

# Understanding crystal nucleation mechanisms: where do we stand? General discussion

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**Kristen Fichthorn** opened the discussion of the introductory lecture by James De Yoreo: I noted that in your potential of mean force (PMF) plots for various particle orientations, fluctuations occur such that different orientations could be preferred at a fixed distance. Could this affect the aggregation outcome?

**James De Yoreo** responded: That's a good point that I had not considered. The gradient of the PMF must impact the average trajectory at any given point. However, at least for the mineral systems we have simulated, both the gradients and the height of the barriers between adjacent minima favor trajectories in which the particles are correctly aligned. The implication is that those particles that happen to be correctly aligned at distances where the interparticle attraction begins to be felt (~100 nm) are most likely to reach the point of contact. Presumably, this also amounts to a torque that rotates particles into alignment as they approach.

**Stephen J. Cox** enquired: Thanks for the very nice talk. My question concerns the mechanism of dipolar interactions between these nanoparticles driving assembly. It seems you are looking at ZnO nanoparticles exposing their basal

faces, which gives rise to the large dipole you have mentioned. How does the system support such a large dipole without incurring a large electrostatic energy penalty?

The basal face of ZnO is a classic type III polar surface in Tasker's classification,<sup>1</sup> and I would have thought that either the surface reconstructs to compensate the dipole, or ions from solution adsorb to the surface and screen the electrostatic interaction between particles.

1 P. W. Tasker, *J. Phys. C: Solid State Phys.*, 1979, **12**, 4977.

**James De Yoreo** replied: I do not know the answer to the question.

**Wenhao Sun** addressed James De Yoreo and Stephen J. Cox: Although polar surfaces and slabs are electrostatically unstable (based on Tasker's criterion of a Tasker Type 3 surface<sup>1</sup>), this is based on the argument of semi-infinite slabs, where the electrostatic energy diverges with slab thickness. Claudine Noguera has shown that the electrostatic energy of a nano-object does not necessarily diverge if the nanoparticle is small;<sup>2</sup> since I believe the electric field lines can close in on themselves.

1 P. W. Tasker, The stability of ionic crystal surfaces, *J. Phys. C: Solid State Phys.*, 1979, **12**(22), 4977.

2 C. Noguera and J. Goniakowski, Polarity in oxide nano-objects, *Chem. Rev.*, 2013, **113**(6), 4073–4105.

**James De Yoreo** replied: I agree. Another point to consider is the extent to which, in the case of particles in solution, electrolytes act to stabilize polarized surfaces.

**Stephen J. Cox** responded: Thanks for pointing out review article by Noguera and Goniakowski.

It is true that the divergence of the electrostatic energy refers to the limit of large crystals. The review highlights that the case for finite size particles is an even more complex affair than for semi-infinite slabs, for example, the electrostatic potential difference across the polar direction can either grow with the thickness of the crystal, or its width, depending on the geometry.

The review does not preclude polarity compensation at the nanoscale, and indeed, states that while polarity compensation mechanisms relevant to semi-infinite slabs may still be relevant, other mechanisms may also be at play.

**Peter Vekilov** asked: You referred to the several hundred debye dipole moment of some of the particles that attach to crystals during their growth as very large. Sick cell hemoglobin (HbS) has dipole moment of about 450 D (ref. 1 and 2) and a size of about 6.5 nm. HbS molecules freely rotate and translate in solution driven by Brownian collisions and they assemble into both sickle cell polymers and crystals. Do these considerations affect in any way the interpretation of the role of the dipole moment of the attaching crystallites in oriented attachment (OA)?

1 D. W. Rodgers, R. H. Crepeau and S. J. Edelstein, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 6157–6161.

2 O. Galkin, W. Pan, L. Filobelo, R. E. Hirsch, R. L. Nagel and P. G. Vekilov, *Biophys. J.*, 2007, 93, 902–913.

**James De Yoreo** answered: I agree that, when compared to dipole moments of all other objects of similar size, several hundred Debye is not anomalously large. In this case, my characterization of the dipole moment as being “large” was in respect to the magnitude of the interparticle force and torque it produces compared to the other interparticle forces at work in this system.

**Peter Vekilov** queried: In your very comprehensive talk, you discuss several examples of nonclassical crystal growth by the association of crystalline, liquid or amorphous precursors. Is this a common theme in the cases that you discussed? Can all these observations be summarized with one or a few sentences? What are the most significant recent achievements in the field of nonclassical growth mechanisms? What questions are still open and may be addressed, with our current tools, in the immediate future?

**James De Yoreo** responded: I do see some common themes. For the sake of brevity I will summarize them at a rather cursory level. The first is that one cannot divorce the observation of what I will generally refer to as crystallization by particle attachment (CPA) from supersaturation. This is, of course, pretty obvious, but, nonetheless, often ignored in the literature. The reason it matters is that the probability of forming clusters or nucleating particles scales strongly with supersaturation (exponential of the inverse supersaturation squared in the classical theory). Thus, one general theme is that strongly driven systems are more likely to exhibit CPA. The second general theme is that solubility matters. Solubilities can span nine orders of magnitude from molar-levels for salts like NaCl to nanomolar levels for oxides. However, the rate of growth by ion addition is proportional to the excess concentration ( $C - C_e$ ), where  $C_e$  is the equilibrium concentration. The proportionality constant (the kinetic coefficient) only varies by approximately an order of magnitude over the full range of solubility and is generally smaller for systems with lower solubility. The consequence is that, even if the supersaturation  $\sim \ln(C/C_e)$  is set to the same level (*i.e.*,  $(C - C_e)/C_e$  approximately constant), the growth rate for a highly soluble phase will be greater by a factor that scales with the product of  $C_e$  and the kinetic coefficient. To take a concrete example, the ratio of the growth rate of KDP to that of hydroxyapatite is about 1010. That means that particle attachment rates would have to be 1010 times faster for KDP than for hydroxyapatite for the relative contribution of CPA to the growth rate to be similar. I've done the calculation for KDP at a typical supersaturation and found that particle attachment rates for  $\sim 5$  nm particles would have to be many times per second, whereas, for hydroxyapatite, the required rate is 1010 times lower. The third theme is that size matters. Because the barriers to attachment tend to be elimination of the hydration layers, the larger the interface, the less likely that thermal energy alone will enable the particles to reach contact.

As far as a summary of all the observations in one or two sentences, I would say that is not possible at any useful level, because, even if one can state a general principle, as I have done above, the details matter and the number of possible outcomes is enormous. The situation is not very different than with nucleation.

The answer to what are the most significant recent achievements in studies of non-classical nucleation would take a great deal of writing to detail. I will highlight two. The first is the direct observation of non-classical pathways by high resolution methods, demonstrating: (i) order can develop gradually, (ii) amorphous clusters can be the stable form below a certain size and thus the system has two critical sizes, one for the amorphous phase and another for the crystalline phase that nucleates in the amorphous clusters, (iii) interfacial gradients drive near surface nucleation, thus biasing systems toward CPA. Some relevant references include ref. 1–3.

- 1 K. C. Cao, J. Biskupek, C. T. Stoppioello, R. L. McSweeney, T. W. Chamberlain, Z. Liu, K. Suenaga, S. T. Skowron, E. Besley, A. N. Khlobystov and U. Kaiser, Atomic mechanism of metal crystal nucleus formation in a single-walled carbon nanotube, *Nat. Chem.*, 2020, **12**(10), 921–928, DOI: [10.1038/s41557-020-0538-9](https://doi.org/10.1038/s41557-020-0538-9).
- 2 L. Houben, H. Weissman, S. G. Wolf and B. Rybtchinski, A mechanism of ferritin crystallization revealed by cryo-STEM tomography, *Nature*, 2020, **579**(7800), 540–543, DOI: [10.1038/s41586-020-2104-4](https://doi.org/10.1038/s41586-020-2104-4).
- 3 G. Zhu, M. L. Sushko, J. S. Loring, B. A. Legg, M. Song, J. A. Soltis, X. Huang, K. M. Rosso and J. J. De Yoreo, Self-similar mesocrystals form via interface-driven nucleation and assembly, *Nature*, 2021, **590**(7846), 416–422, DOI: [10.1038/s41586-021-03300-0](https://doi.org/10.1038/s41586-021-03300-0).

**Jeffrey Rimer** asked: Regarding the observation of high  $\text{Fe}^{3+}$  concentrations in solution in close proximity to crystal surfaces, is this observation consistent with the Boltzmann distribution of counterions near negatively-charged interfaces? Are the concentrations higher than what would be predicted by this theory, which is typically used (along with DLVO theory) to predict the density of ions in the diffuse double layer surrounding charged particles.

**James De Yoreo** replied: The largest contributor to the distribution is the ion–ion correlation term, which I do not believe is included in the DLVO prediction. The details are contained in the supplementary information of ref. 1.

- 1 G. Zhu, *et al.*, Self-similar mesocrystals form via interface-driven nucleation and assembly, *Nature*, 2021, **590**(7846), 416–422, DOI: [10.1038/s41586-021-03300-0](https://doi.org/10.1038/s41586-021-03300-0).

**Jan Sefcik** commented: It is reasonable to expect that dispersion (vdW) interactions between the solid interface and molecules in solution may be an important factor behind concentration profiles at solid–liquid interfaces. Specific effects of organic ligands may be important but there are always dispersion interactions between solution molecules and the solid phase behind the interface. Dispersion interactions would favour one mixture component over another, leading to concentration enhancement of one of the solution components at the interface compared to the bulk concentration far from the interface (see ref. 1).

- 1 D. McKechnie, *et al.*, *J. Phys. Chem. Lett.*, 2020, **11**(6), 2263–2271.

**James De Yoreo** replied: Yes, it is absolutely the case that dispersion forces impact concentration gradients. Moreover, the related ion–ion correlation forces, which arise from density fluctuations in electrolyte solutions, impact these concentration gradients and, in a number of the simulations we have done, come to dominate the interparticle interactions as well. However, the observation that organic functionalization of the surfaces is present in all three cases where near-

surface nucleation or nucleation at a surface in undersaturated conditions is present, suggests that the addition of charged surfaces is an important factor.

**Stéphane Veesler** questioned: My question concerns your slide 27. You spoke about modifying the surface energy and/or supersaturation (*via* a solubility decrease). I did not understand what you meant and how you managed to do that.

**James De Yoreo** answered: What we know is that the nucleation rate is greatly enhanced in the region just near the interface – say within 2 nm. As we do not see any clusters aggregating to form the new particles, we assume that they nucleate classically, that is, ion-by-ion. There are two factors that could enhance the nucleation rate over that of the bulk. The first is the surface energy and the second is the supersaturation. With regard to the surface energy, because the chemistry of the solution and the structure of the solvent are different near the interface, we can imagine that the surface energy is also different. With regard to the supersaturation, we cannot assume that the chemical potential with respect to bulk hematite is different, because the chemical potential must be the same everywhere. It is the need to have uniform chemical potential that leads to non-uniform ion distributions in the first place. However, the solubility of hematite may not be the same in this region of structured solvent and distinct chemistry. So even if the chemical potential of  $\text{Fe}^{3+}$  relative to bulk hematite in bulk solution is uniform, the chemical potential relative to hematite in that interfacial region may be different. We do not know which of these is correct, or if there is another explanation. These are hypotheses.

**Christian Kuttner** enquired: You explained that DLVO theory is fundamental to crystallization. Could you please elaborate on the importance of the secondary minimum and how well it is understood about how it can be manipulated in order to control the process of aggregation?

**James De Yoreo** responded: My comment about DLVO theory was in recognition that CPA is largely a manifestation of colloidal physics with the added complexity of an atomically heterogeneous surface, which imposes structure on the solvent itself. Thus, DLVO theory is a reasonable starting point. The secondary minimum that emerges from DLVO theory provides a place where the approaching particle will have some residence time until it overcomes the intervening barrier to the primary minimum (*i.e.*, contact). During that residence, the particle may have time for Brownian rotation to take it to the minimum energy orientation, which the MD simulations predict is the one for which the particles are crystallographically aligned. From the form of the potential in DLVO theory, one would conclude that the way to manipulate the minimum and the barrier is through the electrolyte concentration and/or strength to alter the electrostatic repulsion, and the solvent to alter the dielectric constant and therefore the van der Waals attraction.

**Rik Drummond-Brydson** asked: In terms of the gaps between nucleating/growing particles (ZnO I think?) and the suggestion that the formation of necks between particles indicates higher supersaturation in that region, how does that square with the indication the structure of the solvent may be more ordered in the

interparticle region? Could the necks form as a result of surface diffusion of say a hydrated surface layer (similar to that seen in particle sintering in the absence of liquid)?

**James De Yoreo** replied: The necking is not a consequence of surface diffusion causing sintering. We know this because we track the particle shape and the conclusion is that the particles are growing during this process. If they were deforming due to surface diffusion of atoms into the gap, the volume would not increase.

**John Harding** opened discussion of the paper by Denis Gebauer: The dipole moment of D<sub>2</sub>O (1.8506 debye) is marginally greater than that of H<sub>2</sub>O (1.8479 debye) – see ref. 1. However the dielectric constant of D<sub>2</sub>O (78.06) is less than that of water (78.39) at 25 °C.<sup>2</sup> This suggests that the screening of electrostatic forces by the solvent would be (slightly) less for D<sub>2</sub>O than for H<sub>2</sub>O and so the stability of ion associates should be slightly greater in D<sub>2</sub>O as the experiments suggest. Also, although the fact that the rates of amorphous calcium carbonate (ACC) precipitation are similar for D<sub>2</sub>O and H<sub>2</sub>O could be explained by saying that ion dehydration and deprotonation have negligible energetic barriers, an alternative explanation (and to my mind more probable one) is that the behaviour in D<sub>2</sub>O and H<sub>2</sub>O is very similar and the energetic barriers (whatever their absolute value may be) are very similar also.

1 S. A. Clough, *et al.*, *J. Chem. Phys.*, 1973, **39**, 2254.

2 G. A. Vidulich, *et al.*, *J. Phys. Chem.*, 1967, **71**, 656.

**Denis Gebauer** responded: It is indeed peculiar that some physical chemical parameters for H<sub>2</sub>O and D<sub>2</sub>O are only marginally different, while the behaviour of various solutes, proteins, and also minerals in the two solvents appear to be strongly affected (see the Introduction of our paper (<https://doi.org/10.1039/d1fd00078k>) for various references). Having said that, I agree that based on the values for the dielectric constants, ion association should be somewhat weaker in heavy than in light water. However, this viewpoint seems to neglect, *e.g.*, entropic effects arising from distinct solvent structures, as discussed in our paper. Moreover, values for the distinct polarizabilities of the waters seem to suggest a different trend in terms of screening effects.<sup>1</sup>

We did not determine the rates for ACC precipitation in the distinct solvents, however, the varying times of nucleation (Fig. 5 in our paper; <https://doi.org/10.1039/d1fd00078k>) suggest that there is a considerable effect on barriers. Since previous studies revealed that the ACC forms *via* the dehydration and solidification of dense liquid precursors,<sup>2</sup> this observation suggests that the solvent change affects dehydration barriers rather than nucleation barriers though.

1 M. N. Rodnikova, *J. Mol. Liq.*, 2007, **136**, 211–213.

2 J. T. Avaro, S. L. P. Wolf, K. Hauser and D. Gebauer, *Angew. Chem., Int. Ed.*, 2020, **59**, 6155–6159.

**Romain Grossier** enquired: In describing ACC, you use the term “amorphous polymorphism in ACCs”, but polymorphism relates to solid crystalline phases,

not amorphous ones, which also share the same chemical composition. As “ACCs” neither have crystalline structure, nor share the same chemical composition(?), using “polymorphism” for such phases is confusing. Would there be another word that could be used? Just “amorphous phases”?

**Denis Gebauer** replied: I agree that the term amorphous polymorphism (or polyamorphism) may be confusing.

The term was introduced by the Ukrainian scientists Palatnik *et al.*,<sup>1,2</sup> who wrote: “By analogy with the phenomenon of polymorphism, well known for crystalline materials, we will refer to the phenomenon of the existence of several such varieties of the amorphous state of the same substance as polyamorphism”.

We have discussed the details of ACC polyamorphism in a previous review article.<sup>3</sup> Since the composition of ACC does not vary for the different forms of ACC, the use of the term polyamorphism seems justified.

In previous papers, we called the distinct forms “proto-structured” ACCs.<sup>4</sup> In the case of biogenic ACCs, the occurrence of distinct structures has been known for decades, using the notion of, *e.g.*, “vateritic” ACC.<sup>5</sup>

1 L. S. Palatnik, Y. A. Bykovskii, P. A. Panckekha, A. G. Dudoladov, V. I. Verchenko and S. V. Marun'ko, *Sov. Phys. Dokl.*, 1980, **25**, 770–772.

2 L. S. Palatnik, Y. A. Bykovskii, P. A. Panckekha, A. G. Dudoladov, V. I. Verchenko and S. V. Marun'ko, *Dokl. Akad. Nauk SSSR*, 1980, **254**, 632–635.

3 J. H. E. Cartwright, A. G. Checa, J. D. Gale, D. Gebauer and C. I. Sainz-Díaz, *Angew. Chem., Int. Ed.*, 2012, **51**, 11960–11970.

4 D. Gebauer, P. N. Gunawidjaja, J. Y. P. Ko, Z. Bacsik, B. Aziz, L. J. Liu, Y. F. Hu, L. Bergström, C.-W. Tai, T.-K. Sham, M. Edén and N. Hedin, *Angew. Chem., Int. Ed.*, 2010, **49**, 8889–8891.

5 L. Addadi, S. Raz and S. Weiner, *Adv. Mater.* 2003, **15**, 959–970.

**Ivo B. Rietveld** addressed Denis Gebauer and Romain Grossier: This is a remark in reference to the previous question asked by Romain Grossier, in which he referred to poly-amorphism and the issues related to its definition.

The semantic part of the problem is that amorphism means “no form”, poly-amorphism is therefore already a contradiction in terms as you cannot count something that is not.

But the scientific issue here goes deeper and is definitely not resolved. Polymorphs are different crystalline phases, with different energetic fingerprints, therefore it will always be possible to pinpoint a transformation of one form into another. When it comes to amorphous or glassy systems, each single one of them will be different from another depending on its history, the point being that to go from one amorphous system to another one can imagine the existence of a continuum of slightly different amorphous layouts connecting all of the amorphous appearances by slightly shifting molecules around into different conformations. This will simply not be possible between crystalline phases, because with a shift of a molecule the unit cell breaks down and a phase change must occur. For an amorphous system a slight shift of a molecule just gives another amorphous system and so on until one “specific” (I would like to say “specific” within our imagination) amorphous phase has been reached. This does not mean that within an amorphous system shifts cannot occur with visible or measurable volume and energy changes, because also volume and energy will be continuums and for example ageing of glassy systems is known to show

“stabilisation” or shifts towards lower energy states. But as long as these states are all interconnected by slight – infinitesimal if you like – changes (of energy, volume, molecular positions), I don’t see the necessity to speak of polyamorphism; it’s a continuum.

There may be an exception though, and that is where the species making up the amorphous material changes, such as changes in charge distributions, hydrogen positions, molecular changes *etc.* (which brings us into another contentious area as we can judge from the salt/co-crystal discussion). In that case, if the identity of the species changes, thermodynamically it will not be possible to speak of the same phase. But should we call this poly-amorphism? I’m not sure.

