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Simulation of Crystallization of Biominerals

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ABSTRACT: Biominerals are crucial materials that play a vital role in many forms of life. Understanding the various steps through which ions in aqueous environment associate to form increasingly structured particles, that eventually transform into the final crystalline or amorphous poly(a)morph in presence of biologically active molecules, is therefore of great significance. In this context, computer modeling is nowadays able to provide an accurate atomistic picture of the dynamics and thermodynamics of possible association events in solution, as well as to make predictions as to particle stability and possible alternative nucleation pathways, as a complement to experiment. This review provides a general overview of the most significant computational methods and of their achievements in this field, with a focus on calcium carbonate as the most abundant biomineral.

1. Introduction

Biomineralization is the process through which organisms direct the formation of minerals. This process is widespread in species belonging to many forms of life, from bacteria through to plants and animals, including human beings. The reasons why natural organisms produce and exploit minerals are numerous, and include: structural support to build exo- and endo-skeletons; defense devices, like spines in echinoderms(1) and raphides in plants; (2, 3) other functional hard tissues, such as otoliths in fish,(4) auditory ossicles in humans, and magnetosomes in magnetotactic bacteria;(5) by-products of other processes, like carbonates from photosynthesis in cyanobacteria.(6) Several pathologies in animals can also involve the undesirable formation of minerals, such as kidney stones[i] and microcalcifications,[ii] or can be caused by an imbalance between mineralization and demineralization processes, like osteoporosis.[iii]

While the above examples of biomineralization are well-known macroscopically, the molecular details of their mechanisms for nucleation and growth are still largely unexplored. Achieving a comprehensive understanding of the fundamental interactions giving rise to such a variety of processes would allow the pathologies linked to the formation of biominerals to be addressed with more effective treatments, and could eventually yield biomimetic approaches for the production of new materials for medical and technological applications. Indeed, organisms have a unique ability to synthesize minerals with precise shapes, sizes, textures, crystallinity and composition (Figure 1); something that is beyond current technology.

[i] https://www.niddk.nih.gov/health-information/urologic-diseases/kidney-stones/definition-facts; The National Institute of Diabetes and Digestive and Kidney Diseases, consulted on July 18th 2017

[ii] http://www.health.harvard.edu/womens-health/calcium-beyond-the-bones, Harvard Health Publications, Harvard Medical School, consulted on July 18th 2017

[iii] https://www.niams.nih.gov/Health_Info/Bone/Osteoporosis/overview.asp; NIH Osteoporosis and Related Bone Diseases, consulted on July 18th 2017

Biominerals span many materials, from elements, through to sulfides, oxides, hydroxides, arsenides, halogenides, arsenates, sulfates, silicates, carbonates, oxalates, and phosphates.(7) Of all biominerals, carbonates and phosphates are the most studied due to their abundance and structural functions in animals and humans. However, other materials are receiving increasing attention, such as silica in diatoms,(8) iron oxohydroxides and sulfides produced by bacteria leading to the deposition of iron in the environment,(9) and oxalates due to the formation of kidney stones.

Knowledge regarding the early stages of biomineralization has advanced considerably during the past two decades for two reasons. Firstly, there have been significant advances in experimental techniques to probe crystallization through developments in microscopy, spectroscopy and crystallography, many of which benefit from synchrotron radiation. Such techniques have allowed greater access to physical and chemical information that is closer to the atomic scale.(10-12) Secondly, alongside the rapid evolution of experimental techniques, there have been major advances in computer simulation, such that it plays an increasing role in providing atomistic models that reveal the fundamental science behind complex phenomena. This is due to the rapid development of cutting-edge computational facilities, coupled to major innovations in methods and algorithms that exploit this growing hardware capability.[iv] While gains in speed for individual CPUs have slowed in the last few years, there remain opportunities for greater performance via massively parallel algorithms.

