

## ON CLASSICAL AND NON-CLASSICAL VIEWS ON NUCLEATION

DENIS GEBAUER\*<sup>†</sup>, PAOLO RAITERI\*\*, JULIAN D. GALE\*\*, and  
HELMUT CÖLFEN\*

**ABSTRACT.** Classical nucleation theory (CNT) is based on the notion of critical nuclei serving as transition states between supersaturated solutions and growing particles. Their excess standard free energy depends on supersaturation, and determines the height of the barrier for phase separation. However, predictions of CNT nucleation rates can deviate from experimental observations by many orders of magnitude. We argue that this is due to oversimplifications within CNT, rendering the critical nucleus essentially a conceptual notion, rather than a truly existing physical entity. Still, given adequate parametrization, CNT is useful for predicting and explaining nucleation phenomena, since it is currently the only quantitative framework at hand. In the recent years, we have been introducing an alternative theory, the so-called pre-nucleation cluster (PNC) pathway. The truly “non-classical” aspect of the PNC pathway is the realization that critical nuclei, as defined within CNT, are not the key to nucleation, but that the transition state relevant for phase separation is based on a change in dynamics of PNCs rather than their size. We provide a summary of CNT and the PNC pathway, thereby highlighting this major difference. The discussion of recent works claiming to provide scientific evidence against the existence of PNCs reveals that such claims are indeed void. Moreover, we illustrate that an erroneous interpretation of the concentration dependence of the free energy has led to a postulated rationalization of the standard free energy of ion pairs and stable ion associates within CNT, which is not sustainable. In fact, stable ion associates are stuck in a free energy trap from the viewpoint of CNT and cannot be considered in a straightforward manner. On the other hand, the notions of the PNC pathway, by dismissing the idea of the CNT-type critical nucleus as a required transition state, overcome this issue. While a quantitative theory of the PNC pathway is eagerly anticipated, the rationalization of experimental observations that are inconsistent with CNT proves its qualitative explanatory power, underpinning great promise towards a better understanding of, for instance, polymorph selection and crystallization control by additives.

Key words: classical nucleation theory; early stages of mineralization; free energy landscapes; liquid-liquid separation; non-classical nucleation; pre-nucleation clusters

### INTRODUCTION

Nucleation, that is, the onset of a first-order phase transition in systems that have become supersaturated, is of enormous importance in various fields, ranging from biology, materials science and medicine to engineering and geology. It is the fundamental step in crystallization and mineralization and, thus, central to many geochemical natural processes (Ruiz-Agudo and others, 2017). Nucleation phenomena are, for instance, important in soil formation, biomineralization (Weiner and Dove, 2003), or acid mine drainage (Banks and others, 1997). While we focus on nucleation mechanisms from aqueous solutions herein, which are illustrated by these examples, the basic issue also affects phase transitions in the gas phase (for example, snow, rain, and cloud formation) or in melts (ice formation, or crystallization of primary silicates). This ultimately connects nucleation with global issues such as climate change, rendering it

\* University of Konstanz, Department of Chemistry, Physical Chemistry, Universitätsstraße 10, 78457 Konstanz, Germany

\*\* Curtin University, School of Molecular and Life Sciences, Curtin Institute for Computation and The Institute for Geoscience Research (TIGeR), PO Box U1987, Perth, WA 6845, Australia

<sup>†</sup> Corresponding Author: denis.gebauer@uni-konstanz.de

highly relevant for most—if not all—geochemical subfields. Consequently, the need for a successful quantitative theory that can be used for the prediction and explanation of phenomena associated with nucleation processes is truly fundamental. The quantitative framework, which has been—and still is—employed most frequently, is without doubt the so-called classical nucleation theory (CNT). CNT is based on ideas that were originally formulated by J. Willard Gibbs (Gibbs, 1876; Gibbs, 1877) and was quantitatively derived for the first time almost a century ago (Volmer and Weber, 1925; Becker and Döring, 1935). Since then, it has emerged as the textbook view on nucleation, which, strictly speaking, has hardly changed until today, due to its simple form and often satisfactory explanatory power. Indeed, CNT can quantitatively rationalize the scaling of nucleation rates with supersaturation ratio or temperature. However, in many cases, predictions of CNT are merely qualitative and differ from experimentally determined nucleation parameters—sometimes by many orders of magnitude (Vekilov, 2010b). In practice, this issue can be addressed by adequate empirical parametrization, thereby not limiting the usefulness of CNT, for instance, in chemical engineering. Significant deviations between predictions of CNT and experiment have also led to new developments. Two-step nucleation theory (ten Wolde and Frenkel, 1997; Vekilov, 2004; Vekilov, 2010a) conveniently explains progressively increased nucleation rates. Here, the initial occurrence of a metastable dense liquid phase reduces the barrier for nucleation of the solid, essentially due to an increased supersaturation level in the intermediate. The nucleation of the solid within the intermediate then also proceeds according to the notions of CNT, ultimately rendering two-step nucleation, in our opinion, a classical framework. Consistently, the existence of *pre-critical* metastable clusters as nucleation intermediates has indeed been invoked for a postulated unification of classical and “non-classical” nucleation theories (Habraken and others, 2013).

#### *Background of Classical Nucleation Theory (CNT)*

The central notion of CNT is the nucleus of critical size (fig. 1, top), which is the relevant transition state separating the metastable supersaturated solution from the growing new phase. Its excess standard free energy determines the probability for nucleation, that is, the height of the nucleation barrier. The major simplification within CNT is that the interfacial tension between the solution and nuclei is that of the corresponding (planar) macroscopic phase interface. In other words, even the smallest nuclei are assumed to behave as if they were macroscopic—the so-called capillary assumption. This oversimplification was realized already upon the first quantitative formulation of CNT (Nielsen, 1964) and might have even deterred Gibbs from being the first to actually write down the basic CNT equations. Today, given the progress in nanochemistry during recent decades (Ozin and others, 2009), the capillary assumption does indeed seem rather naive. For instance, clusters of a few atoms in size behave differently than bulk substances since the quasi-continuous density of states is replaced by a discrete energy level structure on that size scale (Schmid, 1992). Advanced treatments of CNT take, for instance, the size dependence of interfacial tension into account (Dillmann and Meier, 1991), but the fundamental physics of nucleation theories always remain analogous, focusing on thermodynamic barriers and their quantification. In nucleation theories, typically, additional kinetic factors (such as de/hydration, internal molecular rearrangements, *et cetera*) are completely neglected, because they are difficult to quantify. Still, as already pointed out, CNT is a highly useful and simple framework for the explanation and prediction of experimental observations. However, unfortunately, CNT is often considered as a dogmatic framework. It is thus imperative to emphasize and underscore that CNT does not constitute a physical law—it depends on oversimplifying assumptions—and other theories that may be inconsistent with the notions of CNT can certainly be physically sound. On the

