The birth of minerals: from single step to multiple step mechanisms

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ABSTRACT

Mineral formation from ions in aqueous solutions begins with complex initial stages, where amorphous and liquid-like precursors play pivotal roles before crystalline growth occurs. Both classical and non-classical nucleation and growth theories, introduced in previous chapters, offer explanations, each with their own strengths and limitations, for the complex intermediate phases observed in experimental research. Analytical techniques play a critical role in detecting and characterizing precursor phases, offering valuable insights into nucleation and growth mechanisms across various temporal and spatial scales. Molecular dynamics and modelling provide in-depth perspectives on these phases, allowing for a closer examination of their nucleation and growth mechanisms at the molecular level, and revealing the intricate processes that govern their behaviour.

KEYWORDS: amorphous; liquid; liquid-like; precursors; crystallization; pathways.

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INTRODUCTION

We have come to appreciate the astounding intricacy of mineral formation processes from ions in aqueous solutions. The original understanding of these phenomena, stemming from the adaptation of classical nucleation and growth theories introduced in Chapters 1 and 2 of this issue, has increased in complexity due to the discovery of various metastable precursor and intermediate species or phases on pathways to final crystals. These include solute clusters (e.g. prenucleation clusters), nanocrystalline solids, and most notably, amorphous solids and liquid(-like) species. Here, classical nucleation theory describes a simpler, one-step transition from dissolved ions to a stable crystal nucleus. It suggests that nucleation proceeds through a single critical point where a nucleus forms and grows into a crystal. Non-classical nucleation, however, accounts for multiple intermediate stages before the final crystalline phase appears. This conceptual extension reflects the complexity of mineral formation, which classical theory does not fully capture. In aqueous solutions, ion activity and solubility are the key variables that control saturation and mineral formation. While temperature and pressure strongly influence solubility in solutions, the activity of dissolved ions relative to solubility is the variable that drives phase transitions through saturation and supersaturation. The distinction between stable and metastable phases is key, as metastable phases, while not in the lowest energy state, can persist and influence the formation pathway, including the short-lived precursors that appear between dissolved ions and final (stable) solids. A metastable phase persists in a non-equilibrium state that is not the most stable configuration but can remain for extended periods unless disrupted by external factors. The precursors constitute different, often very short-lived, stages in the process from dissolved ions to the final solids. Detection and structural characterization of such precursors is essential for a detailed understanding of crystallization trajectories. This knowledge underpins fundamental research in physical chemistry, Earth sciences and mineralogy, biomineralisation, or advanced synthesis strategies towards functional mineral-based materials (Gebauer and Wolf 2019), such as organic-inorganic composites or mesoporous structures. Recent advances in analytical techniques, such as time-resolved spectroscopy, X-ray scattering and diffraction, and electron microscopy, have indeed enabled the experimental detection and characterisation of metastable states and intermediate phases that were previously difficult to observe. The assembly of materials emerges from the collective motion of atoms and molecules at a nanometric scale, with fast stochastic dynamics (random motions). As such, crystallisation pathways have long served as a testing ground for molecular models and molecular dynamics algorithms, acting as a computational magnifying glass to reveal atomic motions driving material synthesis.

Amorphous precursors

Amorphous precursors often emerge as part of a two-step nucleation process, where an initial amorphous or liquid-like phase forms before transforming into a more stable crystalline phase (Fig. 1). While amorphous precursors are now widely recognized in a wide range of systems, the question of why they arise remains a significant area of research. To date, three fundamentally different mechanisms have been invoked, though, in principle, more than one can occur within a single system. The first mechanism falls well within a classical picture of nucleation (see Chapters 1 and 2) and is a manifestation of Ostwalds's Rule of Stages (Ostwald 1897), which holds that, in a crystallizing system that is supersaturated with respect to multiple polymorphs the appearance of each polymorph will occur independently in the order of increasing stability (i.e., decreasing solubility). Here, a polymorph is a solid material that can exist in more than one form, each having distinct structural and physical properties. Supersaturation occurs when the ion activity product—the effective concentrations of relevant species (for this polymorph) —exceeds the solubility product limit, promoting the formation of polymorphs. Although originally based on empirical observations, this mechanism should be expected from classical nucleation theory (CNT) (Gibbs 1948). In CNT, the free energy barrier to nucleation ΔG^* scales with the third power of the surface energy α, but is inverse in the second power of the supersaturation σ according to $\Delta G^* \sim \alpha^3/\sigma^2$ (Kashchiev 2000). However, there is also a general inverse relationship between surface energy and solubility (Söhnel 1982, Kashchiev 2000). Thus, the energy barrier (ΔG^*) is smallest for the most soluble and thus the least stable of the polymorphs, provided the supersaturation is sufficiently large (Fig. 1a, point C; see also Fig. 5 in Chapter 1). Since the amorphous phase is the most soluble, it is likely to have the lowest surface energy and, thus, the lowest nucleation barrier. The amorphous phase being the most soluble indicates that it is less stable thermodynamically and likely has higher internal energy relative to more ordered crystalline phases. This increased solubility often correlates with lower surface energy, as amorphous phases typically lack the well-defined atomic arrangements that contribute to the surface energy in crystals. Consequently, with lower surface energy, the amorphous phase would have a reduced nucleation barrier, making it easier to form as an initial phase before transitioning to more stable crystalline forms. However, the role of supersaturation cannot be overemphasised. Due to the fact that the crystalline phases have lower solubility, the activity product, essentially the product of the concentrations of ions in solution (adjusted for their activity or effective concentration), of the constituent ions can always be set to a value that sits above the solubility of one or more crystalline phases, but below that of the amorphous phase (Fig. 1a, point A). A macroscopic quantity of the amorphous phase can never be produced under these circumstances. This means that when the solution's ion-activity product exceeds the solubility limit of crystalline phases but remains below that of the amorphous phase, the

