

## General discussion

DOI: 10.1039/c3fd90039h

**Professor Chandler** opened the discussion of the paper by Professor Molinero: Your paper describes molecular simulation results illustrating how low-density liquid water is an unstable fluctuation or transient state appearing in the formation of ice from cold liquid water. In this sense, your results are consistent with conclusions that Dr Limmer and I have drawn from simulations<sup>1,2</sup> and analysis of experiments.<sup>3,4</sup> Nevertheless, as we have heard in Professor Tanaka's introductory lecture, some persist in picturing low-density liquid water as a distinct meta-stable phase that can reversibly transition between and coexist with a higher-density liquid phase. Yet for all the models of water exhibiting ice-like phases that we have examined, Dr Limmer and I find reversible free energy surfaces possessing only one liquid basin. This finding, of course, relates to models consistent with experimental behavior of water. It does not rule out a two-liquid picture for liquids that do not freeze into ice-like solids.

You refer to models that exhibit liquid-liquid transitions? Do these models exhibit crystal states like those of ice?

1 D. T. Limmer, and D. Chandler, *J. Chem. Phys.*, 2011, **135**, 134503.

2 D. T. Limmer, and D. Chandler, *J. Chem. Phys.*, 2013, **138**, 214504.

3 D. T. Limmer, and D. Chandler, *J. Chem. Phys.*, 2012, **137**, 045509.

4 D. T. Limmer, and D. Chandler, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00076A.

**Professor Molinero** responded: The models of Chatterjee and Debenedetti<sup>1</sup> that exhibit liquid-liquid transitions induced by solutes are van der Waals models with an added orientation-dependent hydrogen-bonding contribution. The equations of state for these models derived by Chatterjee and Debenedetti<sup>1</sup> and Truskett *et al.*<sup>2</sup> describe only fluid states, without the possibility of competing crystallization.

1 S. Chatterjee and P. G. Debenedetti, Fluid-phase behavior of binary mixtures in which one component can have two critical points, *J. Chem. Phys.*, 2006, **124**, 154503.

2 T. M. Truskett, P. G. Debenedetti, S. Sastry, and S. Torquato, A single-bond approach to orientation-dependent interactions and its implications for liquid water. *J. Chem. Phys.*, 1999, **111**, 2647.

**Dr Royall** enquired: Is it reasonable to think of an analogy between the phase separation to a bicontinuous network that you see, and colloidal gelation? There the network is driven by demixing into two amorphous phases, such that there is a critical (effective) temperature below which gelation occurs. Of course your system breaks symmetry by crystallising, but I wonder if (1) you see fluctuations of

your "red domains" above  $T = 200$  K, and (2) have you looked at the coarsening behaviour of these domains?

**Professor Molinero** replied: Fluctuations in concentration and in local order of the water molecules are already noticeable above the temperature of maximum crystallization rate of the solutions. The fraction of four-coordinated water molecules and the intensity of the diffraction peak associated with the nanophase segregation increase together as a function of supercooling, as seen in Fig. 6 of a previous paper.<sup>1</sup> Large order fluctuations before crystallization are also observed in the simulations of pure supercooled water.<sup>2</sup> These are equilibrium fluctuations and can be studied in the temperature region where water is metastable with respect to the crystal. Below the temperature of maximum crystallization rate the time scale of crystallization and relaxation of mW water are comparable and the liquid is below its metastability limit. In that temperature region, crystallization is faster than the coarsening of the concentration fluctuations, so the latter cannot be studied.

- 1 L. Le, and V. Molinero, Nanophase segregation in supercooled aqueous solutions and their glasses driven by the polymorphism of water, *J. Phys. Chem. A*, 2011, **115**, 5900–5907.
- 2 E. B. Moore, and V. Molinero, Growing correlation length in supercooled water, *J. Chem. Phys.*, 2009, **130**, 244505.

**Dr Limmer** said: Prof. Molinero has presented her results for a theory of freezing in aqueous solutions and in particular for the temperature of maximum freezing rate as a function of solute concentration. The temperature of this maximal rate reflects the balance between the monotonically decreasing free energy for nucleation and the monotonically increasing free energy for growth. However, the theory that Prof. Molinero has presented neglects any dependence of the crystal growth rate on solute concentration. Is this assumption validated by numerical calculations of the solution diffusivity or viscosity?

**Professor Molinero** replied: The effect of the solute on the viscosity of the solution can be neglected when the glass transition temperature is far below the temperature of ice crystallization. This is always the case for dilute solutions of small molecules. In the case of soluble polymers or high concentration solutions the effect of the solute on the viscosity should be taken into account. As the effect of solutes on the viscosity is not a universal function of the water activity, a scatter of the freezing temperatures as a function of water activity may be expected in highly concentrated solutions. This is actually the case, as seen in Fig.1 in paper by Koop *et al.*<sup>1</sup> Water activity is not sufficient to describe the freezing temperature of very concentrated solutions, dense gels and other systems in which the solute has high concentration and very low mobility. We investigated an exaggerated, cartoonish version of the latter in section C of our paper for this Discussion<sup>2</sup> and concluded that "ice nucleation will be strongly hindered in systems where pure water domains are restricted, because of the topology of the solute distribution (*e.g.* a gel, a polymer) to spaces with characteristic dimensions comparable or smaller than 2 nm."

- 1 Koop, T., Luo, B., Tsias, A. and Peter, T. Water activity as the determinant for homogeneous ice nucleation in aqueous solutions. *Nature* 406, 611??"614 (2000).
- 2 G. Bullock and V. Molinero, *Faraday Discuss.*, 2013, **167**, 10.1039/C3FD00085K.

**Dr Limmer** continued: In a related question, a key parameter in Prof. Molinero's theory of the temperature of maximal crystallization rate,  $T_x(c)$ , as a function of solute concentration,  $c$ , is the enthalpy difference between the liquid and a hypothetical ideal, low density liquid. Within her theory, this parameter is necessary for explaining the larger slope of  $T_x(c)$  relative to the melting temperature,  $T_m(c)$ , that she observes in molecular dynamics simulations. In calculations for a two component lattice gas model with one component undergoing an ordering transition and the other component undergoing diffusion, such a trend can be similarly seen. However, in this case there is no intermediate structured state as assumed in Prof. Molinero's theory. Does this mean that the two observations need distinct explanations, or is it sufficient to include only timescales associated with composition fluctuations to understand  $T_x(c)$ ?

**Professor Molinero** responded: The composition fluctuations control  $T_x(c)$ . I expect that this may be true for the crystallization of a pure component from any mixture. What is distinct for water is that the locus of crystallization, which according to our simulations coincides with the development of nanoscopic domains of pure four-coordinated water, can be predicted from the chemical potential of water, without resorting to solute properties (at least for solutions with water activity above 0.75). It would be interesting to investigate whether the lattice gas model can reproduce the observed collapse of the freezing temperature with solvent chemical potential observed in the experiments for water. The regime for which the freezing temperature approaches the glass transition of the solution is, unsurprisingly, extremely challenging for the study with molecular simulations. Simplified models such as the one you presented at this conference could provide important insight on the interplay between viscosity and freezing in these solutions.

**Professor Angell** asked: What is your opinion about describing the transient water nanophase you describe as an "Ostwald stage" in the spirit of the venerable Ostwald "rule of stages", which states that often a final equilibrium state is achieved not by going directly to it, but instead through several states of decreasing free energy, each kinetically facile compared to the direct transition? Your nanophase is clearly transient, but has it a substantial existence in time? Low temperature phase-separated water seems to be an example of an Ostwald stage on the way to crystallization of water. Put another way, do you see any objection to an amorphous phase serving as a Ostwald stage on the way to a stable crystalline state? No one doubts that the phases Ostwald described are *bona fide* metastable phases.

**Professor Molinero** answered: Homogeneous nucleation usually happens only at high driving forces, when there is a significant free energy gap between liquid and crystal. Under those conditions, a myriad of phases, including disordered ones, could have free energy intermediate between the liquid and the stable crystal state. The phenomenon of two-step nucleation through amorphous precursors is well documented in experiments,<sup>1,2</sup> simulations,<sup>3-7</sup> and theory<sup>7,8</sup> for a variety of systems. The pathway for nucleation, however, is not always controlled by the free energy<sup>9</sup> and in many of the examples of two-step nucleation of crystals, an amorphous phase can assist the nucleation even under conditions for which that phase is not stable (*e.g.* systems above a consolute or critical point<sup>10</sup>). In the

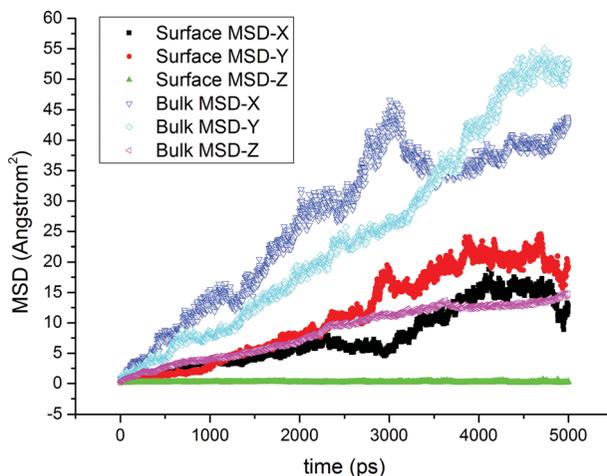
case of the simulations presented in our study of ice crystallization from water-salt solutions,<sup>11</sup> the nucleation of ice can be considered to proceed in two stages, mediated by the formation of the domains of pure four-coordinated water molecules. However, it should be noted that the pure water domains have a short lifetime, comparable to the ice crystallization times, and do not form a true metastable phase.

- 1 P. G. Vekilov, The two-step mechanism of nucleation of crystals in solution, *Nanoscale*, 2010, **2**, 2346–2357.
- 2 D. Erdemir, A. Y. Lee and A. S. Myerson, Nucleation of Crystals from Solution: Classical and Two-Step Models, *Acc. Chem. Res.*, 2009, **42**, 621–629.
- 3 P. Wolde and D. Frenkel, Enhancement of protein crystal nucleation by critical density fluctuations, *Science*, 1997, **277**, 1975.
- 4 N. Duff and B. Peters, Nucleation in a Potts lattice gas model of crystallization from solution, *J. Chem. Phys.*, 2009, **131**, 184101.
- 5 L. C. Jacobson, W. Hujo and V. Molinero, Amorphous precursors in the nucleation of clathrate hydrates, *J. Am. Chem. Soc.*, 2010, **132**, 11806–11811.
- 6 L. Xu, S. V. Buldyrev, H. E. Stanley and G. Franzese, Homogeneous Crystal Nucleation Near a Metastable Fluid–Fluid Phase Transition, *Phys. Rev. Lett.*, 2012, 1–6.
- 7 L. Hedges and S. Whitlam, Limit of validity of Ostwald’s rule of stages in a statistical mechanical model of crystallization, *J. Chem. Phys.*, 2011, **135**, 164902.
- 8 S. Whitlam, Nonclassical assembly pathways of anisotropic particles. *J. Chem. Phys.*, 2010, **132**, 194901.
- 9 B. Peters, Competing nucleation pathways in a mixture of oppositely charged colloids: Out-of-equilibrium nucleation revisited, *J. Chem. Phys.*, 2009, **131**, 244103.
- 10 H. Liu, S. K. Kumar and J. F. Douglas, Self-Assembly-Induced Protein Crystallization, *Phys. Rev. Lett.*, 2009, **103**, 018101.
- 11 G. Bullock and V. Molinero, Low-density liquid water is the mother of ice: on the relation between mesostructure, thermodynamics and ice crystallization in solutions, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00085K.