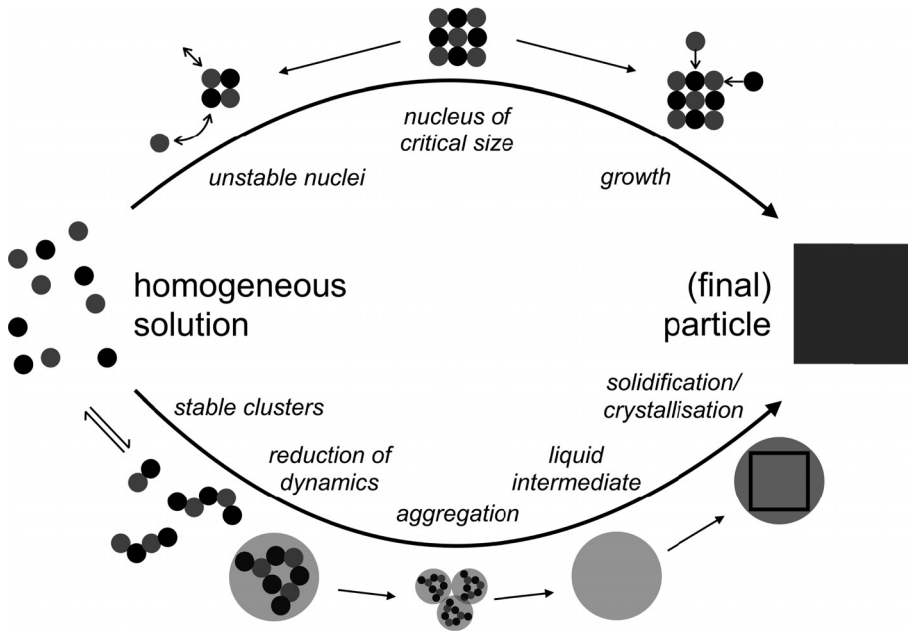


Fig. 1. Schematic illustration of the mechanism of nucleation according to classical nucleation theory (CNT, top) and the pre-nucleation cluster (PNC) pathway (bottom). For different polymorphs or forms, the accessibility depends on the level of supersaturation. The sizes of the different species are system specific and cannot be fully generalized; the critical size (top, middle) for realistic supersaturation levels is typically within tens of ions, that is smaller than approximately 3–4 nm in diameter (Hu and others, 2012). PNCs are similar in size but thermodynamically stable, and thus significantly more abundant than classical (pre-) critical nuclei that cannot occur macroscopically, from a thermodynamic point of view. The smallest sizes of phase separated nano-droplets, which directly emerge from the PNC precursors, are thus also in the lower nanometer regime. Upon aggregation and coalescence, dense liquid droplets with sizes up to several hundred nanometers can be formed (Smeets and others, 2017). Consequently, depending on the kinetics of aggregation and dehydration, the size of solid amorphous intermediates can range from *ca.* 20 nm to hundreds of micrometer in size (Cartwright and others, 2012). For further explanation see the text. Figure and caption reproduced from Gebauer (2018).

other hand, we argue that the critical nucleus should be considered a conceptual notion rather than a species that truly exists. In our opinion, it is inevitable that microscopic, and ultimately atomistic, details of nucleation processes will challenge the notions of CNT.

From the viewpoint of CNT, any associated states smaller than the critical size are higher in standard free energy than the monomeric chemical species ( $\Delta G^0 > 0$ ), which eventually constitute the nascent particles or crystals, that is, atoms, ions, molecules or even polymers like proteins. Obviously, this is not true for calcium carbonate ion pairs, for instance, which are thermodynamically stable with respect to free calcium and carbonate ions [equilibrium constant  $K_{IP} \gg 1$  (Plummer and Busenberg, 1982), with the corresponding standard free energy with respect to free ions,  $\Delta G_{IP}^0 = -RT \cdot \ln K_{IP} < 0$ ; with universal gas constant  $R$ , temperature  $T$ —independent of supersaturation. Here, the definition of thermodynamic stability is based on standard conditions, where the standard concentration is  $c^0 = 1$  mol/L. Even though this seems an unrealistic concentration from the viewpoint of CNT at first glance, especially for hardly soluble minerals like calcium carbonate, it is the relevant state of reference. Herein, this is demonstrated by showing that 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 (see below). In fact, owing to their excess standard free energy, the concentration of (pre-) critical nuclei is minuscule (see below; eqs 7 and 8). An example calculation for specifically chosen, typical parameters at a supersaturation ratio of  $S=10$  equates to one critical nucleus in a volume  $10^8$  times that of planet Earth (Nielsen, 1964). For  $S=20$ , the concentration of critical nuclei then increases by 23 orders of magnitude, which is still a minor molar concentration, but would lead to the occurrence of ten critical nuclei in 1 mL per second (Nielsen, 1964), thereby facilitating nucleation. While this is a nice illustration, and quantitative rationalization of induction times by CNT, it highlights that any species that can be experimentally detected in under- or moderately supersaturated solutions are almost certainly *not* (pre-) critical nuclei, due to their excess standard free energy. This is one of the major reasons why CNT can rarely establish a molecular picture of nucleation [also see Davey and others (2013)]. As soon as we can detect nanoscopic particles or crystal precursors, they are either rather post-critical, or thermodynamically stable species. Since CNT stipulates that ion associates smaller than the critical size are thermodynamically unstable, these species would then be pre-critical *only* from the point of view of size within a CNT perspective. As thoroughly demonstrated herein, thermodynamically stable ion associates cannot play a role on CNT-pathways to nucleation (while they are key in the pre-nucleation cluster pathway, fig. 1, bottom). Also, their existence cannot be rationalized due to the simplifying assumptions underlying CNT thermodynamics—only thermodynamically unstable states can be rationalized and lie on the pathway to the transition state to nucleation that is considered within CNT, the critical nucleus. In fact, the idea that transition states represent merely conceptual notions, owing to the underlying thermodynamics, is well established in chemical kinetics, for instance, based on the treatment of the activated complex (Eyring, 1935). It is seldom realized in nucleation research, however, where detected nanoscopic species have unreservedly been labeled as critical or pre-critical nuclei (Habracken and others, 2013), against all odds arising from fundamental thermodynamic principles, and without quantitative assessment of the corresponding *absolute* population frequencies and/or respective thermodynamics (see below).

Even though thermodynamically stable solute associates, such as ion pairs or complexes, are more abundant than critical nuclei—by many orders of magnitude, under virtually all conditions of under- and (moderate) supersaturation—, CNT neglects stable ion associates as precursors to critical nuclei, because they are viewed as stuck in a free energy trap (De Yoreo, 2013). It is more probable to form a critical nucleus via random collisions of the free monomeric constituents, because they are much closer to the relevant transition state in terms of standard free energy (see below). *Nota bene*, this conclusion is only true within CNT itself. For transition states between the supersaturated solution and the solid other than critical nuclei, there may well be alternative, probable nucleation pathways that start, for instance, from stable solute associates. 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”. This of course does not mean that there is no “non-classical” transition state to nucleation; it rather means that the nucleation barrier is not based on species as defined within CNT, and the structural and thermodynamic properties of these species instead fundamentally differ from those of the macroscopic bulk. However, the term “non-classical nucleation” cannot be strictly defined (Kashchiev, 2000); it has been used for alternative approaches that overcome many of the original, oversimplifying assumptions, but still yield essentially analogous physics to CNT (also see above)—though sometimes only close to the binodal limit (Cahn and Hilliard, 1959).