amorphous phase cannot form macroscopically. It only becomes possible to observe a significant amount of the amorphous phase once the supersaturation reaches a level above its own higher solubility threshold. Unlike crystalline phases, the amorphous phase, with its disordered structure, can form rapidly to reduce supersaturation without requiring strict atomic order. This means that, even when solutions are supersaturated relative to crystalline phases, at sufficiently high supersaturation the amorphous phase becomes kinetically accessible, even when the free energy barrier is equal to or higher than that for the crystalline phases. In other words, this explains why amorphous phases often appear as initial intermediates in crystallization pathways, balancing the need for rapid nucleation with lower structural stability. Only then can the free energy barrier of CNT be overcome, as it scales inversely with the supersaturation. (Kashchiev 2000)(Fig. 1a, point B). This mechanism is often named dissolution-reprecipitation, highlighting that the appearance of the stable phase leads to a concomitant decrease in solute activity below the solubility of the amorphous phase, leading to its dissolution.

The second mechanism arises due to the common inversion of relative phase stabilities occurring in nanoparticle systems; as particle size decreases and surface to volume ratio increases, surface energy contributions begin to dominate. Thus, due to the inverse scaling between surface energy and phase stability, low-stability bulk phases can become stable nanophases (Navrotsky 2011) (Fig. 1b). For example, in the TiO_2 system, bulk rutile is more stable than anatase, but the opposite is true at the nanoscale. In this scenario, the nanoscopic amorphous precursor converts to the crystalline phase by overcoming a second free energy barrier when the particle size is sufficiently large (Fig. 1b). Recent results from Cao et al. (Cao et al. 2020) show this mechanism during the nucleation of a number of metals, including γ-Fe, Au, and Re. In all cases, growing amorphous clusters became stable above one critical size and converted to crystalline phases when they exceeded another, larger size.

In the third mechanism, the amorphous phase is a highly hydrated phase that can either form by a common nucleation process, where ions aggregate to form a solid, or as a dense liquid formed in a binodal or spinodal region, as discussed in the next section. A binodal marks the boundary between a single-phase region and a region where two distinct phases can coexist, while a spinodal represents the limit beyond which phase separation occurs spontaneously and uniformly throughout the system. A dense liquid is an unstable, short-lived phase that contains a high concentration of ions but lacks the ordered structure of a solid. This region may be unstable relative to the uncondensed phase, which refers to a state where species remain dispersed in a solution and have not yet aggregated into a solid or dense liquid phase (Vekilov 2010) (Fig. 1c). Crystallization of the dense liquid proceeds through dehydration accompanied by increased coordination, (i.e., the number of atoms or molecules that surround a central atom in a structure,

influencing the phase and stability of mineral precursors). While this crystallization process has been documented for colloids (Zhang and Liu, 2007), proteins (Houben et al. 2020) and $CaCO_3$ with high Mg content (Liu et al. 2020), the exact nature of the free energy barrier involved in the crystallization step remains debated. Even without knowing the exact free energy surface, spinodals and binodals can be inferred from experimental observations, such as changes in material properties, fluctuations near critical points, and empirical thermodynamic models. Techniques such as scattering, spectroscopy or microscopy detect phase behaviours, providing indirect evidence for these boundaries (Wolf et al. 2008), as we explain further.

