us to establish how the salts drive the system away from the hydrophobic-interaction dominated state.

1. D. T. Bowron and J. L. Finney, *Phys. Rev. Lett.*, 2002, **89**, 215508.
2. D. T. Bowron and J. L. Finney, *J. Chem. Phys.*, 2003, **118**, 8357–8372.
3. D. T. Bowron, J. L. Finney and A. K. Soper, *J. Phys. Chem. B*, 1998, **102**, 3551–3563.

Professor Halling continued the discussion of the paper by Daniel Harries: This follows the previous comment about enthalpy–entropy compensation. There are those who argue that individual values of entropy and enthalpy change are of limited value in condensed phases, and especially aqueous solution. The argument distinguishes a direct process of interest, with a significant Gibbs energy change – and a secondary re-arrangement in the rest of the system, which makes almost no contribution to the Gibbs energy change, but has substantial and almost exactly balancing entropy and enthalpy changes. These will of course contribute to the total measured entropy and enthalpy changes. I am not convinced by this view, but would like to hear your argument. For example, on your enthalpy–entropy plot, classes of species often seem to lie quite close to a line for perfect enthalpy–entropy compensation, with almost the same Gibbs energy change. Thus you would define some class members as enthalpy driven, and others as entropy driven. Are you certain this really means a change in mechanism as we go through the series? An example is the line for polyethylene glycols.

Professor Harries answered: From a purely practical perspective, the entropic and enthalpic contributions that are found experimentally can be regarded as constraints to any mechanism that is offered to explain the overall changes in free energy. The value in making this dissection becomes clear once we notice that chemically distinct classes of cosolutes naturally fall into specific thermodynamic mechanisms (or "fingerprints") by which they change the free energy of a process. For example, we have shown that polymers (PEG and dextran) at low concentrations add to the favorable entropic component of peptide folding, see Fig. 1. At higher polymer concentrations this entropic dominance gives way to enthalpic dominance. Others have also observed a change in behavior at high PEG concentrations. Record and coworkers showed that while low PEG concentrations stabilize a DNA duplex, high concentrations destabilize it.[1] In contrast, the contributions of polyols and sugars to folding are much different, and consist of a favorable enthalpy that is counteracted by unfavorable entropy. Overall, it would be very hard to find a single mechanism that could explain the action of these two classes of cosolutes (polymers *versus* polyols) without directly rationalizing how the energetic contributions could be so different for the two cosolute classes. This has led us to propose that polymers and polyols likely act by different molecular mechanisms, where "mechanism" refers here to the collection of contributions to entropy and enthalpy that make up the total change in free energy. To conclude, similar changes in free energy could result from very different energetic or entropic contributions, and these could become hallmarks of specific underlying forces that determine the overall free energy change.

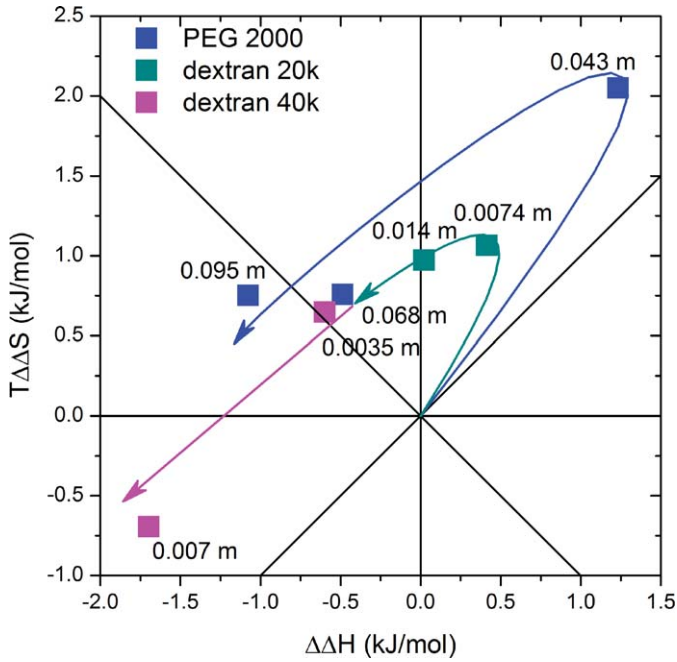

Fig. 1 Contributions of PEG and dextran on the entropic and enthalpic components of peptide folding. Cosolute concentrations are marked next to each of the data points, and arrows follow trends with increasing concentration.