*The Notions of the Pre-Nucleation Cluster Pathway*

In recent years, we have been introducing a new nucleation framework based on thermodynamically stable clusters as fundamental precursors to particles (fig. 1, bottom). Although we have called this “non-classical nucleation” ourselves originally (Gebauer and Cölfen, 2011), we now prefer being unambiguous by referring to it as what it is—the pre-nucleation cluster (PNC) pathway (Gebauer and others, 2014)—, rather than what it is not—classical in the sense of Gibbs, Becker and Döring, Volmer and Weber, or even Cahn and Hilliard. In fact, our original paper on calcium carbonate PNCs (Gebauer and others, 2008) has sparked many lively discussions and hot debate (see for example, *Faraday Discussions* 159 and 179), and led to numerous follow-up studies not only on calcium carbonate, which eventually allowed a formulation of the PNC mechanism (Gebauer and others, 2014). In brief, ions undergo association processes in solution, which are essentially spontaneous from a macroscopic perspective and produce PNCs that are significantly larger than ion pairs, on average. The size distribution of PNCs should relate to that of polycondensation polymers (Flory, 1936; Flory, 1946), constituting a chain-like structural form called dynamically-ordered liquid-like oxyanion polymer (DOLLOP) in case of calcium carbonate (Demichelis and others, 2011), which is highly dynamic. Whether or not this structural form is more general for PNCs of other compounds remains to be shown, while there are some indications for solute associates of organic molecules relating to DOLLOPs (Raiteri and others, 2012). In any case, PNCs formally do not have a phase interface and are solutes, whereby the barrier associated with condensation towards solid-state, bulk-like forms appears to be based on dehydration (Demichelis and others, 2011). The clusters establish a dynamic equilibrium population, which is thermodynamically stable ( $\Delta G^0 < 0$ ), but do not grow without limit. In the PNC pathway, the size of the clusters plays only a secondary role. As the supersaturation increases, more of the larger clusters occur, which can internally develop a higher coordination than in the initially chain-like form (Wallace and others, 2013). This leads to a significant decrease of the dynamics of the clusters, rendering them post-nucleation species that now have an interfacial surface, which is defined by a distinct difference of the dynamics inside of the cluster and the solution (Sebastiani and others, 2016). Thus, cluster dynamics, rather than size, is important for phase separation from the point of view of the PNC pathway. Originally, the phase separation mechanism was interpreted as a spinodal one (Wallace and others, 2013), but taking experimental observations into account, it is more likely a binodal liquid-liquid demixing event (Gebauer and others, 2014). This notion was also corroborated by recent experiments (Sebastiani and others, 2016). Due to the generation of interfacial surface area, which is thermodynamically unfavorable, the as-formed post-nucleation nanodroplets, which directly emerge from the PNC precursors, then aggregate to form larger and larger droplets, thereby minimizing the surface area of the new phase, which eventually dehydrates to form solid, amorphous calcium carbonate (ACC) particles. As opposed to recent claims (Smeets and others, 2017), experimental evidence of the transformation of ion associates into liquid droplets has been achieved by means of THz spectroscopy (Sebastiani and others, 2016) as shown in figure 2. The experiments provided evidence of a distinct change of the dynamics and structure in the hydrogen-bond network of water in the pre-nucleation stage (fig. 2B), which, owing to the shape of the titration curves (fig. 2A) and very long induction times at the corresponding conditions, can only be explained by liquid-liquid separation. Notably, the locus of the respective binodal limit was found to correspond to the solubility threshold of the initially formed amorphous solid (figs. 2A and 2B), strongly underpinning the notion that it indeed forms via the dehydration of initially liquid precursors. Also, a detailed literature review (Gebauer and others, 2014) as well as new original

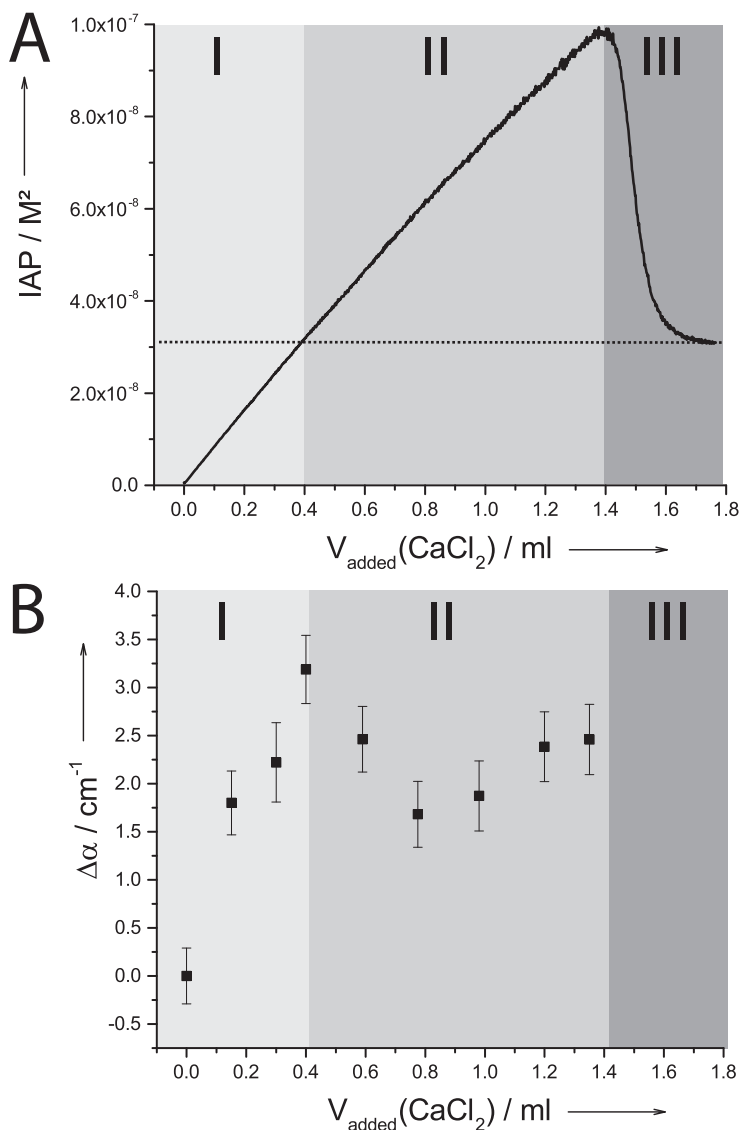


Fig. 2. (A) Development of the ion activity product (IAP) of calcium and carbonate ions obtained from potentiometric titration at constant pH 9.00 upon continuous and slow addition of 10 mM calcium solution into 10 mM carbonate buffer. The horizontal dotted line represents the solubility threshold of proto-calcite ACC (pc-ACC) (Gebauer and others, 2010). (B) Corresponding THz-absorption (2.1–2.8 THz) of samples drawn from the titration experiment. The THz data provide evidence of a distinct structural and/or dynamic transition upon exceeding the solubility threshold of pc-ACC in the pre-nucleation regime (at the transition from regime I to II). The establishment of the solubility threshold after nucleation of the solid occurs in regime III. The induction time for nucleation of a solid at the border between regimes I and II, however, exceeds several hours, and the pre-nucleation THz response cannot be due to the nucleation of solid  $\text{CaCO}_3$  in the spectrometer. Moreover, as the linear parts of the titration profile (A) represent equilibrium stages (Gebauer and others, 2008), the slope of the IAP development is inversely reciprocal to the equilibrium constant describing the binding of the ions. In regime I, ions are bound in PNCs. Upon entering regime II, the THz data provide evidence for a phase transition (B), which must be a liquid-liquid transition; in equilibrium, only a dense liquid can adjust its composition to accommodate the ions via binding, upon a continuously increasing chemical potential in the mother liquid, evident from the continuously increasing IAP (A). By contrast, the solid establishes its solubility threshold in regime III. Figure reproduced with permission from Sebastiani and others (2016).



work (Scheck and others, 2016) suggest that the PNC pathway is a rather common phenomenon in aqueous solutions, also beyond calcium carbonate. For instance, the combination of, *inter alia*, analytical ultracentrifugation and THz spectroscopy showed that the nucleation of tartaric acid crystals involves PNC precursors (Soltani and others, 2017). However, several recent papers claim to provide proof that (i)  $\text{CaCO}_3$  PNCs do not exist, and that (ii) particle formation and aqueous speciation in the  $\text{CaCO}_3$  system can be satisfactorily described by CNT.