**Denis Gebauer** replied: There are many open questions in the rather young field of amorphous polymorphism. From a crystallographic point of view, atomic long-range orders give rise to well-defined crystal shapes and facets on the macroscopic scale. This correspondence principle of atomic structure and macroscopic shape does not occur in amorphous structures; however, short-range structures can certainly be distinct. There is no “absence of structure” in any matter. While the proposed notion of a continuum of amorphous structures may be true in some cases, there is strong evidence speaking against continuous changes of amorphous (short-range) structures for several examples. In amorphous ices, abrupt phase transitions between distinct forms are observed at well-defined pressures and temperatures.<sup>1–3</sup> There is no continuum, but rather a sudden change in properties (*i.e.*, density), and thus, in the structures, even though they are “amorphous”. A related observation can be made in ACCs. For the different proto-structured forms (proto-aragonite,<sup>4</sup> proto-calcite,<sup>5</sup> proto-vaterite<sup>5</sup>), the solubilities depend on temperature, as is the case for crystalline forms. However, ACC polyamorphism is controlled by temperature and pH, whereby the solubilities of the distinct polyamorphic forms do not change continuously. They change discontinuously and abruptly between characteristic values,<sup>6,7</sup> as is also the case for crystals.

1 T. Bartels-Rausch, V. Bergeron, J. H. E. Cartwright, R. Escribano, J. L. Finney, H. Grothe, P. J. Gutiérrez, J. Haapala, W. F. Kuhs, J. B. C. Pettersson, *et al.*, *Rev. Mod. Phys.*, 2012, **84**, 885–944.

2 O. Mishima, *Proc. Jpn. Acad., Ser. B*, 2010, **86**, 165–175.

3 T. Loerting and N. Giovambattista, *J. Phys.: Condens. Matter*, 2006, **18**, R919–R977.

4 M. Farhadi Khouzani, D. M. Chevrier, P. Zhang, N. Hedin and D. Gebauer, *Angew. Chem., Int. Ed.*, 2016, **55**, 8117–8120.

5 D. Gebauer, P. N. Gunawidjaja, J. Y. P. Ko, Z. Bacsik, B. Aziz, L. J. Liu, Y. F. Hu, L. Bergström, C.-W. Tai, T.-K. Sham, M. Edén and N. Hedin, *Angew. Chem., Int. Ed.*, 2010, **49**, 8889–8891.

6 D. Gebauer, A. Völkel and H. Cölfen, *Science*, 2008, **322**, 1819–1822.

7 J. T. Avaro, S. L. P. Wolf, K. Hauser and D. Gebauer, *Angew. Chem., Int. Ed.*, 2020, **59**, 6155–6159.

**Marta K. Dudek** asked: Can you comment on the possibility to use other deuterated solvents to redirect/influence crystallization? Do you think it is possible to use deuterated solvents as a route to elusive polymorphs?

**Denis Gebauer** answered: In my opinion, it is likely that other deuterated solvents can also have distinct effects on crystallization pathways, and it is certainly possible that their utilisation may open up novel routes to elusive



polymorphs. For calcium carbonate formation in light vs. heavy water, we do have some indications for the precipitation of some novel form in the latter solvent (see <https://doi.org/10.1039/d1fd00078k>). However, as we have not studied such effects beyond this system, this is certainly merely speculation on the general, potential use of deuterated solvents in crystallisation.

**Wenhao Sun** remarked: To comment on polyamorphism: there is work in thin-film deposition that shows that you can measure the pair distribution function of amorphous films deposited under different conditions; and that there can be two distinct forms of amorphous regions (indicated by different pair distribution patterns). Then, when this is annealed, it anneals into two different polymorphs. See ref. 1.

I have a question for Denis Gebauer; in your paper, you wrote that  $\text{CaCO}_3$  nucleation is ‘more classical’ in  $\text{D}_2\text{O}$  than it is in  $\text{H}_2\text{O}$ . Can you explain what you mean by it being ‘more classical’, and more generally speaking, can you give us your perspective on what non-classical nucleation means? I think of all the people in the world, your perspective on this matter is of greatest interest.

1 K. H. Stone, *et al.*, Influence of amorphous structure on polymorphism in vanadia, *APL Mater.*, 2016, 4(7), 076103.

**Denis Gebauer** responded: Thanks for your comment on polyamorphism in thin-films.

For a clarification of what we mean by “more classically” in the context of our paper (<https://doi.org/10.1039/d1fd00078k>), I will answer later.

I actually believe that the term “non-classical” nucleation should be avoided, as it is better to describe phenomena by what they are, rather than by what they are not. I thus apologize for using this poorly defined term in our paper (<https://doi.org/10.1039/d1fd00078k>), and will try to elucidate what I actually mean by this in the following. Generally speaking, in my opinion, “non-classical” nucleation means that the pathway from solution to solids does not depend on the formation of a critical nucleus (*i.e.*, as defined within Classical Nucleation Theory, CNT), as a required, fundamental transition state. Thus, “non-classical” processes can involve growth units that are (significantly) larger than monomers, as opposed to CNT and two-step nucleation theory (2S). In other words, “non-classical” nucleation precursors, pre-nucleation clusters (PNCs), are thermodynamically stable (*i.e.*, negative standard free energy of formation) and exist at significant populations as solute species in homogeneous solutions. From the point of view of CNT, it is then a conundrum how these species can participate in phase separation, as they are energetically further away from the required transition state than the monomers,<sup>1</sup> and they are thus neglected. In “non-classical” nucleation, there is an alternative reaction channel that explains the role of stable PNCs, as the event of phase separation does not depend on overcoming a certain size of the clusters but rather on a change in cluster dynamics.<sup>2</sup> Recently, these notions have allowed the development of a quantitative model for the so-called pre-nucleation cluster pathway,<sup>2</sup> which offers both predictive and explanatory powers for the complex phase behavior of the aqueous calcium carbonate system.<sup>3</sup>

1 D. Gebauer, P. Raiteri, J. D. Gale and H. Cölfen, *Am. J. Sci.*, 2018, 318, 969–988.

- 2 D. Gebauer, M. Kellermeier, J. D. Gale, L. Bergström and H. Cölfen, *Chem. Soc. Rev.*, 2014, **43**, 2348–2371.
- 3 J. T. Avaro, S. L. P. Wolf, K. Hauser and D. Gebauer, *Angew. Chem., Int. Ed.*, 2020, **59**, 6155–6159.

**Wenhao Sun** commented: I want to emphasize that we, as a community, should be more careful when we use the word ‘stable’. As Gibbs defines it, stable phase and equilibrium phase are *not* the same thing. Stable means that the internal energy surface,  $U(X)$ , is convex; meaning that the second derivative of  $U$  with respect to extensive variables (volume, entropy, composition) is positive. On the other hand, the equilibrium phase means that the phase is the lowest free-energy phase under those conditions. Metastability means that you can be ‘stable’, but not the lowest free-energy equilibrium phase. Sometimes I see people use the terminology ‘stable’ to indicate persistence of metastable phases, this usage would not be correct. Denis: My question to you is that under  $D_2O$ , we see that the titration curves are slightly flatter than for  $H_2O$ . Does this indicate fewer free nucleation clusters?

**Denis Gebauer** responded: I fully agree with the comment. In addition, there is also kinetic stability (perhaps, better, stabilization), which can add to the confusion. When it comes to thermodynamic stability of pre-nucleation clusters (PNCs), we have used the definition of Gibbs within the constraints of a homogeneous system (as PNCs are solute species that form independent of supersaturation, given by a negative standard free energy of formation  $\Delta G_0$ ). Please also remember that for the definition of the stability of phases, suitable boundary conditions need to be applied. As a hyperbole example, remember that all matter is eventually metastable with respect to some iron isotope.

Regarding your question on the pre-nucleation slopes of the titrations, a flatter slope indicates that fewer free ions are present, *i.e.*, more ions are bound, giving rise to a larger equilibrium constant  $K$  of cluster formation, and with  $\Delta G_0 = -RT \ln K$  a more negative standard free energy of the clusters. Thus, the flatter slopes show that the PNCs become more stable (given a constant pH).

**Aaron R. Finney** enquired: Can the authors expand on the description ‘more-classical’? If the nucleation of  $CaCO_3$  in light water is non-classical, how do the authors reconcile their description of the nucleation in heavy water? Are they suggesting that both CNT-type and non-classical nucleation mechanisms contribute to phase separation according to a relative shifting of the binodal? (They write, “and nucleation of vaterite might occur directly, *i.e.*, ‘classically’.”) The non-classical nature of  $CaCO_3$  nucleation was based partly on a multiple-binding model and the fact that the equilibrium constant for ion association is much bigger than 1 in light water; this is also very much the case in heavy water from the calculations presented. In that case, the model still predicts a population of cluster species before nucleation – are these simply spectator species if the system phase separates more classically, or is it the case that the calculations are performed at conditions that don’t necessarily match with the experiments? In ‘On classical and non-classical views on nucleation’<sup>1</sup> the authors write, “CNT is not a universal framework that can be employed for solute speciation, be it in under- or supersaturated solutions: It already fails at the ion pair” and “In our

opinion, this idea – that is, that the CNT-type critical nucleus is not essential as a transition state for nucleation – is a major criterion rendering nucleation theories truly ‘non-classical’.” In light of this, it would be helpful to clarify the use of terms used to describe the pathways.

1 D. Gebauer, P. Raiteri, J. D. Gale and H. Cölfen, *Am. J. Sci.*, 2018, **318**, 969–988.

**Denis Gebauer** replied: First, I would like to emphasize that we have phrased the statement that calcium carbonate formation might proceed “more classically” in heavy water very carefully. A lot of additional work has to be done to actually show this, and we did mention also alternative explanations for our observation, e.g., that ACC may be simply more transient and was thus not detected by us, but might still occur as an intermediate, formed from PNCs, to vaterite in presence of heavy water.

As we have discussed in the introduction section of our paper (<https://doi.org/10.1039/d1fd00078k>), it might be expected that the classical barrier towards nucleation may be reduced in heavy water. On the other hand, our computer simulations indicate that ion association becomes weaker in heavy water, whereas the cluster size distribution decays quicker than in light water. Taken together (lower barrier for classical nucleation, weakened ion association towards an alternative reaction channel), this could mean that, indeed, stable ion associates might be merely spectators, and might not participate in the nucleation of crystals in heavy water. Still, CNT fails to explain the thermodynamics of stable ion associates, but this would be no issue if these actually did not participate in nucleation. In light of this, the quote from ref. 1 actually addressed contrary claims of Henzler *et al.*<sup>2</sup> that CNT would accurately describe populations of stable ion pairs and clusters. As demonstrated in ref. 1, this notion is not sustainable. Please also see our earlier reply to the question from Wenhao Sun when it comes to a clarification of the use of terms, *i.e.*, “classical” vs. “non-classical”.

1 D. Gebauer, P. Raiteri, J. D. Gale and H. Cölfen, *Am. J. Sci.*, 2018, **318**, 969–988.

2 K. Henzler, E. O. Fetisov, M. Galib, M. D. Baer, B. A. Legg, C. Borca, J. M. Xto, S. Pin, J. L. Fulton, G. K. Schenter, N. Govind, J. I. Siepmann, C. J. Mundy, T. Huthwelker and J. J. De Yoreo, *Sci. Adv.*, 2018, **4**, eaao6283.

**Peter Vekilov** remarked: You emphasized that CNT does not account for the role of solvent in nucleation. In fact, it does. Surface tension, the most important parameter in CNT, is strongly solvent dependent. The interactions between the solute molecules, which determine the supersaturation, the other crucial parameter of CNT, also strongly depend on the solvent chemistry. The activation barrier for attachment of molecules to the nucleus, a part of the kinetic part of CNT, is governed by the detailed structures of the solvent that erect around both the nucleus and the incoming solvent molecules.

**Denis Gebauer** responded: We fully agree. We have described the effects of the solvent on CNT parameters in exactly the same manner in our paper (<https://doi.org/10.1039/d1fd00078k>) and in our talk as Prof. Vekilov summarizes here. Perhaps, the hyperbole statement made in our talk towards the lack of an explicit treatment of solvation in CNT has caused this misunderstanding.

**Ruel Cedeno** questioned: In the experimental measurement of the ion association constant, the pH was set to basic conditions, while in simulations, the solvent essentially assumed a neutral pH (using the flexible SPC model).

Given the sensitivity of calcium carbonate to pH,<sup>1</sup> do you think the disagreement in terms of ion association behavior between the computational and experimental data might have also originated from this discrepancy in pH? Would it be useful to employ reactive forcefields which allows bond-breaking and bond-forming of water to account for this?<sup>2,3</sup>

1 D. J. Tobler, J. D. Rodriguez Blanco, H. O. Sørensen, S. L. S. Stipp and K. Dideriksen, *Cryst. Growth Des.*, 2016, **16**, 4500–4508, DOI: [10.1021/acs.cgd.6b00630](https://doi.org/10.1021/acs.cgd.6b00630).

2 J. D. Gale, P. Raiteri and A. C. T. van Duin, *Phys. Chem. Chem. Phys.*, 2011, **13**, 16666–16679, DOI: [10.1039/C1CP21034C](https://doi.org/10.1039/C1CP21034C).

3 W. Zhang and A. C. T. van Duin, *J. Phys. Chem. B*, 2017, **121**, 6021–6032, DOI: [10.1021/acs.jpcc.7b02548](https://doi.org/10.1021/acs.jpcc.7b02548).

**Denis Gebauer** answered: We agree that pH is an important parameter in calcium carbonate formation, and the difference in pH between simulations and experiments may in part be a reason for the disagreement. However, please note that the pH in the simulations can be assumed to be basic rather than neutral, as the excess of carbonate (without added bicarbonate) would correspond to a pH value of around 14. Having said that, we did not add OH<sup>-</sup> ions, which would probably start to interfere with calcium carbonate formation at this pH level.

Please note that we also performed simulations at pH 8.5 (again, as given by the carbonate/bicarbonate ratio) to investigate the system's pH dependence, resulting in, *e.g.*, more elongated clusters than at pH 14 and a shift towards smaller clusters.

A reactive force field, if it represents the aqueous calcium carbonate system correctly, would be great to study these systems. However, the slow speed of these force fields heavily counters their usefulness. While we already struggle to reach realistic solution conditions with an atomistic model, these seem to be yet out of reach employing a reactive force field.

**Sten O. Nilsson Lill** asked: In the MD-simulations, did you allow the O–H or O–D bonds to change upon interactions or were they kept fixed? Did you use any particular algorithm, for example SHAKE or something similar?

**Denis Gebauer** replied: We used the SPC/fw model.<sup>1</sup> This means the bonds are unbreakable and not interchangeable. However, they are not kept at a fixed length like in the SPC/E model, but can oscillate freely following a harmonic potential. The same holds true for the angle. So, no SHAKE algorithm, *etc.*, was used.

Details about the force field can be found in ref. 2 in the supplementary information.

1 Y. Wu, H. L. Tepper and G. A. Voth, *J. Chem. Phys.*, 2006, **124**, 024503.

2 P. Raiteri, R. Demichelis and J. D. Gale, *J. Phys. Chem. C*, 2015, **119**, 24447–24458.

**Alexei Kiselev** enquired: The effects of H<sub>2</sub>O/D<sub>2</sub>O mixtures on the crystallization of calcium carbonate presented in your paper (<https://doi.org/10.1039/d1fd00078k>) are really fascinating and open a whole new world of interesting problems. My initial question was going to be about the specific role of HDO in

the crystallization process, but now I realize that this issue could require another paper or a series of papers to answer this question. So I reformulate my question in the following way: when the mixtures of H<sub>2</sub>O/D<sub>2</sub>O are prepared, did you check if the concentrations of the intermediate species (HDO, H<sub>2</sub>O, D<sub>2</sub>O, DH<sub>2</sub>O<sup>+</sup>, HD<sub>2</sub>O<sup>+</sup>, DO<sup>-</sup>, HO<sup>-</sup>, etc.) have reached equilibrium? I am asking because the Bernal and Fowler model of water expects the lifetime of a water molecule to be on the timescale of several hours, so one would expect that the equilibrium concentration of HDO could require a very long time to achieve.

1 J. D. Bernal and R. H. Fowler, A theory of water and ionic solution, with particular reference to hydrogen and hydroxyl ions, *J. Chem. Phys.*, 1933, **1**, 515–548, DOI: [10.1063/1.1749327](https://doi.org/10.1063/1.1749327).

**Denis Gebauer** answered: Thanks for your positive comment. To answer your question: no, we did not verify whether or not the light/heavy water mixtures had equilibrated prior to our measurements (though they likely were equilibrated, as we used stock mixtures and did not prepare the solutions for each experiment freshly). While I think that equilibration of the mixtures and corresponding potential effects have to be taken into account in future studies, I am not sure whether or not the Bernal and Fowler model is consistent with the (later?) measurements of proton diffusion coefficients and H–D exchanges, which show that these processes are very fast.

**Peter Vekilov** addressed Denis Gebauer and James De Yoreo: Professor Gebauer stated that he avoids using the term nonclassical nucleation because all nucleation complies with the general framework of Gibbs' theory. Gibbs proposed a thermodynamic framework applicable to the nucleation of liquid droplets in supersaturated vapors based on the Second law of thermodynamics, which he reformulated in terms of thermodynamic potentials so that it can provide guidance for systems held under isothermal and isobaric constraints, common in the laboratory, nature, and industry. As all processes in the universe comply with the Second law, it is no mystery that all nucleation processes fit Gibbs' general framework. Classical theory of crystal nucleation, however, includes a thermodynamic part, based on adapting Gibbs' ideas to ordered solids, and a kinetic part developed by L. Farkas, L. Szilard,<sup>1</sup> M. Vomer,<sup>2</sup> R. Becker, W. Döring,<sup>3</sup> and Y. B. Zeldovich<sup>4</sup> in the 1930s and 40s. The main thermodynamic assumptions of classical theory are that both the structure and the faceting of the nucleus replicate those of large crystals. In consequence, the surface free energy of the nucleus equals that of large crystals (and is thus size-independent) and its contribution to the nucleation barrier scales with the nucleus surface area. These assumptions are referred to as the “capillary approximation” and they are readily violated at high supersaturations, at which the nucleus is so small that both its structure and faceting diverge from those of large crystals.<sup>5</sup> More recently, deviations from the classical thermodynamic assumption have been recorded at moderate supersaturations. These include non-equilibrium nucleus shapes<sup>6,7</sup> or nucleus sizes of one molecule that lead to barrier-free nucleation,<sup>8</sup> akin to spinodal decomposition. The central assumption of the kinetic part of classical theory of crystal nucleation is that molecules join the nucleus individually. Whereas the value of the nucleation barrier does not depend on the pathway by which the molecules assemble into a nucleus, the rate of nucleation must. A

cluster comprised  $n + m$  molecules can assemble in multiple ways from the collision of two clusters of any combination of sizes  $n$  and  $m$  (Fig. 1a). Clusters of more than one molecule, however, possess larger surface and carry greater surface free energy. As a result, their concentration is low and the probability of a collision of any cluster with another cluster of size  $m$  is insignificant if  $m$  is greater than one. Guided by the low concentration of clusters with  $m > 1$ , classical theory presumes that nucleation proceeds as successive assembly of single molecules (Fig. 1b), as suggested in the first comprehensive model of crystal nucleation, by L. Farkas,<sup>1</sup> where this argument was attributed to L. Szilard. The “Szilard postulate” is perhaps the most consequential assumption of the classical theories of both nucleation and growth of crystals. Numerous recent examples of growth by association of ordered and disordered mesoscopic and microscopic precursors constitute violations of the Szilard postulate during crystal growth.<sup>9–12</sup> The most heavily discussed nonclassical mode of crystal nucleation involves violation of a corollary of the Szilard postulate, that the molecules join the nucleus directly from the solution. Crystals have been found to nucleate in liquid droplets, amorphous aggregates, mesoscopic clusters, and other solute assemblies.<sup>13–35</sup> A possible explanation of why two-step nucleation hosted by a precursor with high solute concentration is preferred to direct nucleation in the dilute solution centers on the lower surface free energy between the nucleus and its environment during two-step nucleation.<sup>36</sup> For a more detailed discussion of nonclassical nucleation scenarios, see our recent review.<sup>37</sup>

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2 M. Volmer and W. Schultze, *Z. Phys. Chem., Abt. A*, 1931, **156**, 1–22.