The aim of this review is to present an overview of the current state-of-the-art of computer simulation in the field of biomineralization. One of the motivations, is that there have been important developments in our understanding of the mechanisms of mineral nucleation and growth over the last few years where the synergy between experiment and simulation has played a key role. While many of the new concepts have emerged from

[iv] www.top500.org

biomineral systems, the implications extend more broadly throughout crystallization. After introducing the most commonly used computational approaches, their application to the study of biomineral nucleation and growth will be presented. Since it is not possible to comprehensively review all systems, calcium carbonate will be used as the primary example, given that this is the most abundant biomineral and often the most studied. However, many of the challenges identified are generally applicable to other biominerals.

2. Methods

It is beyond the scope of this review to provide a description of all computational methods that have been applied to biomineralization and biominerals.(13) Here we provide an overview of the approaches most relevant to crystallization at the atomic level, while noting that other techniques can treat processes at longer length- and time-scales (Figure 2). Indeed biomineralization as a complete process requires a multiscale perspective to understand the influence of the biological environment, which includes effects such as confinement within the cell and control of transport properties that regulate ion concentrations. By focusing on the atomic level, only a subset of factors can be explored; for example, how direct coordination of organics to the mineral ions or particles controls nucleation and/or growth. Furthermore, to gain insight into the mechanisms of biological influence it is necessary to first study the abiotic case as a point of reference.

When considering the available atomistic methods, the most accurate are those that start from an explicit quantum mechanical (QM) description, namely *ab initio* or first-principles methods. Such approaches provide information regarding the electronic structure of a system, but are computationally expensive and, so far, have been more often applied to the study of bulk minerals and their surfaces (e.g. Refs. (14-16)) *in vacuo*, rather than the wet conditions relevant to growth. Given that many biominerals are wide gap insulators, the details of the electronic structure are often less important than the ability to determine accurate forces with

which to explore configuration space. Arguably the most valuable feature is the ability to reliably treat bond breaking/formation during crystallization. In particular, proton transfer is often coupled with crystal growth; for example, while bicarbonate (HCO_3^-) is the dominant species at environmental pH in the worlds' oceans, the mineral that is precipitated contains carbonate (CO_3^{2-}). Whilst this can be described using *ab initio* molecular dynamics (AIMD),(17) in which electronic structure methods are coupled to classical techniques for the time evolution of the nuclei, there are some challenges. Currently the cost of AIMD typically restricts the system that can be studied to less than 1,000 atoms and the time sampled rarely exceeds 1 ns. Therefore, while spontaneous reactive events, including proton transfer, are theoretically possible, in practice they are unlikely unless explicitly forced.

Aside from the limitations due to the computational cost of QM methods, there are also issues related to the approximations introduced to solve the equations, such as the choice of Hamiltonian. While methods based on Kohn-Sham Density Functional Theory (DFT) provide accurate results for the structure and most properties of crystalline(14-16) and amorphous(18) solids, they fail where van der Waals interactions are important, including phase stability, the structure and interaction of ions with their solvation sphere, and water-surface interactions.(19, 20) The worst case is liquid water, where functionals can lead to a description that is over-structured, has a low self-diffusion coefficient and an elevated melting temperature. Empirically, it is found that increasing the simulation temperature (often by 30 K) combined with the use of hybrid functionals and van der Waals corrections improves the results,(21) though some contemporary meta-GGAs may offer results that are accurate as expected without a treatment of nuclear quantum effects.(22) An overview of the progress of *ab initio* simulation of liquid water is available elsewhere.(23)

By far the most frequent atomistic techniques for the simulation of crystallization are force field methods. Because of the low cost of evaluating forces, this permits extensive use

of molecular dynamics to sample longer times and much larger system sizes than QM-based techniques. Despite the inherent empiricism, interatomic potentials can be accurate, provided the process does not involve a chemical reaction, and the force field has been carefully parametrized.(24, 25) One limitation for most force fields is the inability to properly account for pH and therefore for mineral precursor speciation. If only one protonation state is dominant at biological pH (e.g. $C_2O_4^{2-}$ oxalate) then speciation can be disregarded. In other cases (e.g. for phosphates where $H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$ can all occur) a practical approach is to include the species at concentrations corresponding to the relevant pH. (24) Reaching equilibration will be slower, since reactions that should involve proton transfer have to occur instead through diffusion and ion exchange. Several approaches are available to allow partial or full reactivity;(26, 27) however, they have yet to be parametrized and validated for biomineral-water interfaces.