#### DISCUSSION

Here, we would like to focus on three recent papers (Carino and others, 2017; Smeets and others, 2017; Henzler and others, 2018), and take the opportunity, taking all of the above into account, to demonstrate that no sustainable scientific evidence against the PNC pathway has in fact been reported. Moreover, when explaining those results within the framework of CNT, we think that substantial errors were made. First, it is important to point out that solute association in the calcium carbonate system can be described by different models, given that they can describe experimental binding data [see Kellermeier and others (2014) for details]. As Smeets and others (2017) correctly realize, the experimentally observed, linear binding profiles are consistent with different speciation models, from ion pairs to PNCs, so additional evidence is required, for instance, on the size of the forming species, in order to be able to arrive at an accurate speciation. However, they claim that the correct prediction of experimental binding data by a speciation program (Felmy and others, 1984), which only takes ion pairs into account, would go against the notion of PNCs. The latter is clearly a contradiction of the former, and thus does not rule out the existence of PNCs. On the other hand, the example formation of a defined  $(\text{CaCO}_3)_6$  cluster population with a seemingly arbitrary equilibrium constant was used to show that non-linear binding profiles would be obtained (Henzler and others, 2018), as opposed to experiments. However, the specific speciation model examined in the work of Henzler and others (2018) was never previously used for describing PNC formation, as it is indeed not in accord with experimental binding data, or the previously introduced notion of underlying equal binding constants for all association steps upon PNC formation (Gebauer and others, 2014; Kellermeier and others, 2014). Dynamic light scattering (DLS) measurements on pre-nucleation states exhibited only a minor scattering intensity, which was interpreted as additional proof against the existence of PNCs (Smeets and others, 2017). However, this observation can be equally well explained by insufficient data quality due to the dilute solutions and the instrument used. In our opinion, this is actually the likely explanation, as the DLS data were acquired on a Malvern Instruments Zeta Sizer (Smeets and others, 2017), which is a benchtop device for routine measurements, but even single ions, ion pairs and small clusters can be detected by DLS when advanced instrumentation is employed, and sufficiently high concentrations are accessible (Georgalis and others, 2000). The argument of Smeets and others (2017) could thus be caricatured by claiming that their data would rule out the existence of ions and ion pairs in the solutions as well [regarding the debate on the existence of ion pairs, also see Gal and others (1996)]. Along similar lines, X-ray absorption near edge spectroscopy (XANES) data on dilute calcium carbonate solutions were only tested against the occurrence of defined populations of a  $(\text{CaCO}_3)_6$  cluster (Henzler and others, 2018), which, again, does not reflect the PNC notion (see above). Furthermore, it appears that the signal-to-noise ratio was insufficient to exclude significant populations of clusters larger than ion pairs with a dynamic, decaying size distribution according to the notions of Flory, reflecting the PNC notion more accurately. In another work (Carino and others, 2017), it was shown that “classical” species such as ion pairs could describe experimental binding profiles, which alone is inconclusive regarding the existence of PNCs as already reasoned above.

Kinetic modeling further suggested that the species relevant for growth upon the establishment of the post-nucleation solubility threshold were monomeric ions, based on the obtained diffusion coefficients (Carino and others, 2017). However, this does not rule out the possibility that the initial ACCs were formed via dehydration of liquid droplets according to the PNC pathway, because the as-formed solidified primary ACC particles could also grow predominantly via single ions towards the initial establishment of the solubility threshold.

#### *Unfounded Criticism of Analytical Ultracentrifugation*

Despite the lack of sufficient experimental evidence against PNCs, analytical ultracentrifugation (AUC) data, which do provide proof of the existence of species significantly larger than ion pairs in the pre-nucleation stage (Gebauer and others, 2008; Pouget and others, 2009), were fundamentally challenged (Smeets and others, 2017). It should be noted that AUC is an absolute, first principle technique, which was historically used to demonstrate the existence of macromolecules (Svedberg and Fåhræus, 1926). As opposed to light scattering or cryo-TEM, every species in the system is detected upon fractionation in the AUC, which is likely why AUC can detect PNCs in dilute solutions as opposed to many other techniques (Gebauer and Cölfen, 2011). In fact, even single sedimenting ions can be detected in rather dilute systems (Gebauer and others, 2008). The skepticism against the AUC evidence for PNCs is essentially threefold (Smeets and others, 2017). First, it was highlighted that we had used the densities of amorphous  $\text{CaCO}_3$  (ACC) and ikaite for calculating the average size of DOLLOPs, but that the density of the PNCs of this structural form would likely be smaller than those of the references. This is almost certainly true, but the argument completely overlooks that the dependence between sizes and densities obtained from sedimentation coefficients is reciprocal (Mächtle and Börger, 2006; Gebauer and others, 2008; Planken and Cölfen, 2010). That is, if the density of the PNCs was lower than that of ACC (it very likely is), then their average size would be somewhat larger than the lower size limit of *ca.* 2 nm given in the original work (Gebauer and others, 2008). Second, it was argued that the sedimenting PNCs had experienced a concentration gradient due to sedimentation through the cell, thereby generating supersaturation. This is a misunderstanding of the AUC sedimentation velocity methodology, because sedimenting species migrate through the original solution with essentially unchanged ionic equilibrium concentrations (as the boundary for the small ions naturally follows behind all larger species). In fact, the evaluation of binding equilibria by means of AUC is well established, there is simply no interference of the claimed gradient effects (Lebowitz and others, 2002). Third, we evaluated the broadening of the sedimenting boundaries over time and thereby obtained diffusion coefficients, which were used to calculate the average size of PNCs, independent from that obtained from assumed densities and measured sedimentation coefficients, employing the Stokes-Einstein equation. While there might be issues with the Stokes-Einstein equation if the solvent viscosity is increased at the solute surface (Zhang and others, 2016), the argument of a postulated, complete break-down of the equation (Smeets and others, 2017) is certainly not true. The point really is that the solvent viscosity near a solute surface is similar to that of the hydration layer, and thus larger than that of the bulk solvent, perhaps by a factor of 2, which becomes important especially at small sizes. Since the viscosity enters the denominator of the Stokes-Einstein equation, the viscosity correction would roughly double the size of the PNCs determined via diffusion coefficients. In the original work (Gebauer and others, 2008), the average PNC diameter was 2.1 nm from sedimentation coefficients, and 0.9 nm from diffusion coefficients, bringing the independently determined values into better accord when the viscosity is corrected. For very small species with radii up to 3 to 5 Å, an empirical correction formula for the hydrodynamic radius from the Stokes-Einstein equation was



given by Schultz and Solomon (1961), who were misleadingly cited by Smeets and others (2017) postulating a “break-down” of the equation at small solute sizes. However, the PNC sizes are larger than the limit of 5 Å. Also, the formula would correct the hydrodynamic radius to larger values similar to the effect of increased solvent viscosity of a hydration layer. Furthermore, it has to be realized that we determined the diffusion coefficients of the ions as well and calculated their size in the same way. Our AUC analyses thus had an internal reference, which provided values for the free, hydrated ions that were consistent with the literature (Gebauer and others, 2008). The determined diffusion and sedimentation coefficients are independent because they were obtained from the broadening and movement of sedimenting boundaries over time, respectively, and yielded similar respective sizes, as well as size differences between single ions and PNCs. Another argument was made that the AUC data did not account for the impact of cluster size distribution (Smeets and others, 2017). This is due to the rapid dynamics of PNCs, which constantly form and dissociate (Demichelis and others, 2011) on a time scale, which is by far faster than that of an AUC experiment that takes several hours. As already well established for rapidly and reversibly interacting proteins, in this case, a time averaged sedimentation coefficient distribution is obtained (Howlett and others, 2006). Thus, none of the arguments proposed to date invalidate the notion that the AUC analyses do indeed prove the existence of PNCs.

#### *Initial Formation of Amorphous Intermediates versus Vaterite*

Besides the arguably unjustified criticism of AUC data, another peculiar point concerns the initial solid phase to be observed during titration experiments. In the original experiments (Gebauer and others, 2008) this was found to be ACC, though in what was argued to be a re-production of the same procedure it was claimed that vaterite had formed directly instead of solid ACC (Smeets and others, 2017). The initial solubility product was in fact significantly higher than that of vaterite also in the experiments of Smeets and others (2017), and appears to be consistent with the reported value for proto-calcite ACC (Gebauer and others, 2008; Gebauer and others, 2010). It must also be noted that the solubility of ACC reported in the literature (Brečević and Nielsen, 1989), which was never exceeded in the titrations, is not necessarily the relevant form of ACC forming under the conditions in the titration experiments, as discussed in detail elsewhere (Cartwright and others, 2012). Smeets and others (2017) proposed that we had not corrected for activities and CO<sub>2</sub> in- and out-gassing in the original work (Gebauer and others, 2008), but the fact that their more thorough treatment yielded the same solubility threshold underpins that our initial simplifying assumptions are indeed valid—as already shown elsewhere (Kellermeier and others, 2014). The assignment of the solubility threshold, which is significantly higher than the literature value of vaterite, to vaterite by Smeets and others (2017) is thus arbitrary and most questionable, also from the point of view of thermodynamic modeling supporting the initial occurrence of ACC (Carino and others, 2017). It moreover contradicts recent experiments on the locus of the liquid-liquid binodal limit and the formation of ACC from liquid precursors, with and without trace amounts of polycarboxylic acids (Sebastiani and others, 2016). The latter findings were also entirely disregarded in the discussion of the formation of a dense liquid phase during their work (Smeets and others, 2017). In this context, the criticism by Smeets and others (2017) that our extensive work on silica-stabilized ACC (Kellermeier and others, 2012) could be explained by silica precursors alone (Carcouët and others, 2014) fails to mention that we did find these silica precursors too, but that their occurrence is pH dependent, and that numerous reference experiments at relevant pH levels actually strongly underpinned our interpretation for the role of CaCO<sub>3</sub> clusters during ACC formation also in the systems containing silica.