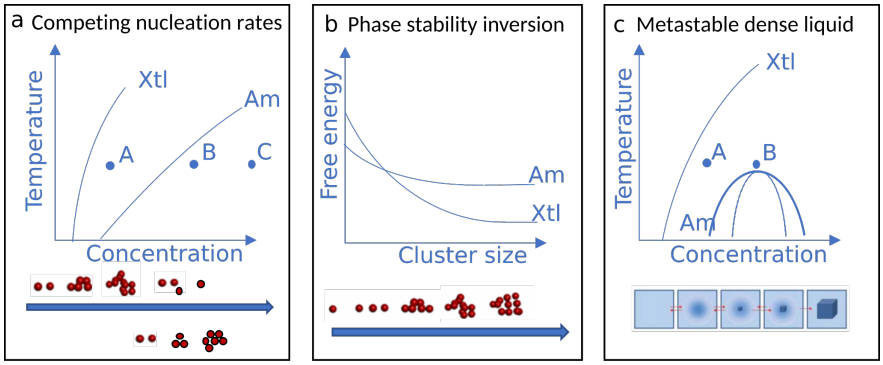

Fig. 1. *Three fundamentally different mechanisms have been proposed to explain two-step nucleation processes. (a) Crystalline (Xtl) and metastable amorphous (Am) phases nucleate independently of one another at relative rates that depend on the relative magnitudes of α^3/σ^2. At point A in the diagram, only Xtl can nucleate. At point B, both nucleate, but the barrier to nucleating Am is greater despite its smaller a due to the low value of σ. At point C, σ relative to Am is now large enough that the barrier to nucleating Am is now smaller due to its smaller value of α. As illustrated below the diagram, although Xtl will likely nucleate after Am, once it does, Am dissolves away. (b) When the stability of Am and Xtl invert at small cluster size and fluctuations are large enough to ensure the low energy phase will dominate, Am will nucleate first and remain stable until it reaches the size where the relative stabilities revert to the bulk relationship. Then Am converts to Xtl as illustrated below the diagram. (c) When the phase diagram contains a hidden spinodal region, Am is an amorphous dense liquid phase that is unstable relative to the uncondensed phase and thus it can only exist microscopically and transiently. Formation of Am in or near the spinodal region — particularly near the critical point B — provides a lower barrier to nucleation of Xtl than at point A between that region and the phase stability line for Xtl. Xtl then forms from Am, as illustrated below the diagram.*

Liquid-like or amorphous?

The crystallization of solids from liquid or molten parent phases is ubiquitous in the natural world. However, the notion that the onset of crystallization may proceed through various short-lived and microscopic liquid or liquid-like states is not widely appreciated across the geoscience

and other research landscape. While liquid states are recognized intermediates in the crystallization of proteins, polymers, and various other synthetic systems of interest to materials science, compelling evidence of liquid or liquid-like mineral precursors is restricted to a handful of experimental model systems and theoretical predictions.

Perhaps the most compelling evidence for the existence of dense liquid phases at conditions relevant to natural systems is documented by studies of the occurrence of liquid-liquid phase separation (LLPS) in aqueous hydrothermal fluids at temperatures approaching the critical point of water (374 °C; 221 bar). This phenomenon was first noted by Secoy (Secoy 1950), who discovered that at temperatures exceeding ~285 °C, aqueous solutions of uranyl-sulfate separate into two distinct liquid phases, one enriched in uranyl-sulfate, the other depleted. Yang and Pitzer (Yang and Pitzer 1989) later concluded that the declining dielectric constant of water approaching its critical temperature could induce sufficiently concentrated 2:2 electrolyte solutions to undergo LLPS and exhibit a lower critical solution temperature (LCST) above ~200 °C. In this context, the LCST refers to the specific temperature above which a solution separates into two distinct liquid phases. Some systems may exhibit an upper solution critical temperature (UCST) instead of (i.e., hexane-nitrobenzene mixtures) or in addition to a LCST (i.e., nicotine-water mixtures). Practically, this implies that dense liquids may act as mineral precursors in systems where the liquid-liquid coexistence curve exists at conditions that exceed the solubility of the solid phase. It is important to note that in the uranyl-sulfate system, the two liquid phases that form are stable in subcritical solutions; meaning, they coexist where the ion-activity product of either liquid phase does not exceed the solubility product of solid uranyl-sulfate, and where supercritical fluid is not present. This means that while these liquids are stable relative to one another under certain conditions, they are not direct precursors to crystallization below the critical point of the water rich phase. In other words, the dense uranyl sulfate liquid is not supersaturated with respect to crystalline uranyl sulfate, so the latter phase cannot nucleate in the subcritical region of the phase diagram. However, further investigations into the phase behaviour of metal-sulfate brines have revealed that metastable dense liquids are also common in these systems (Figure 2a, 2b) and can conceivably act as precursors during crystallization (Wang et al. 2013).