3 R. Becker and W. Döring, *Ann. Phys.*, 1935, **416**, 719–752.

4 Y. B. Zel'dovich, *Acta Physicochim. URSS*, 1943, **18**, 1–22.

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8 L. F. Filobelo, O. Galkin and P. G. Vekilov, *J. Chem. Phys.*, 2005, **123**, 014904.

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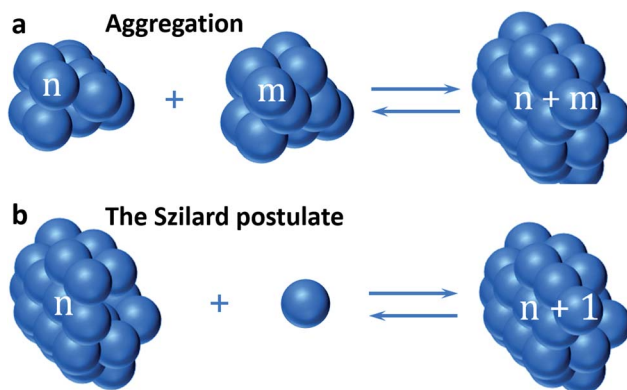


Fig. 1 Models of molecular assembly into nuclei. (a) Aggregation of two clusters comprised of  $n$  and  $m$  molecules, respectively. (b) Cluster growth by association of single molecules.

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- 37 P. G. Vekilov, in *Crystallization via Nonclassical Pathways Volume 1: Nucleation, Assembly, Observation and Application*, American Chemical Society, 2020, vol. 1358, ch. 2, pp. 19–46.

**James De Yoreo** commented: This is an excellent exposition of the issue of “non-classical” nucleation.

**Denis Gebauer** added: I would like to make two additions. Firstly, it has recently been demonstrated that CNT is able to reproduce quintessential features of two-step nucleation pathways,<sup>1</sup> suggesting that they should not be labeled as “non-classical nucleation”. Secondly, the theories of the pre-nucleation cluster (PNC) pathway and two-step nucleation (2S) are fundamentally distinct. The former considers thermodynamically stable solute clusters (*i.e.*, negative standard free energy of formation) as the species that are fundamental to phase separation. According to the PNC pathway, phase separation is then not based upon overcoming a certain critical size, but on dynamical changes within stable solute clusters.<sup>2</sup> There is thus an alternative reaction channel to solids that does not rely on critical nuclei as transition states. The PNC theory does not rely on assigning interfacial free energies to nanoscopic species and can thus explain significant

populations of nucleation precursors (as opposed to CNT and 2S),<sup>3</sup> and with it, also growth modes that rely on species larger than monomers. Please also consider our earlier replies to the questions from Wenhao Sun and Aaron R. Finney.

1 D. Kashchiev, *J. Cryst. Growth*, 2020, **530**, 125300.

2 D. Gebauer, M. Kellermeier, J. D. Gale, L. Bergström and H. Cölfen, *Chem. Soc. Rev.*, 2014, **43**, 2348–2371.

3 D. Gebauer, P. Raiteri, J. D. Gale and H. Cölfen, *Am. J. Sci.*, 2018, **318**, 969–988.

**Christine Kirschhock** asked: You observe a stepwise behavior in function of increasing D<sub>2</sub>O content. Considering that H–D exchange is very fast, is it possible that the crystallising phase is affecting the distribution of isotopes and therewith influencing the speciation in the solvent?

**Denis Gebauer** answered: I think that it is possible, however, we can to a large extent only speculate about how the distribution of isotopes is affected during the early stages of calcium carbonate formation. Having said that, our simulations do indicate that heavy water molecules might have a stronger attraction to calcium cations than light water. This might lead to a bias towards heavy water hydration of mineral constituents already at rather low heavy water contents, which may be a clue to understand the stepwise behavior that we have observed in our experiments.

**Romain Grossier** opened discussion of the paper by Aaron R. Finney: Question 1: you show that CNT agrees well with your simulations for low supersaturations, but differs for “higher” supersaturations. As CNT was developed only for low supersaturation, it's not surprising it fails at higher supersaturations. So, in a way, could we see your simulation as a measure of the supersaturation bandwidth on which CNT is usable?

Question 2: you asked for experimentation data that could be used as force fields benchmarkings. Would solubility (NaCl here) be a pertinent benchmarking to test force fields?

**Aaron R. Finney** responded: Question 1: Classical Nucleation Theory (CNT) can be applied to predict nucleation rates at any level of supersaturation below the limit of solution stability (when considering solutions), *i.e.*, throughout the metastable zone, beyond which spinodal decomposition occurs. At high levels of supersaturation in macroscopic metastable solutions, many nuclei may be present and determining the rates for nucleation in order to compare with CNT predictions may be challenging. In simulations, the number of nucleation events is limited due to finite size effects and following the time evolution of the largest cluster is sufficient to estimate nucleation rates even at the high end of supersaturation in the metastable zone.<sup>1,2</sup>

For the system and force field under investigation in our paper, several other simulation groups established the supersaturation limits for the metastable solution zone at room temperature and pressure ( $S = 1$  and 4.1 at  $b(\text{NaCl}) = 3.7$  and 15 mol kg<sup>-1</sup>, respectively); see, *e.g.*, ref. 3 and 4. At  $S = 3.2$ , simulations indicated that crystal nucleation occurs in a single step *i.e.*, there is concerted growth in the size of crystalline regions in emerging clusters and the total size of



the clusters.<sup>4</sup> At  $S = 3.7$ , far into the metastable zone, our investigation of an extended reaction coordinate space, where we do not presuppose the pathway for the emergence of crystalline order in this space, indicates that a range of nucleation pathways are available for phase separation. Analysis of the free energy landscape presented in Fig. 4A of our paper (<https://doi.org/10.1039/d1fd00089f>) shows that the most probable pathway (highlighted by the red dashed line) involves first an enriching of the ion density in liquid-like transient clusters – which are apparent in solution throughout the entire metastable zone – before restructuring of the ions occurs to form a crystalline lattice. Given the evolution of the system along orthogonal degrees of freedom in the reaction coordinate space at early times during nucleation, we describe this as a two-step pathway.

We speculate that in a macroscopic system, a myriad of possible pathways could contribute to phase separation in the metastable zone and that a shift in the most likely pathway from one- to two-step could occur in the range  $S = 3.2$ – $3.7$ . In addition, we suggest that the notion of a critical cluster be extended to an ensemble of critical clusters which can display a broad range of structures and include sizeable, disordered domains depending upon the reaction conditions.

If one considers the capillarity approximation as a central tenet of CNT, then all the pathways we identify deviate from the theory predictions because the smallest clusters emerging in solution do not have the same properties as the final bulk crystal phase with well-defined facets and chemical ordering. Nonetheless, if one allows for the fact that the smallest clusters on the pathway to crystals can have some intermediate structure and energetic properties between dispersed ions in solution and the final bulk crystal phase, we can reconcile the pathways we observe with the concepts associated with established nucleation theories which follow, *e.g.*, Becker–Döring statistics and the application of (single barrier) transition state theory to a phase separation process. See, *e.g.*, ref. 5. One crucial aspect here is that the lowest energy pathway is represented by an arc length on a multi-dimension manifold which appropriately defines the free energies of states in a reaction coordinate characterising nucleation.

Recent thermodynamic and kinetic frameworks were proposed to describe the driving forces and pathways to nucleation in an extended reaction coordinate, similar to those adopted in our study. One such framework from Kashchiev<sup>6</sup> adopts a composite cluster model which includes an inner crystalline phase surrounded by a metastable amorphous phase embedded in a solution. Depending upon the relative supersaturations between the solution, metastable and crystalline phases, different crystallisation pathways may be observed. In this framework, there is no size dependence on the interfacial tension or chemical potential difference between phases; hence, the author provides theorised energy landscapes for one- and two-step pathways (see Fig. 3 in ref. 6) making full use of CNT. While the energy landscapes we compute are consistent with those theorised elsewhere,<sup>6</sup> we cannot definitively say that the mechanisms we observe are “classical” or “nonclassical” not least because these terms are used somewhat subjectively; indeed, this was evident during this *Faraday Discussions* meeting. In our paper, we opted to refer to one- or two-step pathways as these terms better describe the structural evolution of the system. Nonetheless, we do not find evidence that the mechanisms for NaCl nucleation are inconsistent with the *essence* of established nucleation theories.

Question 2: for studies of crystallisation, or dissolution for that matter, measurements of solubilities and rates for these processes are extremely useful to compare simulation results to and to gauge the range of environmental conditions where the models perform adequately.

For NaCl, the experimental solubility of  $6.15 \text{ mol kg}^{-1}$  seems well established; however, there is still limited experimental data on the NaCl(s) homogeneous nucleation rate. A comparison of the rates from seeding simulations to experimental data was provided by Zimmermann *et al.*<sup>1,2</sup> Heterogeneous nucleation rates covering a range of supersaturations are also hard to come by and any input from experimental groups on this front would be most welcome.

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6 D. Kashchiev, *J. Cryst. Growth*, 2020, **530**, 125300.

**Peter Vekilov** opened discussion of the paper by Yuki Kimura: The nucleus is a cluster at the top of an energy barrier. You stated that there is not a free energy barrier for the formation of a nucleus during dissolution. What do you mean by nucleus during dissolution?

**Yuki Kimura** answered: You are right. To avoid confusion, it might be better to refer to the cluster with the maximum energy as the critical nucleus. That is why we chose our terminology with care in our paper (<https://doi.org/10.1039/d1fd00125f>). If I used the term nucleus for the dissolution process, that was my mistake. Perhaps I may have said nucleus in my talk, but I usually refer to it as particles.

In the paper, we defined particles as objects that have a clearly distinguishable interface with the solution and clusters as objects that do not. I use the terms nucleus and nucleation only for the formation processes that may have critical nuclei due to density fluctuation.

**Gábor Schuszter** asked: During your experiments applying TEM as the observation technique, you are bombarding water with high energy electrons which can cause radiolysis just like in the case of cloud chambers used for alpha particle detection. How can you make sure that this does not affect nucleation? Did you observe any change in the mechanism while varying the accelerating voltage?

**Yuki Kimura** replied: Radiolysis of water by electron irradiation leads to the formation of ions and radicals, and changes in pH. These reach equilibrium on a millisecond time scale. The most effective way to reduce the effect of electron irradiation on nucleation is to lower the electron dose rate. This can be done by observation at low magnification, image processing to brighten dark images, *etc.* However, the effects of the electron beam cannot be completely eliminated, and observation at high magnification is also necessary to visualize small nuclei. It should be considered in conjunction with other techniques such as optical microscopy.

We did not find any significant differences during the observations with two different acceleration voltages of 200 and 300 kV.

**Rik Drummond-Brydson** queried: As far as I understand the dose limits quoted in the paper are for damage of solid (dry) calcium carbonate I think. In the liquid cell, radiolysis of water is the key factor and at a dose rate of  $40 \text{ e}^- \text{ \AA}^{-2} \text{ s}^{-1}$  (this is  $40\text{--}150 \text{ MGy s}^{-1}$ ) you would expect big pH changes for pH 7 and above – with (amongst other things) pH reducing and causing increased solubility of all calcium carbonate polymorphs and also ACC. I think you see this at longer times in your experiments and the behaviour and eventual dissolution is caused by the electron beam. However doesn't this mean that conditions in solution are changing during observation. I think the dose rate needs to be lowered by roughly two orders of magnitude to make conditions more stable.

**Yuki Kimura** responded: You are right. The pH of the solution is decreased by the radiolysis of water by electron beam irradiation. As a result, the solubility of calcium carbonate increases. If we can reduce the electron dose rate from  $1000\text{--}10\,000 \text{ e}^- \text{ nm}^{-2} \text{ s}^{-1}$  to  $10\text{--}100 \text{ e}^- \text{ nm}^{-2} \text{ s}^{-1}$ , the pH decrease will be very small, making the experiment more ideal. To achieve this, we recently developed an algorithm of machine learning to enhance the image clarity. This will lower the electron dose rate by 3–4 orders of magnitude.<sup>1</sup> Please look forward to our next report.

1 H. Katsuno, Y. Kimura, T. Yamazaki and I. Takigawa, *Microsc. Microanal.*, 2022, **28**, 138–144, DOI: [10.1017/S1431927621013799](https://doi.org/10.1017/S1431927621013799).

**Michael Anderson** questioned: Can you do electron diffraction on the particles in real time?

**Yuki Kimura** answered: Unfortunately, it is not possible to perform particle imaging and electron diffraction simultaneously in real time. However, it is possible to acquire (observe *in situ*) electron diffraction alone in real time.

**Michael Anderson** asked: Can you distinguish between an amorphous solid state and a liquid state or is this too difficult?

**Yuki Kimura** responded: For example, it can be inferred from whether two particles merge when they are in contact, or from the difference in contrast when comparing particles of the same size.

**Stéphane Veesler** enquired: In the video you presented, sometimes fringes appeared and disappeared. Are they artefacts or are they real?

**Yuki Kimura** replied: They are real. There are two different kinds of objects formed. One is two dimensionally nucleated thin crystal on the membrane of the liquid cell. Another one is a three dimensionally nucleated tiny particle. I assume that you are talking about the fringes of the thin crystal.

**Jutta Rogal** addressed Aaron R. Finney and Denis Gebauer: Concerning the PMF for ion dissociation, it was shown<sup>1</sup> that the distance between ions is not a good reaction coordinate and that the solvent degrees of freedom need to be considered in the free energy barrier. How confident are you about the PMF? (This actually also applies to the PMF for CaCO<sub>3</sub> in Denis Gebauer's paper).

1 P. L. Geissler, C. Dellago and D. Chandler, *J. Phys. Chem. B*, 1999, **103**, 3706.

**Aaron R. Finney** replied: We agree that a reaction coordinate defined using a single variable quantifying the distance between ions ( $r$ ) is not always sufficient to understand mechanisms for ion association or to evaluate the free energy barriers for such processes. Often an additional collective variable, *e.g.*, quantifying the hydration level of the cation, is at least required to investigate these reactive features, as was shown in other biased simulation studies.<sup>1,2</sup> This is particularly necessary if the timescale for solvent exchange in the first ion coordination spheres is slow relative to the simulation times.

From the potential of mean force (PMF) calculation, our principle objective was to determine the equilibrium constants for ion association ( $K_a$ ) which can be compared with experiments and used in the multiple-binding model for speciation<sup>3</sup> before nucleation, as we discussed. To calculate  $K_a$ , an integral over  $\exp(\text{PMF}(r)/k_B T)$  is performed and a  $4\pi r^2$  factor is included to account for the increasing phase space volume as a function of the distance between ions,  $r$ . So long as the PMF profile is converged in the reaction coordinate space, the value of  $K_a$  is accurately evaluated and a single variable,  $r$ , is sufficient to establish the relative probabilities of bound and unbound ion pair states. In this regard, we performed 5 ns of simulations for each of the umbrella windows sampled. We are very confident that these are converged, not least because the uncertainty bars shown in Fig. 1E of our paper (<https://doi.org/10.1039/d1fd00089f>) are very small on the energy scale provided. Furthermore, the PMF is a very close match to those evaluated elsewhere in the literature.<sup>4</sup>

1 P. Raiteri, *et al.*, *J. Phys. Chem. C*, 2015, **119**, 24447–24458.

2 A. R. Finney, *et al.*, *Chem.–Eur. J.*, 2019, **25**, 8725–8740.

3 D. Gebauer, *et al.*, *Science*, 2008, **322**, 1819–1822.

4 C. Zhang, *et al.*, *Nat. Commun.*, 2020, **11**, 3037.

**Denis Gebauer** added: We are aware of this problem. That is why the original PMF for the force field was measured as a 2D PMF of ion-distance and cation–water coordination number, see Fig. 3 in ref. 1. This was found to be a better representation for a PMF between ions.<sup>2,3</sup>

We compared the reduction of the 2D PMF to 1D with a PMF that was computed in 1D and found them to be very similar. Thus, we decided to limit our calculations for D<sub>2</sub>O to 1D PMFs. Please also note that we use these 1D PMFs only for comparison and for an assessment of relative binding strengths. We do not investigate the underlying dissociation mechanism. This would indeed need a 2D PMF.

1 P. Raiteri, R. Demichelis and J. D. Gale, *J. Phys. Chem. C*, 2015, **119**, 24447–24458.

2 A. J. Ballard and C. Dellago, *J. Phys. Chem. B*, 2012, **116**(45), 13490–13497.

3 R. Gotchy Mullen, J.-E. Shea and B. Peters, *J. Chem. Theory Comput.*, 2014 **10**(2), 659–667.

**Jutta Rogal** continued discussion of the paper by Aaron R. Finney: Do you have an idea of how the relative path probability of the different nucleation mechanisms depends on, for example, the supersaturation?

**Aaron R. Finney** responded: We have not explicitly evaluated the pathways for nucleation at lower levels of supersaturation, although this is perhaps the focus of future work. From the Markov state model we present, at  $S = 3.7$  the thermodynamic barrier for the most-probable two-step pathway is  $2k_{\text{B}}T$  lower in energy than the one-step pathway, but both of these types of mechanisms contribute to the phase separation and there is a myriad of possible routes from supersaturated solutions to crystals.

Previous computational studies using the same force field have shown that at  $S = 3.2$ , the growth in the size of crystalline regions in clusters during nucleation linearly correlates with the size of the clusters, and nucleation was described as one-step.<sup>1</sup> It is important to note that close inspection of the cluster population distribution at small cluster sizes in those studies (see middle panel of Fig. 7 of ref. 1) appears to indicate that a relatively wide range of (largest) cluster sizes are accessible to the system before significant crystalline order emerges. In support of the one-step mechanism at  $S = 3.2$ , the results from seeding simulations indicated that the rates for crystal nucleation – determined within a CNT framework from the rates at which crystal seeds grow or shrink in simulations – can be aligned to the NaCl crystal nucleation rates determined in experiments when an appropriate definition for ions within crystalline embryos is adopted.<sup>2</sup>

In light of these results, we speculate that there is a change in the most likely pathway from predominantly one- to two-step between  $S = 3.2$  and  $3.7$  in the metastable zone. However, we also speculate that in a macroscopic system, two-step pathways could contribute to phase separation – albeit with often lower probability than one-step pathways (below the proposed transition region in  $S$ ) – across the entire metastable zone. This is because, as shown in Fig. 1B of our paper (<https://doi.org/10.1039/d1fd00089f>), extended ionic networks are observed with increasing probability as  $S$  is increased, matching the results from recent experiments.<sup>3</sup> Our earlier work,<sup>4</sup> considering NaCl(aq) solutions at carbon surfaces, indicates that interfaces promote the formation of extended liquid-like networks, which raises the possibility that two-step pathways dominate the phase separation at lower levels of  $S$  than in the bulk solutions in the case of heterogeneous nucleation.