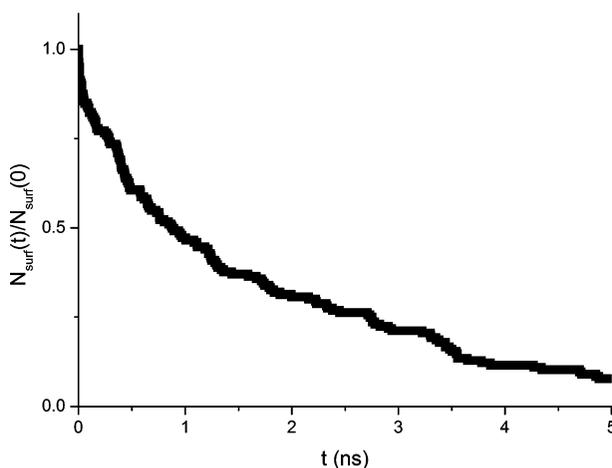
**Professor Wang** opened the discussion of the paper by Mr Cox: During the movie of the nucleation on kaolinite, you mentioned that the second layer of ice formed first and the first layer remained as a liquid longer. Do you know if the first layer is actually a liquid or a transient ice with more fluctuations than the second layer? Did you measure the lateral diffusion constant for the first layer of water? Did it actually move around?

**Mr Cox** replied: I must first of all clarify that in the particular movie that I showed (movie 1 of the ESI), I meant to convey the fact that the water molecules in the second layer occupied positions close to the final ice structure before those in the first. To answer fully whether or not the first layer is a “transient ice” during the transition period, I would need a working definition of such a material. As the system is undergoing a phase transition, I imagine that this would be a difficult property to define and obtain meaningful time averages of.

The question of diffusion in the first layer compared to the rest of the water is interesting, and in Fig. 1 below, I show the mean square displacement (MSD) of the water molecules in the first layer compared to those of the water above, in the supercooled state. The figure clearly shows that diffusion is slower in the first layer than in the water above (referred to as “bulk”, even though the vapour interface is present). I also show a plot (Fig. 2) showing the fraction of water molecules that stay in the first layer as a function of time. It is clear that there is exchange of water molecules between the first layer and those above. As such, the diffusion for individual layers is difficult to compute for long times.



**Fig. 1** Mean squared displacement (MSD) for bulk (open symbols) and first layer (filled symbols) water molecules. Note that “bulk” includes contributions from the water molecules at the liquid/vapour interface. The MSD in each Cartesian direction has been calculated separately. We can clearly see that diffusion is slower in the first layer compared to the rest of the system. If a molecule joins or leaves the first layer (see Fig. 2), it is excluded from making any further contribution to the calculation. These results are averages over 5 trajectories, each of 5 ns in length.



**Fig. 2** Survival probability of water molecules in the first layer.  $N_{\text{surf}}(0)$  is the number of water molecules in the first water layer.  $N_{\text{surf}}(t)$  is the number of water molecules in the first layer at time  $t$  that have never left the first layer. Clearly there is exchange of water molecules between the first layer and those above, making the diffusion of individual water layers difficult to compute. These results are averages over 5 trajectories, each of 5 ns in length.

To compute the MSD for the first layer, as soon as a water molecule leaves the first layer it is excluded from making any further contribution to the calculation. Similarly, a water molecule that joins the first layer from the bulk no longer contributes to the bulk MSD. The data presented in the plots here are averages over 5 trajectories of 5 ns each.

**Professor Wang** asked: Did you study the effect of the thermostat on the nucleation kinetics? Ice formation is an exothermic process. Standard thermostats, such as the Nose–Hoover chain cannot properly capture the true energy dissipation kinetics. Did you investigate if the thermostat you are using is a sufficient approximation to the true nucleation kinetics?

**Mr Cox** responded: No, we have not carried out a study on the effect of the thermostat on the nucleation kinetics. It is correct to say that thermostats such as the Nose–Hoover chain cannot properly capture true energetic dissipation kinetics when applied globally as we have done, as one may reasonably expect heat flow between the embryonic ice nucleus and the substrate. However, I would expect the biggest effect of the thermostat is to change the rate of nucleation, which we are unable to measure in our simulations. I would not expect the thermostat to affect the main conclusions presented, especially the unexpected observation of prism rather than basal face growth when nucleation is induced on kaolinite.

**Dr Henchman** enquired: Do you expect defects on the surface of kaolinite play an important role in ice crystallisation?

**Mr Cox** answered: Yes, I would expect defects on the kaolinite surface to play an important role in ice nucleation. Certainly, kaolinite particles can be highly defective (see, for example, the AFM images of Bickmore *et al.*<sup>1</sup>) and the presence of defects at the surface is often said to be one of the requirements for a particle to be a good ice nucleating agent (see *e.g.* ref. 5 and 9 of the main article). The exact mechanisms by which surface defects enhance ice nucleation is, however, unclear and warrants further study. From a computational point of view, there are two main challenges that need to be overcome, on top of those already mentioned in the paper pertaining to the proper sampling of ice nucleation: (i) characterisation of the surface defects and (ii) describing the water–defect interaction. Point (i) could be achieved through well controlled surface science experiments or through structure prediction methods (or a combination of the two). Point (ii) will depend on the type of defects that form, but it is unclear whether or not simple point charge force fields, such as those used in this study, will be adequate. It is likely that an *ab initio* method would be required, but this even with these methods challenges still exist, as we have discussed previously.<sup>2,3</sup>

1 B. Bickmore *et al.*, *Am. Mineral.*, 2002, **87**, 780.

2 J. Carrasco, A. Hodgson and A. Michaelides, *Nature Mater.*, 2012, **11**, 667.

3 J. Klimeš and A. Michaelides, *J. Chem. Phys.*, 2012, **137**, 120901.

**Professor Molinero** remarked: Large ice crystals grown from deeply supercooled water are usually rich in stacking faults.<sup>1–3</sup> If stacking faults were also pervasive at the temperatures for which kaolinite nucleates ice, would they impair the effect that a small ice nuclei have on the control of the morphology of macroscopic ice crystals?

1 E. B. Moore and V. Molinero, Is it cubic? Ice crystallization from deeply supercooled water, *Phys. Chem. Chem. Phys.*, 2011, **13**, 20008–20016.

2 T. L. Malkin, B. J. Murray, A. V. Brukhno, J. Anwar and C. G. Salzmann, Structure of ice crystallized from supercooled water, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 1041–1045.

3 W. F. Kuhs, C. Sippel, A. Falenty and T. C. Hansen, Extent and relevance of stacking disorder in “ice I(c)”, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 21259–21264.

**Mr Cox** replied: The stacking faults discussed in ref. 1–3 occur exclusively along the *c*-axis of ice, resulting in a mixture of hexagonal and cubic stacking arrangements (referred to as “ice  $I_{SD}$ ” in reference 2). The result of the basal face of kaolinite studied in our simulations is to give a preferential growth along the prism face of ice, which is orthogonal to the *c*-axis. I would therefore not expect stacking faults to impair the effect of small ice nuclei on the macroscopic crystal morphology. The question of what effect changes in structure of a small ice nucleus can have on the macroscopic morphology is not fully known, and is a speculation based on the findings of our work. It is worth noting, however, that the experiments of Evans<sup>4</sup> suggest that  $\beta$ -AgI is able to affect the *phase* of ice that forms at different pressures, which can be seen as an extreme example of the effect that we suggest ice nucleating agents can have on the macroscopic structure of ice.

- 1 E. B. Moore and V. Molinero, Is it cubic? Ice crystallization from deeply supercooled water, *Phys. Chem. Chem. Phys.*, 2011, **13**, 20008–20016.
- 2 T. L. Malkin, B. J. Murray, A. V. Brukhno, J. Anwar and C. G. Salzmann, Structure of ice crystallized from supercooled water, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 1041–1045.
- 3 W. F. Kuhs, C. Sippel, A. Falenty and T. C. Hansen, Extent and relevance of stacking disorder in “ice I(c)”, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 21259–21264.
- 4 L. Evans, *Nature*, 1965, **206**, 822.

**Mr Limmer** said: Mr. Cox has shown how correlations within the water on the kaolinite surface can affect the preferred nucleation pathways for ice crystallization away from the surface. Platinum, which is similar to kaolinite in forming a water adlayer that is hydrophobic, is also regarded as a good surface for growing single ice crystals. Are there reasons to believe that the formation of a hydrophobic interface is related to these surfaces particular ice nucleating abilities? What does this imply for ice nucleation at a liquid–vapor interface?

**Mr Cox** responded: The question of how a surface’s ice nucleating ability is related to the hydrophobicity of its adlayer is interesting, but as we have only been able to nucleate ice in the presence of kaolinite, we are unable to determine with any certainty what this relationship is (*i.e.* a study in which ice nucleation at a variety of surfaces that form different types of adlayer is required). See also comment 514 from Prof. Molinero.

**Professor Molinero** continued: We studied the heterogeneous nucleation of ice on a variety of carbon surfaces and found that, similar to what was reported by Mr. Cox for kaolinite, liquid water layers at the surface of graphitic surfaces. We found a correlation between increased layering of liquid water at a carbon surface and the surface ability to promote ice nucleation.<sup>1</sup> It is an open question whether layering of liquid water occurs on all surfaces that promote heterogeneous ice nucleation.

- 1 L. Lupi, A. Hudait and V. Molinero, Heterogeneous nucleation of ice on carbon surfaces, under review.

**Dr Royall** commented: You mention in your paper and at the end of your talk some concerns about finite size effects. In fact, you say that homogenous nucleation is not observed the case of the larger system of 768 molecules, but that it is in the smaller system of 192, suggesting that finite size effects through the periodic boundaries are influencing nucleation. Can you comment on whether it is

possible to, for example, go to deeper quenches or even shallower quenches where the dynamics are faster?