Careful force field parametrization is the key for classical simulations to be realistic. When it comes to model development, a challenge is often finding sufficient reliable experimental data to fit, and hence QM methods are often used to obtain structural parameters and interatomic interactions. The process of deriving force fields for mineral crystallization has been described elsewhere.(28, 29) Here we stress a few key points. First, the importance of using a good water model. As discussed later, the dynamics and thermodynamics of water interacting with other species plays a pivotal role during nucleation and growth. Therefore, the model needs to be accurate in reproducing the density, diffusion coefficient, pair distribution functions, and dielectric constant of liquid water. Second, the parameters describing the interactions between water-mineral, mineral-biomolecules and water-biomolecules need to be tuned to reproduce properties such as the hydration free energy and the structure of the solvation sphere of ions and molecules. Many early force fields for minerals were fitted with a focus on solid-state structure and mechanical properties, whereas

for studies of crystal growth the key quantities relate to the thermodynamics, and the solubility in particular. Often models are used without characterizing this quantity, leading to results that are at an unknown saturation state and may not be meaningful at the conditions simulated.

One the challenges during parametrization is often the lack of experimental solvation free energies. While there are several experimentally and theoretically derived hydration free energies for simple anions (e.g. acetate), values for more complex molecules (e.g. amino acids) in the appropriate protonation state to the pH can be scarce.(30) To compensate for the missing experimental data, QM calculations are often performed to estimate the free energy of solvation. However, the range of values can be wide and dependent on the functional, basis set, solvation model used, and thermodynamic reference state. For many anions this is compounded by them being multiplied charged (e.g. CO_3^{2-}) and therefore not existing in the gas phase, rendering the solvation reaction purely hypothetical.

Tuning parameters for the mineral-biomolecule and water-biomolecule interactions is also challenging. Again, there is a lack of experimental data against which the model can be fitted or validated. While numerous established biomolecular force fields exist that are extensively applied in bioscience, no general parametrization is available for the interactions between biomolecules and biominerals. Consequently, different studies have selected popular force fields such as CHARMM,(31) AMBER,(32) OPLS(33) and GROMOS(34) to describe the organic component. Despite biomolecular force fields already including the interactions between most of the relevant species in solution (i.e. metal cations) and organic molecules, there is often compromise in merging a model developed for mineral-aqueous systems with a standard biomolecular force field. Often the water models are different and so errors occur due to the change of parameters. More importantly, many cross-terms are missing and so are typically approximated via combination rules without validation.

Above we highlight the extremes of atomistic models, namely *ab initio* QM and force fields. However, there are a range of intermediate methods that trade quantum mechanical rigor and the introduction of parameters for computational speed, including density functional tight binding (DFTB), and semi-empirical quantum mechanics (e.g. MNDO, PM6, etc). While there has been use of these techniques for minerals, only a limited number of studies are relevant to biominerals. In some cases, they have been used for pre-optimization of structures prior to more accurate calculations.(35) Ultimately an improved description of the interaction of ions as they crystallize from aqueous solution into a solid will require treatment of dipolar and quadrupolar polarizability. This is not just true for highly charged anions, but also for water that has to evolve from the liquid state to environments where it experiences strong local electric fields. Therefore use of approximate descriptions of quantum mechanical phenomena, be it from tight binding, MNDO-derived methods,(36) or sophisticated force fields such as AMOEBA,(37) is likely to become increasingly important.