Liquid precursors are also considered to play a role in the formation of some calcium carbonate biominerals. This inference arises largely from the pioneering work of Gower and Odom (Gower and Odom 2000) on the polymer-induced liquid-precursor (PILP) process, during which the presence of polyaspartic acid induces LLPS in solutions supersaturated with respect to amorphous calcium carbonate (ACC). Further explorations of the microscopic character of PILP suggest that the precursor phase is an evolving complex fluid that can contain particles of

microscopic ACC. On this basis, microscopically smooth morphological features observed in images obtained from high resolution scanning electron microscopy (SEM) and X-ray photoelectron microscopy (PEEM) have been interpreted as evidence of a liquid precursor in the mineralization of spines from the sea urchin *Strongylocentrotus purpuratus* (Stifler et al. 2021). Theoretical studies on the early stages of calcium carbonate mineralization also indicate that the formation of a dense liquid is possible in the absence of stabilizing polymers. Although direct observations consistent with the existence of a wholly inorganic dense liquid phase are somewhat open to interpretation, there is increasing indication for such phases in the experimental literature (Wolf et al. 2008; Zou et al. 2017; Smeets et al. 2017). While Wolf et al. (2008) provided compelling evidence of a liquid-liquid phase separation in calcium carbonate systems, other studies, such as that of Zou et al. (2017), proposed the locus of a spinodal line based on particle size distributions, without direct observation of a liquid phase. Together, these studies support the potential existence of dense liquid mineral precursors.

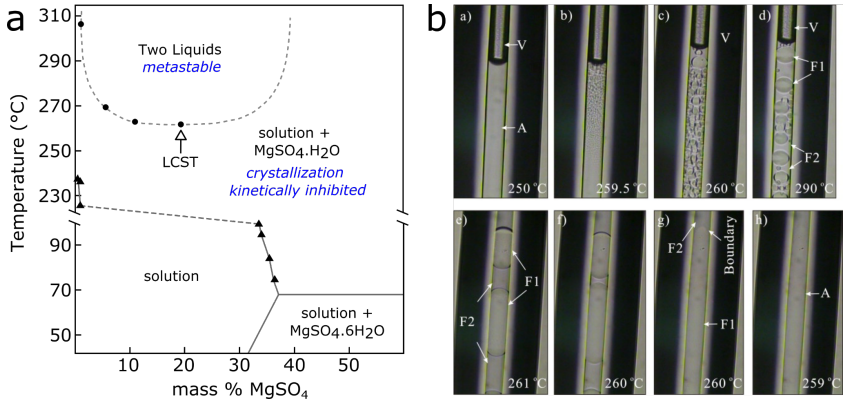

Fig. 2. Experimental observation of metastable LLPS. (a) Simplified representation of the phase relations in the $MgSO_4$-H_2O system, modified after Wang et al., 2013. The metastable dense liquid forms at temperatures in excess of the LCST and may precede the formation of magnesium sulfate monohydrate (kieserite), whose solubility curve is denoted by triangular symbols. (b) Optical microscopy images. Reversible formation of a dense $MgSO_4$ liquid at the vapour saturation pressure of the solution above 259 °C. V: vapour phase; A: aqueous phase; F1: fluid phase 1; F2: fluid phase 2. For scale: the images show fused silica capillary tubing with 300 µm outer diameter, 100 µm inner diameter. Reprinted with permission from Elsevier (Wang et al. 2013).