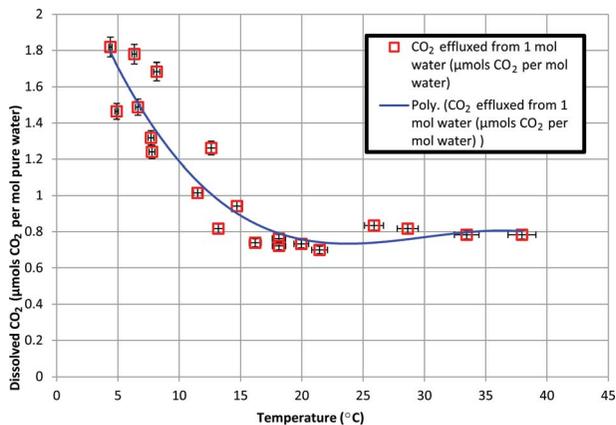
**Mr Cox** responded: This is a reasonable suggestion and one that we have investigated. We mention in the paper that we tried a temperature range of 190–220 K for TIP4P/2005 water (15.5  $\mu\text{s}$  total trajectory time). We also tried using the TIP4P/ice water model at 240 K (2  $\mu\text{s}$  total trajectory time) but we did not see any nucleation. As the computational expense of these calculations is quite large, we have not investigated a wider temperature range.

**Mr Durham** asked: Does your simulation allow you to include traces (around 100  $\mu\text{mol}$  per mol) of a non-water molecule such as  $\text{CO}_2$ , to see if a clathrate or hydrate could exist as part of the hexagonal matrix?

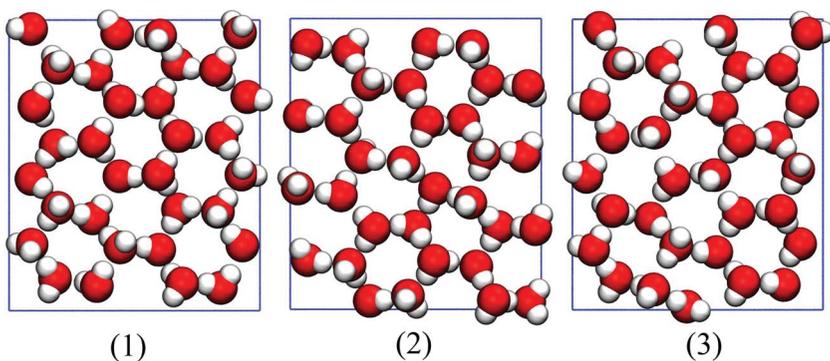
The question derives from Poster 35 (The  $\text{CO}_2$ –water equilibrium at partial pressures below 101.325 kPa: eighty years of measurements), which seeks an explanation for unexpectedly high  $\text{CO}_2$  concentrations in UK rainwater and snowfall.

**Mr Cox** answered: Our ice nucleation simulations would not allow us to include trace gases at the concentrations that you specify. So far, we have only been able to observe ice nucleation in systems containing 192 water molecules, periodically repeated in  $x$  and  $y$ . Including just one gas molecule would therefore lead to a concentration of approximately 5 mmol per mol. Even in the larger systems that we investigated (768 water molecules, where ice nucleation was not observed) the concentration would still be a little over 1 mmol per mol. To reach a low concentration of 100  $\mu\text{mol}$  per mol, 10 000 water molecules would be required for every gas molecule. To my knowledge, no heterogeneous ice nucleation simulations involving atmospherically relevant minerals have been performed on such large systems.

**Mr Durham** communicated in reply: Your oral response tallies with Dr Salzmann's view afterwards, *i.e.* "space in ice"<sup>1</sup> is confined to the hexagonal matrix illustrated in your simulation, and despite its 10% additional molar volume gained at freezing, ice would only be capable of transporting a species as small as helium. This view accords with my own analyses of snowfall and of ice created in  $\text{CO}_2$  solutions, but fortuitously this conference illustrated the potential for space in liquid water. An upward deflection in the slope of the molar volume of water begins around 25 °C, whereafter between zero and –34 °C the volume increases by 2.5%.<sup>2,3</sup> Could this offer solvent "space" that is less constrained than the ice matrix? The smooth curve of  $\text{CO}_2$  solubility in liquid water at partial pressure of 101.3 kPa is not visibly deflected.<sup>4</sup> However at a partial pressure of 45 Pa of  $\text{CO}_2$  (still half again higher than cloud partial pressure), the solute gas would be present at a much lower concentration, and a 2.5% increase in molar volume of the solvent might be relatively more available to the solute molecule. Some encouragement is provided by additional post-conference values for Fig. 5 of Poster 35, showing solubility inverse to temperature (see Fig. 3). If this curve continues to track molar volume into the supercooled zone, cloud water would have a significant dissolved content at the temperature of ice nucleation.



**Fig. 3** Aqueous solubility of carbon dioxide at a partial pressure of c. 45 Pa of the gas, in the temperature range 4–38 °C. In the lower part of this range solubility is showing as inverse to temperature. Values are RMS averages of 60 readings at Li-Cor's 3% confidence level.

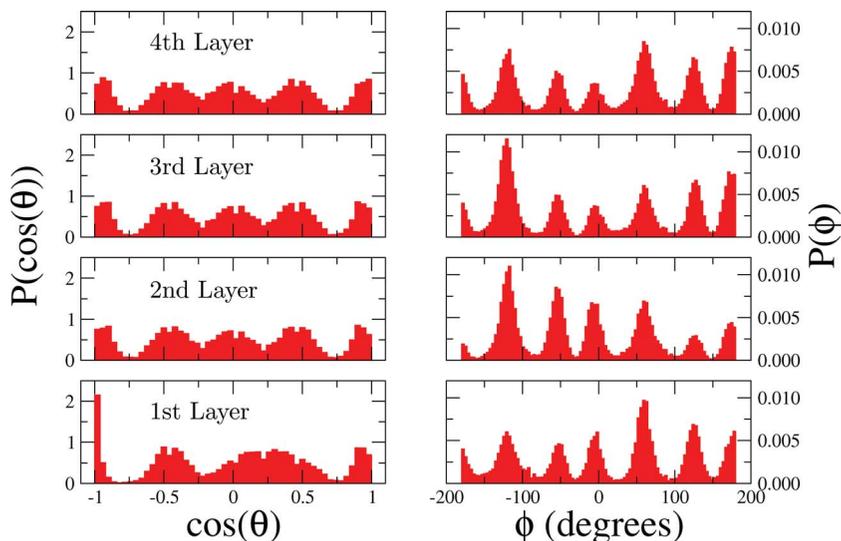


**Fig. 4** Snapshots of ice that forms in the first (1), second (2) and third (3) layers in one of our simulations. Here we can see that the ice that forms is not a proton ordered phase.

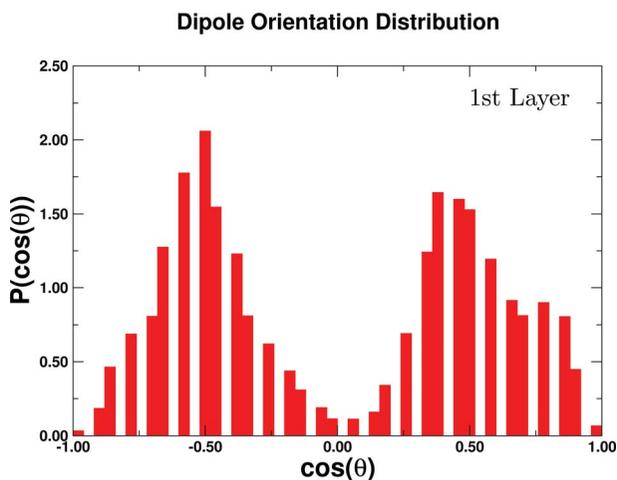
- 1 W. H. Bragg, The crystal structure of ice, *Proc. Phys. Soc. London*, 1921, **34**, 98.
- 2 D. E. Hare and C. M. Sorensen, The density of supercooled water. II. Bulk samples cooled to the homogeneous nucleation limit, *J. Chem. Phys.*, 1987, **87**, 4840.
- 3 V. Holten and M. A. Anisimov, Entropy-driven liquid–liquid separation in supercooled water, *Sci. Rep.*, 2012, **2**, 1–7, <http://dx.doi.org/10.1038/srep00713>.
- 4 P. Scharlin, *IUPAC Solubility data series v 62*, Oxford, 1996.

**Professor Wales** queried: I wonder if there might be additional proton ordering of the ice 1h oxygen framework near to the surface? There could be some interesting electrostatic effects here that might reflect additional structure.

**Mr Cox** communicated in reply: This is an interesting question and something we had not looked into. To answer this, I have included snapshots from the 1st, 2nd and 3rd layers of ice that form in one of our simulations (Fig. 4(1), (2) and (3), respectively). From these pictures, we can clearly see that we are not forming a distinct proton ordered phase of ice. However, we have investigated this in more detail, and I include two other figures. Fig. 5 shows the zenith and azimuth O-H



**Fig. 5** Zenith ( $\theta$ ) and azimuth ( $\phi$ ) angle distributions of O–H bonds. For the zenith angle,  $\cos(\theta) = -1$  corresponds to an O–H bond directed towards the surface and  $\cos(\theta) = +1$  corresponds to an O–H bond directed away from the surface. From the plots of  $P(\cos(\theta))$  (left panels) we can see that there is a preference for O–H bonds in the first layer to be directed towards the surface that is absent in the above layers. The distribution for  $0 \leq \cos(\theta) \leq 0.5$  in the first layer, however, is much broader than in the other layers (see Fig. 6). The azimuth angle is not significantly different between layers.



**Fig. 6** Zenith angle distribution of water dipole moments in the first layer. For dipoles oriented towards the surface,  $\cos(\theta) = -1$ . Clearly the distribution is bimodal (average value of  $\cos(\theta) = 0.007$ , standard deviation = 0.551).

bond angle distributions for the 1st to 4th layers of ice (averaged over 44ns). The zenith angle ( $\theta$ ) is defined such that  $\cos(\theta) = -1$  corresponds to an O–H bond directed towards the kaolinite surface, and  $\cos(\theta) = +1$  corresponds to an O–H bond directed away from the kaolinite. From the distribution of  $\cos(\theta)$  ( $P(\cos(\theta))$ ) plots in Fig. 5, we can clearly see a peak at  $\cos(\theta) = -1$  in the first layer that is

absent in the other layers. This means the surface is inducing a preference for the water molecules to direct an O–H bond directly towards the surface. This is consistent with previous density functional theory calculations.<sup>1,2</sup> However, the distribution for  $0 \leq \cos(\theta) \leq 0.5$  in the first layer is broadened compared to the other layers and this is reflected in the dipole orientation distribution in the first layer (Fig. 6). Here we can clearly see a bimodal distribution centred around zero (the average value of  $\cos(\theta) = 0.007$ , standard deviation = 0.551). If we look at the azimuth angle ( $\varphi$ ) distributions in Fig. 5 we can also see that the kaolinite does not induce any significant changes in the OH orientations in the plane of the surface (the plots are slightly noisy due to the small lateral dimension used). The azimuth angle is defined such that  $\varphi = 0$  corresponds to an O–H bond laying parallel to the  $a$ -axis of the kaolinite conventional unit cell. To summarise, we do not form a fully proton-ordered phase, but the kaolinite does induce a preference for O–H bonds to be directed towards the surface.