1. D. B. Knowles, A. S. Lacroix, N. F. Deines, I. Shkel and M. T. Record Jr, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 12699–12704.

Mr Marslaek opened the discussion of the paper by Mikael Lund: If you have multiple binding constants, for different ions, how do you deal with sampling in the Monte Carlo simulation? Do you consider exchange moves at the same binding site, for example?

Professor Lund responded: In the presented scheme, ions can compete for binding sites and the site-to-bulk swap move is sufficient to explore all possible configurations. Site–site exchange moves are currently not implemented but may indeed improve sampling of tightly bound, competing ions.

Dr Vila Verde opened the discussion of the paper by Bob Eisenberg: How do we reconcile the fact that in biology, specific ion effects occur at ion concentrations as high as 20 M, when outside of a biological context they only occur for lower concentrations?

Dr Eisenberg replied: I do not know if the specific ion effects are in fact similar in detail.

Of course, many different mechanisms can produce the same result. An amplifier is an amplifier whether it is made of vacuum tubes (valves in UK English), bipolar transistors, or FETS.

Dr Goodman added: Bob Eisenberg has explained how his view of ion interactions is inconsistent with some other people's. However, as Philip Ball has pointed out, different phenomena emerge at different length scales and at different levels of complexity. If a molecular system is made ten times bigger, it may well be more

than ten times as complicated, as co-operative effects which are impossible for simple systems become possible. For example, the discussion between Bruce Gibb and Dor Ben-Amotz about whether curvature affects binding led to the observation that small systems like tertiary butyl alcohol, cannot create concave binding surfaces and so generalisations based on the analysis of concave molecules may not be applicable to very simple systems. Observations on isolated carboxylates, or carboxylates in flat surfaces may well lead to different conclusions to multiple carboxylates carefully positioned to make ion channels, for which large synergistic effects lead to different behaviour. As a result, the apparent difference of opinion between Bob Eisenberg and Werner Kunz may really be consistent opinions about different systems. Therefore, you are both correct.

Dr Eisenberg replied: I agree.

Professor Levin continued the discussion of the paper by Mikael Lund: The simulation proposed uses the Yukawa potential which is based on linear Poisson–Boltzmann equation. How realistic is this? Near a charged protein residue the field can be quite large so that the non-linear effects can be very important.

Professor Lund replied: I agree that the Debye–Hückel approximation is simplistic and will break down at strong coupling conditions. However, the approach has previously been critically compared with explicit ion MC simulations (F. Luíf, S. B. Da Silva, B. Jönsson and R. Penfold, *Protein Sci.*, 2001, **10**, 1415–1425; and A. Kurut, B. A. Persson, T. Åkesson, J. Forsman and Mikael Lund, *J. Phys. Chem. Lett.*, 2012, **3**, 731–734) and for 1 : 1 salt and typical charge densities found in proteins, linearised PB seems fully applicable. In multivalent salt, however, the method can/will be qualitatively wrong.

Let me add that the idea of treating Hofmeister ion binding as a two-state process is not limited to implicit salt models, but can be readily incorporated in explicit salt MC simulations using a grand canonical scheme that does not suffer from the approximations made in PB theory, linearised or not (C. Labbez and B. Jönsson, *Applied Parallel Computing: State of the Art in Scientific Computing*, Springer, Berlin–Heidelberg, 2007,vol. 4699, pp. 66–72).

Professor Jungwirth asked: Your Monte Carlo model very nicely puts interactions of ions with proteins and amino acid protonation/deprotonation on the same conceptual footing. Of course, protonation is a chemical reaction and proton concentrations are typically orders of magnitudes lower than those of Hofmeister salts. Consequently, the (de)protonation effect effects are more dramatic than the Hofmeister effects, which may have led some early researchers (*e.g.*, Loeb and Kunitz, 1923) to disregard the latter.

Professor Lund replied: I have two comments on this:

(1) For reasonably short ranged, attractive potentials of mean force between the ion and a motif, a binding constant can be defined, regardless of the nature and depth of the minimum.

(2) On one hand pH – *i.e.* the proton concentration – can be used to tune the charge state of acids and bases at low ionic strengths. On the other hand, more weakly bound ions require higher bulk concentrations to promote a fully bound site. This consequently leads to screening of long range electrostatic interactions, whereby the charge change may not be as significant for intermolecular interactions as for charge changes induced by changes in the bulk proton concentration.