*PNCs and Computer Simulations*

Computer simulations, based on molecular dynamics, have also featured as evidence in the debate regarding calcium carbonate nucleation since they can provide both an atomistic picture of what is occurring in solution and potentially even quantitative data, such as free energies and equilibrium constants. Demichelis and others (2011) presented data that showed that a dynamic equilibrium distribution of cluster sizes was possible based on DOLLOPs, that gave way to phase separation at high pH and/or concentration. Smeets and others (2017) have repeated these simulations over an extended range of conditions and have identified the phase separation as the formation of a dense-liquid phase, as proposed by others previously, while at low concentrations they also find a distribution of cluster sizes, though the rate of decay of population with size is greater than in the earlier work. This latter study suggests that the equilibrium constant for ion pair association is actually lower than for ion pairing itself, and that the stronger binding seen in Demichelis and others (2011) may be representative of the behavior in a dense-liquid phase, rather than dilute solution, due to the high concentrations. Of course, unbiased simulations at low concentrations carry a higher degree of uncertainty due to diffusion limitations and truncation of the equilibria due to the finite number of ions. A key point is that while the equilibrium constant for ion pair association in dilute solution appears now to be smaller than previously thought, it still remains greater than unity (that is association is stable, but the cluster size distribution decreases with increasing numbers of ion pairs due to the mM concentration of ions multiplying the equilibrium constant).

Henzler and others (2018) have also made a significant step toward overcoming the challenges of performing simulations under conditions where ion concentrations are low through the introduction of coarse-grained techniques. Here the association of cations and anions is described by potential of mean force curves determined from atomistic techniques, which allows the explicit description of water to be removed, thereby leading to a dramatic increase in length- and time-scales that are accessible. Based on this approach, it is argued that the use of the free energy curve from a hybrid of quantum mechanics, molecular mechanics and continuum electrostatics (DFT+MM/CE) leads to a “predominance of ions and ion pairs”, while the original molecular mechanics alone yields a cluster distribution that has a high concentration of larger clusters. While the existence of a systematic error in the molecular mechanics is not in doubt, since it has been shown to overestimate the free energy of ion pairing by  $\sim 4$  kJ/mol (Kellermeier and others, 2016), there are also uncertainties and approximations within the approach of Henzler and others (2018) that need to be appreciated. Firstly, the method relies on aligning the interaction curves of the DFT calculation with the molecular mechanics, and then again at the boundary of the latter with the continuum model. Examination of figure 3A [reproduced from Henzler and others (2018)] shows that the DFT and MM curves do not align well and are almost orthogonal at the end of the DFT data, while there is a discontinuity in the slope of the MM at the boundary with the continuum. Both of these result in a significant quantitative uncertainty and ultimately a rather arbitrary scale factor in the equilibrium constants. Secondly, the MM curve used is not fully converged as can be seen by comparison with a similar curve published for the model that does indeed overlay the continuum result at the boundary [fig. 3B, reproduced from Raiteri and others (2015)]. Thirdly, the approximation that the  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  ions interact with each other according to the highly-screened form computed in water, as a high dielectric solvent, is only valid for small hydrated clusters and progressively breaks down as the system tends toward dense species. In effect, the model is inconsistent with CNT since it cannot properly describe the solid phases of calcium carbonate. The key point to take away is that all computational methods have their issues and the quest for

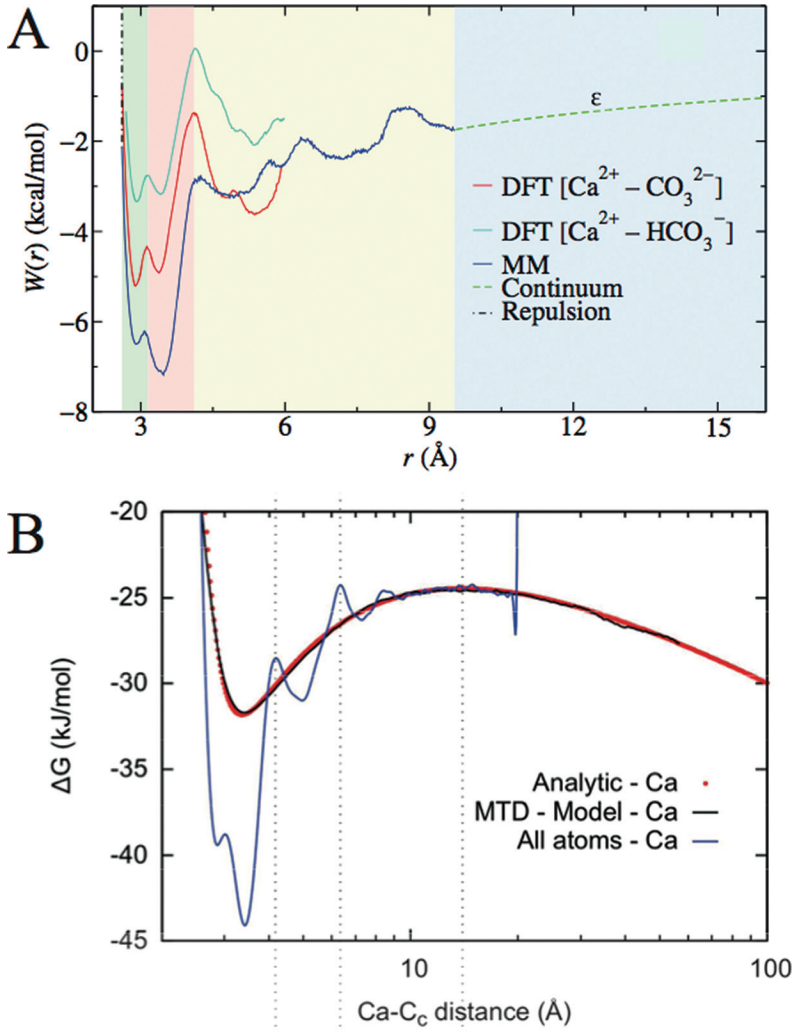


Fig. 3. (A) The computed potentials of mean force for calcium carbonate ion pairing from Henzler and others (2018). The color-coded areas correspond to the bidentate bound state (light green), monodentate bound state (light red), solvent-shared bound state (light yellow) and aqueous calcium (light blue). (B) For comparison, the data of Raiteri and others (2015) for the  $\text{Ca}-\text{CO}_3$  pairing free energy calculated using well-tempered metadynamics for the fully atomistic system (blue line), for a model system of two doubly charged point particles (black line), and the analytic solution for the model system (red dots). Reproduced with permission from Henzler and others (2018) (A) and Raiteri and others (2015) (B).

quantitative accuracy is an on-going challenge. However, while there may be variability in the values, all studies to date agree that the equilibrium constants for ion pair association are greater than one (that is, negative standard free energies) for calcium carbonate.