CHARACTERIZATION OF PRECURSORS

The challenge

The microscopic and ephemeral nature of liquid precursors presents multiple challenges to their characterization. While crystalline phases are often readily distinguishable by microscopy because the distinct interfacial tensions exhibited by each of their crystal faces results in recognizable and characteristic crystal habits, the single interfacial tension exhibited by amorphous and liquid phases drives them to assume a similar spherical morphology. Further, structural characterization of liquid and amorphous phases generally informs most upon the short-range order present in these materials rather than the dynamic character of their constituent species. The difference between a dense liquid and an amorphous solid lies in molecular mobility: a dense liquid retains mobility on millisecond-to-second timescales, observable with dynamic light scattering or NMR, while an amorphous solid is essentially rigid. Techniques like X-ray or neutron scattering can confirm the lack of dynamic fluctuations characteristic of an amorphous solid. However, although certain methods may be capable of distinguishing between the liquid and solid states, often short lifetimes and high detection limits may inhibit detection in systems of geochemical interest that contain sparingly soluble phases. However, the observed mode of phase separation can be informative in some circumstances. In systems that contain metastable dense liquid phases, LLPS can occur at concentrations exceeding that of the binodal line (Fig. 1c, 2a). At supersaturations between the binodal and spinodal lines, LLPS proceeds via nucleation of the dense liquid phase. As the dense liquid drops that form under these conditions represent only a small volume fraction of the solution, their appearance is expected to be indistinguishable from that of an amorphous solid in this regime (Fig. 3c). However, at supersaturations that exceed the spinodal, LLPS proceeds via uphill diffusion (e.g., diffusion of ions from low to high concentration) and the solution is expected to develop a microstructural fabric (Fig. 3b) characteristic of spinodal decomposition (Wolf and Gower 2017). Therefore, confirming the presence of a metastable dense liquid precursor may be most readily accomplished under conditions where the solution can be observed as it is driven beyond its stability limit (i.e., the spinodal).

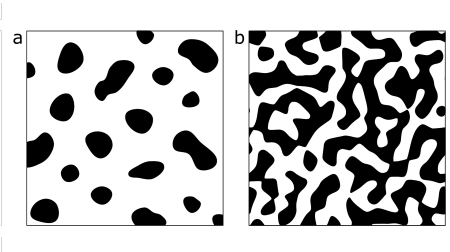

Fig. 3. Schematic diagrams of emergent morphological patterns during spinodal decomposition in a binary system. (a) Isolated domains form when the separating component is locally in low abundance. This outcome

is difficult to distinguish from nucleation solely on the basis of morphology. This is because isolated domains in (a) and possible nucleated phases both appear as discrete, rounded structures, unlike the continuous lamellar pattern in spinodal decomposition in (b). Side-by-side comparison highlights these subtle morphological differences. (b) A characteristic lamellar pattern emerges when the spatially averaged concentrations of the separating components are roughly equal. Under certain circumstances, b can evolve towards a. (Kim et al. 2016).

Experimental approach

The leap in understanding the nature of precursor species has become possible thanks to synergistic feedback between the development of suitable analytical techniques and the realisation that amorphous and liquid-like intermediates have been far more ubiquitous than it had been previously expected. The first group of detection strategies relies on ex-situ characterisation (Fig. 4). Here, one samples intermediate products along the reaction trajectory, where the specific moments of interest are decided based on heuristic criteria (Fig. 4a). Typically, the as-obtained materials from different stages must be quenched, so that the phases separate from the mother solution, thereby a phase transformation is "halted" for subsequent investigation, e.g., by centrifugation and filtration, or cryo-quenching (Fig. 4b). In the next step various analytical methods, X-ray diffraction and scattering, electron microscopy or vibrational spectroscopies provide structural and morphological information about the revealed phases (Fig. 4b, c). The choice of methods is truly vast, and restricted only by accessibility and the level of detail of information to be gained. We highlight just a few. In particular, diffraction and scattering have always been "go-to" methods because they inherently discriminate among crystalline and amorphous materials and provide extensive multi-length-scale structural information (Fig. 4d) (Karafiludis et al. 2023). Likewise, electron microscopy methods probe the structure and morphology of amorphous intermediates over different length-scales, from several microns to sub-nanometre.

The main advantage of an ex situ approach lies in its conceptual simplicity: we just have to observe the reaction and extract products of interest in a time-resolved manner as they show up (Fig. 4a). The ex situ strategies also exhibit numerous obvious disadvantages, just to name a few: reaction kinetics need to be sufficiently slow for handling; it is easy to omit important stages. A significant drawback is the fact that a "quenched" intermediate product is likely to be altered in the course of processing (such as filtration, or even quenching itself), and can further evolve and consequently may not be representative of the actual reaction pathway. We can remedy this flow, by performing quenching rapidly and with the aim to preserve the system as closely as possible to a native state. Probably the best way to achieve this is by freezing followed by transmission

electron microscopy analysis (cryo-TEM) (Saha et al. 2012; Houben et al. 2020; Karafiludis et al. 2023). Here, an educt is rapidly frozen in liquid ethane so that a liquid solution with embedded transitional phases forms vitrified amorphous ice. Such a sample is further studied with advanced microscopy approaches to reveal morphology, composition, and atomic structure of intermediates.