1 H. Jiang, *et al.* *J. Chem. Phys.*, 2019, **150**, 124502.

2 N. E. R. Zimmermann, *et al.*, *J. Chem. Phys.*, 2018, **148**, 222838.

3 H. Hwang, *et al.*, *Chem. Sci.*, 2021, **12**, 179–187, DOI: [10.1039/D0SC04817H](https://doi.org/10.1039/D0SC04817H).

4 A. R. Finney, *et al.*, *Chem. Sci.*, 2021, **12**, 11166–11180, DOI: [10.1039/D1SC02289J](https://doi.org/10.1039/D1SC02289J).

**Sarah Price** remarked: This is a comment on the earlier question about deuterating other solvents.

The effects of deuterating other solvents could be even larger. The thermodynamically stable form of isotopically normal pyridine (pyridine- $h_5$ ) between the melting point (231 K) and 5 K is the complex structure of form I ( $Pna2_1$ ,  $Z' = 4$ ), and it can be cooled or heated within this temperature range without undergoing any phase transitions. In contrast the thermodynamically stable form of pyridine-

d5 below 215 K is the simpler phase II ( $P2_12_12_1$ ,  $Z' = 1$ ).<sup>1,2</sup> Such a difference in crystal structures seems likely to affect the structure of pyridine as a solvent.

I would like to comment on force-field validation.

It would be good if all computational papers were very explicit about the evidence for their force-field or functional and simulation method being appropriate for the study. The force-fields that can be used in most molecular dynamics simulations are usually inadequate for crystal structure prediction studies. So knowing whether the force-field used for nucleation studies can reproduce the known polymorphs, and how the calculated relative stability compares with experiment, can be important in drawing conclusions from the simulation results. It can be harder to test the solute–solvent force-field, for example by comparing the calculated and observed solubility. However, defining the supersaturation relative to the calculated solubility with the force-field, as was done in this paper (<https://doi.org/10.1039/d1fd00089f>), can go a long way towards compensating for the limited accuracy of the force-field.

- 1 S. Crawford, M. T. Kirchner, D. Blaser, R. Boese, W. I. F. David, A. Dawson, A. Gehrke, R. M. Ibberson, W. G. Marshall, S. Parsons, *et al.*, Isotopic polymorphism in pyridine, *Angew. Chem., Int. Ed.*, 2009, **48**(4), 755–757.
- 2 N. Giordano, C. M. Beavers, B. J. Campbell, V. Eigner, E. Gregoryanz, W. G. Marshall, M. Peña-Álvarez, S. J. Teat, C. E. Vennari and S. Parsons, High-pressure polymorphism in pyridine, *IUCrJ*, 2020, **7**, 58–70, DOI: [10.1107/S2052252519015616](https://doi.org/10.1107/S2052252519015616).

**Kevin Roberts** added: I have no comments to add regarding the issue of solvent deuteration.

Regarding the issue of force-field validation, I would comment that existing forcefields are broadly speaking quite appropriate for modelling organic systems. Whether crystal structure prediction (CSP) is an optimal test of forcefields validation is perhaps an open question as it predicts an energy landscape for bulk crystal structures governed by the periodic boundary conditions for a fully formed microscopic crystal structure. In contrast, the structural chemistry at nucleation is though more likely to be governed by the surface free energies of molecular clusters. In this, many of their constituent molecules will be under-coordinated and hence translationaly displaced with respect to their bulk crystallographic structures and where, for flexible molecules, conformational variability molecule-to-molecule can also be expected. As the clusters grow the variability can be expected to decrease as the longer-range forces from the bulk structure dominate, see *e.g.* ref. 1. Hence, it is a challenge for CSP to predict structures with forcefields without some allowance for the size-dependent simulation of structural energetics.

- 1 R. B. Hammond, K. Pencheva and K. J. Roberts, Structural variability within, and polymorphic stability of, nano-crystalline molecular clusters of L-glutamic acid and D-mannitol, modelled with respect to their size, shape and 'crystallisability', *CrystEngComm*, 2012, **14**, 1069–1082.

**Matteo Salvalaglio** commented: Comparing experimentally measurable quantities, such as solubility, with estimates obtained from molecular simulations is a complex and often challenging task. This challenge reflects an inherent tension between two complementary aspects of molecular simulations that must simultaneously be satisfied to obtain precise and accurate estimates of

macroscopic observables. These two aspects are the accuracy of the model used to represent the molecular system of interest and the quality of the sampling of the configurations accessible to that system in the relevant thermodynamic conditions for the problem.

The tension between these two aspects originates from the fact that the accuracy in the model used to represent a system drastically affects its computational cost and, thus, its application in calculations that require extensive sampling. This means that expensive and accurate models are impractical for simulating large systems for long timescales. The dual aspect of this issue is that often we can converge ensemble properties *via* sampling only for simplified and inherently inaccurate models. This tension naturally leads to identifying acceptable compromises every time simulations are deployed, especially in the context of crystallization. Such compromises depend on the system studied, the process investigated, and ultimately, the research question at the heart of a simulation campaign. Studying crystallization from complex liquids with molecular dynamics requires large systems, long timescales and often complex sampling algorithms to overcome activated events. In this case, favouring sampling under controlled conditions (*i.e.* known solubility of the model solute in the model solvent) is a typical choice, see, for instance, Aaron R. Finney's paper (<https://doi.org/10.1039/d1fd00089f>) and the discussion of it and Jutta Rogal's paper (<https://doi.org/10.1039/d1fd00099c>). The calculation of accurate lattice energies sits on the opposite end of the spectrum, where minute energy differences can drastically change conclusions. Thus, expensive electronic structure methods are needed to answer specific research questions. Awareness of this tension between model accuracy and sampling allows one to formulate better research questions, find more effective compromises in the choice of models, and ultimately extract usable insight on length and timescales inaccessible to direct experimental observation.

**Joonsoo Kim** continued discussion of the paper by Yuki Kimura: In the video, the brightness of the big crystal in the bottom left corner is fluctuating. Is that an artefact or change in the crystallinity?

**Yuki Kimura** replied: Diffraction contrast is the significant factor affecting crystal contrast in TEM images. Electron beams are diffracted when they are incident parallel to the crystal plane. If the diffracted electron is blocked by an aperture, the contrast becomes stronger (the crystal becomes darker). When the image is formed without being blocked by an aperture, the electron beam returns to a position slightly displaced from the crystal by the focal point. This location becomes brighter. The main reason for the fluctuating brightness of the crystal in the lower left is probably that the orientation of the crystal has changed slightly because it is in aqueous solution.

**Wenhao Sun** continued the general discussion: To address the earlier questions on validating theoretical models; I would just like to add that if we are using molecular dynamics potentials, it is important that the potentials are programmed to have the proper physics. For example, if you are studying a chemical reaction involving bond breaking, your potential should be able to account for that. If you are hoping to capture stericity or bond angles, your potential should

account for that. There has been this discussion about theorists *vs.* experimentalists in this session thus far, which from my perspective, has mostly been experimentalists hoping that theorists can provide insights that are more actionable than we have so far. I have a criticism for the experimentalists, and perhaps for our community in general. **It would be best if our community decreased our reliance on the “Energy Landscape” picture**, which envisions a chemical reaction as a ball rolling on an energy landscape with hills and valleys; where valleys indicate the metastable or stable states (thermodynamics) and the hills represent the activation energy barriers (kinetics). This picture was developed for changes in molecular conformations, but **it is not proper to apply the energy landscape picture to solid-state transformations such as nucleation**, where the number of atoms are changing throughout the course of the reaction. There are two major arguments for this: (1) the *y*-axis is a generic ‘energy’ term, but energy is an extensive quantity, meaning it depends on the mol number of atoms being considered. For the ‘valleys’, which represent stable phases, we might have units of  $\text{kJ mol}^{-1}$ . However, for the barriers, the units of the barriers depend on the mechanism. If the barrier is a nucleation barrier, it should be energy per nucleus. If it is a diffusion barrier, the units would be energy per atomic hop. **It is not meaningful to put on a single figure both the bulk energies and the barriers at the same time.** (2) The reaction coordinate is often very vaguely defined. It sometimes refers to time, sometimes applied reaction conditions, sometimes a conformation. As a theorist, this is very frustrating, because **if you can’t say what it is, we cannot calculate it.**

The problem with the energy landscape picture is that it is conceptually lazy and theoretically non-rigorous. Overall, it would promote theory/experimental collaboration if instead of using the energy landscape cartoon, we altogether moved to more precise descriptions of both the *y*-axis and the *x*-axis when we discussed thermodynamics and kinetics. It is frustrating for me to see a beautiful experimental observation in the literature, that has a discussion section with an ‘energy landscape’ rationalization of the mechanisms. Remember that scientific theory should be falsifiable. If the energy landscape picture can describe everything, then it cannot predict anything. The energy landscape picture is not a robust foundation to build a theory of nucleation or crystallization.

**Denis Gebauer** responded: I am not sure if I can generally agree with your comment. Even though nucleation theories consider barriers per nucleus, the barrier itself corresponds to a transition state that we can formally assign a standard free energy, and with it, also an equilibrium constant (in analogy to the valleys in free energy landscapes). It should thus always be possible, within the framework of a theory, to convert or normalize the energetics so as to illustrate barriers and valleys in the same diagram. Having said that, in my opinion, the free energy landscape illustrations are often used for the lack of a better description or quantitative expression, and I have seen this in both experimental and theoretical papers. I still believe that these illustrations are useful for demonstrating a principle idea, and they should be taken as what they are – cartoons (as you have rightfully pointed out). This cartoon perspective is then also often useful to illustrate basic differences between theories, without claiming any explanatory or predictive power going beyond that intention. I am sorry to have caused frustration by using these cartoons in our own works, however, the actual purpose



always was to spark discussions, *e.g.*, on proper reaction coordinates, and to catalyse the development of new models. Finally, please note that for the illustration in ref. 1, we can now actually say what it is in terms of a quantitative model,<sup>2</sup> and look forward to theoreticians calculating it.

1 D. Gebauer, P. Raiteri, J. D. Gale and H. Cölfen, *Am. J. Sci.*, 2018, **318**, 969–988.

2 J. T. Avaro, S. L. P. Wolf, K. Hauser and D. Gebauer, *Angew. Chem., Int. Ed.*, 2020, **59**, 6155–6159.

**Kristen Fichthorn** remarked: Having accurate force fields is, of course, important in modelling experiments. However, force fields usually have deficiencies and even quantum calculations are not always accurate. As we listen to the talks presented here, we will see that experimentalists do not always know what they are measuring and they often have to do many different types of experiments to reach a conclusion. Theory is valuable in suggesting new mechanisms, new possibilities, revealing trends, and helping experimentalists tell their stories, not just predicting data to high accuracy. After all, theoreticians are not just accountants.

**Ruel Cedeno** opened discussion of the paper by Alexander Van Driessche: Sulfate ions have been shown to form large solvation shells in water.<sup>1</sup> To some extent, this also occurs with strontium ions.<sup>2</sup> These shells may cause charge shielding which could reduce the sensitivity of the probes. This effect might be more pronounced at lower dosing rate since such solvation shells are more well defined in dilute solutions. Conversely, the hydration numbers decrease in concentrated solution.<sup>3</sup> Could this phenomenon play a role in the apparent consumption of neutral species (prenucleation clusters) during nucleation at low dosing rate which is not detected by the ion selective electrode (ISE)?

1 J. T. O'Brien, J. S. Prell, M. F. Bush and E. R. Williams, *J. Am. Chem. Soc.*, 2010, **132**, 8248–8249, DOI: [10.1021/ja1024113](https://doi.org/10.1021/ja1024113).

2 P. D'Angelo, V. Migliorati, F. Sessa, G. Mancini and I. Persson, *J. Phys. Chem. B*, 2016, **120**, 4114–4124, DOI: [10.1021/acs.jpcc.6b01054](https://doi.org/10.1021/acs.jpcc.6b01054).

3 A. V. Dighe and M. R. Singh, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 23954–23959, DOI: [10.1073/pnas.1910691116](https://doi.org/10.1073/pnas.1910691116).

**Alexander Van Driessche** replied: It is unlikely that the solvation shells described in the above works play a significant role in our measurements. The solvation shell for Sr<sup>2+</sup> ions described in the source provided<sup>1</sup> was derived from a XANES measurement taken of a bulk liquid (volume unspecified) which would have averaged the absorption spectra across all Sr atoms present in the solution. Thus, the absorption spectrum must be primarily providing information on what we, in our work, considered “free” Sr<sup>2+</sup> ions as they are the majority species – in other words, we are measuring the hydrated Sr<sup>2+</sup> ions with the ISE. It is also important to note that the particularly stable hydration shells of  $n \sim 40$  for SO<sub>4</sub><sup>2-</sup> ions found in ref. 2 may change with supersaturation in a manner not dissimilarly to the  $41 < n < 47$  solvation shells simulated for glutamic acid.<sup>3</sup> It is a much larger leap in logic to presume that the measured and simulated hydration shell of  $n = 8$  described in the Sr XANES study would necessarily be subject to the same effects. It is not theoretically impossible that there are some strongly hydrated Sr ions that are shielded from the ISE and participate in the early stages of nucleation.

However, there is no data in our experiment, or any that we know of in literature, that would support this assertion.

- 1 P. D'Angelo, V. Migliorati, F. Sessa, G. Mancini and I. Persson, *J. Phys. Chem. B*, 2016, **120**, 4114–4124, DOI: [10.1021/acs.jpcc.6b01054](https://doi.org/10.1021/acs.jpcc.6b01054).
- 2 J. T. O'Brien, J. S. Prell, M. F. Bush and E. R. Williams, *J. Am. Chem. Soc.*, 2010, **132**, 8248–8249, DOI: [10.1021/ja1024113](https://doi.org/10.1021/ja1024113).
- 3 A. V. Dighe and M. R. Singh, *PNAS*, 2019, **116**, 23954, DOI: [10.1073/pnas.1910691116](https://doi.org/10.1073/pnas.1910691116).

**Aaron R. Finney** questioned: In the paper (<https://doi.org/10.1039/d1fd00092f>), you indicate that at the lowest dosing rates, the transmittance probe detected the formation of a *new phase* before the consumption of dispersed ions indicated by the change in the ISE signal (*i.e.*, the inflection point in the LaMer diagram). You suggest that this phase emerges *via* consumption of neutral species *e.g.* ion pairs. What is the nature of this phase and can you comment on the possible comparison between the bound species (prenucleation clusters) in  $\text{SrSO}_4(\text{aq})$  *cf.*  $\text{CaCO}_3(\text{aq})$ ? Furthermore, how does your model differentiate between the nanoclusters and dense liquids/amorphous phases (see Fig. 1 in the paper; <https://doi.org/10.1039/d1fd00092f>) that could be involved in the nucleation pathway?

**Alexander Van Driessche** responded: Neither the probes used in the titration experiments, nor the model used in this work have the implicit capacity to precisely determine the nature of the (pre)nucleation species present during the nucleation process. In fact, the model is completely independent of any chemical information about what is nucleating. It provides only information about the relative density of nucleating phase as it traverses a free energy landscape from a perfectly mixed fluid state to a thermodynamically stable solid phase. Similarly, the potentiometric and optic techniques used in the titrations serve primarily to characterize the solution, *i.e.* the fluid that is not nucleating. For example, the ion selective electrode characterizes the quantity of unbound  $\text{Sr}^{2+}$  ions in the solution, but does not provide a direct counting or characterization of the ions consumed in the reaction – any measures of those atoms are derived through subtraction from measurements carried out in the absence of any nucleation reaction (or pre-nucleation events). The most information gleaned about the nature of the phases formed during the titrations comes from the photometric sensor, and that is limited to a few minor details. We know that the phase is different enough from the solution surrounding it to form a light-scattering interface, and we can recover a limited qualitative measure of the concentration of those interfaces (higher turbidity = more interfaces). From the ISE data, and the information gleaned about bound species concentration, we could also attempt to extract some binding constants for the bound species and their dependence on time and supersaturation, but this analysis would create estimations blind to the actual structure and number of atoms contained in the bound species. Thus, we do not wish to speculate too extensively on the nature of the first formed phases. To obtain more insight on the nature of the transitory precursor cluster species other tools are necessary. For example, the structural properties of  $\text{CaSO}_4$  precursor clusters have been derived by combining *in situ* high-energy X-ray diffraction experiments and molecular dynamics (MD) simulations.<sup>1</sup>

The pathway monitored by ISE and the photometric sensor is comparable to the pathway observed for  $\text{CaCO}_3$  (ref. 2) using an identical setup and similar to the pathway revealed for  $\text{CaSO}_4$  using *in situ* X-ray scattering techniques.<sup>3,4</sup> The only aspect that awaits experimental confirmation is the existence of  $\text{SrSO}_4$  and  $\text{CaSO}_4$  prenucleation clusters in the undersaturated regime, as has been shown for  $\text{CaCO}_3$  prenucleation clusters. In any case, our model does predict the existence of a prenucleation population for undersaturated solution conditions. The model used in this work (initially introduced in ref. 5) reveals that before crossing the nucleation barrier a kinetically induced cluster population appears; yet this does not represent a true thermodynamic phase. Eventually, the system will cross the barrier and a new phase will nucleate with a density close the final one. Noteworthy, after crossing the nucleation barrier the system will continue to (slowly) evolve approaching the ideal density of the crystalline phase. Taking into account this pathway, the population of kinetically induced clusters would correspond to nanoclusters/PNCs shown in Fig. 1 in the paper (<https://doi.org/10.1039/d1fd00092f>), and are clearly differentiated in our model because these occur on the pathway before crossing the nucleation barrier. The first nucleated phase could potentially correspond to an amorphous phase because the density of this phase has not yet reached unity. Of course, this is one interpretation of the results and cannot be unequivocally confirmed from the current model. To do so, the simulations need to be run considering crystallinity as the variable, and not density as was done in the current work. In any case, the behavior observed in our model has been observed experimentally using *in situ* scattering during  $\text{CaSO}_4$  precipitation at room and high temperature.<sup>3,4</sup> In these experiments, nanosized clusters formed first and subsequently aggregated into an amorphous phase (which is the first phase detected by an optical sensor). Eventually, a crystal structure develops through the reorganization of the nanosized clusters inside the disordered phase. Noteworthy, this reorganization continues long after the apparent precipitation reaction has finished. This is akin to what we observed in our model, where once the new phase has nucleated it will continue to evolve approaching the final density.