#### *Flawed Interpretation of CNT Thermodynamics*

The last, but most critical point that must be addressed is the conclusion that Smeets and others (2017), “[...] demonstrate that the bound calcium in solution is predominantly present in the form of ion pairs alongside a population of clusters

stochastically formed from the association of ions/ion pairs, of which the abundance decays rapidly with increasing cluster size in accordance with CNT.” This seems equivalent with the idea that, “[. . .] ion pairs are the fundamental unit leading to nucleation of the first  $\text{CaCO}_3$  condensed phase”, while clusters form “[. . .] at a rate, in relative numbers, and with energies of formation that are expected from CNT” (Henzler and others, 2018). In our opinion, the essential conclusion is fundamentally flawed. It is crucial to realize that, as already discussed above, CNT can be regarded as a transition state theory, where pseudo-equilibria towards the formation of the critical nucleus are formulated. This is, at first, largely analogous with the treatment of the activated complex (Eyring, 1935). Critical free energies from CNT are clearly standard free energies, because they are obtained by comparison of the interfacial standard free energy of the nuclei, represented by that of the macroscopic case, with the driving force for phase separation. In other words, in analogy to the theory of the activated complex (Eyring, 1935), the CNT nucleation rate is proportional to the equilibrium constant for the formation of the critical nucleus, whereas the corresponding standard free energy is derived from macroscopic material properties. In the specific case of calcium carbonate, the driving force for phase separation is given as the affinity  $\sigma$  (fig. 4);

$$\sigma = RT \cdot \ln[a(\text{Ca}^{2+})a(\text{CO}_3^{2-})] + \Delta G_{\text{sp}}^0 \quad (1)$$

where  $a(i) = \gamma \cdot c(i) / c^0$  is the activity of the ions  $i$ , and  $\Delta G_{\text{sp}}^0$  is the standard free energy of the free ions in equilibrium with the nucleated phase, which can be calculated from the solubility product  $K_{\text{sp}}$  (fig. 4);

$$\Delta G_{\text{sp}}^0 = -RT \cdot \ln K_{\text{sp}} \quad (2)$$

Note that  $\sigma < 0$  and  $\sigma > 0$  for the case that phase separation is thermodynamically impossible and possible, respectively, as opposed to the sign convention of  $\Delta G$ . Within CNT, this approach is valid for all nuclei sizes, the critical nuclei merely represent a special state that provides a maximum in  $\Delta G^0$ , at which the surface and bulk contributions to the standard free energy of nuclei are balanced. Henzler and others (2018), however, quote free energies of the identified associated states with respect to low millimolar concentrations, that is, not with respect to standard conditions (fig. 5). Their motivation for using this unconventional point of reference appears to be based on the realization that standard free energies do not show whether a reaction is possible ( $\Delta G < 0$ ) or impossible ( $\Delta G > 0$ ) at any given concentration. The concentration dependence of the corresponding reaction free energy  $\Delta G_{\text{IP}}$  for the example of calcium carbonate ion pairing is given by;

$$\Delta G_{\text{IP}} = \Delta G_{\text{IP}}^0 + RT \cdot \ln \left[ \frac{a(\text{CaCO}_3^0)}{a(\text{Ca}^{2+})a(\text{CO}_3^{2-})} \right] = \Delta G_{\text{IP}}^0 + RT \cdot \ln Q \quad (3)$$

$$\text{with } \Delta G_{\text{IP}}^0 = -RT \cdot \ln K_{\text{IP}} \quad (4)$$

where  $\Delta G_{\text{IP}}^0$  and  $K_{\text{IP}}$  are the standard free energy and equilibrium constant of ion pair formation, respectively. It can be seen immediately from equation (3) that for any activities of free ions and ion pairs that result in values  $Q > K_{\text{IP}}$  or  $Q < K_{\text{IP}}$ , the free energy of reaction would be  $\Delta G_{\text{IP}} > 0$  or  $\Delta G_{\text{IP}} < 0$ , and ion pairs would dissociate or free ions associate, all respectively, until the state of equilibrium  $Q = K_{\text{IP}}$  is established and  $\Delta G_{\text{IP}} = 0$ . In this sense, the free energy of reaction can be used to assess into which direction a process proceeds at any given concentration, but it is not a criterion for thermodynamic stability. Rather, Henzler and others (2018) introduce unconventional reference concentrations, say,  $c' = 0.0001$  mol/L. For the example of the

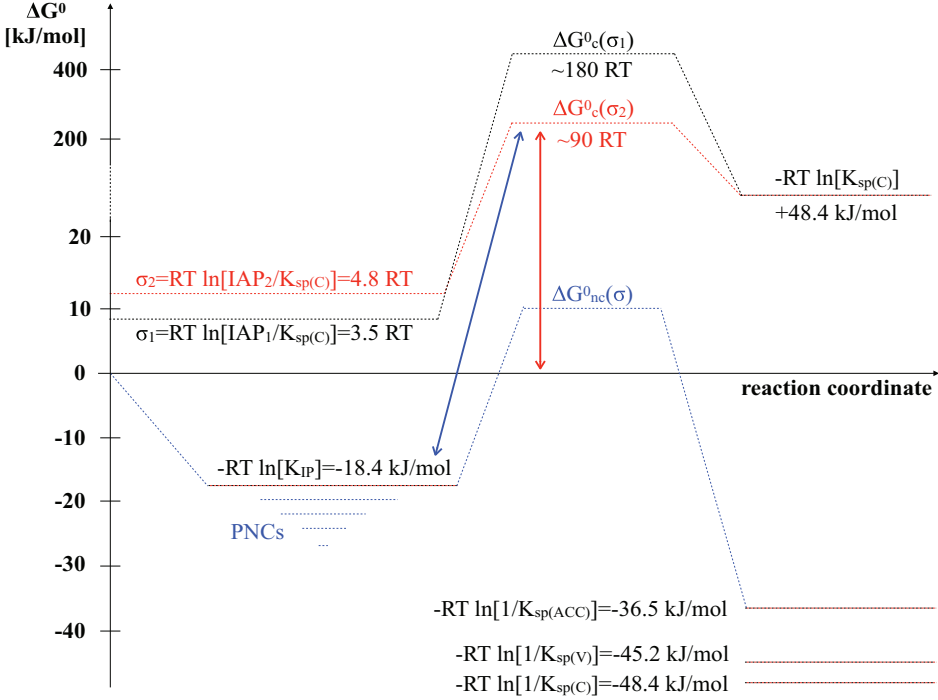


Fig. 4. Standard free energy landscape for the nucleation of calcite according to CNT (black and red levels) in contrast to the PNC pathway (blue) at 25 °C. The solubilities of calcite ( $K_{sp(C)}$ ), vaterite ( $K_{sp(V)}$ ) and amorphous calcium carbonate ( $K_{sp(ACC)}$ ) were taken from Brečević and Nielsen (1989), the value for the equilibrium constant for calcium carbonate ion pairing  $K_{IP}$  was adopted from Plummer and Busenberg (1982). Note that the solubilities of proto-structured ACCs are considerably lower than the disordered form included here (Cartwright and others, 2012), whereas metastable aragonite (A) was left out for the sake of clarity ( $-RT \ln(1/K_{sp(A)}) = -47.58$  kJ/mol at 25 °C). The supersaturation examples and corresponding CNT barriers to homogeneous calcite nucleation were adopted from Hu and others (2012). According to CNT, the standard free energy of the formation of the critical nucleus depends on supersaturation (eq 7); as the ion activity product (IAP) increases from  $IAP_1$  to  $IAP_2$ , the barrier is progressively reduced from  $\Delta G_c^0(\sigma_1)$  to  $\Delta G_c^0(\sigma_2)$ , eventually facilitating nucleation. Note, however, that the homogeneous CNT-barriers to calcite nucleation are tremendous and at odds with experimental observations, as discussed in detail by Hu and others (2012). The standard free energy of the final state for CNT is expressed in terms of that of the free ions, which are unstable with respect to the forming solid ( $\Delta G^0 > 0$ ). As opposed to the standard free energy of the critical nucleus, the standard free energy of ion pairs (characterized by the ion association constant  $K_{IP}$ ) and of the final solid do not depend on supersaturation. PNCs are associates of ion pairs, which are progressively more stable with increasing size, as schematically indicated by the blue cluster of standard free energy levels. Note that the quantitative values depend on the model of PNC formation and a showcase scenario is shown. From the viewpoint of CNT, these states sit in a free energy trap, as the CNT barrier (bold red double-headed arrow) is increased by at least  $RT \ln K_{IP}$  toward a much larger barrier (bold blue double-headed arrow), rendering the formation of a CNT-like critical nucleus from stable ion associates thermodynamically improbable. The transition state within the PNC pathway is not a CNT-like critical nucleus, the free energy of which ( $\Delta G_{nc}^0(\sigma)$ ), however, arguably depends on supersaturation too. For the sake of clarity, liquid-liquid separation and dehydration of liquid intermediates towards the final solid according to the PNC pathway are summarized within one major step characterized by  $\Delta G_{nc}^0(\sigma)$ , which is likely subdivided into a more complex standard free energy landscape with multiple barriers that remain to be quantified. This may or may not include a rate determining step, as suggested in this illustration. The final stage according to the PNC pathway is represented by the standard free energy of the solid with respect to the free ions ( $\Delta G^0 < 0$ ).