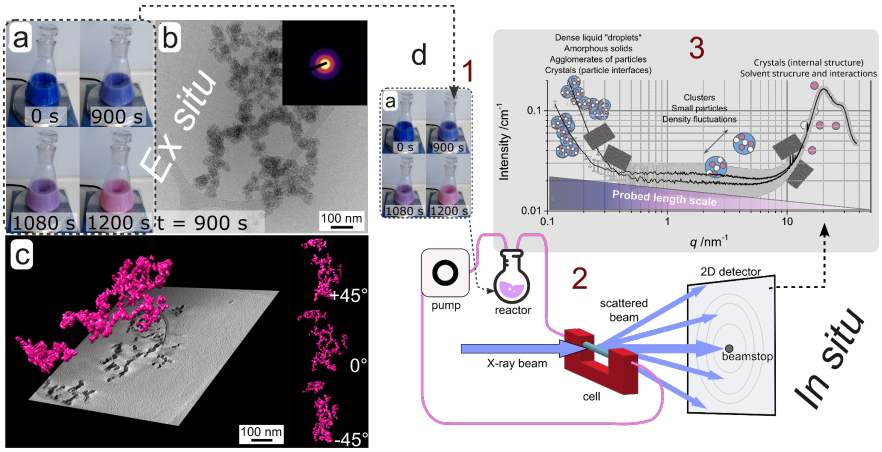

Fig. 4. Comparison of the selected in and ex situ approaches and their intersections. (a)-(c) Cryo-TEM analysis: (a) Temporal colour changes in solution, which can be used to identify the stages of the processes ; (b) cryo-TEM snapshot of the observed structures and the associated electron diffraction pattern which indicates an amorphous character of the observed particles; (c) temporary structure as a 3D volume rendering from a cryo-electron tomography reconstruction. In (d) the evolving system from (a), marked as 1, can be followed in situ with X-ray scattering methods in (2). In (2) The solution is continuously pumped through a capillary cell and scattering patterns are collected as snapshots with high time resolution (typically 100 ms to 1 s). In (3), a recorded scattering pattern represents intensity as a function of scattering vector q (proportional to a reciprocal length unit). *Each pattern in (3) contains collective information about features present in the solution, spanning from molecular length scales, to sub-microns. These features are schematically shown in (3) and include (but are not limited to): molecular structure of amorphous and crystalline species (including the actual solvent structure), size or shape of small clusters and particles (primary building blocks), larger assemblies of primary building blocks (agglomerates, droplets etc.), large interfaces, and possibly interactions among all the above including phase separation processes and local density fluctuations. With permission from the American Chemical Society, adapted from (Karafiludis et al. 2023).*

Considering the limitations of ex situ strategies, the research community has developed various complementary in situ methods, which improve observation of the evolution of precursor phases (Fig. 4a, d). The simplest approaches comprise time-resolved pH- and ion-selective probes, turbidity, conductivity, light scattering or even optical microscopy methods (Gebauer et al. 2008; Wang et al. 2013; Lauer et al. 2023; Karafiludis et al. 2023). Although these techniques are relatively information-poor, they provide a good proxy for exploring the kinetics of a crystallisation pathway. Thus, they allow for discovery of moments of interest, when amorphous phases may form, which in turn can be further confirmed and characterised with advanced ex situ analyses. However, truly in situ approaches deliver multifaceted kinetic, morphological or structural information. Therefore, one would, if possible, revisit the advanced ex situ methods, and apply them in an in situ and time-resolved manner. In this regard, various time-resolved spectroscopic methods, such as Raman, infrared (IR) or even UV-Vis etc. constitute powerful tools, which yield qualitative and quantitative structural information at a molecular scale. Spectroscopic methods are highly applicable to numerous chemical systems and can be implemented cost-effectively with standard equipment. However, the extension of the applicability of X-ray diffraction/scattering and electron microscopy to amorphous solids in aqueous environments requires novel (and expensive) technology. For instance, laboratory X-ray sources cannot provide sufficiently high flux, to characterise the evolution of mineral phases with a meaningful time resolution. Instead, synchrotron-based scattering instruments equipped with fast and sensitive plate detectors are utilised in the field (Fig. 4d). Although the kind of structural information provided by laboratory and synchrotron-based machines may be quite similar, the synchrotrons revolutionised in situ characterisation thanks to achievable time scales. Just to illustrate: the best laboratory instruments can probe information from solids in solutions with a temporal resolution of a few minutes at best, more typically several hours. On the other hand, modern scattering and diffraction beamlines approach several milliseconds, while time resolution of <1 s is routinely achieved. Moreover, synchrotron radiation sources enable use of complementary local structure methods suitable for the characterisation of amorphous and liquid-like phases, e.g. X-ray absorption spectroscopy (XAS) and high-energy X-ray diffraction with pair distribution function analysis (PDF) (Stawski et al. 2019). Nonetheless, although synchrotron methods allow for multi-length-scale structural characterisation including at the atomic level, such information practically always has a global character: it originates from an entire probed volume of the crystallising system. It is very challenging to probe local interfaces, e.g. liquid-solid, or determine the presence or the role of small population species, e.g. prenucleation clusters. For such a level of structural sophistication, electron microscopy would be, in principle, far better suited. The issue arises that EM operates under high-vacuum conditions, which effectively removes any aqueous component, thereby likely altering the nature