- 1 T. M. Stawski, A. E. S. Van Driessche, R. Besselink, E. H. Byrne, P. Raiteri, J. D. Gale and L. G. Benning, *J. Phys. Chem. C*, 2019, **123**, 23151–23158, DOI: [10.1021/acs.jpcc.9b04268](https://doi.org/10.1021/acs.jpcc.9b04268).
- 2 P. I. Schodder, M. B. Gindele, A. Ott, M. Rückel, R. Ettl, V. Boyko and M. Kellermeier, *Phys. Chem. Chem. Phys.*, 2022, **24**, 9978–9989, DOI: [10.1039/D1CP05606A](https://doi.org/10.1039/D1CP05606A).
- 3 T. M. Stawski, A. E. S. Van Driessche, M. Ossorio, J. D. Rodriguez-Blanco, R. Besselink and L. G. Benning, *Nat. Commun.*, 2016, **7**, 11177, DOI: [10.1038/ncomms11177](https://doi.org/10.1038/ncomms11177).
- 4 T. M. Stawski, R. Besselink, K. Chatzipanagis, J. Hövelmann, L. G. Benning and A. E. S. Van Driessche, *J. Phys. Chem. C*, 2020, **124**, 8411–8422, DOI: [10.1021/acs.jpcc.0c01041](https://doi.org/10.1021/acs.jpcc.0c01041).
- 5 M. A. Durán-Olivencia, P. Yatsyshin, S. Kalliadasis and J. F. Lutsko, *New J. Phys.*, 2018, **20**, 083019, DOI: [10.1088/1367-2630/aad170](https://doi.org/10.1088/1367-2630/aad170).

**Alan Hare** enquired: Given that in Fig. 3 in the paper (<https://doi.org/10.1039/d1fd00092f>) the U-shaped curve  $\rho_0$  tends towards a CNT limit, are you now able to draw any inference concerning the cluster shape? (I realise that this question could have a binary answer.)

**Alexander Van Driessche** answered: Clusters (or density fluctuations) are assumed to be spherically symmetric in mesoscopic nucleation theory (MeNT), consequently we only observe a spherical shape. Recently, a further generalization

of MeNT has been put forward by Lutsko<sup>1</sup> allowing also for non-spherically symmetric shapes to be considered. However, the resultant formalism equations are considerably more complicated than the ones involved in the spherically-symmetric MeNT.

1 J. F. Lutsko, *New J. Phys.*, 2018, **20**, 103015, DOI: [10.1088/1367-2630/aac174](https://doi.org/10.1088/1367-2630/aac174).

**Stéphane Veessler** queried: Nucleation is a localized phenomena there is a resolution issue by using a global measure such as a conductimetric one to detect it. You do not know where nucleation will occur. Cannot we say that the  $\Delta t$  you observed is (by analogy) the growth time to a detectable size, this growth time being classically used in the interpretation of induction time measurement experiments?

**Alexander Van Driessche** responded: Indeed, it is important to consider growth when describing the nucleation events in the titration experiments. We do consider that the photometric sensor detects nucleation not at the formation of the first light scattering interface, but at the moment when the concentration/size of particles (and thus total amount of interfaces) present is significant to overcome the resolution limits of our sensor. Similarly, the ion selective electrode only detects nucleation when the change in free ion counts is significant enough to generate measurable change in the potential on the probe membrane – this would theoretically happen after the concentration of bound ions is greater than the detection limit for  $\text{Sr}^{2+}$  ions in solution. If we consider that the sensitivity threshold of both probes are comparable, then even if we do not detect only nucleation, but a combination of nucleation and growth, our main observation is still valid because when the photometric sensor is detecting the formation of a new phase, the ion selective electrode does not detect any change. Hence, the nucleation (and growth) of this new phase is mainly consuming bound species. We can also conduct a thought experiment where there is a distinct difference in detection limits for the probes. In this thought experiment, the nucleation process is assumed to be identical (classical) across all concentration ranges. We imagine classical nuclei that grow in size and number until the amount of interface present is enough to be detected by the photometric sensor. Some time later the concentration of nuclei increases until enough ions have been removed from the solution to be measure potentiometrically. This is a perfectly reasonable explanation for the results of a single titration with one supersaturation rate. However, if this were the complete picture for how nucleation happens in this system, we would expect the sequence of particle detection to remain unchanged regardless of the rate of ion addition. We would anticipate the same probes to have the highest sensitivity regardless of nucleation and growth rates. This is not what we see in the experiments.

**Joonsoo Kim** requested: Intuitively, larger nanoclusters should be more present at higher supersaturation but based on the observation in this work, larger nanoclusters are involved in the nucleation process when the concentration is low. May I ask your perspective?

**Alexander Van Driessche** replied: The involvement of large clusters in the nucleation pathway (step 1, Fig. 3 in the paper; <https://doi.org/10.1039/d1fd00092f>) mentioned in this work requires us to first reconsider the definition of “cluster”. Within the classical realm, namely Classical Nucleation Theory (CNT), clusters are thought to be small replicas of the final stable phase. This definition (also referred to as “capillary approximation”) leads to one of the main sources of problems for CNT, since it imposes the simplest possible nucleation pathway. Within the Mesoscopic Nucleation Theory (MeNT) framework, the concept of cluster is defined as the excess density with respect to the mother phase. This general definition, which lies at the core of MeNT, allows for the construction of a much more detailed theory of nucleation with enough flexibility to consider a much richer family of cluster and nonclassical nucleation pathways, and not just the classical kind. Within this context, the larger clusters (*i.e.* large-size–low-density fluctuations) that appear at the onset of the nucleation pathway obtained from MeNT, and are discussed in this work, are much larger in size than one molecular radius, but have an inner density that is much lower than the final phase and close to the mother phase. In this sense, the large clusters reported in this work are not replicas of the final phase. Instead, they are density fluctuations which extend several molecular radii in size, but whose intensity (or simply termed, average inner density) is very low. These initial large “clusters” are present in the nucleation pathway predicted by MeNT irrespective of the supersaturation.

**Matteo Salvalaglio** asked: The MeNT allows to describe nucleation using a multidimensional reaction coordinate space function of density and radius of the nucleus. Would it be possible to extend this approach to introduce additional parameters, such as measure of the order in the clusters/nuclei? Do you think there is a practical limit in the number of descriptors considered in this framework?

**Alexander Van Driessche** responded: Indeed, MeNT allows including as many order parameters as one might consider relevant to model nucleation. Some examples of other reaction coordinates considered under MeNT can be found, *e.g.*, in a previous work by Durán-Olivencia and Lutsko.<sup>1</sup> Additionally, a further extension of MeNT to consider other order parameters was carried out by Lutsko<sup>2</sup> in recent years. This new extension provides a roadmap to systematically develop nucleation theories considering all types of cluster geometries and reaction coordinates. Although there is no easy rule of thumb, or practical limit, to decide the optimal number of descriptors, we have observed that the complexities of the resultant equations grow exponentially with the number of order parameters.

1 M. A. Durán-Olivencia and J. F. Lutsko, *Phys. Rev. E*, 2015, **91**, 022402, DOI: [10.1103/PhysRevE.91.022402](https://doi.org/10.1103/PhysRevE.91.022402).

2 J. F. Lutsko, *New J. Phys.*, 2018, **20**, 103015, DOI: [10.1088/1367-2630/aae174](https://doi.org/10.1088/1367-2630/aae174).

**Ivo B. Rietveld** opened discussion of the paper by Joop ter Horst: It is clear that the detection rates of the crystals are very different in the case of seeding and in the case that no seeding is taking place. But how can we be sure that the seeded solutions are not simply showing crystal growth of the seeds; how can we prove

that secondary nucleation is taking place (and how is secondary nucleation defined exactly)? And to go a bit further still, is there a possibility to decide whether CNT is valid in these nucleation experiments? Or are other processes possible, or even more likely (besides secondary nucleation)?

**Joop ter Horst** answered: As we operate the continuous antisolvent crystallization process for over 6 residence times and each residence time a lot of product mass is formed compared to the seed mass used, substantial secondary nucleation must be happening in order to create new crystals of the same chirality. Secondary nucleation follows a completely different mechanism compared to Classical Nucleation Theory or other primary nucleation mechanisms.

**Aurora Cruz-Cabeza** enquired: I really enjoyed this contribution Joop, thank you. I was wondering if the way the anti-solvent is added to the crystalliser matters to the resulting nucleation mechanisms (primary *versus* secondary).

**Joop ter Horst** responded: Thanks Aurora. I showed in the paper that secondary nucleation is dominant over primary nucleation. The secondary nucleation rate, whether it is shear- or attrition-based, is determined by the stirrer speed, number of crystals present and the bulk supersaturation, among others. The primary nucleation rate in continuous antisolvent crystallization is determined by the local supersaturation at the points where solution or antisolvent is mixed with the bulk solution. In order to increase the importance of primary nucleation one indeed can try to increase the local supersaturation in the continuous process by changing the local mixing. One way to create extreme local supersaturations is by pre-mixing the antisolvent and solution inflows before they enter the crystallizer. This might then result in a dominance of primary over secondary nucleation. There are many different ways to mix process streams and it would be very interesting to investigate the relative dominance of the nucleation mechanisms based on the local mixing conditions (and on parameters influencing secondary nucleation rate).

**Ian Ford** questioned: Are the crystals sufficiently fragile to break up to produce seeds for secondary nucleation under the typical shear stresses in the flow or impact with the mixer? The fragility must depend on crystal size as well as intrinsic strength. Is there experimental evidence for this?

**Joop ter Horst** replied: We find experimental evidence for secondary nucleation in the continuing enantiopurity of the product. As we operate the continuous antisolvent crystallization process for over 6 residence times and each residence time a lot of product mass is formed compared to the seed mass used, substantial enantioselective secondary nucleation must be happening in order to create new crystals of the same chirality.

**Weronika Kras** asked: I was wondering whether it is possible for the two enantiomers to have formed a solid solution with a crystal structure resembling one of the enantiomers?

**Joop ter Horst** responded: There are three main possibilities for enantiomers to crystallize from a racemic solution. (1) As a racemic compound, which is a 1 : 1 co-crystal of the two enantiomers. This happens for about 90% of the chiral compounds. (2) As a conglomerate, a physical mixture of chirally pure crystals, which happens for about 10% of the chiral compounds. When a chiral compound forms a conglomerate preferential crystallization and crystallization-enhanced deracemization techniques such as Viedma ripening can lead to an enantiopure crystalline product. (3) As a solid solution, for which the two enantiomers can both (more or less) occupy the same lattice position. This happens only occasionally.

In our paper (<https://doi.org/10.1039/d1fd00098e>) we used sodium bromate which is a compound that forms achiral ions in solution, but crystallizes in a chiral space group and therefore can form left- and right-handed crystals. So in effect it is a conglomerate system.

**Alfred Y. Lee** enquired: A single crystal seed strategy is described. Can the author comment on the potential impact of the shape and particle size of the seeds on the crystallization outcome and the solid-state properties of the end product? Does it not matter since it is an attrition-based secondary nucleation mechanism?

**Joop ter Horst** replied: The main parameters influencing secondary nucleation rate, whether it is shear- or attrition-based, are stirrer speed, crystal number and size, and the bulk supersaturation, among others. The size is important in attrition-based secondary nucleation as above a certain crystal size the crystals will have difficulty following the flow patterns close to the turbulently mixed stirrer region. Large particles then have a higher chance to collide with the stirrer blades and be attrited. The shape can also have an effect: as an example, needle crystals might be easier to break in the case of attrition-based secondary nucleation. In the paper (<https://doi.org/10.1039/d1fd00098e>) we focused on the enantiomeric excess of the product in comparison to the seed crystals. We did not yet look further into the effect of seed size and shape on the antisolvent crystallization of sodium bromate.

**Jan Sefcik** remarked: Both mechanical impact and fluid shear may be important in inducing secondary nucleation. While it is often assumed that mechanical impact and related crystal attrition is the main cause of secondary nucleation, it has been observed that fluid flow around a suspended crystal can also lead to secondary nucleation,<sup>1–3</sup> so attrition is not necessary. When antisolvent crystallisation is used, the manner of antisolvent addition can influence primary nucleation kinetics.<sup>4</sup> There may be intermittent heterogeneities in local composition due to differences in local mixing environments, although significant supersaturation overshoots over values corresponding to fully mixed conditions are not expected in antisolvent crystallisation systems.<sup>5</sup>

1 C. Y. Sung, *et al.*, *AIChE J.*, 1973, **19**, 957–962.

2 J. Wang and J. Estrin, *Chem. Eng. Commun.*, 1996, **152–153**, 275–286.

3 M. Yousuf and P. J. Frawley, *Cryst. Growth Des.*, 2018, **18**, 6843–6852.

4 L. A. I. Ramakers, *et al.*, *Cryst. Growth Des.*, 2020, **20**, 4935–4944.

5 R. Miller, *et al.*, *Cryst. Growth Des.*, 2022, **22**, 2192–2207.

**Dezsó Horváth** queried: Based on Kondepudi's findings<sup>1</sup> in the crystallization of sodium chlorate, the suppression of primary nucleation is essential in maintaining the asymmetry during the competition between L- and D-crystals, besides enhancing secondary nucleation. Does this mean that in your system the residence time in the tank reactor has to be kept shorter than the induction time associated with primary nucleation?

1 D. K. Kondepudi, R. J. Kaufman and N. Singh, Chiral symmetry breaking in sodium chlorate crystallization, *Science*, 1990, **250**(4983), 975–976, DOI: [10.1126/science.250.4983.975](https://doi.org/10.1126/science.250.4983.975).

**Joop ter Horst** answered: I should indeed have added a references to Kondepudi's nice paper.<sup>1</sup> In their batch-wise evaporative crystallization experiments it is not so much the suppression of unselective primary nucleation but rather the enhancement of chirally selective secondary nucleation that is responsible for the single chirality product from stirred solutions. The primary nucleation of the first crystal with a specific chirality gives rise to secondary nucleation of all other crystals having the same chirality.

In our continuous antisolvent crystallization experiments we use single chirality seed crystals to steer the product outcome to the same chirality as the seed crystals. This actually proves that a secondary nucleation mechanism is at play in this process, as only such a mechanism can be chirally selective due to the presence of the parent seed crystals. In the continuous process the seed material is gradually removed with the outgoing suspension flow. New crystals are created by secondary nucleation since chirality is maintained more than 6 residence times. These secondary nuclei have grown to undergo attrition or shear induced secondary nucleation to provide new crystals in the crystallizer.

While the bulk supersaturation is relatively low, in antisolvent crystallization at the local mixing points of antisolvent and solution with the bulk the local supersaturation is quite high. So it is actually quite surprising that no unselective primary nucleation takes place, as proven by the single chirality product, also after more than 6 residence times. It indicates that the primary nucleation rate in this process is very low, despite the high local supersaturations. The small number of primary nuclei of the other chirality that may form may be removed from the crystallizer before they can induce large amounts of secondary nuclei of the other chirality. Therefore it appears that such a continuous process can be operated to produce an enantiopure product much longer than any induction time measured in a batch-wise manner.

1 D. K. Kondepudi, R. J. Kaufman and N. Singh, Chiral symmetry breaking in sodium chlorate crystallization, *Science*, 1990, **250**(4983), 975–976, DOI: [10.1126/science.250.4983.975](https://doi.org/10.1126/science.250.4983.975).

**Helen Wheatcroft** asked: You state that the secondary nucleation rate is at least 6 orders of magnitude greater than the primary nucleation rate at typical industrial crystallisation conditions. How much variation is expected in the ratio of the primary and secondary nucleation rates as a function of supersaturation?

**Joop ter Horst** responded: It is conventionally thought that with increasing supersaturation, primary nucleation will become dominant over secondary



nucleation. What we see in our experiments is that if the flow rates increase at the same antisolvent fraction, at some point we lose control over enantiopurity and the product moves towards racemic composition (Fig. 8 in the paper; <https://doi.org/10.1039/d1fd00098e>). However, this point is well beyond what we had expected. Fig. 9 of the paper tells us that this loss of control happens as the suspension density in the crystallizer (related to productivity) goes down with time. This coincides with an increase in supersaturation. Due to the build up of supersaturation in the crystallizer, at some point nucleation occurs, which is probably unselective primary nucleation that compromises the product chirality.

**Helen Wheatcroft** enquired: Following up on the discussion of whether the secondary nuclei are generated by shear in the slurry or attrition by impact with the vessel and agitator, I wondered how large a secondary nucleus needs to be to grow? Does the critical nucleus size for primary nucleation also apply to secondary nucleation?

**Joop ter Horst** answered: A secondary nucleus formed by attrition might contain stress or strain, increasing somewhat the solubility of those small attrition fragments. Another effect might be that for small attrition fragments (<1 micron) indeed their total interfacial energy becomes a contributing factor and would also increase the solubility of the small fragments, similar to the primary nucleus in classical nucleation theory. Due to both causes the needed supersaturation to grow the attrition fragments must exceed a certain lower boundary limit. This lower boundary limit of the supersaturation is therefore indeed partly related to the primary nucleus size in classical nucleation theory.

**Kristen Fichthorn** opened discussion of the paper by Helmut Cölfen: Theoretical studies of the ordering of hard rods and nanowires reveal that various ordered phases can occur, depending on the aspect ratio.<sup>1–3</sup> This may also affect nucleation. Have you looked at the ordered phases that occur once aggregates have formed?

1 R. Blaak, D. Frenkel and B. M. Mulder, *J. Chem. Phys.*, 1999, **110**, 11652.

2 K. Zhao, C. Harrison, D. Huse, W. B. Russel and P. M. Chaikin, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2008, **76**, 040401(R).

3 D. Triplett and K. A. Fichthorn, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2008, **77**, 011707.

**Helmut Cölfen** responded: It is well known for liquid crystals that ordered phases form once a critical aspect ratio of the particles is reached. A preordering in solution may affect nucleation. However, in our system, we did not observe any order in the heterogeneously nucleated patches (scanning electron microscopy) while the particles themselves showed ordering on a TEM grid upon drying. The reason for the missing order for our heterogeneously nucleated particle superstructures is most likely the countercharged particles/surface, which have an attractive potential so that particles immediately stick to the surface once nucleation takes place without the possibility for orientational corrections. However, in other systems than the one with the attractive particle–surface interaction we have used in our study, I can well imagine that also ordered phases

will form after heterogeneous nucleation. This is for example the case, when mesocrystals are formed.

**Johanna Marie Galloway** queried: Would it be possible to look at binary charged systems? Either a mixture of oppositely charged particles, or particles displaying different charges on different faces? Most colloidal self-assembly systems focus on equidimensional spherical or cube like particles, it would be really interesting to see what could be assembled with nanorods.

**Helmut Cölfen** answered: We would be able to also look at binary charged systems with our combined light microscopy/UV-Vis spectroscopy technique. Nucleation rates could be determined for these binary charged systems as for the presented gold nanorods since we detect nucleated assemblies when they have a size about a micrometer and become visible in light microscopy. I agree that it is interesting to observe the structure of the nanorod self-assemblies. In our case of the countercharged gold nanorods and surfaces, no order could be observed in the self-assembled structures, which we attribute to the charge–charge attraction, hindering orientational corrections of the nanorods on the surface to increase the order in the self-assembled structures.

**Christian Kuttner** enquired: Mechanistically, do the gold nanorods exclusively assemble at the surface or are there any indications to assume that the assembly takes place in the liquid phase as well? Could the aggregation of nanorods be considered reversible to some extent or is it a classical hit-and-stick mechanism?