1 X. L. Hu and A. Michaelides, *Surf. Sci.*, 2007, **601**, 5378.

2 X. L. Hu and A. Michaelides, *Surf. Sci.*, 2008, **602**, 960.

**Dr Haji-Akbari** enquired: Have you studied homogeneous nucleation in the bulk?

**Mr Cox** replied: No, we have only studied the thin films. This provided the easiest way by which we could compare homogeneous and heterogeneous ice nucleation.

**Dr Haji-Akbari** asked: Based on your successful trajectories that correspond to homogeneous nucleation of ice in a free-standing thin film, do you have any statistics on the likelihood of crystallization occurring in the subsurface in comparison to bulk crystallization, *i.e.* crystallization initiating in the center of the film? This question is due to conflicting results in the literature on this matter. For instance, Vrbka and Jungwirth<sup>1</sup> study thin films of pure water; they use a six-site potential and observe a higher probability of sub-surface crystallization due to the violation of electrostatic neutrality in the surface. On the other hand, Lu *et al.* study thin films of mW and observe a higher probability of bulk (center) nucleation compared to subsurface nucleation.

1 L. Vrbka and P. Jungwirth, *J. Phys. Chem B*, 2006, **110**, 18126.

2 Y. Lu *et al.*, *J. Phys. Chem. B*, 2013, **117**, 10241.

**Mr Cox** responded: We have not investigated whether homogeneous ice nucleation occurs in the subsurface or bulk. As we have only investigated 30 Å thick films, our simulations offer little in the way of trying to resolve this issue.

**Dr Haji-Akbari** asked: How thick is the film in which homogeneous nucleation is studied?

**Mr Cox** answered: The homogeneous films are approximately 30 Å thick.

**Professor Wynne** continued the discussion of the paper by Professor Molinero: In your simulations,<sup>1</sup> the ions were modelled as effective single particles rather

than as ion pairs. However, experimental work by the Bakker group<sup>2</sup> has shown cooperative effects where water molecules form short chains between cation and ions, and our own work<sup>3</sup> has shown the presence of tetrahedrally hydrogen-bonded “Walrafen” pentamers between  $\text{Li}^+$  and  $\text{Cl}^-$ . Therefore, the question is whether the single-particle representation of the ions is an accurate representation of the relevant physics.

- 1 G. Bullock and V. Molinero, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00085K.
- 2 K. J. Tielrooij, N. Garcia-Araez, M. Bonn and H. J. Bakker, *Science*, 2010, **328**, 1006–1009.
3. D. A. Turtton, C. Corsaro, D. F. Martin, F. Mallamace and K. Wynne, *Phys. Chem. Chem. Phys.*, 2012, **14**, 8067–8073.

**Professor Molinero** responded: According to our analysis, ions have a colligative effect on the freezing temperature of water. Colligative properties depend on properties of the solvent, but not of the solute. In a colligative property, the only property of the solute that matters is its ability to tune the chemical potential of the solvent (*i.e.* the water activity). Fig. 1 of our paper<sup>1</sup> shows quantitative agreement of the melting temperature of ice in water–LiCl mixtures (experimental) and mW–S mixtures (simulations) for concentrations up to 15 mol% of ions. As the temperature of melting and enthalpy of melting of pure mW water are in good agreement with the experiments, the agreement on the melting temperatures implies a good description of the water activity as a function of solute content. These results indicate that the simulation model, while surely too simple for the study of the hydration structure, captures the relevant physics for the study of the crystallization of solutions.

- 1 G. Bullock and V. Molinero, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00085K.

**Professor Chandler** commented: Professor Molinero’s paper presents a number of interesting results showing how crystallization follows from fluctuations into low-density liquid configurations of water. She also shows how the amorphous solids appear when rapid cooling arrests these fluctuations. As I have already noted, her results are consistent with independent theoretical results that Dr. Limmer and I have described elsewhere.<sup>1–3</sup> Some have questioned whether these results are peculiar to the mW model of water. I believe that any reasonable model one for which the liquid prefers local tetrahedral structure and for which the liquid crystallizes into an ice-like structure will exhibit the same general behaviors shown to us by Professor Molinero. Different models will produce qualitatively different behaviors only to the extent that they are examined at different corresponding states. The usual models applied in molecular simulation studies of water all behave similarly when put at the same corresponding states.<sup>2,4</sup>

- 1 D. T. Limmer, and D. Chandler, *J. Chem. Phys.*, 2012, **137**, 045509.
- 2 D. T. Limmer, and D. Chandler, *J. Chem. Phys.*, 2013, **138**, 214504.
- 3 D. T. Limmer, and D. Chandler, 2013, arXiv:1306.4728.
- 4 D. T. Limmer, and D. Chandler, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00076A.

**Professor Tanaka** responded: We agree that the link of locally favoured or mesoscopic structures formed in a liquid with the equilibrium crystalline structure<sup>1</sup> is a very interesting issue. Recently we studied this problem for hard spheres, soft spheres, and mW water. In the case of hard spheres, we have two

important structural features: crystal-like bond orientational ordering (fcc- and hcp-type) and icosahedral ordering.<sup>2</sup> We found that the presence of mesoscopic ordering, whose rotational symmetry is consistent with the equilibrium crystal, leads to the lowering of the nucleation barrier<sup>3,4</sup> and the pre-existing order selects crystal polymorphs to be formed.<sup>4,5</sup> We found a similar link between the pre-existing order and the crystal to be formed in soft Gaussian core liquids and mW water. In the case of water, we also found that the locally favoured structures characterized by the translational order in the second shell have a structural feature consistent with the ice structure as well as that inconsistent with it,<sup>6</sup> which leads to an interesting effect on ice crystallization.<sup>7</sup>

- 1 H. Tanaka, Bond orientational order in liquids: Towards a unified description of water-like anomalies, liquid–liquid transition, glass transition, and crystallization, *Eur. Phys. J.*, 2012, **E35**, 1.
- 2 M. Leocmach and H. Tanaka, Roles of icosahedral and crystal-like order in hard spheres glass transition, *Nat. Comm.*, 2012, **3**, 974, DOI: 10.1038/ncomms1974.
- 3 T. Kawasaki and H. Tanaka, Formation of crystal nucleus from liquid, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 14036–14041.
- 4 J. Russo and H. Tanaka, The microscopic pathway to crystallization in supercooled liquids, *Sci. Rep.*, 2012, **2**, 505, DOI: 10.1038/srep00505.
- 5 J. Russo and H. Tanaka, Selection mechanism of polymorphs in the crystal nucleation of the Gaussian core model, *Soft Matter*, 2012, **8**, 4206–4215.
- 6 J. Russo and H. Tanaka, Understanding water's anomalies with locally favored structures, 2013, arXiv:1308.4231.
- 7 J. Russo, F. Romano and H. Tanaka, unpublished.

**Professor Wang** remarked: We have a water model (presented in poster 7) that seems to indicate a more stable transient LDL conformation.<sup>1,2</sup> The neighbor averaged  $q_6$  order parameter for the metastable LDL configurations calculated with our model is very different from that of ice-Ih even for the first solvation shell waters. If a substantial reorientation is required to transform LDL to ice-Ih, do you anticipate the mW model will underestimate the transition barrier?

- 1 E. Pinnick, S. Erramilli and F. Wang, Predicting the melting temperature of ice-Ih with only the electronic structure information as input, *J. Chem. Phys.*, 2012, **137**, 014510.
- 2 Y. Li, J. Li and F. Wang, Liquid–liquid transition in supercooled water suggested by microsecond simulations, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 12209–12212.

**Professor Molinero** responded: Within the framework of Classical Nucleation Theory (CNT), the barrier for crystal nucleation depends only on thermodynamic quantities that reflect the competition of the higher stability of the bulk crystal and the free energy cost of the interface between crystal nucleus and liquid mother phase. These quantities are the density, the excess free energy of the liquid with respect to the crystal and the liquid-ice surface tension. These properties are all well reproduced by the mW model, down to the lowest temperatures for which experimental data is available. This has been elaborated upon and discussed in detail in the supporting information a previous paper.<sup>1</sup> However, as the free energy barrier in CNT depends on the cube of the surface tension, a quantitative assessment of the agreement would require data on the dependence of the liquid–ice surface tension with temperature and radius of the crystallites, which to my knowledge is not available for mW nor experimental water.

- 1 E. B. Moore and V. Molinero, Structural transformation in supercooled water controls the crystallization rate of ice, *Nature*, 2011, **479**, 506–508.

**Professor Anisimov** enquired: Could the low-density water be also the mother of molecular clustering in solutions of hydrotropes at low temperatures?

**Professor Molinero** replied: Low-density water is, generally, solutophobic although it is a better solvent for small hydrophobic species than high temperature water.<sup>1</sup> It would be interesting to investigate whether hydrotropes can be dissolved in low-density amorphous ice. The study of Ben-Amotz and coworkers in this Faraday Discussion<sup>2</sup> suggests that the structure of the clusters of TBA and water may resemble the one of “blobs”, clusters of solutes at solvent-separated distances, which are precursors for the homogeneous nucleation of clathrate hydrates.<sup>3</sup> While water in clathrates is, like in ice, tetrahedrally coordinated, the local order of water in blobs is different from that in low-density water.

- 1 D. Paschek, How the liquid–liquid transition affects hydrophobic hydration in deeply supercooled water, *Phys. Rev. Lett.*, 2005, **94**, 217802.
- 2 D. Ben-Amotz *et al.*, *Faraday Discuss.*, 2013, **167**, DOI: C3FD00086a.
- 3 L. C. Jacobson, W. Hujo and V. Molinero, Amorphous precursors in the nucleation of clathrate hydrates, *J. Am. Chem. Soc.*, 2010, **132**, 11806–11811.

**Professor Mallamace** asked: In your work, the water activity is a central quantity; what is the correct experimental way to have a proper measure of it?

**Professor Molinero** answered: The water activity is defined as the ratio between the vapor pressure of water in the mixture and the vapor pressure of pure water at the same temperature. That definition provides a way to measure water activity by determining the vapor pressure of water. Alternatively, the water activity can be determined from the equilibrium melting temperature using eqn 1 in our paper<sup>1</sup> or a more accurate integration that accounts for the temperature dependence of the enthalpy of melting. We took the second route for the determination of the water activity in the simulations.