of the analysed samples. This problem has driven the development of in situ liquid-cell TEM (LC-TEM) (Nielsen and De Yoreo 2017; Pu et al. 2020). In LC-TEM, essentially, a regular microscope is used in combination with a special sample holder. Such a holder comprises two window membranes of ultrathin SiN_4 or graphene so that the investigated liquid environment is sandwiched between the windows at a nominal distance of ~100-1000 nm. A very thin window material, together with a relatively thin sample are sufficiently transparent for electrons to pass through and enable characterisation, yet the solution remains separated from the microscope's low pressure. In this way, one can, at least in theory, harness all the advantages of TEM in real-time, without altering the liquid sample. Nonetheless, this is only a wishful assumption due to several factors. Firstly, a probed sample environment is geometrically highly confined, which has been shown to actually affect the crystallisation pathway and change the stability of amorphous phases. Secondly, beam-sample interactions need to be considered, and the very fact of imaging with the beam is likely to change the pathway. Consequently, LC-TEM is an innovative tool to study transitional phases at the nanoscale and in situ, probably the best one we have right now, but the results obtained with this technique need to be analysed comparatively with other methods. This highlights the notion that no single technique can truly reveal the complex nature of mineralisation pathways.

BEYOND EXPERIMENTS

When investigating multistep nucleation and growth processes involving disordered precursors, the current experimental methods are not best suited to delivering information at very short temporal and spatial length scales. While several computational approaches, such as Monte Carlo simulations, static Density Functional Theory (DFT), and computational fluid dynamics, may provide valuable insights into mineral nucleation, molecular dynamics (MD) simulations are particularly suitable for studying multistep nucleation processes. This is because MD allows the explicit modelling of atomic motions and interactions over time, capturing the dynamic and collective behaviours that are crucial for understanding the formation and evolution of amorphous and liquid-like precursors. In this regard, to appreciate the role of molecular dynamics, it is essential to clarify the current challenges limiting their application and the research questions about multistep processes that can be tackled with molecular simulation methods (Finney and Salvalaglio 2024).

The first challenge is posed by the scale of the simulations necessary to capture self-assembly events, which is common to other areas of scientific inquiry (e.g. computational biophysics). The emergence of clusters (either ordered or disordered) from a supersaturated liquid is a collective

process that, even at the nanometric scale, involves thousands to millions of group units. As such, solving the first challenge is represented by the necessary trade-off between computational efficiency and accuracy of the models employed to propagate Newton's equations of motion. State-of-the-art nucleation simulations in multicomponent systems employ classical approximations of intermolecular interactions, usually neglecting reactivity. Although the advent of machine learning methods applied to the development of force fields significantly alleviates this challenge, an efficiency gap still favors the application of simplified (and inherently inaccurate) classical models. The minimal requirement for approximate molecular models to study nucleation is that the force field can capture the structure, density, and stability of the investigated crystal phase. Ideally, the phase diagram for the deployed model, in the conditions investigated, should be known, as well as liquid phase properties such as solvation thermodynamics and diffusion coefficients. The second challenge is related to the ability to sample long timescales associated with overcoming high free energy barriers typical of assembly events. A free energy barrier represents the energy threshold that must be overcome for a phase transition or nucleation event to occur. The higher the free energy barrier compared to the thermal energy of a system, the exponentially slower a given transformation is. The thermal energy is a measure of the number of attempts to cross the barrier; therefore, the larger the difference between the barrier and the thermal energy, the more attempts are required, increasing the simulation time. In multistep nucleation, this challenge is exacerbated by the fact that the same system might undergo more than one activated transition on its way to generate a crystalline phase, and often, methods relying on a preconceived notion of nucleation mechanism fall short in appropriately considering multiple steps, including the formation of disordered intermediates, i.e., amorphous or liquid-like phases. Finally, even large-scale molecular simulations explicitly model volumes much smaller than experimental ones. Finite-size thermodynamic effects can impact the thermodynamics and kinetics of the assembly of precursors and crystalline phases alike, and they need to be carefully considered to extract information that can be translated to the experimental scale.