Identifying a thermodynamically accurate method for computing the energies and forces of a combined organic-inorganic system is just one part of the challenge of simulating biomineralization: Equally daunting is the need to sample the full configuration space in order to determine meaningful free energies. For example, the stable conformation of a biomolecule may be different in aqueous solution from that at a mineral surface, and this problem increases rapidly with the length of the backbone and number of sidechains. To further exacerbate this, the dynamics of water exchange at metal cations can be slow, and this often determines the rate of crystal growth events or attachment of biomolecular functional groups to surface sites. For example, water exchange at Ca^{2+} in bulk water is relatively rapid with a timescale of a few hundred picoseconds. However, when this ion is incorporated into the surface of mineral this process can slowed by several orders of magnitude.(38) For other metal ions, such as Mg^{2+}, even a single water exchange is too slow to observe during a

simulation.

To deal with energy barriers that take too long to overcome in unbiased MD (i.e. those >> kT), two main approaches can be adopted. Replica exchange MD allows accelerated sampling of different states through running several independent simulations in ensembles with different temperatures or Hamiltonians, and periodically exchanging replicas between them. Instead free energy methods are usually applied when not only the states, but also the thermodynamic difference between two or more states needs to be analyzed.(39) Amongst those widely applied to biomineralization, some of them (e.g. metadynamics, umbrella sampling) work through adding a bias that drives rare events, while others implement a perturbative approach or rely on thermodynamic integration along a pathway of interest. The challenge of these methods is defining the reaction coordinates (collective variables) along which to sample the free energy. Multi-dimensional sampling can be performed when two or more collective variables need to be sampled simultaneously (e.g. to determine a well-converged ion pairing free energy, often both the water coordination number of the cation and the ion pair distance are sampled), though the cost increases exponentially with the number of variables.

An alternative approach to simplifying simulation of biomineralization is to use coarse-graining. This can vary from reduction of the biomolecule to a smaller number of interaction sites, through to simplifying the water model. Given that most biominerals have low solubilities, saturation occurs at concentrations of the order of mM or less. Therefore, any approach that reduces the cost of water, as the most numerous species, will lead to a dramatic speed-up, even before accelerating the exploration of configuration space. An example of this approach is the use of the Rosetta model to study binding at mineral surfaces,(40) while the advent of the DryMARTINI(41) force field also offers possibilities. It is important to caution

that since many mineral surfaces lead to the formation of ordered water structure, coarse-grained models must take explicit account of this to be reliably predictive.

3. An Example of a Biomineral: Calcium Carbonate

To provide specific examples of the simulation of processes relevant to biomineralization at the atomic level, we now focus on calcium carbonate nucleation and growth starting from ions in aqueous solution. This system has attracted widespread interest because of the complexity and diversity of possible pathways to crystallization that may include stable pre-nucleation clusters, liquid-liquid phase separation, amorphous precursors and competition between polymorphs on route to the stable mineral form.(24, 25, 42)

3.1. Solution speciation

The starting point for crystallization of calcium carbonate is a solution that contains the ions Ca^{2+} and CO_3^{2-}. However, the carbonate ion can often be present at low concentration near neutral pH, and is generated via the equilibrium with bicarbonate, which in turn arises due to dissolved carbon dioxide. While formally carbonic acid (H_2CO_3) is the parent species, this only plays a minor role in an aqueous environment.(43)

Although *ab initio* studies of carbonate equilibria have been performed, the cost of QM calculations has prevented most studies from going beyond simple speciation. Therefore, it is necessary to handle the carbonate/bicarbonate equilibrium within classical simulations since both species are typically present. A common approach is to run multiple simulations with different calcium/carbonate ratios(24) and then infer the effect of pH on the process. However, the same species, e.g. carbonate, may have a different pK_a in different environments. Andersson et al.(44) have estimated the pK_a for bicarbonate at different sites relevant to the calcite surface using cluster calculations and an implicit solvent model, leading to values ranging from -6 to +7.5. Although the absolute values for equilibrium constants may be significantly affected by the methodology used, *i.e.* the absence of explicit solvent

molecules, this study indicates that bicarbonate may deprotonate more easily when embedded in the mineral surface or simply surrounded by Ca^{2+} ions. This could have important implications for species at low pH, where there is a large concentration of bicarbonate, which can transform to carbonate when bound to calcium. Therefore, the inability of classical force fields to simulate reactivity may lead to artefacts that are hard to quantify.