**Helmut Cölfen** replied: We can be sure that the observed nanoparticle assembly only takes place at the particle surface because we place our functionalized mica surface on top of the nanoparticle dispersion. This avoids erroneously detecting nanoparticle assemblies, which may have formed in solution and sedimented onto the mica surface as heterogeneously nucleated particle assembly. Due to the charge–charge attraction between nanorods and functionalized mica surface, we can assume the aggregation of the nanorods at the surface to be irreversible. Support for this assumption comes from the observed lack of order in the nanoparticle assemblies in Fig. 2 in our paper (<https://doi.org/10.1039/d1fd00087j>) as a result of a lack of orientational correction possibilities for the nanorods in the assemblies.

**Alan Hare** asked: I realise that we are looking at 2D pictures of the nanorod, but if in 3D I allow myself the luxury of imagining a cylinder topped by a hemisphere at either end, then in  $\text{nm}^2$  its surface area is  $4\pi r^2$  times the aspect ratio; or, for a radius of 20 nm, a multiple of exactly  $1600\pi \text{ nm}^2$ . Given that we are concerning ourselves with heterogeneous nucleation, can you comment on the relationship between aspect ratio and surface area?

**Helmut Cölfen** answered: The surface area of the nanorods is not really relevant for the consideration of their attachment at a surface during heterogeneous nucleation since for the contact with a surface, only one face will be relevant and its contact area is proportional to the aspect ratio as long as the nanorods attach with a side face, which is the case here.

**Alan Hare** queried: In Fig. 3 in the paper (<https://doi.org/10.1039/d1fd00087j>), “slope” vs. supersaturation, we see non-linearity: a flattening-off of the curve, apparently; so that when  $\sigma$  has reached 4 or 5, there is little or no further increase in the nucleation rate. Could there be a secondary effect at work, perhaps?

**Helmut Cölfen** replied: Yes, this indeed seems to be the case, especially for the lowest aspect ratio of 1.75. The nature of a secondary effect is yet unknown.

**Rik Drummond-Brydson** questioned: For nanorods you have to consider rotational as well as translational diffusion coefficients. Could the differences in kinetics observed for different aspect ratio rods be due to the respective mobilities of the different aspect ratio rods on the surface as they self-assemble to form their superstructure?

**Helmut Cölfen** responded: On the surface, rotational diffusion is limited due to the limited degrees of freedom of a nanorod to rotate. Since we have charge attraction between the surface and the nanorods, also the translational mobility should be decreased in our case. But in principle, the higher diffusion coefficients of the shorter aspect ratio rods and thus their higher mobility on the surface support the self-assembly.

**Joonsoo Kim** asked: In Fig. 2 in the paper (<https://doi.org/10.1039/d1fd00087j>), the surface is not completely covered with AuNRs. If surface–AuNR interaction is based on charge, shouldn't the NPs cover the surface completely and later form aggregates? Is that really happening but just not visible in the SEM image?

**Helmut Cölfen** answered: The detected self-assembled aggregates are predominantly particle monolayers. If sufficient particles would be added, one could imagine a monolayer coverage with the nanorods because of their charge attraction to the surface but the number of added particles was not yet high enough to reach a full surface coverage in our case. If the surface would be fully covered by the nanorods, we would see this in SEM, because we are able to see single nanorods.

**Christine Kirschhock** enquired: You use surfactants to stabilise the gold particles and use surfactant desorption to initiate their assembly. Do you expect transport phenomena related to surfactant ad- and de-sorption to affect the particle–particle interactions in a size dependent manner?

**Helmut Cölfen** replied: Yes, partial surfactant desorption initiates the particle assembly and their heterogeneous nucleation on the negatively charged surface. It is unknown if and in how far the surfactant ad/desorption correlates with the particle size with respect to aspect ratio. If that would be the case, the particle–particle interactions would be size dependent.

**Matthew Bennett** asked: Surfactants affect the viscosity of the solvent. Does this have an effect upon crystallisation?

**Helmut Cölfen** answered: If the viscosity of the solvent changes for whatever reason, this will have a direct influence on the diffusion of the nanoparticles as can be quantified *via* the Stokes–Einstein equation. This can then have an effect on crystallization, as the supply of building units is changed, which can even change the growth mode for example to diffusion controlled if the particle diffusion is significantly decreased. A slow particle supply would allow for orientational corrections of the nanoparticles in the nucleated crystal superstructure and can lead to an ordered superstructure while a fast supply can have the opposite effect.

**Alan Hare** opened discussion of the paper by Alexei Kiselev: Given the beautiful Fig. 2 in your paper (<https://doi.org/10.1039/d1fd00115a>), is it true to say that the joint pyramidal apex has physicochemical significance and that ice nucleation begins at that point? If not, then what has been drawn as an inverse double pyramid could be drawn as a hexagonal prism, instead; in which case, could nucleation perhaps begin at a vertex of the hexagon?

**Alexei Kiselev** replied: As discussed in the paper (<https://doi.org/10.1039/d1fd00115a>), the ice nucleation indeed initiates at the apex of a double pyramid; so this point (or rather, the underlying feature on the feldspar surface) has physicochemical significance: it is the nucleation site. The shape of the double pyramid is a result of the ice growth process in the confined geometry of the sucrose layer. Due to the slow rate of crystal growth, we could visually follow the evolution of the crystal shape right after the nucleation event.

**Peter Vekilov** enquired: Your discussion of how feldspar, a common aluminosilicate, enhances ice crystal nucleation from vapor is fascinating. I have a small technical question. Even though feldspar may be main mineral in the earth's crust, is it indeed heavily represented in atmospheric dust, where it may interact with emerging ice nuclei?

**Alexei Kiselev** answered: Feldspars are indeed widely represented in the atmospheric mineral dust aerosol. The major components of atmospheric mineral dust are clay minerals (approx. 60%), quartz (approx. 25%), and feldspar (approx. 12%), but only feldspars have been shown to be ice nucleation active. For details, please refer to ref. 1

1 B. J. Murray, D. O'Sullivan, J. D. Atkinson and M. E. Webb, Ice nucleation by particles immersed in supercooled cloud droplets, *Chem. Soc. Rev.*, 2012, **41**(19), 6519–6554.

**Peter Vekilov** asked: Do all faces of feldspar crystal equally boost ice crystal nucleation? Are there correlations between the structures of the feldspar faces that support ice crystal nucleation and the ice faces that line them? Do you have an idea about the chemical interaction between water molecules in ice and the feldspar moieties that help the observed nucleation effect?

**Alexei Kiselev** responded: Not all crystalline faces perform equally well in nucleating ice. As you can see in Fig. 5 of our paper (<https://doi.org/10.1039/d1fd00115a>), the ice nucleation (IN) efficiencies of cleavage planes (001) and

(010) are almost identical, whereas ice nucleation on the substrate prepared along the (100) crystal plane is enhanced by almost 5 °C. Neither face has a noteworthy match to the crystal structure of ice, if taken in its ideal “undisturbed” state. However, the atomistic modeling has shown<sup>1</sup> that the presence of OH<sup>-</sup> groups on the surface of the (100) face creates a flexible layer that can reduce the mismatch between the feldspar framework and prismatic face of ice. A similar layer would be also present at the other surfaces of feldspar, but the highest energy gain (and thus, the lowest free energy barrier for ice nucleation) is achieved on the (100).

1 A. Kiselev, *et al.*, Active sites in heterogeneous ice nucleation—the example of K-rich feldspars, *Science*, 2017, 355(6323), 367–371.

**Thomas Whale** enquired: The work of Holden *et al.*<sup>1</sup> demonstrates that different active sites on feldspar nucleate ice in the deposition and immersion modes and provided a range of ideas regarding what might lead to this. Does this work provide any insight to what the differences might be?

1 M. A. Holden, J. M. Campbell, F. C. Meldrum, B. J. Murray and H. K. Christenson, Active sites for ice nucleation differ depending on nucleation mode, *Proc. Natl. Acad. Sci. U. S. A.*, 2021, 118, e2022859118, DOI: [10.1073/pnas.2022859118](https://doi.org/10.1073/pnas.2022859118).

**Alexei Kiselev** replied: This is an excellent comment showing that there are still gaps in our understanding of heterogeneous ice nucleation mechanisms. Our work was strictly focused on immersion freezing so I can only offer some thoughts in this respect. The deposition and immersion modes of ice nucleation differ in many ways, but the most important difference is in the availability of water for ice nucleation and growth. In deposition (or pore condensation and freezing (PCF)) mode, some water has to be adsorbed on the surface or form liquid pockets in pores and cracks prior to nucleation onset; whether the sites of condensation coincide with the most active ice nucleation sites is not guaranteed. On the contrary, in immersion mode, all surface defects are equally accessible to water, making nucleation on the most active IN sites highly probable. The other aspect could be that liquid water present in abundance (in immersion mode) would modify the surface properties in general or the properties of the IN sites in particular, by *e.g.* cation exchange or formation of a macroscopic electric double layer (EDL). In deposition mode, such a thing would not happen or could have a different effect on ice nucleation due to confinement effects.

**Thomas Whale** remarked: It is worth noting that not all ice nucleation sites on feldspar are active in only one mode – Holden *et al.*<sup>1</sup> showed that some sites are effective in both modes.

1 M. A. Holden, J. M. Campbell, F. C. Meldrum, B. J. Murray and H. K. Christenson, Active sites for ice nucleation differ depending on nucleation mode, *Proc. Natl. Acad. Sci. U. S. A.*, 2021, 118, e2022859118, DOI: [10.1073/pnas.2022859118](https://doi.org/10.1073/pnas.2022859118).

**Sarah Price** asked: How does the nucleation of ice on feldspars lead to snowflakes?

**Alexei Kiselev** answered: Formation of a snowflake is a multistage process. In a mixed-phase tropospheric cloud, it starts with the nucleation of ice in a supercooled cloud droplet. This is where the ice nucleation particle (INP) is needed to facilitate freezing, and feldspar can be a very efficient INP. In a water-saturated environment, the frozen droplet would grow rapidly *via* the water vapor diffusion (Wegener–Bergeron–Findeisen mechanism) and at a certain point too heavy to be kept in an updraft, falling down as a result. On its way to the ground, the ice particle travels through the regions of various ambient temperature and of high and low vapor saturation, where the growth rate of crystal faces of ice varies strongly. This is how the typical dendrite structure of a snowflake appears. So, in principle, feldspar by itself is not responsible for formation of snowflakes, but its unique ability to facilitate freezing of water at low super-cooling is beneficial for formation of large snowflakes in the moderately supercooled convective tropospheric clouds.

**Ilaria Sandei** enquired: Feldspars and other minerals have been proven to be efficient ice nucleating materials and the paper (<https://doi.org/10.1039/d1fd00115a>) adds new and interesting insights about the nature of the active planes of feldspars and their effect on ice nucleation and growth. However, overall, the main characteristics that make a material a good ice nucleator are still unclear. In most cases, it has been suggested that a crystallographic match between ice and a material is necessary for this nucleation activity but, in other cases, it has also been shown that less oxidised materials can nucleate ice better than more oxidised ones<sup>1</sup> or that differences in surface roughness can be other important factors in this behaviour.<sup>2</sup> This leads to questions whenever the match between the crystalline lattices of ice and the mineral is the only property that makes a material a good ice nucleator.<sup>3</sup> Would the investigation of the ice nucleating activity of engineered substrates (obtained, for example, by modifying the morphology or the charge distribution of an otherwise neutral material) help to understand better, even in the case of feldspars or other minerals, if their activity results from a contribution of different surface features?

1 T. F. Whale, *et al.*, *J. Phys. Chem. Lett.*, 2015, **6**, 3012–3016.

2 L. Lupi, *et al.*, *J. Am. Chem. Soc.*, 2014, **136**(8), 3156–3164.

3 M. Fitzner, *et al.*, *J. Am. Chem. Soc.*, 2015, **137**(42), 13658–13669.

**Alexei Kiselev** replied: This is an excellent question. The problem with ice nucleation experiments involving natural substrates is that so many factors are affecting their IN behavior at the same time: surface topography, crystal structure, surface chemistry, charge distribution. In a real world experiment, disentangling the individual contributions of these factor is almost impossible. If we were able to manufacture an artificial substrate with fixed properties where we could vary only one thing at once, the role of individual factors would become more clear. To some extent, this is what we have tried recently by chemically modifying pure “gem-quality” potassium-rich feldspar to induce a series of cracks where certain morphological features become exposed, see ref. 1.

1 A. A. Kiselev, *et al.*, *Atmos. Chem. Phys.*, 2021, **21**(15), 11801–11814.

**Jan Sefcik** opened discussion of the paper by Nick Pellens: How would the proposed mechanism lead to the spherical shape of the submicron particles observed? What could explain the differences between the sodium and caesium outcomes? Perhaps the spherical shape of particles hints that surface tension plays a role in their formation. This would indicate the presence of liquid–liquid phase transitions which may be involved in particle formation pathways.<sup>1,2</sup> Liquid–liquid phase transitions can also be sensitive to the nature of cations present in solutions.<sup>3,4</sup>

1 J. Šefčík and A. V. McCormick, *Catal. Today*, 1997, **35**, 205–223.

2 K. Z. Gaca and J. Sefcik, *J. Colloid Interface Sci.*, 2013, **406**, 51–59.

3 S. J. Taylor, *et al.*, *Langmuir*, 2014, **30**, 10231–10240.

4 S. J. Taylor, *et al.*, *Langmuir*, 2015, **31**, 13571–13580.

**Nick Pellens** responded: The CrystalGrower simulation software presents the opportunity to gain deeper insights in the stability of the crystal surface during zeolite crystallisation *via* kinetic Monte Carlo simulations.<sup>1</sup> Comparison of the synthesis experiments with these simulations suggests that zeolite crystallisation in the presence of sodium or cesium cations results in a different crystal surface energy landscape. In the presence of sodium, crystal growth proceeds preferably *via* specific surface sites (large  $\Delta\mu_b$ ), while for cesium all surface sites seem to have a comparable stability (small  $\Delta\mu_b$ ). The proposed crystallisation mechanism explains such behaviour. Only in the presence of cesium cations, reaction-limited crystallisation kinetics are observed and crystal growth proceeds in pre-equilibrium conditions. In other words, due to the high mobility of Cs cations, fast removal of the cation from the surface freezes a purely statistical distribution of active surface sites. Because redissolution is hindered by the high mobility of Cs cations, nanosized isotropic crystals are perceived to be stable in the Crystal-grower simulations (small  $\Delta\mu_b$ ). Even for the synthesis of an identical zeolite framework, crystal morphologies remain dissimilar depending on the cation that is present. Because these observations are closely related to presence or absence of dissolution–reprecipitation chemistry during and after crystal growth, at this moment, it seems unlikely that a cation-dependent surface tension during the phase transition preceding nucleation plays a direct role in determining the crystal morphology of HSIL zeolite crystallisation. Our recent study for HSIL zeolite crystallisation in the presence of sodium, potassium, and cesium cations shows that ion pairs in aluminosilicate-alkali synthesis liquids determine the zeolite aluminium content and topology, further suggesting an invariable nucleation pathway.<sup>2</sup>

1 M. Anderson *et al.*, Predicting crystal growth via a unified kinetic three-dimensional partition model, *Nature*, 2017, **544**, 456–459; A. R. Hill *et al.*, Crystalgrower. A generic computer program for Monte Carlo modelling of crystal growth, *Chem. Sci.*, 2021, **12**, 1126–1146.

2 K. Asselman, *et al.*, Ion-pairs in aluminosilicate-alkali synthesis liquids determine aluminium content and topology of crystallizing zeolites, *Chem. Mater.*, 2022, DOI: [10.1021/acs.chemmater.2c00773](https://doi.org/10.1021/acs.chemmater.2c00773).

**Jeffrey Rimer** queried: My question pertains to the properties of the clusters: is the chemical formula or general size and composition (Si, Al, and alkali content) known, and is the population uniform or is there a distribution of oligomeric species? This leads to the second question involving the chemical reaction used to

model pre-nucleation cluster (PNC) addition: if there is a distribution of clusters, is this reflected in the chemical formula? Is the number of alkali metals released per PNC addition known or is there variability?

**Nick Pellens** answered: The chemical formula, size and composition of the identified ion-pair pre-nucleation are determined by the combination of several characterization tools. Based on conductivity measurements,  $^{23}\text{Na}$  NMR and chemical modelling, it is feasible to quantify the fraction of sodium cations engaging in ion-pairing. Hydrogen electrode pH measurements provide an accurate probe to determine the global extent of (alumino)silicate deprotonation, measured *via* the depletion of hydroxide ions in the synthesis liquid. We performed  $^{27}\text{Al}$  and  $^{29}\text{Si}$  NMR measurements on the synthesis liquids to determine the (alumino)silicate speciation. Combined, this information led to the identification of ion-paired aluminosilicate 4 ring-alkali cations complexes. These 4 rings contain one or two aluminate centers based on the chemical composition of the synthesis liquid, as reflected in the zeolite framework Si/Al ratio. Based on the assumptions of crystallization *via* condensation of solute pre-nucleation clusters, a structural and compositional relation is implied between the liquid-state pre-nucleation clusters and the forming crystal nucleus. Therefore, it is not directly observed, but implied by the wealth of our experimental observations that this relation between the cluster properties and the crystallizing zeolite stands. The derived model is based solely on the first condensation step between a liquid-state PNC and a growing crystal nucleus, as this step should be rate-limiting. Therefore, the model is based on a single alkali metal and hydroxide ion release. However, our experimental observations show that a single ion-paired aluminosilicate 4 ring, on average and depending on the sample composition, ion pairs to 3–7 alkali metals due to the full deprotonation of silicate centers. We are in the progress of publishing these experimental results and, as a result, the related experimental evidence will become available in the near future.<sup>1</sup>

1 N. Pellens *et al.*, Nucleation of porous crystals from ion-paired pre-nucleation clusters, *Chem. Mater.*, 2022, DOI: [10.1021/acs.chemmater.2c00418](https://doi.org/10.1021/acs.chemmater.2c00418); K. Asselman *et al.*, Ion-pairs in aluminosilicate-alkali synthesis liquids determine aluminium content and topology of crystallizing zeolites, *Chem. Mater.*, 2022, DOI: [10.1021/acs.chemmater.2c00773](https://doi.org/10.1021/acs.chemmater.2c00773).

**Michael Anderson** asked: Your work is on very well-defined systems that are not the same as a gel synthesis or a templated synthesis of a zeolite. Is it possible to broaden out such studies to these systems?

**Nick Pellens** responded: Currently, we do not have experimental observation regarding gel or templated zeolite synthesis. Therefore, it is only possible to speculate regarding the general applicability of the observed crystallization kinetics. The main limitation is enabling a molecular investigation that is necessary to construct surface chemistry approximations of the crystallization kinetics. The well-defined physicochemical properties of HSIL zeolite synthesis liquids enable performing such research. To the best of our knowledge and at this moment, it is not possible to directly apply this research strategy to more complex zeolite synthesis systems. However, we are working on enabling *in situ* conductivity measurements for gel synthesis, that is inorganic zeolite crystallisation at a higher supersaturation compared to crystallisation from HSIL synthesis liquids.



**Michael Anderson** enquired: In the Na system you saw 3 or 4 different phases – do you know if these phases were successive or appeared at the same time?