- 1 G. Bullock and V. Molinero, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00085K.

**Professor Chandler** opened the discussion of the paper by Dr Royall: I wonder whether your focus on specific static structural features in a glass forming material can be valid. As you know, an order parameter distinguishing a glass from its melt must measure deviations from equilibrium ergodic behavior. In particular, a measure of local structure at a given time, call it  $a(t)$ , has the time average

$$\bar{a} = (1/t_{\text{obs}}) \sum_{t=1}^{t_{\text{obs}}} a(t).$$

Taking the averaging time,  $t_{\text{obs}}$ , to be large, the quantity  $\bar{a}$  is the equilibrium average,  $\langle a \rangle$ , when the system is at equilibrium. A glass transition occurs at a stage where  $\bar{a}$  changes notably from  $\langle a \rangle$ . In other words, a glass is characterized by an arrested fluctuation that is far from equilibrium, a behavior for which  $\bar{a} - \langle a \rangle$  serves as an appropriate order parameter.

This observation is the basis for the so-called s-ensemble method for preparing glassy states in molecular simulation. Ref. 1–4 provide illustrations. In contrast to  $\bar{a} - \langle a \rangle$ , the behavior of  $a(t)$  at one point in time does not distinguish equilibrium

from non-equilibrium, which brings me to my question: to what extent can you justify characterizing glass, as you attempt in your paper, through a specific choice of  $a(t)$  (like the instantaneous density of a certain type of cluster) rather than through a time average of that choice?

- 1 L. O. Hedges, R. L. Jack, J. P. Garrahan and D. Chandler, *Science*, 2009, **323**, 1309–1313.
- 2 R. L. Jack, L. O. Hedges, J. P. Garrahan and D. Chandler, *Phys. Rev. Lett.*, 2011, **107**, 275702.
- 3 T. Speck, and D. Chandler, *J. Chem. Phys.*, 2012, **136**, 184509.
- 4 T. Speck, A. Malins and C. P. Royall, *Phys. Rev. Lett.*, 2012, **109**, 195703.

**Dr Royall** replied: Your points about the  $s$ - and  $\mu$ -ensembles are quite right in that context, and in the latter case for sure, when the time-averaging was taken to one frame, the transition disappeared. Certainly, as these and other papers demonstrate, a transition exists in trajectory space. However, this does not in my view exclude the possibility of a random-first-order transition<sup>1</sup> behaviour of cooperatively re-arranging regions, or indeed concepts such as geometric frustration.<sup>2</sup> Such scenarios are based on static quantities. Now our data, like many others, show a disparity between the static and dynamic correlation lengths. I believe that this may be related to an increase in the dynamic correlation length near the mode-coupling transition.<sup>3</sup> What is clear is that at deeper supercoolings, the increase of the dynamic correlation length in the  $T > \text{TMCT}$  regime is not maintained.<sup>4,5</sup> I thus expect a coincidence of structural and dynamical lengths at the  $T_g$ .

- 1 V. Lubchenko and P. Wolynes, *Ann. Rev. Phys. Chem.*, 2007, **58**, 235–266.
- 2 G. Tarjus, *et al.*, *J. Phys.: Condens. Matter*, 2005, **17**, R1143–R1182.
- 3 P. Charbonneau and G. Tarjus, *Phys. Rev. E.*, 2013, **87**, 042305.
- 4 M. T. Cicerone and M. D. J. Ediger, *Chem. Phys.*, 1995, **103**, 5684–5892.
- 5 L. Berthier, G. Biroli, J.-P. Bouchaud, L. Cipelletti, D. El Masri, D. L'Hôte, F. Ladieu and M. Pierno, *Science*, 2005, **310**, 1797–1800.

**Professor Chandler** remarked: There is a general structural feature that distinguishes glass from melt. It is the distribution of distances between excitations. Excitations in glass and glass-forming materials are localized to regions where particle configurations can reorganize.<sup>1</sup> These regions are soft spots in an otherwise rigid material. Aging of the glass, or relaxation of the melt, follows from the dynamics of those excitations or soft spots.<sup>2</sup> At equilibrium, *i.e.*, in the melt, the spatial distribution of soft spots is like that of particles in a dilute gas.<sup>1</sup> In contrast, when the melt is driven out of equilibrium to form glass, a non-equilibrium correlation length,  $\ell_{ne}$ , emerges. The distribution of distances between excitations changes from exponential in the melt to non-exponential in the glass.  $\ell_{ne}$  is the most probable distance in the non-exponential distribution. This length can be large. The more stable the glass the larger it is.<sup>3</sup> It may be that one can relate the equilibrium concentration of excitations to average densities of locally ordered clusters, but it is the arrangement of those excitations (not simply their concentration) that distinguishes non-equilibrium glass from its equilibrium melt. Stillinger and Torquato have also noted that a large correlation length distinguishes a melt from its glass.<sup>4</sup>

- 1 A. S. Keys, L.O. Hedges, J. P. Garrahan, S. C. Glotzer, and D. Chandler, *Phys. Rev.*, 2011, **X1**, 021013.
- 2 D. Chandler and J. P. Garrahan, *Annu. Rev. Phys. Chem.*, 2010, **61**, 191–217.

3 A. S. Keys, J. P. Garrahan and D. Chandler, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 4482–4487.

4 E. Marcotte, F. H. Stillinger, and S. Torquato, *J. Chem. Phys.*, 2013, **138**, 12A508.

**Dr Royall** replied: Thank you for this point. I agree that excitations can provide a mechanism for relaxation and that their distribution – and the “surging” events between excitations – may form a key structural aspect to relaxation upon deep supercooling. I should like to add that we expect structural signatures of “fast” regions also to be accessible to our techniques, which may correspond to these excitations.

**Professor Barrat** asked: Do you have any idea of the local mechanism that governs the dissolution or disappearance of long lived clusters?

**Dr Royall** replied: The clusters come and go through thermal fluctuation. We define them through a bond network, and can have redefinitions of the bond network on timescales shorter than the  $\alpha$ -relaxation time. However we ignore these “breakages” of a cluster if the same particles are again found in the cluster within the  $\alpha$ -relaxation time.

We believe that the locally favoured structure (LFS), the longest-lived cluster, is a geometrically stable arrangement. For example, the 11A bicapped square antiprism which is the LFS for the Kob–Andersen mixture has a full shell around the centre particle. Within the Kob–Andersen stoichiometry, we almost always find a small particle at the centre of the 11A cluster, and this can act to minimise strain.

Conversely, in the Wahnstrom binary Lennard-Jones mixture, the LFS is the icosahedron.<sup>1</sup> Again the shell is full, but here the stoichiometry is 50–50 such that the shell is around 50–50 large and small particles, while in the Kob–Andersen system the 80–20 stoichiometry means the shell is predominantly large particles.

In hard spheres, the LFS is a cluster we term 10B.<sup>2</sup> These are icosahedra missing three particles. However, we have some evidence that, at deeper supercooling, more icosahedra form and 10B are “broken icosahedral fragments”.

In short, we think the long-lived structures are mechanically robust. This is implied by a filled shell and low strain. These properties will be satisfied by different geometries depending on the system under consideration.

1 A. Malins *et al.*, *J. Chem. Phys.*, 2013, **138**, 12A535.

2 J. Taffs *et al.*, *Soft Matter*, **9**, 297.

**Dr Perera** commented: There are currently three competing theories of the glass transition, from what is reported in a recent review (random first order transition, frustration and facilitation).<sup>1</sup> The RFOT predicts the hypothetical existence of an ideal glass, made of mosaic of domains; hence suggesting that glasses would be a mosaic of domains. To what extent do you think that the sort of elementary clusters that you report could be used to support the picture provided by RFOT?

1 L. Berthier and G. Biroli, *Rev. Mod. Phys.*, 2011, **83**, 587.

**Dr Royall** replied: Random first-order theory is proposed to become dominant in the temperature range between the mode-coupling transition and a lower

temperature transition to an “ideal glass”. At the moment, this regime is hard to access with computer simulation (or other techniques that provide a similar degree of detail such as particle-resolved studies of colloids). I believe that the reason that our measures of structural and dynamic lengths do not coincide may reflect the mode-coupling transition somehow leading to the former increasing strongly. What is clear is that the dynamic correlation length seems unlikely to maintain its rate of increase in the temperature regime above the mode-coupling transition. This is because it has already increased to around 10 particle diameters (see our paper and many others), yet, indirect measurements on molecules at the molecular glass transition<sup>1–3</sup> show a similar length-scale, despite the fact that the molecular glass transition corresponds to a relaxation time around ten orders of magnitude greater than the mode-coupling transition. This view is further supported by recent computer simulations which suggest a change in the growth of the dynamic correlation length around the mode-coupling transition.<sup>4,5</sup>

I would therefore like to think that, in the temperature regime below the mode-coupling transition, the structural and dynamic correlation lengths coincide and fast-moving cooperatively re-arranging regions correspond to clusters distinct from the bicapped square antiprisms, but the bicapped square antiprisms are found in dynamically slow regions. Thus, at lower temperatures than we can access with our simulations, our structural and dynamic lengthscales will coincide, and the mosaic state consists of slow bicapped square anti-prism-based CRRs and fast CRRs of different local structure.

1 M. T. Cicerone and M. D. Ediger, *J. Chem. Phys.*, 1995, **103**, 5684–5892.

2 S. Tatsumi, S. Aso and O. Yamamuro, *Phys. Rev. Lett.*, 2012, **109**, 045701.

3 L. Berthier, *et al.*, *Science*, 2005, **310**, 1797–1800.

4 W. Kob, S. Roldán-Vargas and L. Berthier, *Nature Phys.*, 2011, **8**, 164–167.

5 E. Flenner and G. Szamel, *J. Chem. Phys.*, 2013, **138**, 12A523.

**Professor Angell** asked: Would it be reasonable to see the glass formed in the laboratory (*i.e.* one that has lost its entropy by cooling at rates that are quite unachievable in computer simulation) as being not merely a percolated structure, but a totally jammed structure made of units like the ones you describe? Glass transition would then correspond to the unjamming, by local excitations, occurring under completely solid-like diffusivity conditions ( $D \approx 10\text{--}22 \text{ m}^2\text{s}^{-1}$ ); the glass transition occurs on time scales many orders of magnitude below the limits of present day calculations. Would that be a viable way of looking at the declustering during heating?

**Dr Royall** responded: I think what you suggest is a most attractive scenario. Of course, since the dynamical regime accessible to simulation is far from the molecular glass transition, inferences from numerical data are necessarily speculative. However, we<sup>1</sup> and others<sup>2</sup> have devised means of preparing simulation data which by some measures appears closer to the molecular glass transition. These papers suggest a saturation in the number of clusters. We have studied the break-up of the clusters upon effective heating (specifically, by removing the biasing field that generated the clusters). Our results suggest a melting transition concurrent with disintegration of the clusters. A more detailed analysis might well reveal the kind of behaviour you suggest and we will look into it.