calcium carbonate ion pair, this can be demonstrated using the value of Plummer and Busenberg (1982) of  $K_{IP} = 1660$  at 25 °C, yielding;

$$\Delta G_{IP}^0 = -RT \cdot \ln K_{IP} = -18.4 \text{ kJ/mol} \quad (5)$$



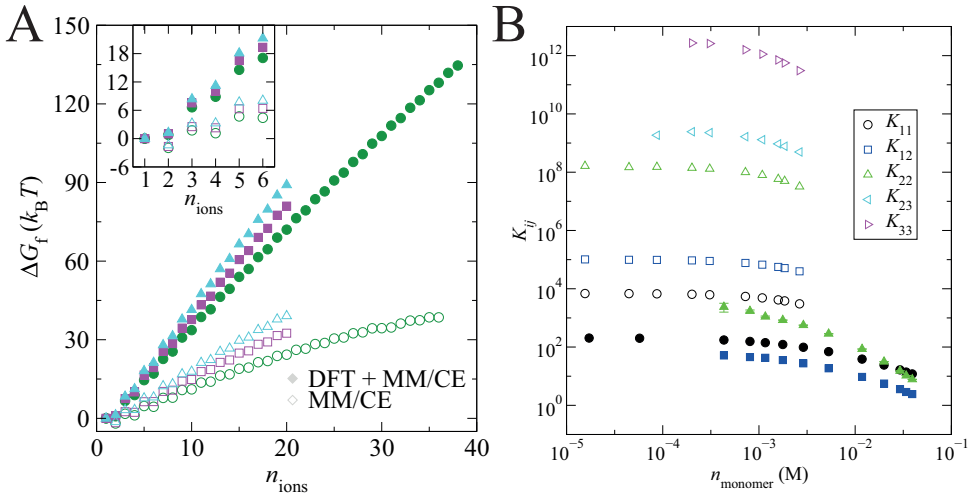


Fig. 5. (A) Free energy of formation of  $\text{CaCO}_3$  clusters versus cluster size, reproduced with permission from Henzler and others (2018). Dark green, magenta, and cyan symbols correspond to total  $\text{CaCO}_3$  concentrations of 17.22, 7.264, and 3.719 mM, respectively. Henzler and others (2018) erroneously state that these results represented the free energy landscape for nucleation, but the free energies do not refer to standard conditions, as fundamentally required. This is obvious from (B) the computed equilibrium constants for the formation of small  $\text{CaCO}_3$  clusters versus equilibrium concentration of monomers, also reproduced with permission from Henzler and others (2018). Filled symbols denote DFT + MM/CE results, and open symbols indicate MM/CE results. Note that all equilibrium constants are larger than unity (A), and thus at odds with positive standard free energies, compare equations (4), (5) and (6). Obviously, Henzler and others (2018) use irrelevant, unconventional and low concentrations as state of reference, that is, the low mM concentrations quoted above.

$$\Delta G'_{\text{IP}} = -RT \cdot \ln \left[ K_{\text{IP}} \cdot \frac{c'}{c^0} \right] = +4.45 \text{ kJ/mol} \quad (6)$$

Consequently, the concentration dependence of the free energy then leads to formally positive free energies of the associated states found, at non-standard conditions [fig. 5A, reproduced from Henzler and others (2018)]. These must not be compared with predictions of CNT, which uses  $c^0=1$  mol/L as a point of reference (see above). Otherwise, for instance, the expression for the affinity would have to be corrected to  $c'$  in equations (1) and (2). According to CNT, the standard free energy of the critical nucleus  $\Delta G^0_c$  can be calculated from the affinity  $\sigma$  (eq 1) and the interfacial standard free energy  $\alpha$  according to (fig. 5);

$$\Delta G^0_c = B \frac{\alpha^3}{\sigma^2} \quad (7)$$

where  $B$  is a constant depending on the properties of the nucleus (Hu and others, 2012). Since, independent of supersaturation,  $B>0$  and  $\alpha>0$ , and  $\sigma>0$  for the case that phase separation is possible (eq 1), the standard free energy of the critical nucleus is always positive,  $\Delta G^0_c>0$ . Corresponding considerations apply for all pre-critical states. It follows that the equilibrium constant for critical nuclei is:

$$0 < K_c = e^{-\frac{\Delta G^0_c}{RT}} \ll 1 \quad (8)$$

This is the thermodynamic reason for the minuscule concentration of (pre-) critical nuclei already discussed in the introduction, and equations (1), (7) and (8) make us realize that the equilibrium constant for the formation of (pre-) critical nuclei does depend on supersaturation (fig. 5);

$$K_{p/c} = f(\sigma) \quad (9)$$

where the equilibrium constant for the formation of (pre-) critical nuclei  $K_{p/c}$  is a function  $f$  of the affinity  $\sigma$  (eq 1). Thus, strictly speaking, CNT does not meet the fundamental requirement of the constancy of equilibrium constants, but, in a way, does not violate thermodynamics, as the formation of (pre-) critical nuclei is thermodynamically impossible ( $\Delta G_c^0 > 0$ ) from a *macroscopic* point of view anyway (Nielsen, 1964). In other words, whether the corresponding equilibrium constant  $K_{p/c}$  is  $10^{-10}$  or  $10^{-20}$  is macroscopically irrelevant, at least from the point of view of absolute populations, which remains essentially zero. The fluctuations towards the formation of (pre-) critical nuclei occur microscopically, and are very rare both in time and space. The dependence of equilibrium constants of (pre-) critical nuclei within CNT underpins that these species can only be conceptual.

Since the equilibrium constants of associated states found by Henzler and others (2018) were in fact all larger than unity [fig. 5B, reproduced from Henzler and others (2018)], the corresponding relevant *standard* free energies are indeed all negative ( $\Delta G^0 = -RT \cdot \ln K$ ), which cannot be rationalized by CNT (eq 7). The positive non-standard free energies shown in figure 5A are irrelevant, the species are clearly thermodynamically stable from the point of view of standard free energies and cannot be rationalized within CNT. As already outlined above, from the viewpoint of CNT, these states are stuck in a free energy trap (bold blue versus red double headed arrows in fig. 4). Moreover, CNT stipulates that the standard free energy of nuclei depends on supersaturation, that is, the ionic activity product (eqs 1 and 7). Equation (9) then highlights that the formation of any species, the thermodynamics of which are to be consistent with CNT, should be characterized by an equilibrium constant that increases towards 1 with increasing concentration, as opposed to the findings of Henzler and others (2018) (fig. 5B; the slight decrease of equilibrium constants at high concentrations is probably due to activity effects). This is in fact proof that the stable associated states identified by Henzler and others (2018) are squarely inconsistent with CNT.