Mr Patko asked: I have a question in two parts mostly related to the treatment of anion binding as an analog to pH changes (*i.e.* site specific proton binding).

1. In your treatment of anions to be the complete analog to protons for your model of specific site binding for biological macromolecules, you assume the near instantaneous on/off kinetic behavior that is generally reserved for proton exchange and electron transfer reactions. Please explain why this assumption should also valid for anions, particularly those that are slow to lose their solvation shells? In short can anion binding truly be considered as a true fast on/off type analog as is the case for pH? 2. The use of the Monte-Carlo-type simulation mostly precludes the impact of the time-dependent solvent interactions upon the conformational changes and attributes them primarily to the impact of the site specific anion binding (ignoring any solvent shell and kinetics associated with each site binding itself as described in part one of my question). Can you please explain why these underlying assumptions needed to handle the actually more complex anion site-specific binding with this simplified on/off type binding mode are a good trade-off? The simplification is presumably made in the interests of computational efficiency and to develop a systematic reduced-model for macromolecular ion binding that can use some of the convenient and familiar language of pH dependent site binding.

Professor Lund replied: Statistical mechanics show that equilibrium or static properties can be rigorously separated from dynamics and, consequently, the kinetics of solvation or any time-dependent property have no influence on the studied thermodynamic properties. In the model, solvation is accounted for *implicitly* by the experimental ion-motif dissociation constant (pK_d), although we assume that solvation of a motif is unchanged from the model compound and when incorporated in a protein. This is often a good approximation (M. Lund, B. Jönsson and C. E. Woodward, *J. Chem. Phys.*, 2007, **126**, 225103), although for deeply buried sites, solvation changes may need to be considered as commonly done in more advanced Poisson–Boltzmann protein models – see for example the APBS project.

Professor Bakker opened the discussion of the paper by Nico van der Vegt: In your calculations of the salt bridges between the carboxylate groups and the different cations (overview in Table 2 from the paper) you find that for Li^+ the fraction of free ions is less and the fraction of mono-coordinated salt bridges is higher than for Na^+. From this systematic, one would expect that the fraction of di- and tri-coordinated salt bridges would also be larger for Li^+ than for Na^+. However, in your calculations you find that the fractions of di- and tri-coordinated salt bridges are smaller for Li^+ than for Na^+. Can you explain this? Could this observation be related to some steric effect, the mono-coordinated salt bridge with Li^+ somehow giving less opportunity for a second contact with a carboxylate than the mono-coordinated salt bridge with Na^+?

Dr Rodriguez-Ropero replied: As shown in Table 2, the population of mono-coordinated salt bridges is higher for Li^+ than for Na^+, but for di- and tri-coordinated salt bridges this situation is inverted and the population of such salt bridges is higher for Na^+ than for Li^+. This is mainly because of two reasons: (i) steric effects; the Na^+ cation is bigger than the Li^+ cation and it fits better between 2 or 3 carboxylate groups belonging to 2 or 3 neighbouring chains leading to the formation of, respectively, di- and tri-coordinated salt bridges. (ii) The hydration shell around the Li^+ cation is stronger than for Na^+, thus the energy penalty of losing one water molecule in Li^+ is higher. This higher energy penalty makes the formation of di- and tri-coordinated salt bridges slightly more difficult for Li^+ than for Na^+.

Professor Goodall continued the discussion of the paper by Bob Eisenberg: In your paper you presented the challenge to the community to identify a specific set of experimental measurements that operationally define "the Hofmeister Effect", to make a physical model of the setup of those experiments, and to compare with experiments in a range of solutions and concentrations. I think this is a really interesting proposal, and suggest that there would be enthusiasm from the biopharmaceutical community if you looked at the role of Hofmeister ions in stabilising antibody formulations. Every drug company has an interest in biotherapeutic proteins: these

species are formulated at high concentration in solution, where they must be stabilised against aggregation and precipitation, so any insights from the Hofmeister community would be welcomed.

Dr Ball asked: You mentioned that finite-size effects are typically neglected in theoretical treatments. We know that effects such as crowding can be important in the cell, for example altering binding constants and kinetics. Is this the sort of thing you're referring to?