**Nick Pellens** replied: In an earlier experiment, we performed an *ex situ* crystallization experiment to gain more insight into the stability of various zeolite frameworks. For the here-studied Na sample, at a synthesis temperature of 60 °C and slightly lower supersaturation (Si/Al = 18), it was observed that the three crystalline phases (FAU, GIS, SOD) appeared at the same time. The three different phases remained stable up to 120 h at 60 °C, but after 168 h, GIS formation was preferred over the FAU and SOD frameworks. In addition, at a higher synthesis temperature of 90 °C ( $t = 168$  h), a mixture of the GIS/ANA frameworks was observed in the solid product. Pushing the synthesis temperature to 160 °C ( $t = 168$  h) resulted in a full conversion into an ANA zeolite product. Based on these arguments, we conclude that dissolution–reprecipitation processes are responsible for our observations of zeolite polymorphism in Na-based HSIL zeolite synthesis.

**Alan Hare** asked: On seeing a curve like the conductivity curve in Fig. 3 in your paper (<https://doi.org/10.1039/d1fd00093d>), it is almost intuitive to think “this is Avrami”, but did you make this assumption? Alternatively, were you able to *infer* Avrami, and *then* fit the curve to the data? I note that you acknowledge Kolmogorov’s mathematics.

**Nick Pellens** responded: In the case of HSIL zeolite crystallisation, only a small fraction of the liquid solidifies into a crystalline phase. Therefore, the Avrami model was not inferred as is difficult to motivate the direct implementation of the crystallisation kinetics that was derived for the case of a total liquid to solid conversion. Instead, a reasonable set of assumptions was formulated to derive the crystallisation kinetics in the case of the incongruent transformation of only a fraction of the initial system with different compositions of solid and liquid phases. The fact that the resulting equation ( $P_N(t) = 1 - \exp[-Gt^2]$ ) is of the same form as the Avrami model is rooted in the assumption of fast liquid dynamics and kinetics in the homogeneous liquid phase, compared to the surface processes.

**Alan Hare** enquired: In the model of increasing zeolite fraction (Section 3.2.2; <https://doi.org/10.1039/d1fd00093d>), your simplification (2) leapt off the page at me because many years ago Sam Levine (R.I.P.), when trying to teach us colloidal stability theory, made the very assertion: “chemical equilibrium does exist [in the liquid, throughout crystallisation]”. Nearly 50 years on, my question today has mutated only slightly: not “does equilibrium exist” but “does it *have* to exist”? If equilibrium *doesn't* exist, how does this alter the system description? Does the problem become intractable; or does it now have a different solution?

**Nick Pellens** answered: For the derivation of the kinetic model, fast dynamics in the liquid state compared to the growth reactions is assumed. With this assumption the liquid phase has a homogeneous composition throughout and the *in situ* conductivity measurements report the accurate state of the growth medium. This way, no depletion effects or change of liquid state speciation

needed to be accounted for, which would result in more complex crystallization kinetics, also accounting for the pre-equilibria reactions within the liquid. For example, if the liquid growth medium would be far from chemical equilibrium, the crystallization kinetics might be limited by the supply of suitable ion-paired pre-nucleation clusters, and the lifetime of these clusters must be longer than the time needed to pass the depletion zone. However, considering the nature of the used aluminosilicate fluids, a fast interchange between cation-paired oligomeric species can safely be assumed, so that the probability that a viable pre-nucleation cluster is formed is the same everywhere in the liquid, including the surface of a growing crystal. It also needs to be pointed out that a very low aluminium content was used for our study, so that the global change of the liquid state concentrations, especially the hydroxide content did not change significantly during crystal growth. This allowed focusing on the processes on the surface of the growing crystal, as the chemical state in the liquid only changed marginally.

**Alan Hare** asked: Regarding the pre-nucleation clusters: do you think that the nucleation mechanism could be consistent with a single energy-barrier model (such as that mentioned by Alexander Van Driessche in the context of  $\text{SrSO}_4$ ; <https://doi.org/10.1039/d1fd00092f>)? I am wondering if there might be a continuous distribution of local energy minima.

**Nick Pellens** responded: In Na HSILs, the liquid zeolite crystallization medium has an experimentally determined liquid density of  $1.3\text{--}1.5\text{ g ml}^{-1}$  and laboratory XRD measurements show that these liquids already exhibit local order. The ion-paired pre-nucleation clusters, thus, exist in a liquid medium that has similar properties to the crystallizing solid (average density  $\approx 1.4\text{ g ml}^{-1}$ ). The nucleation pathway proposed by Alexander Van Driessche is based on model systems with a large density gap between the solution and solid phases. Therefore, it is not possible to state whether or not this pathway can apply to HSIL zeolite nucleation.

**Matteo Salvalaglio** opened discussion of the paper by Romain Grossier: In your paper (<https://doi.org/10.1039/d1fd00090j>) you present new exciting experimental results on nucleation rate measurement within small sessile droplets. At such small size, the measured nucleation rates are affected by the droplet size, as you demonstrate experimentally and report in the manuscript. In the past, you have provided a general theoretical framework for understanding the nucleation of crystalline particles in droplets that don't exchange mass with a surrounding reservoir.<sup>1</sup> Can you use your own theoretical description to interpret the data gathered in this paper in order to extrapolate to the macroscopic limit the rates measured in small volumes?

1 R. Grossier and S. Veessler, *Cryst. Growth Des.*, 2009, **9**(4), 1917–1922.

**Romain Grossier** replied: Before interpretation of the here presented data, determining rates  $J$  as defined by CNT would imply to model more accurately the contraction dynamics, *i.e.* getting droplet volume evolution with time  $V(t)$  (as stated in the paper: “An exact and precise model is complex and out of the scope of this paper, and will be the object of specific studies later on.”). This is an

ongoing, unpublished up to now, work.<sup>1</sup> It would then be possible to accurately calculate  $J$ , based on the here presented data. Then, before trying to extrapolate to larger volumes, I would first use this approach (and maybe collect more data on many other droplet sizes) to answer the following question: should depletion during nucleation be taken into account (thereby proving a thermodynamical aspect of confinement as developed in ref. 2)?

1 R. Cedeno, R. Grossier, N. Candoni, A. Flood and S. Veessler, *Evaporation Dynamics of Sessile Saline Microdroplets in Oil*, 2022, <https://hal.archives-ouvertes.fr/hal-03440976>.

2 R. Grossier and S. Veessler, Reaching one single and stable critical cluster through finite-sized systems, *Cryst. Growth Des.*, 2009, 9(4), 1917–1922, DOI: [10.1021/cg801165b](https://doi.org/10.1021/cg801165b).

**Peter Vekilov** asked: You have collected an enormous set of data on rock salt crystal nucleation. What new insights on the nucleation of sodium chloride from aqueous solutions does this data set provide?

**Romain Grossier** answered: The aim here is first to present a novel approach for measuring highly time-resolved induction times (without interference of a time to grow to detectable size), able to provide large (statistically representative) reproducible datasets.

And second, to propose first steps, in analyzing data (such as removing outliers) without any nucleation mechanism inference.

Still, if the direct goal was not to learn about NaCl nucleation process, we measured, quantitatively, the (statistical) extent of confinement effects: smaller systems sustain higher supersaturations before nucleation occurs. This large dataset could be useful in simulations addressing such situations for “benchmarking” purposes, or to test different nucleation theories.

**Sarah Price** requested: Please can you comment on the location of the nucleation within the droplet. From the coffee-ring effect, I would have expected nucleation to tend to occur at the edge of the boundary between the droplet and the surface.

**Romain Grossier** responded: The exact location of nucleation is not attainable to us regarding spatial and temporal resolution, and also because of the large Marangoni flows that appear when nucleation occurs. Still, exact location (which interface? – contact line, PMMA surface or oil/droplet interface) could be studied in addressing more droplet sizes and see how  $J$  scales with diameter and its different exponents.

**Liam Hunter** enquired: During the generation of your droplet arrays do you assume that the micropipette is not in contact with the PMMA surface or does the size of the generated droplets make it necessary to be in contact? Can you comment on the effect of any pipette induced scratching of the PMMA substrate on the subsequent nucleation behaviour of the arrays? I assume that any surface defects generated this way would become apparent in your heterogeneity/memory tests. The low image acquisition rate may make it difficult to determine the point of nucleation; in your opinion does the nucleation generally seem to preferentially occur at one of the interfaces or is it homogeneous?

**Romain Grossier** replied: The micropipette needs to be in contact with the surface to generate droplets, and details on the generation mechanism can be found in ref. 1. Regarding potential scratching of the PMMA surface by the micropipette, under the same experimental conditions (PMMA on glass coverslip, spin coating conditions, “femtotip I” glass capillary), we never had any sign of a potential effect, and while observing resulting crystals in SEM<sup>1</sup> (Fig. 4 for example) no apparent scratching was seen. Despite this, we cannot rule out sub-resolution scratches, but one must have in mind the micropipettes have a small wall thickness (#200 nm), making them highly flexible (and fragile) and not prone to apply large forces so that to scratch PMMA. Regarding detection of such potential surface defects with our heterogeneity/memory tests, it may not help in detecting a “heterogeneity” that is uniformly distributed in droplets (or on the surface they rely on): if the micropipette uniformly scratches the PMMA surface during its course, each droplet would have the same “amount” of scratched surface, thereby no reasons to induce a particular behavior in only a subset of droplets. The tests we implemented are more effective to detect embedded heterogeneities (“impurities”) which should not uniformly distribute among droplets, or PMMA surface defects localized only in some droplets, in fact any heterogeneity that would always give lower nucleation barrier to specific droplets.

You are right our image acquisition rate does not allow to determine the nucleation point, but higher acquisition rates would not give us such opportunity: spatial resolution to detect “critical cluster” is unattainable and growing crystals in the first seconds are subject to a lot of jittering due to large Marangoni flows induced by large concentration gradients inside the droplets when nucleation occurs, *i.e.* they could travel anywhere else in the droplet before being detected.

For your last question, I would prefer not to give an opinion, but if I had to, I would certainly preferentially bet on heterogeneous nucleation process. Now, it still has many “interfaces” on which nucleation could occur, and information could be obtained by studying a larger set of droplet diameters to see if induction time scales with their diameter (contact line), or with diameter squared (PMMA surface and droplet/oil interface) or diameter cubed (volume embedded heterogeneities – or homogeneous).

1 R. Grossier, Z. Hammadi, R. Morin, A. Magnaldo and S. Veessler, Generating nanoliter to femtoliter microdroplets with ease, *Appl. Phys. Lett.*, 2011, **98**, 091916, DOI: [10.1063/1.3560453](https://doi.org/10.1063/1.3560453).

**Jan Sefcik** commented: The term “induction time” is normally used to represent the time it takes from the point of reaching a given degree of supersaturation until the point of observing the first sign of crystal formation, while remaining at constant composition and temperature (see ref. 1, 2 and the paper of Joop ter Horst presented earlier (<https://doi.org/10.1039/d1fd00098e>)). Therefore it may be potentially misleading to use induction times to describe nucleation outcomes while supersaturation is time-dependent, for example in a cooling experiment. It would be useful to clarify terminology used to precisely describe crystallisation processes and their (stochastic) outcomes.

1 N. Javid, *et al.*, *Cryst. Growth Des.*, 2016, **16**, 4196.

2 M. L. Briuglia, *et al.*, *Cryst. Growth Des.*, 2019, **19**, 421.

**Alexei Kiselev** remarked: About the possibility of multiple nucleation events in the microscopic solution droplets: there are two arguments against it. First, decreasing the observation volume reduces the probability of having an impurity in the droplet which could induce nucleation at low supersaturation. Second, the probability of nucleation (which is a product of volume-specific nucleation rate and volume) is reduced in small droplets compared to larger volumes. Both factors lead to higher supersaturation than can be achieved in small droplets. On the other hand, if nucleation starts at higher supersaturation, the rate of crystal growth immediately after nucleation would also be higher, rendering the probability of a second nucleation event inside the same microdroplet negligible. In fact, this chain of arguments is exactly the reason why homogeneous nucleation should rather be studied in the system of many possibly small identical sample volumes. In view of this argument, the system presented by Dr Grossier is very well suited for such studies.

Several similar systems were reported recently, based on the principle of subdivision of sample volume.<sup>1,2</sup>

- 1 N. Reicher, L. Segev and Y. Rudich, The Weizmann Supercooled Droplets Observation on a Microarray (WISDOM) and application for ambient dust, *Atmos. Meas. Tech.*, 2018, **11**, 233–248, DOI: [10.5194/amt-11-233-2018](https://doi.org/10.5194/amt-11-233-2018).
- 2 A. Peckhaus, A. Kiselev, T. Hiron, M. Ebert and T. Leisner, A comparative study of K-rich and Na/Ca-rich feldspar ice-nucleating particles in a nanoliter droplet freezing assay, *Atmos. Chem. Phys.*, 2016, **16**, 11477–11496, DOI: [10.5194/acp-16-11477-2016](https://doi.org/10.5194/acp-16-11477-2016).

**Lucia Maini** asked: In your experiments you've got nice cube-like crystals in the first crystallization, but in the second crystallization the shape is totally different. Is it possible to obtain cube-like crystals also in the second or third crystallization?

**Romain Grossier** replied: Nice cube-like crystals were obtained at step 0, where droplets were left (still under oil layer) at ambient %RH, higher than 10% as used in step 2. Nucleation had time to occur before reaching too high supersaturations which promotes dendritic growth. Also, there is enough supersaturated solution left to complete smoothening to the cubic habit. So, cube-like crystals could certainly be obtained in larger droplets (images of the videos here are extracted from 60 pL droplets array) and/or in less dry conditions during step 2.

**Stéphane Veesler** noted: In the experiment presented here, relative humidity (RH) cycling (deliquescence–recrystallization), at the end of the crystallization step we observed one dendritic single crystal per droplet. The dendrite is the growth form, and there is no more solution (complete drying) at the end of the experiment to return to the equilibrium form, *i.e.* the cube. In other experimental conditions, in which complete drying was slower, we observed this return to the equilibrium form.<sup>1</sup>

- 1 Z. Hammadi, N. Candoni, R. Grossier, M. Ildefonso, R. Morin and S. Veesler, Small-volume nucleation, *C. R. Phys.*, 2013, **14**, 192–198.

**Romain Grossier** added: I agree.

**Gábor Schuszter** enquired: You mentioned that a mass flow controller will be purchased to more precisely adjust the RH of the air in contact with the covering oil layer. How thick is the oil layer above the droplets? What is the distance separating the droplets? Since the droplets are open to each other as well and not only to the air, do you see any communication (*i.e.*, collective behavior) between them?

**Romain Grossier** answered: Here, the oil layer thickness is 0.8 mm, as stated in the supplementary information file (note there is a typo error in the paper; <https://doi.org/10.1039/d1fd00090j>).

At step 0, while generating droplets to obtain the initial crystal array, droplet-to-droplet distance is a function of pressure applied in the micropipette and its velocity on the surface, as described in ref. 1. Resulting crystals, then have positions distributed around the initial droplets' centers. Step 1 (deliquescence of crystals) will then place the resulting droplets roughly centered on the crystals' initial positions.

You're right in saying droplet-to-droplet communications can occur, and this was the purpose of a previous study<sup>2</sup> where we explained their nature and developed the image processing technique we here use. This image processing technique is able to show droplet-to-droplet crystal nucleation mediated interactions: Fig. 3 in the paper clearly shows such individual interactions. Knowing how to measure such interactions, and parameters (oil chemistry, thickness, viscosity, above oil layer %RH) to either enhance or avoid them, we here developed and characterized two %RH controller module versions, which both revealed efficient removal of droplet-to-droplet interactions. Plus, other individual dynamics (see section "Distribution of characteristic time points"), not occurring through crystal nucleation, are addressed through the use of a dimensionless time. Finally, since we're able to both detect and get rid of droplets' interactions, we see no reason to focus on droplet-to-droplet distances.

- 1 R. Grossier, Z. Hammadi, R. Morin, A. Magnaldo and S. Veessler, Generating nanoliter to femtoliter microdroplets with ease, *Appl. Phys. Lett.*, 2011, **98**, 091916, DOI: [10.1063/1.3560453](https://doi.org/10.1063/1.3560453).
- 2 R. Grossier, V. Tishkova, R. Morin and S. Veessler, A parameter to probe microdroplet dynamics and crystal nucleation, *AIP Adv.*, 2018, **8**, 075324, DOI: [10.1063/1.5034443](https://doi.org/10.1063/1.5034443).

**Sten O. Nilsson Lill** opened discussion of the paper by Vivek Verma: For the hydrogen bond propensity (HBP), did you model both Gly and Digly as zwitterions? If yes, they both would interact *via* an ion-ion interaction and this is much stronger than H-bonding. I would suggest to look into the HBP for Digly again if this was modeled as a zwitterion, the value should be very similar to that found for Gly.

**Vivek Verma** responded: Yes, HBP was modelled for both Gly and Digly as zwitterions. Since Digly has two oxygens and two nitrogens compared to only the one nitrogen and one oxygen of glycine, the HBP of the terminal N and O on both Gly and Digly would be similar while the central N and O of Digly will have different HBP. Now, I am not sure which N and O of Digly are participating in the H-bonding with silica and I guess this will have to be varied with molecular dynamics simulations. We have started working on the MD simulations of the

same systems to gain more insight on the heterogeneous nucleation mechanism and see which groups are participating in the H-bonding.

**Ruel Cedeno** enquired: My question is on the possible effect of glass beads on the hydrodynamics of the system. Studies have shown that as the shearing rate increases, the pre-exponential factor can also increase in certain hydrodynamic regimes due to the increased advective transport towards the nucleus.<sup>1</sup> The presence of glass beads during agitation (as in grinding experiments) can induce more turbulence in the system leading to higher shear rates. This is supported by your crystal images where more fine particles are seen in the case with glass beads.

Is it possible that the observed increase in prefactor is related to the increase in shearing rate rather than the H-bonding capability of the beads? To eliminate the interference of hydrodynamics, it would be interesting to compare the functionalized glass beads against inert beads in order to see the templating effect at identical hydrodynamic conditions.

1 F. Mura and A. Zaccone, *Phys. Rev. E*, 2016, **93**, 042803, DOI: [10.1103/PhysRevE.93.042803](https://doi.org/10.1103/PhysRevE.93.042803).

**Vivek Verma** replied: The increased shear rate could potentially increase the nucleation rate:  $J = A \exp(-\Delta G_c^*/RT)$

Based on the dimensional analysis of the above equation, nucleation rate ( $J$ ) and pre-exponential factor ( $A$ ) should have the same unit. For  $J$  this is usually expressed as the number of nuclei of a size greater than the critical nucleus size generated per unit volume and per unit time (no. of stable nuclei  $\text{m}^{-3} \text{s}^{-1}$ ). On this basis,  $A$  may be defined as the total number of nuclei (or clusters) of any size generated per unit volume and per unit time (no. of pre-critical and stable nuclei  $\text{m}^{-3} \text{s}^{-1}$ ). The exponential factor is the fraction of these pre-critical nuclei which can advance to the critical size. So, it could be possible that shear would be leading to more nucleation sites. But, I still believe that H-bonding is responsible for the nucleation to start despite the increase in nucleation sites. H-bonding provides the driving force for the crystal nucleus to form leading to the crystal propagation.