This realization then also reveals that ion pairs cannot be the fundamental species underlying the formation of (pre-) critical nuclei within a CNT perspective. When we express the supersaturation ratio in terms of ion pairs as  $S_{IP}$ , we obtain for the case of calcium carbonate;

$$S_{IP} = \frac{a(\text{CaCO}_3^0)}{a(\text{CaCO}_3^0)_{eq}} \quad (10)$$

where index 'eq' indicates the respective activity in equilibrium with the nucleated phase. With the definition of the ion pairing constant;

$$K_{IP} = \frac{a(\text{CaCO}_3^0)}{a(\text{Ca}^{2+}) \cdot a(\text{CO}_3^{2-})} \quad (11)$$

we obtain;

$$S_{IP} = \frac{a(\text{CaCO}_3^0)}{a(\text{CaCO}_3^0)_{eq}} = \frac{K_{IP} \cdot a(\text{Ca}^{2+}) \cdot a(\text{CO}_3^{2-})}{K_{IP} \cdot a(\text{Ca}^{2+})_{eq} \cdot a(\text{CO}_3^{2-})_{eq}} \quad (12)$$

In equation (12),  $K_{IP}$  cancels, because it is independent of concentration, and, with it, supersaturation. For CNT-like (pre-) critical nuclei, this would not be the case because the corresponding equilibrium constant would vary in the numerator, but stay constant in the denominator (eq 9). We hence obtain;

$$S_{IP} = \frac{a(\text{CaCO}_3^0)}{a(\text{CaCO}_3^0)_{\text{eq}}} = \frac{a(\text{Ca}^{2+}) \cdot a(\text{CO}_3^{2-})}{a(\text{Ca}^{2+})_{\text{eq}} \cdot a(\text{CO}_3^{2-})_{\text{eq}}} = S \quad (13)$$

and realize that the formulation of the supersaturation ratio based on stable ion associates such as ion pairs,  $S_{IP}$ , is identical to the supersaturation ratio based on the free ions,  $S$ . This highlights that CNT neglects stable ion associates, and any species the excess standard free energy of which does not depend on supersaturation, so the implementation of stable ion pairs or PNCs within CNT is anything but straightforward.

#### CONCLUSIONS

There is no experimental evidence against the existence of PNCs in the works discussed above (Carino and others, 2017; Smeets and others, 2017; Henzler and others, 2018). We are also not aware of any other studies where this would be the case. Moreover, Henzler and others (2018) erroneously interpreted the concentration dependence of the free energy of ion associates. This yielded formally positive values for free energies of associated states with respect to unconventional, low reference concentrations that cannot be directly compared to predictions of CNT, which in turn refers to standard conditions. Clearly, the existence of stable ion pairs is beyond CNT, where stable species are neglected in terms of supersaturation (eqs 12 and 13). The standard free energy of stable ion associates is independent of supersaturation, while the standard free energy of (pre-) critical nuclei is not. This is the essence of CNT, where this quantity represents the height of the barrier for nucleation depending on supersaturation, and the existence of stable ion associates thus cannot be considered within the latter. It should also be noted that the general shape of cluster size distributions does not allow the assignment of the sign of the standard free energy for the formation of the different species—which is, in essence, the same misunderstanding of Henzler and others (2018). Rather, the above considerations underpin that relative populations are irrelevant for assessing the thermodynamics of associated states, as opposed to prominent claims made (Henzler and others, 2018). *Absolute* population frequencies can be evaluated based on the law of mass action and provide corresponding standard free energies, which show whether or not a given species is stable ( $\Delta G^0 < 0$ ) or unstable ( $\Delta G^0 > 0$ ) within a (pseudo-) equilibrium perspective. Only the latter, unstable species are formally consistent with the notions of CNT but can—very likely—never be detected experimentally. Consistently, abundant species like polycondensation polymers are thermodynamically stable, while exhibiting a decaying size distribution (Flory, 1936). On the other hand, non-standard free energies obtained by simulations, for instance, must be appropriately corrected if they are to be compared to CNT.

Last, but not least, we would like to point out that CNT does remain useful for describing and predicting nucleation processes, including calcium carbonate, given adequate parametrization. In fact, it is the only quantitative framework currently at hand, but it should be emphasized that the molecular picture of CNT is merely a conceptual notion. The formation of CNT species is macroscopically impossible, which means that they cannot be experimentally observed on the microscopic level, for example, using cryogenic transmission electron microscopy. The probability for this is, in fact, extremely low, considering the very small volumes probed and the minuscule concentrations of (pre-) critical nuclei. A quantitative theory of the PNC pathway, on

the other hand, is eagerly anticipated. The complex species that can be experimentally observed in calcium carbonate nucleation are certainly “non-classical”, considering structure, thermodynamics, and their role in phase separation, in the sense outlined above. In our opinion, the qualitative explanatory power of the PNC pathway is very appealing already today, as opposed to the notions of CNT. We would like to point out merely three aspects here. First, the link between the stability of PNCs and that of distinct forms of ACCs can mechanistically rationalize the phenomenon of amorphous polymorphism in ACC, promising a better understanding of crystalline polymorph selection (Cartwright and others, 2012; Gebauer and others, 2014). We do not see how this is possible within the framework of CNT, based on supersaturation and unstable nuclei alone. Moreover, the pH dependent formation of different forms of ACC as shown in the original work (Gebauer and others, 2008; Gebauer and others 2010) was neglected by Smeets and others (2017) when erroneously assigning the solubility of the initial phase to vaterite, which is in turn expected to be independent of pH. Second, the strong inhibitory effect of minute amounts of polycarboxylates on the nucleation of calcium carbonate (Gebauer and others, 2009; Sebastiani and others, 2016) can hardly be explained classically: the concentration of the polymer is too low for significant calcium complexation, thus not lowering supersaturation. On the other hand, spontaneous adsorption of the polymer on the nascent nuclei would inevitably reduce their interfacial free energy, facilitating nucleation (eq 7). Within the PNC pathway, however, and taking recent results into account (Bewernitz and others, 2012; Sebastiani and others, 2016), the observed effects can be nicely explained by the stabilization of liquid-like intermediates due to the superadsorbent properties of the polycarboxylate molecules incorporated in the dense liquid phase, thereby inhibiting its dehydration towards the formation of intermediate amorphous solids. In analogy to the above discussion of the pure system (fig. 2), titrations in combination with THz spectroscopy did show that liquid-liquid separation occurred also in the presence of poly(carboxylic acids), whereas the liquid-liquid binodal limit remained unchanged and minor amounts of the polymer considerably stabilized, kinetically, the liquid-liquid separated state (Sebastiani and others, 2016). In light of these quantitative results, the very recent suggestion that such ‘polymer induced liquid precursors’ (PILPs, which actually represent polymer-stabilized states) would really consist of solid nanoparticles of circa 2 nm in size, cross-linked by the polymer (Xu and others, 2018), are arguably unfounded. Third, the addition of spectator ions, which reduce the activity coefficient of free calcium and carbonate ions in solution, also significantly inhibits nucleation, although the supersaturation was formally increased (Kellermeier and others, 2014). From a CNT perspective, the inhibition could be explained by an increased interfacial tension in the presence of the spectator ions, which is doubtful. Rather, the fact that nucleation occurred at constant levels of *bound* calcium carbonate was striking, a purely thermodynamic effect based on reduced ionic activity coefficients, suggesting that bound calcium carbonate instead of the free ions was relevant for nucleation. It should be noted that the observed inhibition occurred at nominally higher levels of supersaturation, which do remain identical when formulated based on the ion pair (eqs 12 and 13) as opposed to the claims of Henzler and others (2018). The latter authors have also used this very specific observation of nucleation occurring at constant levels of bound calcium carbonate for underpinning the validity of CNT, owing to an erroneous interpretation of the concentration dependence of free energies, which in turn assigned the bound calcium carbonate fraction to an unstable population, by mistake, as discussed in detail above. The realization of this mistake might be a transition state towards dismissing the idea of a universal generality of CNT for solute speciation, and eventually accepting the promises of the novel ideas of the PNC pathway in nucleation research.

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