3.1.1. Ion pair formation

Bjerrum and Fuoss performed pioneering work to explain the degree ion association in an electrolyte solution of singly charged ions by connecting the equilibrium constant with the screened electrostatic interaction between the ions;(45)

$$K_a = C_0 = \int_{R_A}^{R_B} 4\pi r^2 e^{-\beta \emptyset(r)}\, dr, \tag{1}$$

where $\beta=1/k_BT$, $\varphi(r)$ is the potential of mean force (PMF) acting between the positive and negative ions. In Eq. (1) C_0 is the standard state concentration of the ion pair in the units of the PMF, *e.g.* 1 mol/L or 6.022×10^{-4} atoms/Å^3. The upper limit of the integral is the distance beyond which the ion pair is considered to be dissociated and is chosen to be the Bjerrum length (R_B), defined as the distance at which the screened electrostatic interaction between the ions is equal to -2 k_BT. Although, this definition may seem arbitrary it has a clear physical meaning and the Bjerrum radius corresponds to the distance at which the association free energy of two point particles interacting via a screened electrostatic potential, plus configurational entropy, exhibits a maximum:

$$\Delta G(r) = \emptyset(r) - k_B T \ln(4\pi r^2). \tag{2}$$

The lower limit of the integral in Eq. (1) is an adjustable parameter and can be interpreted as the minimum distance at which the ions can be found. This framework has been used to interpret experimentally measured association constants for electrolytes in different solvents.(45-47) Observed discrepancies between theory and experiment were ascribed to uncertainties in the contact distance and to charge-dipole interactions, plus higher moments, which could be included as a correction to the PMF.

With the reliable calculation of the PMF has become routine with force fields(48-50) and is becoming feasible using QM methods.(51-53) It is worth noting that the pairing free energy obtained from the most common MD approaches, such as umbrella sampling,(54) steered MD, (55) or metadynamics,(56) is only defined to within an additive constant. Finding this constant, which is irrelevant where differences between states are calculated, becomes a key step when the absolute association constant is required. Indeed, a rigid shift of the PMF in Eq. (1) can lead to any value of the association constant. The way to determine this additive constant is to align the PMF from MD simulations to the expected long-range limit for two point particles interacting via a screened electrostatic potential. Although this is trivial for force field simulations, where systems of several nm in size can be used and the PMF accurately calculated up to the Bjerrum radius,(50) it may not be possible for AIMD as only small simulation boxes can be afforded and the PMF may only extend to the solvent-shared state where oscillations due to the structure of the solvent are still present.(52, 53)

The calculation of the ion pair association free energy is useful to assess the accuracy of a force field, as it indicates whether the thermodynamics are consistent with experiment. For example, consider calcium-carbonate ion-pair formation. In early MD works using both classical and polarizable force fields(49, 57, 58) the association free energy between Ca^{2+} and $CO_3^{=}$ was predicted to be in the range -39/-35 kJ/mol at room temperature; significantly more exothermic than the reported experimental value of -17 kJ/mol.(59, 60) A more refined force

field(50, 60), designed to target the solubility of calcite, better reproduces the experimental data and allows exploration of the full atomistic association dynamics (Figure 3). This is particularly important when force fields are used to shed light on complicated problems, such as the existence and stability of pre-nucleation clusters(24, 42) or of liquid-liquid phase separation(25) as discussed below.

3.1.2. Pre-nucleation clusters

Gebauer *et al.*(42) have demonstrated that stable association of ions (i.e. $K_a > 1$) beyond ion pairing occurs prior to nucleation of calcium carbonate (Figure 4). This has led to broader debate as to whether mineral formation from homogeneous solution follows classical nucleation theory,(61) and raised questions as to what the nature of any pre-nucleation clusters might be. For most biominerals, experimental observation of pre-nucleation clusters is challenging because of their low solubility, which limits the concentration of such species in solution. Although Cryo-TEM can potentially provide evidence for the existence of pre-nucleation clusters,(62, 63) the size of these species is close to the detection limit of the technique and definitive conclusions cannot be made without stabilizing agents.(64) Therefore, it is necessary to rely on indirect experimental evidence and/or computational methods.