Dr Eisenberg replied: I have discussed these matters at length in my paper and in other publications[1–4] and it is best not to try to deal with all that here in brief. Crowding is easy to see and document for bio-ions like sodium, potassium, calcium, and chloride in and near nucleic acids, binding proteins, ion channels, enzymes and even near electrodes in electrochemical systems of our technology. Here extreme crowding producing number densities greater than 10 molar is easy to document. For reference, solid NaCl is 37 molar. Other forms of crowding produced by the general density of structures in the cytoplasm are obviously also important because they are likely to produce excess free energies that change all electrochemical properties of these ions. Since some of these ions are signalling ions, whose activity controls biological function the way a gas pedal controls the speed of a car; the consequences could be profound. This kind of crowding is harder to quantify and document but indeed may be very important, as Dr Ball hints.

1. B. Eisenberg, Crowded charges in ion channels, *Adv. Chem. Phys.*, 2007, 77–223; also available at http://arxiv.org as B. Eisenberg, Life's solutions are not ideal, http://arxiv.org/abs/1105.0184.
2. B. Eisenberg, Mass action in ionic solutions, *Chem. Phys. Lett.*, 2011, **511**, 1–6.
3. B. Eisenberg, Life's solutions. A mathematical challenge, 2012, http://arxiv.org/abs/1207.4737.
4. B. Eisenberg, Living devices: The physiological point of view, 2012, http://arxiv.org/abs/1206.6490.

Professor Halling added: Can I follow your suggestion that we should think what we can learn from observing the results of evolutionary selection, and suggest a possibly interesting example. These are the halophile proteins, which are adapted to work in about 4 M salts, usually KCl. One feature they share is an unusually large number of surface carboxyl groups. However, I also have to offer a warning. The halophile community state that enzymatic activity in 4 M salt is a special property of these enzymes. In fact however quite a few "normal" enzymes have been found to be active, even activated, in concentrated salts. So such activity is not a unique property of halophile proteins.

Dr Eisenberg replied: Thank you for the information.

Dr Konvalinka continued the discussion of the paper by Mikael Lund: It is well known that in biological systems (intracellular organels, budding virus particles *etc.*), the local concentration of ions could be very high. At the same time, in some of these compartments the local concentrations of polar groups of biomolecules could be extremely high as well and the number of available water molecules actually limited. Could a theory describe the behaviour of ions in such highly crowded environments, far away from ideal aqueous solutions?

Professor Lund replied: If an ion-binding site is exposed to a strong electric field from the surrounding or is significantly desolvated compared to the model compound representing the used pK_d value, then the current scheme will be less applicable as also discussed in the answer to Yan Levin's questions earlier.

We have however used this level of theory to study phase transitions in concentrated solutions of charged proteins (30 *v*/*v*%) and achieved semi-quantitative agreement with experiment (A. Kurut, B. A. Persson, T. Åkesson, J. Forsman and Mikael Lund, *J. Phys. Chem. Lett.*, 2012, **3**, 731–734).

Dr Rodriguez-Ropero added: Proteins are definitively a valid source of inspiration even in the design of synthetic systems like the diblock copolymer brush we are presenting today. In this sense we can, for instance, place the binding sites in specific positions within a polyelectrolyte brush so that their relative positions and local concentrations resemble those found in selective channel proteins or enzymes. In this way we could enhance the selectivity of the system towards a specific ionic species.

Professor Jungwirth commented: It is important to distinguish between (typically weak) Hofmeister salt effects and (typically strong) ion binding situations, *e.g.*, in enzymes or ion channels. The example brought from the audience concerning solubility and stability of strongly charged enzymes of halophilic bacteria falls into the former category and can be rationalized in terms of increasing protein charge leading to higher solubility in high salt and in terms of the higher affinity of Na^+ compared to K^+ to carboxylic groups at the protein surface. There may be situations lying at the fringes between the above two ion binding regimes and these may actually be very interesting to study.

Dr Goodman noted: A comment on Bob Eisenberg's abstract: "Chemistry is about chemical reactions". This is true, and chemical reactions are so complex that for many cases qualitative analyses may be all that is available. These are useful in themselves, even without a mathematical framework.

Dr Eisenberg replied: I agree.

Dr Goodman added: Sometimes I do not care how fast something goes, as long as A goes to B. This may represent a very complex and surprising transformation. Qualitative analyses can be very useful in the absence of an underlying mathematical theory.