I agree, that this could be cross verified by using the inert templates and we have already started working on using the inert beads along with the different functionalised beads to see the effect of different functional groups on the heterogeneous nucleation.

**Aurora Cruz-Cabeza** asked: Your microscopic image (Fig. 5 in the paper; <https://doi.org/10.1039/d1fd00101a>) shows very small fines for the samples obtained by crystallisation with glass beads. Glass beads will have a significant impact on breakage and attrition thus enhancing secondary nucleation. How can you ascribe your results to heterogeneous nucleation on beads rather than secondary nucleation?

**Vivek Verma** answered: I agree that the microscopic image shows some fines and they could potentially be due to the secondary nucleation from the impact of glass beads happening after the nucleation event. The main aim of the study was to observe the change in the induction time in the absence and the presence of

glass beads. And the experimental data suggests that there was reduction in induction time in the presence of glass beads confirming the effect of the heterosurface and hence the propagation of heterogeneous nucleation. The induction time value was later used to obtain the nucleation rate and subsequently thermodynamic and kinetic parameters. The next phase of the study will investigate the secondary nucleation phenomenon.

**Helen Wheatcroft** questioned: Was the pH controlled in the experiments with and without the glass beads? As you mention in the introduction to the paper (<https://doi.org/10.1039/d1fd00101a>), the pH is known to affect the rate of crystallisation of glycine (ref. 47 in the paper; ref. 1 here). How was pH controlled or monitored in your experiments and did the addition of the glass beads cause a change in the solution pH?

1 C. S. Towler, R. J. Davey, R. W. Lancaster and C. J. Price, *J. Am. Chem. Soc.*, 2004, **126**, 13347.

**Vivek Verma** responded: We did not measure the pH before and after the experiments but I think the pH did not change much during the experiments. From a different study in the group we know it is difficult to change the pH of Gly and Digly due to the zwitterion effect. And secondly, the crystallisation of Gly and Digly will be influenced if the pH variation is significant. But having said that, I will measure the pH to see the change in pH with and without glass beads. Also I will use a different amount of glass beads to see the change in overall pH and if that has any effect on the nucleation behaviour.

**Jan Sefcik** said: Primary nucleation kinetics can depend on both glass–solution surface area<sup>1</sup> and agitation/fluid shear.<sup>2</sup> It would therefore be interesting to investigate varying the surface area of the beads and stirring in the nucleation experiments involving suspended beads.

1 C. Forsyth, *et al.*, *Cryst. Growth Des.*, 2016, **16**, 136.

2 M. J. Vesga, *et al.*, *CrystEngComm*, 2019, **21**, 2234.

**Vivek Verma** answered: Yes, I agree that nucleation kinetics will depend on both glass bead size and agitation rate. I am currently using a Design of Experiment approach to look at the effects of different glass bead size and agitation rate on the nucleation rate of glycine and diglycine.

**Aaron R. Finney** continued discussion of the paper by Nick Pellens by communicating: During the discussion, you listed criteria for species in solution before nucleation to be labelled prenucleation clusters. One of these was “stability”. What specifically is meant by stability in this context and how is this determined in your work? Clusters are also predicted from classical nucleation theory and a key difference between these clusters and those proposed in the prenucleation cluster pathway is that the probability of “classical” clusters decays rapidly as a function of increasing size below some critical size, beyond which growth occurs. Why can't the clusters be classical?

**Nick Pellens** communicated in response: The paper (<https://doi.org/10.1039/d1fd00093d>) and discussion of the work refer to the stability of nm-scale ion-



paired pre-nucleation clusters by the observation of their room-temperature stability in HSIL zeolite synthesis liquids. By  $^{27}\text{Al}$  and  $^{29}\text{Si}$  NMR measurements, it is verified in a nearly identical sample with a slightly lower aluminate content ( $\text{Si}/\text{Al} = 18$ ) that liquid-state (alumino)silicate oligomer speciation remains unchanged, independent of whether the speciation is probed directly after sample preparation, or after resting the sample for 14 days at room temperature. The ion-paired aluminosilicate 4 ring oligomers, acting as pre-nucleation clusters, thus are stable observables, as probed by quantitative high-resolution NMR spectroscopy. Within the time period of weeks, these pre-nucleation clusters are kinetically stable and only condense to crystallize zeolites when exposed to a hydrothermal treatment. While we refer to ion-paired pre-nucleation clusters, these consist of aluminosilicate four ring oligomer anions, ion-paired to alkali metal cations. Classical nucleation theory states that these clusters should be highly unstable due to their small size. However, we show that these oligomers are stabilized by ion-pairing, something classical nucleation theory cannot account for. In addition, the impact of crystal surface stability on HSIL zeolite crystallisation, evidenced by the presented work, depends on the ion that is present, not solely on the minimization of the interfacial surface. Based on these considerations, while the observed experimental data and crystallization kinetics could be compatible with the classical theory it seems more appropriate to tackle the crystallization behaviour from the viewpoint of molecular (in)stability.

**Zhiyu Liao** continued discussion of the paper by Yuki Kimura by communicating: In the section ‘The role of ACC as a precursor’, it says ‘Comparing ACC and solution, the structure of ACC is closer to the structure of calcite than to that of the solution’, but later it is suggested that ‘the ACC behaves in a fluid manner’. This is kind of contradicting; do you have evidence to support the former statement? Or is it an assumption?

**Yuki Kimura** communicated in reply: Because the existence of high fluidity is contrary to the observed fact that ACC particles do not fuse to each other, we reject that the ACC behaves in a fluid manner. Instead, another possibility has been suggested: that there are many water-filled voids in the ACC. Part of the supporting data indicating the structure of ACC is closer to the structure of calcite than to that of the solution is very low interfacial energy between ACC and calcium carbonate crystals, which has been described in the first paragraph of ‘The role of ACC as a precursor’ section.

**Zhiyu Liao** continued discussion of the paper by Romain Grossier by communicating: In the paper, as well as during the discussion, it's stressed that only a single crystal is formed within each microdroplet. However, from Fig. 2 (bottom right; <https://doi.org/10.1039/d1fd00090j>), it seems there is more than one crystal formed in the droplet. What does it really imply when you have a single crystal or multiple crystals in each droplet, does it indicate heterogeneous or homogeneous crystallisation?

**Romain Grossier** communicated in response: In Fig. 2 in the paper, bottom right, there is only one single crystal: its fast growth implies a dendritic growth mechanism with possible subsequent smoothening if enough supersaturated

solution is still available. Due to both droplet size and high supersaturation (fast growth), it is nearly impossible for a second nucleation event to occur before the first has entirely depleted the solution. At least, experimentally, under the here presented conditions, we never had more than a single crystal.

Equivalent droplets have been used previously where we did induce nucleation with a “controlled heterogeneity”: a sharp tungsten tip.<sup>1</sup> In these experiments, yes, it was possible to obtain several crystals due to the heterogeneous nucleation mechanism. But identifying emergence of multiple crystals with a strict signature of a heterogeneous nucleation mechanism is a step I won't take.

1 R. Grossier, Z. Hammadi, R. Morin and S. Veessler, Predictive nucleation of crystals in small volumes and its consequences, *Phys. Rev. Lett.*, 2011, **107**, 025504, DOI: [10.1103/PhysRevLett.107.025504](https://doi.org/10.1103/PhysRevLett.107.025504)

**Marko Ukrainczyk** continued discussion of the paper by Joop ter Horst by communicating: Secondary nucleation mechanism: primary *vs.* secondary nucleation is investigated in small scale vial experiments and nicely exemplified with seeded fed-batch and continuous antisolvent crystallisations of sodium bromate deracemisation in this paper (<https://doi.org/10.1039/d1fd00098e>). The secondary nucleation mechanism responsible for the dominance over primary nucleation is briefly mentioned in this work, namely due to attrition/shear. However, it is hard to expect attrition is the dominant mechanism in the performed seeding crystallisation experiments using a single crystal to induce secondary nucleation. When a crystal seed is introduced into supersaturated solution a pronounced secondary nucleation event is observed. As discussed after the talk, this was also apparent after seeding supersaturated solution with a single crystal without stirring, where no significant attrition/shear should be expected. Recent work published by BMS<sup>1</sup> focused on the shear and attrition contribution of secondary nucleation to particle size distribution (PSD) control *via* population balance modeling (PBM). In contrast, this work investigated scale-dependency of secondary nucleation, by quantifying shear forces *via* impeller power number and impeller pumping number and introducing them in the PBM model to describe the secondary nucleation contribution, in order to model and gain control over PSD over different scales (lab and pilot/manufacturing). However, recent work shed light on the secondary nucleation mechanism (supported by both computational and experimental evidence<sup>2</sup>) and pointed to a surface catalytic activity mechanism *via* “loose” 2D nucleation on a crystal surface followed by detachment of the loosely bound nucleus from a surface into the solution. The energy barrier for such a surface 2D nucleation is significantly lower as opposed to primary (3D) nucleation in the bulk of a solution. Also such a 2D surface (crystal seed templated) nucleation could also be the dominant reason for nucleation control in terms of preferential polymorphism or chiral handedness deracemisation *via* crystallisation, as opposed to uncontrolled primary nucleation in the bulk of the solution where other undesired polymorphs/enantiomers can form. Such a surface catalytic nucleation mechanism can offer a better explanation for the dominant secondary nucleation event (studied/discussed in Prof. Joop ter Horst's paper) and thus the polymorph or chiral purification outcome (facilitated by templated nucleation on the crystal seed surface of the desired polymorph or enantiomer). It is expected (and in fact observed in our experimental reality) that

such a secondary nucleation mechanism will be proportional to (beside supersaturation and temperature) the crystal seed-bed surface area and shear mixing, rather than solely attrition caused by the impeller or crystal/crystal impacts (dependent on agitation rate and crystal brittleness). Industrial relevant secondary nucleation after seeding is well established in industrial crystallization, where crystal seed is introduced in supersaturated solution to trigger (secondary) nucleation where often about 30% of the solute/batch is nucleated/crystallized at this early stage of the process (seeding stage followed by aging period). Such a secondary nucleation strategy upon seeding is often utilized to gain polymorph control (ensuring the desired form throughout the crystallisation process), and product PSD and morphology control. Therefore, fundamental knowledge of secondary nucleation is important, but it is less studied in the literature, in comparison to primary nucleation, due to experimental complexity and decoupling the problem of primary *vs.* secondary nucleation.

The application note on enantiomeric purification control (addressed in this paper) is indeed novel and much welcomed to be able to increase throughput/productivity of the deracemisation process, in comparison to Viedma ripening. How is the product purity affected by such a seeded process at relatively high supersaturations (to induce secondary nucleation after seeding), in comparison to the aging near equilibrium Viedma ripening process?

- 1 T. Rosenbaum, L. Tan and J. Engstrom, Advantages of utilizing population balance modeling of crystallization processes for particle size distribution prediction of an active pharmaceutical ingredient, *MDPI Processes*, 2019, 7, 355, DOI: [10.3390/pr7060355](https://doi.org/10.3390/pr7060355).
- 2 J. Anwar, S. Khan and L. Lindfors, Secondary crystal nucleation: nuclei breeding factory uncovered, *Angew. Chem., Int. Ed.*, 2015, 54, 14681, DOI: [10.1002/anie.201501216](https://doi.org/10.1002/anie.201501216).

**Joop ter Horst** communicated in reply: In our experiments we see enantioselective secondary nucleation occurring. We indeed do not make definite conclusions about the secondary nucleation mechanism. However, the fast rotating tip of the stirrer and the large seed crystal combined with the supersaturation create optimal conditions for attrition-based secondary nucleation. Anyway, we leave the door open to other enantioselective secondary nucleation mechanisms such as shear-induced secondary nucleation.

For deracemization processes the main product purity aspect is the enantiopurity, often quantified in the enantiomeric excess. The paper shows that under the right conditions the enantiomeric excess is maintained at close to 100% in a continuous antisolvent crystallization process. The impurity rejection of other impurities is not investigated. A major source for poor impurity rejection<sup>1</sup> can be agglomeration but this was not observed. Another source might be inclusion formation. Anyway, while impurity rejection in continuous antisolvent crystallization might be compromised by the high local supersaturation, in Viedma ripening the harsh grinding conditions might lead to incorporation of all kinds of impurities into the chiral product as well.

- 1 S. J. Urwin, G. Levilain, I. Marziano, J. M. Merritt, I. Houson and J. H. Ter Horst, A structured approach to cope with impurities during industrial crystallization development, *Org. Process Res. Dev.*, 2020, 24(8), 1443–1456, DOI: [10.1021/acs.oprd.0c00166](https://doi.org/10.1021/acs.oprd.0c00166).

**Jacek Zeglinski** continued discussion of the paper by Aaron R. Finney by communicating: Can you see in your simulations that solvent/water molecules

are entrapped in those transient disordered clusters of NaCl ions. If this is the case, is this observed in low density clusters only, or maybe also in the high density assemblies? Would you consider such entrapped water molecules to be highly impactful on the energy barriers to nucleation/crystallisation of NaCl? Can those solvent-induced energy barriers be quantified with MD simulations?

**Aaron R. Finney** communicated in response: The low-density ionic networks are highly hydrated and the water molecules – as well as ions for that matter – in these liquid-like clusters are in dynamic pseudo-equilibrium with the surrounding environment; therefore, while water is associated in the clusters, these can exchange readily with dispersed water molecules on the simulation timescales. Water molecules cannot be described as ‘trapped’ on this basis, in contrast to water molecules in amorphous solids or crystals with extremely slow diffusion. In brute force simulations, the solvent-exchange dynamics determines the efficiency of sampling different ion solvation states, and that was not an issue in the case of NaCl(aq) simulated for  $10^1$ – $10^2$  ns.

As the size of transient clusters increases, we observe significant heterogeneities in the local chemical ordering of ions, and dense ion regions emerge which are internally dehydrated. Characterisation of the size of the dense regions in the clusters (see Fig. 1C of our paper; <https://doi.org/10.1039/d1fd00089f>) makes use of a first-coordination sphere criterion, such that  $n$  here represents the scaled number of ions with five-fold or more counter-ions in direct contact. We do not observe, therefore, entrapped water molecules in the high-density regions where crystallisation occurs.

Exchange of water molecules with counter-ions must contribute to the energy barriers associated with nucleation. After all, in the assembly of ions into an ion-rich amorphous precursor or directly into a crystal, solvent removal is a crucial step. In the case of the two-step pathways we identified in our work, the energy landscape in Fig. 4A (see the red dashed line; <https://doi.org/10.1039/d1fd00089f>) indicates first an increase in the size of the high ion density region in clusters, as  $n^{\text{sph}}$  increases when  $n^{\text{sph}}(q6) = 0$ . This is directly associated with solvent removal and carries an energy penalty. Though not presented explicitly in our paper, we used nudged elastic band calculations to determine the free energy profiles along the most likely two-step pathway, and while the barrier for the reordering of ions in the dense regions dominates the nucleation barrier, the removal of water in a first-step significantly contributes to the free energy change for nucleation (*i.e.* beyond thermal energy).

It is possible, therefore, to compute barriers for solvent removal in simulations and these are correlated with increased ion–ion coordination. In our approach, we performed a range of enhanced sampling and seeding simulations to provide an ensemble of initial configurations to initiate trajectory swarms using unbiased molecular dynamics; from these we constructed a Markov state model to determine the free energy landscape and the barriers for nucleation. We also note that in Fig. 1E of our paper we computed the potential of mean force for ion-pairing in the dilute limit, where the thermodynamic energy barrier for ion desolvation during the formation of a contact ion pair is around  $4k_{\text{B}}T$ . While this is only an approximate estimate for the barrier – an accurate estimate here requires sampling of an extended reaction coordinate – it offers a framework to compute the energy barriers for solvent removal around single ions. Additional

computational methods for enhanced sampling and calculation of energy barriers, and challenges therein, are discussed in ref. 1 and 2.

1 G. C. Sosso, *et al.*, *Chem. Rev.*, 2016, **116**, 7078–7116.

2 K. E. Blow, *et al.*, *J. Chem. Phys.*, 2021, **155**, 040901.

**Sten O. Nilsson Lill** continued discussion of the paper by Joop ter Horst by communicating: Did you try any other antisolvents than ethanol, for example a chiral alcohol, and explore its impact on the kinetics of the control of enantiomeric excess/deracemization?

**Joop ter Horst** communicated in reply: We did not try other antisolvents for sodium bromate. It is an interesting idea to try though. Solubility measurements of chiral compounds in chiral solvents by the group of Heike Lorenz, however, did not indicate large changes (see ref. 1). I expect that the creation of supersaturation by the antisolvent is more important than its chirality in determining the outcome of the antisolvent crystallization.

1 S. K. Tulashie, H. Lorenz and A. Seidel-Morgenstern, Solubility of mandelic acid enantiomers and their mixtures in three chiral solvents, *J. Chem. Eng. Data*, 2010, **55**(11), 5196–5200, DOI: [10.1021/jc1006955](https://doi.org/10.1021/jc1006955).

**Alfred Y. Lee** continued discussion of the paper by Vivek Verma by communicating: In Fig. 5 in the paper (<https://doi.org/10.1039/d1fd00101a>), there is significant particle size differences between the homogeneous and heterogeneous cases for both compounds. Was there agitation in each system? If yes, how can you conclusively rule out that secondary nucleation is not driving the enhanced nucleation and contributing as the dominant factor? In terms of the fines shown in both heterogeneous examples, is this akin to media milling?

**Vivek Verma** communicated in response: The overall particle sizes of glycine and diglycine crystals are almost the same, but they appear different due to different scale bars on each image. Yes, both the homogeneous and heterogeneous solutions were agitated and that could potentially lead to some fines especially in the heterogeneous case as visible in Fig. 5 in the paper (<https://doi.org/10.1039/d1fd00101a>) and that could be due to the secondary nucleation from the impact of glass beads happening after the nucleation event. Though we are not negating the secondary nucleation effect, the main aim of the study was to observe the change in the induction time in the absence and the presence of glass beads. And the experimental data suggests that there was reduction in induction time in the presence of glass beads confirming the effect of the heterosurface and hence the propagation of heterogeneous nucleation. The induction time value was later used to obtain the nucleation rate and subsequently thermodynamic and kinetic parameters. The next phase of the study will investigate the secondary nucleation phenomenon.

**Alfred Y. Lee** continued discussion of the paper by Romain Grossier by communicating: The RH cycling process for deliquescence–recrystallization is analogous to metastable zone width determination *via* heating and cooling. In the

latter, it has been described in the literature<sup>1</sup> that the thermal history of the solution may influence the metastability. Curious to know if there is a similar effect noted in the RH cycling process – perhaps an effect with elevated RHs (akin to overheating), or possibly a crystallization memory effect due to the repetitive RH cycling?

1 K. Hussain, G. Thorsen and D. Mathe-Sorensen, *Chem. Eng. Sci.*, 2001, **56**, 2295.

**Romain Grossier** communicated in reply: Fig. 5 (cycles nucleation rank comparison) in the paper (<https://doi.org/10.1039/d1fd00090j>) was designed for that purpose. If one has in mind that we here have only two cycles in interpreting it, it does not show any memory effects. More cycling may have to be done to specifically address the question.

## Conflicts of interest

There are no conflicts to declare.