- 1 T. Speck *et al.*, *Phys. Rev. Lett.*, 2012, **109**, 195703.
- 2 S. Singh *et al.*, *Nature Mater.*, 2013, **12**, 139–144.

**Professor Anisimov** opened the discussion of the paper by Dr Sefcik: What makes your system different from the TBA solution where the mesoscale inhomogeneities originate from hydrophobic impurities? Are your aggregates closely related to the existence of the solid phase?

**Dr Sefcik** communicated in reply: We investigated aqueous solutions of glycine and DL-alanine. These are the two simplest amino acids, where for glycine there is no side group present that would provide a hydrophobic character and DL-alanine has a single methyl side group. So one clear difference between these small amino acid molecules and TBA is an absence of hydrophobic functional groups. This would be expected to limit propensity of these amino acids to associate with hydrophobic impurities and/or stabilise corresponding nanoemulsions present in TBA aqueous solutions.<sup>1</sup> We believe that mesoscale clusters observed in aqueous amino acid solutions are not directly related to the existence of solid phases, but rather are thermodynamically stable features of liquid phases well below saturation concentrations (*i.e.*, well above solubility temperatures) with respect to the corresponding solid phase.

1 D. Subramanian *et al.*, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00070b.

**Professor Wynne** remarked: The data in the first panel of Fig. 4 in your paper<sup>1</sup> shows very distinct jumps at a supersaturation of 0.1 and 1, respectively. Are these jumps associated with some type of phase transition, for example, something akin to a liquid–gas transition involving the molecular cluster you have spoken about? If these are phase transitions, why are there two of them and why are they occurring at these particular concentrations?

1 A. Jawor-Baczynska, B. D. Moore, H. S. Lee, A. V. McCormick and J. Sefcik, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00066D.

**Dr Sefcik** replied: There are two distinct jumps in mesospecies concentrations in DL-alanine solutions as seen in Fig. 4. We believe that the first jump at supersaturation of about 0.1 is due to a phase transition between a molecular fluid (including small molecular clusters with hydrodynamic diameters up to 1 nm) and a cluster fluid. Above this concentration (a critical concentration for cluster formation), the thermodynamically stable state of the solution is a mesostructured liquid containing solute-rich domains (*i.e.*, mesospecies). Why the critical concentration is at this particular value, we cannot explain at present, but it would presumably depend on details of molecular interactions in a given system. We note that small molecular clusters were found to be present in amino acid solutions under all conditions investigated here and their formation is apparently not subject to a significant energy barrier. However, there appears to be a considerable energy barrier for the formation of mesoscale clusters.

Regarding the second jump at supersaturation of 1, we believe that this is in fact related to the presence of metastable conditions in solutions supersaturated with respect to the solid crystalline amino acid and therefore it is not an indication of a phase transition in the liquid phase itself. This is supported by

observations of filtered supersaturated solutions after subsequent tumbling (Fig. 4a in our paper here and further unpublished data), where the number concentrations of mesospecies obtained were similar to those for undersaturated solutions at comparable tumbling times, but significantly lower than those observed in unfiltered solutions prepared by direct dissolution of crystalline solids. This shows that mesospecies populations in supersaturated solutions are pathway dependent and thus not in full thermodynamic equilibrium (unlike those in undersaturated solutions).

**Dr Alexander** asked: Can you go through, in terms of the clusters reappearing, what you think about the effects of walls, and how the energy of agitation affects the system? Where are the clusters hiding?

**Dr Sefcik** replied: Mesoscale clusters observed in both glycine and DL-alanine solutions can be removed by nanofiltration with suitable filters. When such filtered solutions are mechanically agitated for extended periods of time, the mesospecies population reappears with the same mean size and number concentration as before. This indicates that while the mesospecies appear to be an equilibrium feature of the solution, there is a substantial energy barrier to their formation. Since agitation including gentle mechanical contact with glass container walls appears to enhance cluster reformation, we speculate that formation of clusters (*e.g.*, through a nucleation process) might be more favourable at container walls (through heterogeneous nucleation). Resulting clusters then may be detached from walls through mechanical contact and appear in the bulk solutions where they can be detected by light scattering and Brownian microscopy (NTA).

**Dr Vila Verde** enquired: Do you know the composition of your droplets?

**Dr Sefcik** communicated in reply: We have not yet been able to quantify the composition of nanodroplet mesospecies observed in aqueous amino acid solutions. Based on observations from glycine and DL-alanine aqueous solutions<sup>1,2</sup> we believe that mesospecies in these two-component solutions correspond to solute-rich liquid cluster domains with solute concentrations higher than those in surrounding bulk solution.

1 A. Jawor-Baczynska, B. D. Moore, H. S. Lee, A. V. McCormick and J. Sefcik, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00066D.

2 A. Jawor-Baczynska, J. Sefcik and B. D. Moore, *Cryst. Growth Des.*, 2013, **13**, 470–478.

**Professor Angell** commented: The clusters you describe, delicate assemblies that can be easily destroyed by shear stresses, as in filtration or ultrasonic agitation, but then reappear after a certain time, remind me a lot of the controversial “Fischer clusters”, discussed a lot by polymer and glassformer liquid scientists over the years. Would it not be possible to examine the reassembly process more quantitatively to see if they follow the equations of nucleation and growth? There are a variety of experiments that can be carried out to quantify this process. For instance one can carry out combinations of probes to separate nucleation kinetics from nucleation + growth combinations.

**Dr Sefcik** communicated in reply: It would be certainly very interesting to perform kinetic investigations of nucleation and growth of thermodynamically stable mesoscale clusters observed in aqueous amino acid solutions. In this context it is also worth highlighting experimental work by Sedlak on kinetics of formation of mesoscale clusters in a range of solution systems<sup>1</sup> as well as recent investigations to distinguish mesoscale liquid clusters from nanobubbles.<sup>2</sup>

1 M. Sedlak, *J. Phys. Chem. B*, 2006, **110**, 4339–4345.

2 M. Sedlak and D. Rak, *J. Phys. Chem. B*, 2013, **117**, 2495–2504.

**Dr Vila Verde** opened the discussion of the paper by Dr Alexander: In Fig. 4, the signal decreases between  $S = 0.95$  and  $S = 1.35$ . You state that this decrease is significant; however, the decrease is small and the uncertainty associated with each point is large, so one could just as easily interpret the data to mean that the signal stays approximately constant in that range. Can you elaborate on why you are confident that the observed decrease in signal is significant? Would it be feasible to perform more experiments so that the statistical uncertainty associated with each point could be reduced?

**Dr Alexander** responded: In our paper we have tried to be reasonably careful with the language used, so we refer to the changes as apparent rather than definite. As you rightly point out, the difference in the means between the points at  $S = 0.95$  and  $1.35$  is not statistically significant (2-tailed  $t$ -test,  $P = 0.15$ ); the difference between the points at  $S = 1.75$  and  $1.86$  is significant ( $P < 10^{-4}$ ). More measurements in the region of  $S = 1$  are desirable. This should be done by improving detection of the SHS signal from the particles relative to the HRS background. For example, simply increasing the laser power would favour collection of the HRS signal, which is monotonic (dashed line in Fig. 4 of our paper). One possible strategy would be to use optical trapping of these particles, which we have demonstrated in principle but as yet have not combined with the SHS experiment.

**Professor Ben-Amotz** asked: How many times were each of the experiments repeated in order to obtain the error bars on each of the points in Fig. 4 of your paper? Note that it is always worrisome when removing one point from a set of data points is sufficient to make an apparent feature disappear.

**Dr Alexander** replied: For the data in Fig. 4 of our paper the number of repeat measurements per point ( $n$ ) for  $S < 0.5$  was  $n = 5$ , and for  $S > 0.6$  was  $n = 8$  to  $14$ . The error bounds shown represent 95% confidence intervals ( $2\sigma$ ). The repeat experiments at different concentrations were conducted in no particular order to avoid systematic errors.

**Professor Wales** asked: What are the error bars for the supersaturation values?

**Dr Alexander** replied: We believe the largest source of uncertainty in our supersaturation values stems from the accuracy of the solubility data rather than experimental uncertainties from preparation of samples. A rudimentary analysis comparing solubility data from several sources suggests that the uncertainties in

our supersaturations is <1%, certainly within the boundaries of the symbols used in Fig. 4 of our paper.

**Professor Walker** commented: The data in Fig. 4 show an increase in integrated signal as a function of concentration. Larger signals might result from either more small particles generating the second harmonic response or a smaller number of particles that grow in size.

Are the authors able to assess which mechanism is operative? (Or another mechanism altogether?) Have the authors attempted to measure SH signals using different polarization combinations in order to perhaps learn more about the structures that are responsible for the observed signals?

Along those lines, the authors acknowledged that particle growth may be occurring around trace contaminants in the solution. One way to test this hypothesis might be to intentionally seed their solutions with a chiral contaminant and then look for chiral SH signal. (Garth Simpson at Purdue has been one of the most active scientists in this area; see, for example ref. 1). Finally, work by Ken Eisenthal and coworkers might be relevant to the authors' results. Specifically, Eisenthal noticed that spherical particles in bulk solution could generate a surprisingly large SH response. A number of papers have been published over the past 10 years looking at nanoparticles, styrene beads and vesicles (see ref. 2 for an example).

1 G. Simpson *et al.*, *Cryst. Growth and Des.*, 2008, **8**(8), 2589–2594, DOI: 10.1021/cg700732n.

2 K. Eisenthal *et al.*, *J. Phys. Chem. C*, 2008, **112**(40), 15809–15812, DOI: 10.1021/jp8047168.

**Dr Alexander** replied: The possible mechanisms for change in SH signal that we outline in our paper are about as far as we can go with speculation for now. Measurements of the angular dependence of the scattering, and the dependence on polarization as Professor Walker suggests would be very useful. The idea for the chiral experiment is particularly innovative. We have not yet attempted such measurements.

**Professor Molinero** asked: Is it possible to distinguish between liquid and crystal states of the mesoscopic urea–water aggregates using second-harmonic scattering? Do you observe evidence of crystallization of the mesoscopic aggregates in the experiments at high supersaturation?

**Dr Alexander** replied: We did not see any evidence of crystal nucleation in our samples, which we found can remain metastable for months. In our paper<sup>1</sup> we demonstrate that the signals come from localised particles, floating in solution, but we are not able to say whether these are crystalline or not.

1 M. R. Ward, S. W. Botchway, A. D. Ward and A. J. Alexander, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00089C.

**Professor Wynne** enquired: In the trapping experiment you have described,<sup>1</sup> would it be possible to send one of the trapping lasers, after it has passed through the sample, to a spectrometer to perform a Raman spectroscopy experiment? This would allow you to ascertain that the scattering centres are indeed urea.