To address these challenges, a range of modelling approaches have emerged in the literature, which can be broadly classified into two groups. The first includes methods that employ molecular simulations to compute parameters that enter independently established theoretical frameworks describing the nucleation process. A typical example is seeding methods used to obtain parameters, such as surface tension, that are then used to inform nucleation rate theories, such as CNT. The second approach involves simulation methods that act as computational experiments. In these simulations, the emergence of a new phase is modelled from the atomistic dynamics of an out-of-equilibrium parent phase. This approach enables the sampling and discovery of nucleation mechanisms without relying on a predefined theoretical framework. The

former class includes methods typically more computationally affordable and preferred when the nucleation mechanisms are known. The latter enables discovering potentially new and unexpected processes, contributing to the assessment of existing theoretical frameworks and, if necessary, developing new ones. Adopting methods that belong to this second class of unseeded methods can allow one to investigate the emergence of intermediates via LLPS, amorphous intermediates, or the formation of pre-nucleation clusters in solution. However, it is essential to note that simulations ("in silico" approaches, as in silicon chips) become particularly robust when validated against suitable laboratory ("in vitro") control experiments. This combination ensures that theoretical predictions are verified and refined by real-world observations, enhancing the reliability and applicability of the computational models.

In this context, aqueous NaCl systems represent one of the mineralizing solutions that is simplest to model, thus providing one of the earliest and most adopted case studies for developing and testing methods based on molecular simulations to study crystal nucleation from solution. Importantly, NaCl provides a level playing field to compare simulation protocols since most of the studies in the literature have been performed using the Joung-Cheatham (JC) classical force field that uses the SPC/E water model (Finney and Salvalaglio 2024). Using this model, Jiang et al. have obtained information on both the nucleation mechanism and nucleation kinetics as a function of supersaturation (Jiang et al. 2019), discovering that at low supersaturation, NaCl crystals assemble directly into a rocksalt structure (halite)~~,~~ independent~~ly~~ of their size and that the level of crystallinity of the nuclei has a strong influence on their lifetime and probability of growing. This suggests that the nucleation process at moderate supersaturations follows a mechanism that can be described by CNT and does not involve the appearance of intermediates. However, using unseeded simulations, Jiang and co-workers discovered that the situation changes when the limit of solution stability is approached. In such conditions, the nucleation mechanism switches to a two-step process, and dense amorphous salt clusters act as intermediates along the crystallisation pathway. The fact that at the limit of solution stability, the nucleation of NaCl follows a non-classical, multistep pathway has been further corroborated by the work of Bulutoglu et al. (Bulutoglu et al. 2022). The nucleation mechanism of NaCl was analysed by performing free energy calculations and by developing a theoretical approach based on the composite-cluster model able to interpret the atomistic simulation results. Finney et al. 2024, used a combination of unseeded exploration of the space of nuclei configurations (i.e., where the system is allowed to evolve without predetermined structures) and construction of kinetic models from seeded simulations. They demonstrated that below the solution stability, both a two-step and single-step mechanisms can coexist (Finney and Salvalaglio 2024). So far, moving beyond NaCl to complex systems like $CaCO_3$ introduces challenges, including multiple polymorphs and more intricate speciation and ion-solvent interactions. These effects, together

with its poor solubility, have so far inhibited a quantitative prediction of nucleation rates, limiting the scope of most of the studies to the qualitative exploration of early-stage precursors.

CONCLUSIONS

The research on multistep pathways in nucleation and crystallization has significantly advanced our understanding of complex mineral formation processes. A central focus of this research has been on the role of intermediate phases, such as amorphous solids and liquid-like precursors, which often serve as key steps in the transition from dissolved ions to stable crystalline forms. Despite these significant advancements, we are still far from establishing a comprehensive quantitative theory that can expand the Classical Nucleation Theory. The complexity of multistep pathways presents a formidable challenge, particularly in predicting when and why systems follow these pathways. The current models, while useful, fall short of offering a simple and universal explanation for the observed phenomena. The ability to predict multistep pathways and understand the underlying reasons for their occurrence remains elusive. This highlights the need for continued research and refinement of our theoretical frameworks to achieve the same level of functionality as Classical Nucleation Theory (CNT). Future efforts should focus on integrating experimental observations with advanced computational models to develop a more robust predictive capability. This endeavour holds the promise of not only advancing our fundamental understanding, but also enabling the design of new materials and processes with tailored properties, and for deepening our insights into the evolution natural Earth systems. Intermediate phases may play a key role in geochemical cycling by controlling the mobility and distribution of elements in Earth's crust and oceans, sediment formation, or even the evolution of planetary crust.

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