1 M. R. Ward, S. W. Botchway, A. D. Ward and A. J. Alexander, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00089C.

**Dr Alexander** answered: The image in Fig. 5 of our paper was taken while the particle at the centre was being held in a dual optical trap; full results of these experiments have yet to be published. We have looked at urea and several other solutes at high concentrations using Raman microscopy without trapping, and we found that the signal is dominated by the background solute. A combined trapping and Raman spectroscopy experiment may indeed help to unravel the nature of these particles.

**Professor Wynne** asked: You mention experiments on vanillin solutions in water, however, this system is known to display a liquid–liquid phase separation.<sup>1</sup> Do you believe that supersaturated aqueous urea solutions also crystallise through a liquid–liquid phase separation process along the lines of the theory proposed for protein crystallisation<sup>2</sup> by Frenkel?

1 M. Svard, S. Gracin and A. C. Rasmuson, *J. Pharm. Sci.*, 2007, **96**, 2390–2398.

2 P. Tenwolde and D. Frenkel, *Science*, 1997, **277**, 1975–1978.

**Dr Alexander** responded: The analogy of the liquid–liquid phase separation is very tempting. There is now quite a lot of circumstantial evidence for objects floating around in two-component ionic and small-molecule systems. These objects are intrinsic and not foreign to the system; let's call them clusters. I would say that these clusters are not liquid in the sense that they have restricted internal degrees of freedom; I would view them more as disordered liquid-crystalline or glassy objects, which may include molecules of solvent.

**Dr Perera** commented: In our simulations of aqueous urea, we found that urea aggregates into domains, just like other hydrophilic molecules like DMSO or formamide. However, the experimental observation of the concentration fluctuations, as measured by the Kirkwood–Buff integrals, are surprisingly ideal looking.<sup>1</sup> It seems that the urea clusters are formed while the mixture exhibit very little concentration fluctuation. In view of this, you may want confirm this behaviour by studying DMSO or formamide aqueous mixtures, which are soluble over the entire concentration range.

1 E. Matteoli and L. Lepori, *J. Chem. Phys.*, 1984, **80**, 2856.

**Dr Alexander** communicated in reply: We agree.

**Professor Angell** asked: Did I miss something? Did you show us a phase diagram for the urea–water system? Can you tell us what it looks like? Does it have binary compounds or is it just a simple eutectic?

**Dr Alexander** communicated in reply: The urea–water system exhibits simple eutectic behaviour.<sup>1</sup> There is only one known crystal form of urea at ambient pressures.<sup>2</sup>

1 M. Egal, T. Budtova and P. Navard, *Cellulose*, 2008, **15**, 361–370.

2 F. J. Lamelas, Z. A. Dreger and Y. M. Gupta, *J. Phys. Chem. B*, 2005, **109**, 8206–8215.

**Professor Angell** communicated: If the latter, it may possible that there are complex crystal structures hiding just below the eutectic, as there are in the case of the Au–Si system that is a simple, but enormously deep, eutectic (at half the  $T_m$  of gold, the lower melting component).

**Professor Wynne** opened a general discussion of Professor Anisimov, Dr Alexander and Dr Sefcik's papers: A number of papers discussed in this meeting so far<sup>1–3</sup> appear to show the existence of very large molecular clusters in solution. My gut feeling is that these huge clusters should be thermodynamically unstable, however, the experiments appear to demonstrate that they are stable and reform after filtering. Non-classical nucleation theories have been proposed—to understand crystal nucleation of calcium carbonate—that involve stable pre-nucleation clusters.<sup>4–6</sup> Could it be that the clusters discussed here are related to pre-nucleation clusters?

- 1 D. Subramanian, C. T. Boughter, J. B. Klauda, B. Hammouda and M. A. Anisimov, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00070B.
- 2 A. Jawor-Baczynska, B. D. Moore, H. S. Lee, A. V. McCormick and J. Sefcik, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00066D.
- 4 M. R. Ward, S. W. Botchway, A. D. Ward and A. J. Alexander, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00089C.
- 4 D. Gebauer, A. Voelkel and H. Coelfen, *Science*, 2008, **322**, 1819–1822.
- 5 A. Dey, P. H. H. Bomans, F. A. Mueller, J. Will, P. M. Frederik, G. De With and N. a. J. M. Sommerdijk, *Nat. Mater.*, 2010, **9**, 1010–1014.
- 6 D. Gebauer and H. Coelfen, *Nano Today*, 2011, **6**, 564–584.

**Professor Anisimov** replied: I feel that mesoscale aggregates reported in the literature may belong to different physical phenomena. In some systems they reappear after filtration. In other systems (such as TBA–water) they emerge only in the presence of a hydrophobic impurity.

**Dr Alexander** responded: These may be stable clusters, or they may be impurity particles that have passed the filtering. We note that our estimates of the number density of these objects given in our paper are very similar to those measured by Jawor-Baczynska *et al.*,<sup>1</sup> who demonstrated that these objects can be reformed after removal by filtering.

- 1 A. Jawor-Baczynska, B. D. Moore, H. S. Lee, A. V. McCormick and J. Sefcik, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00066D.

**Dr Sefcik** added: Indeed we believe that it is likely that mesoscale clusters observed in amino acid aqueous solutions are involved in non-classical nucleation pathways to formation of solid crystalline phases. It may well be the case for other solutions, such as calcium carbonate, as well. However, these non-classical nucleation pathways might be more complex than involving just a single pre-nucleation cluster “species”. For example, there might be a distribution of cluster sizes and their densities and internal arrangements in a mutual equilibrium (or non-equilibrium, especially in supersaturated solutions). For example, recently we showed<sup>1</sup> that glycine mesospecies (nanodroplets) need to be significantly larger than their most typical average size in order to effectively participate in glycine nucleation, and that mechanical agitation can be used to enhance coalescence of smaller nanodroplets into larger ones.

1 A. Jawor-Baczynska, J. Sefcik, B. D. Moore, *Cryst. Growth Des.*, 2013, **13**, 470–478.

**Professor Ben-Amotz** remarked: It would be useful to more precisely define what we mean by “mesoscopic order” so as to distinguish the formation of such structures from micelle formation (and low-order aggregation processes), as well as and from the sort of fractal-like structures that would be expected to appear in non-aggregating random mixtures. For example, one might consider restricting the use of the term mesoscopic order to situations in which a system contains sub-micron structures that are significantly larger than 1 nm, with a well-resolved characteristic length scale (as opposed to a broad fractal-like distribution of aggregate sizes spanning the entire nm to micron regime). The latter characteristic length scale could perhaps further be associated with the existence of a well-resolved low-Q peak in X-ray or neutron scattering spectra. The latter definition might thus provide an experimental means of distinguishing unique mesoscopic structures from fractal-like random contact structures, which would presumably not give rise to a well defined low-Q peak, but would rather produce a broad and relatively featureless low-Q background.

**Professor Anisimov** replied: I agree with your comment.

**Dr Sefcik** addressed Dr Alexander: In your paper you mentioned Rayleigh scattering measurements on urea aqueous solutions, revealing presence of particles at concentrations on the order of  $10^8 \text{ cm}^{-3}$ . Have you been able to estimate their hydrodynamic diameters? Also, have there been any previous DLS measurements on urea solutions? Regarding filtration of hot urea solutions, have you tried different filters (in terms of filter materials and pore sizes) and if yes, were there any effects on your observations? Your observations of 2-photon fluorescence decay in concentrated glycine solutions is intriguing and while it may indeed be due to trace impurities as mentioned, it would be worth following up.

**Dr Alexander** replied: The analysis of our scattering measurements on nearly saturated solutions of urea is still in progress. We have found a very recent study that we did not cite in our paper: this article reports on static and dynamic light scattering for dilute aqueous solutions of urea ( $S \sim 0.04$ ).<sup>1</sup> The conclusions strongly support our own findings, in particular the number densities of the native particles that they observe. The study also concludes that these particles are not nanobubbles. We have tried different types of filter. Smaller-pore filters (20 nm) appear to decrease the number of particles. Different filter materials also appear to have an effect, *e.g.*, poly(tetrafluoroethene) filters appear to reduce the number of particles, but the particles are never removed entirely. All of these results are in accordance with your own measurements on amino acids.<sup>2,3</sup>

1 M. Sedlak, D. Rak, *J. Phys. Chem. B*, 2013, **117**, 2495, DOI: 10.1021/jp4002093.

2 A. Jawor-Baczynska, J. Sefcik and B. D. Moore, *Cryst. Growth Des.*, 2013, **13**, 470–478.

3 A. Jawor-Baczynska, B. D. Moore, H. S. Lee, A. V. McCormick and J. Sefcik, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00066D.

**Dr Vila Verde** addressed Dr Sefcik: Proving your system is free from impurities, and thus that the mesoscale structures that you see are not related to the presence

of impurities, is difficult. It would be instructive to take the opposite approach and put small amounts of impurities in your systems, and investigate what happens to the size and stability of the mesostructures.

**Dr Sefcik** responded: This is indeed worth investigating, although one would need to choose a specific impurity (or impurities) to focus on. However, we also assessed potential effects of impurities on formation of mesoscale species indirectly by observations of mesospecies in solutions prepared after purification by nanofiltration and/or recrystallization.

**Professor Wales** asked: What sort of impurities did you consider?

**Dr Sefcik** replied: We assessed potential effects of impurities on formation of mesoscale species indirectly by observations of mesospecies in solutions prepared after purification by nanofiltration and/or recrystallization. As reported in our paper here,<sup>1</sup> filtration of DL-alanine solutions by either hydrophobic (PTFE) or hydrophilic (alumina) nanofilters removes virtually all observable mesospecies and yet they reappear after a certain period of time with the same mean size and number concentration as before. If any impurities were associated with mesospecies in original solutions, they would be expected to be at least partially removed together with original mesospecies and therefore their total amount should decrease. Significantly, filtration does not lead to a quantitative change in mesospecies populations once equilibrium is re-established (see Fig. 4 and 5 in our paper). Furthermore, as we reported previously,<sup>2</sup> purification of glycine by repeated recrystallization was performed to check for effects of impurities on mesospecies formation in glycine solutions, and again the same size and number concentration of mesospecies were found irrespective of the degree of purification. While we were not able to detect any specific impurities by the HPLC analysis, it can be reasonably expected that concentrations of any impurities present in glycine would significantly decrease after each recrystallization step, and therefore we believe that it is unlikely that impurities play a significant role in formation of mesospecies in these solutions.

1 A. Jawor-Baczynska, B. D. Moore, H. S. Lee, A. V. McCormick and J. Sefcik, *Faraday Discuss.*, 2013, **167**, DOI: 10.1039/C3FD00066D.

2 A. Jawor-Baczynska, J. Sefcik and B. D. Moore, *Cryst. Growth Des.*, 2013, **13**, 470–478.

**Dr Perera** commented: I would like to draw the attention of everyone on the remarkable work of Yoshikata Koga<sup>1</sup> from UBC, Vancouver, Canada, who is a calorimetrist. He measures the vapour pressure and enthalpy of solutions, and computes second derivatives of enthalpy with respect to the concentration of solutes. He reports an anomalous behaviour of such derivative at very low concentration of solute, under the form of a peak. When all anomalies for a variety of solutes are plotted in the  $(T, x)$  plane, where  $T$  is the temperature and  $x$  the solute mole fraction, then the different lines seem to converge to a single temperature for  $x = 0$ , independent of the nature of the solute, and pointing to an intrinsic property of water at  $T = 70$  °C approximately. This temperature seems very close to the sound anomaly in water, but the relation to this anomaly and solute universal clustering scheme is unclear at present.

1 Y. Koga, *Solution Thermodynamics and its Application to Aqueous solutions*, Elsevier, Amsterdam, 2007.

**Professor Molinero** addressed Dr Sweatman: Is there an agreement on what is the physical origin of the stabilization of mesoscopic aggregates and whether these aggregates are stable only for specific intermolecular interactions?

**Dr Sweatman** communicated in reply: I am not aware of broad agreement at this early stage, but our work with the SALR model, which extends the work of others on the SALR model to lower density regions of the phase diagram where micelle-like behaviour is observed, demonstrates that competition between short-range attraction and long-range repulsion does generate thermodynamically stable mesostructures.

We have submitted this work for peer review elsewhere. We expect the same basic physics underlies many aggregation phenomena in liquid mixtures, and suggest that it can also provide an explanation for reversible non-classical, or two-step, crystal nucleation processes (reversible in the sense that SALR clusters can be thermodynamically stable, so that by adjusting solvent conditions one can go from macroscopic crystal to a micelle-like phase, in principle). The physical origin of these competing short and long-range effective interactions could be quite varied, depending on the specific chemistry of each system.

Regarding some of the details of our work, we devised a thermodynamic model to describe the cluster fluid phase of the SALR system. We discovered later that our thermodynamic model is similar to theories of micellization, which are usually used to model surfactant aggregation. However, unlike typical micelle theories, our approach has no empirical or fitted parameters – it is entirely self-contained. The model parameterizes the cluster fluid phase in terms of 4 parameters: the overall bulk density, the cluster density, the liquid “body” density of a cluster, and the “background” vapour density.

Each cluster is taken to be spherical and identical. Entropy and energy density functions are designed that when minimised yield the equilibrium behaviour. The model predicts, as you might expect, micelle-like behaviour.

We map the phase diagram at low density for some combinations of SALR fluid parameters. We find the cluster fluid phase is stable over a range of SALR parameters, and locate the “critical cluster concentration”, analogous to the critical micelle concentration, below which a uniform vapour phase with pre-clusters is stable. We also locate a cluster vapour to cluster liquid first-order transition, which is driven by a depletion effect (vapour is depleted between clusters as they approach each other). The cluster size and body-density hardly vary with bulk density, but they are sensitive to the relative strength of attraction *vs.* repulsion; their size gradually diverges and their body-density increases as attractions become dominant. We suggest that at higher bulk densities the cluster fluid will transform to some form of modulated aggregate phase, perhaps a cluster solid. We also suggest that when the cluster body-density becomes sufficiently high (when attractions dominate) that clusters will be solid. Finally, we provide a simple approximate formula for the cluster size, which depends on the pair potential and radial distribution (rdf) function of a bulk system with the same density as the cluster body-density. Because of this explicit link between cluster size and rdf we argue that the cluster size will change, and possibly

diverge, when clusters solidify; *i.e.* we provide a mechanism for a non-classical crystal nucleation process from within liquid-like clusters.

That is, we suggest macroscopic crystals can grow out of microscopic liquid-like clusters. We compare the model's predictions at one state point (deep within the cluster fluid phase) with Monte Carlo simulations, which demonstrate the cluster fluid is thermodynamically stable.

**Professor Evans** continued: There has been much discussion about the type of model used in the nice poster by Dr Sweatman *et al.* describing the phase behaviour of a simple (one-component) fluid shown to exhibit an interesting “cluster phase” at low densities. I am familiar with the model and its origins. The idea of using a “mermaid” pair potential, *i.e.* one with an attractive head and a repulsive tail is certainly not new in colloid science. This type of potential is also termed SALR. Steric effects in colloids plus depletion give rise to a short ranged, strongly attractive effective colloid–colloid interaction. And if the colloids are charged screening effects will give rise to a slowly decaying repulsive tail whose decay length depends on the Debye length of the solvent. It is not difficult to see that such competing effective interactions will have two length scales associated with them: the range of the attraction and that of the repulsion. The interesting cases have the attraction shorter ranged than the repulsion. Dr Sweatman cites the paper by Archer and Wilding.<sup>1</sup> This paper provides a fine introduction to the relevant literature and how such models came about. Papers by Imperio and Reatto<sup>2</sup> were important in illustrating the range of (meso)phases than can occur for such models. Archer and Wilding discuss a vapour to spherical cluster phase and, importantly, the sensitivity of phase behaviour to the height of the repulsive tail. Dr Sweatman appears to have taken substantial steps forward by identifying further transitions. Of course the key issue is whether this type of model is relevant to the systems investigated in several papers that were presented here. Are there ingredients in the physical chemistry of these particular aqueous solutions that would lead one to argue for a mapping to the type of model considered by the colloid community? This is not at all clear to me.

1 A. J. Archer and N. B. Wilding, *Phys. Rev. E*, 2007, **76**, 031501.

2 For example, A. Imperio and L. Reatto, *J. Chem. Phys.*, 2006, **124**, 164712.

**Dr Perera** commented: Following the remark of Professor Evans on the clustering in SALR interactions (Short range Attraction and Long range Repulsion) in one component systems, I would like to point out that we tried to extract such interaction as a bridge term from integral equation techniques (using the  $g(r)$  from simulations) from aqueous-binary mixtures. Unfortunately, our findings were inconclusive, to our disappointment, since the molecular structure induces oscillations that prevent us from observing the long range repulsion in an unambiguous fashion. However, since the SALR repulsion is often very small compared to the short range attraction, we do not exclude that a LR mechanism is hidden behind the clustering observed in aqueous mixtures.

**Professor Evans** replied: It is important to recognize that the LR tail can have a very small amplitude but still influence significantly the phase behaviour and in particular the preponderance for clustering. That is why the studies of simple

models are important in identifying the underlying physics. I am not surprised that it is difficult to pick up such subtleties in simulation or integral equation analyses of 'realistic' aqueous mixtures. It is an optimistic man or woman who attempts to invert pair correlation functions in such systems in order to identify a slow repulsive decay.

**Dr Meech** concluded the discussion of Dr Alexander's paper and communicated: In Fig. 4 the case is made for a distinction between the hyper-Rayleigh scattering and the second harmonic emission from the urea solution. Since an arbitrary scale factor is involved and the shapes of the model and the data are not radically different some additional mechanism for the distinction might help. The measurement recalls an earlier study second harmonic generation from an isotopic solution of structures which locally lack an inversion centre – in that case the purple membrane.<sup>1</sup> In that case the SHG was distinguished from HRS through the polarisation dependence and the spatial distribution of the emission (relative to the fundamental pump direction). Similar measurements could provide useful support for the mechanism proposed here.

1 P. Allcock, D. L. Andrews, S. R. Meech and A. Wigman, Doubly Forbidden Second Harmonic Generation From Isotropic Suspensions of The Purple Membrane, *Phys. Rev. A*, 1996, 53, 2788–2791.

**Mr Ward** communicated in reply: Professors Meech and Walker make important points in this discussion. The experimental signals are quite challenging to collect, but even simply obtaining the angular dependence of the scattering could distinguish between incoherent hyper-Rayleigh scattering (HRS) and coherent second-harmonic generation (SHG) contributions. In our paper we were only able to infer the latter mechanism from the observation that large signals appear to come from floating particles.

**Miss Rhys** concluded the discussion of the paper by Dr Sefcik by communicating: The methods that have been used in this paper have either precluded or provided a more vague measure of the hydrodynamic diameter of any small molecular clusters. For the dynamic light scattering measurements, the paper states that the peak at 0.005 ms, found in Fig. 1, corresponds to objects up to 1 nm, and so this would mean that you are likely unable to accurately distinguish between monomers and the formation of very small clusters. As you quote, the technique is more sensitive towards larger sizes. Glycine is an amino acid known to favour the formation of dimers, which is mentioned in the references you quote (ref. 5 and 39), and so should be contributing to this higher small cluster peak seen in the dynamic light results. In any work your group has completed, or in any other studies you are aware of, is small-scale clustering such as that seen in glycine also known/thought to be formed in D/L-alanine? In reference to Fig. 1, it is interesting that the comparison between D/L-alanine and glycine solutions, though having nearly the same *S* value, is between one solution that is under the solubility limit for the amino acid (glycine), and one that is over the solubility limit (D/L-alanine). With that in mind and the fact that Fig. 2 suggests a higher concentration of mesospecies for D/L-alanine, has a critical concentration for formation of mesospecies been established for glycine and what would be the

likely cause for any similarity/difference to the value determined for D/L-alanine ( $\sim 10 \text{ mg ml}^{-1}$ )?

**Dr Sefcik** communicated in reply: Dynamic light scattering is able to detect hydrated monomers and/or small molecular clusters in DL-alanine solutions. This can be seen from a (relatively small) autocorrelation function decay at delay times below 0.01 ms in Fig. 1a. This decay can be seen much better after filtration of mesoscale clusters, which is similar to observations from glycine solutions.<sup>1</sup> It is an interesting question whether it would be possible to quantitatively distinguish contributions of dimers (or larger molecular clusters) from those of hydrated monomers, by analysis of autocorrelation function decays in filtered DL-alanine solutions. We have not attempted that but it might be worth trying.

In Fig. 1 we show DLS autocorrelation functions for undersaturated DL-alanine and glycine solutions at a similar value of supersaturation ( $S = 0.75$ ). The concentration of DL-alanine was  $125 \text{ mg ml}^{-1}$  (not 225 as erroneously stated in the originally submitted manuscript) and the concentration of glycine was  $180 \text{ mg ml}^{-1}$  as indicated. The critical concentration for formation of mesospecies in DL-alanine solutions was found to be around  $17 \text{ g ml}^{-1}$  (corresponding to  $S = 0.1$ ), while for glycine it is in a similar region of concentrations.<sup>2</sup>

1 A. Jawor-Baczynska, J. Sefcik and B. D. Moore, *Cryst. Growth Des.*, 2013, **13**, 470–478.

2 Manuscript in preparation.