Classical MD has been used by several groups to probe the association of calcium and carbonate ions in aqueous solution.(24, 65, 66) In earlier works the force fields available gave a description of $CaCO_3$ that was many orders of magnitude less soluble than the real mineral and so rapid formation of persistent clusters was observed. More recent work using a thermodynamically calibrated force field gave quite different results. Here dynamic formation of ion pair associates was observed that were named DOLLOP.(24) In these species, the ion pairs come together to form chains that rapidly evolved between linear, branched and ring arrangements. Using the radius of gyration as a collective variable, the free energy landscape

was mapped for one size of cluster, showing that many different configurations were within ambient thermal energy of each other. This flat landscape, combined with the absence of significant barriers, explained the dynamic evolution of DOLLOP. Under conditions of moderate pH a dynamic equilibrium distribution of cluster sizes could be obtained, allowing the equilibrium constant for ion pair association to be fitted. This led to a value that was comparable to ion pair formation itself, which supports the multiple binding model used to interpret the experimental binding data. Crucially, DOLLOP species rapidly vary in size and the ions remain able to exchange with those in solution, consistent with being pre-nucleation species. At elevated pH (i.e. increased carbonate/bicarbonate ratio) and concentration the breakdown of equilibrium was observed leading to phase separation.

The initial simulations of DOLLOP species were based on unbiased MD in which different concentrations of ions were placed in a periodic box of water. Due to the low solubility of $CaCO_3$, the systems studied were all significantly supersaturated, which was necessary to overcome the limitation of ion diffusion rates relative to feasible simulation length. However, this raises the question as to whether the equilibria observed are relevant to dilute solution or instead representative of ion association within a dense liquid phase that is proposed to also exist? While systematic determination of association constants under dilute conditions is viable for one or two ion pairs, this becomes impractical as cluster size increases for two reasons. Firstly, the number of collective variables needed to unambiguously capture the complex structural landscape increases. Secondly, in order to equate the free energies to standard conditions it is necessary to connect the landscape to a well-defined limit, such as separation into isolated ions. For $CaCO_3$ species this limit is only reached at distances of hundreds of Angstroms, even for small clusters, making the space to be sampled prohibitively large. Here it may be necessary to turn to coarse-grained (CG) simulations to access realistic concentrations and determine equilibrium constants for ion association. However, a careful

parameterization of the CG potential is required to avoid over-binding of the ions due to the lack of explicit water, which may lead to unphysically dense clusters.

An alternative approach to sampling cluster stability has been taken by Wallace *et al.*.(25) Here a modified version of the Kawska-Zahn approach(67) was used to incrementally grow a cluster one ion pair at a time in a sequence of steps that involve annealing the cluster in the presence of water using replica-exchange. The free energy was then determined using the 2PT method(68) based on the vibrational density of states. In line with previous simulations and experiment an approximately linear decrease in free energy with cluster size was observed, though the slope of the free energy differs from that determined previously. This arises because the 2PT method was applied to the cluster, but for reasons of computational expediency neglected the change in free energy of the surrounding solvent. In the absence of any magic islands of stability, and given the high diffusivity of the ions within the cluster, it was proposed that liquid-liquid phase separation is the likely pathway to the first nucleation event, with this dense liquid phase being the precursor to precipitation of amorphous calcium carbonate as the first solid.

3.2 Nucleation

One of the most challenging events to understand in biomineral systems is nucleation, and calcium carbonate is no exception. To date the majority of simulation studies in this field have focused on heterogeneous nucleation in which the mineral forms on an existing surface.(69) Self-assembled monolayers of organics that present an array of carboxylate groups to solution offer a natural template for nucleation of crystals, given a suitable epitaxial match for a surface facet that exposes calcium ions. In biological environments there will be plenty of suitable functional groups that match those used in templating studies. However, these are unlikely to present as an ordered array, and so the driving force for heterogeneous nucleation will be reduced.

Even more challenging is the question of whether and how homogeneous nucleation might occur. Given that any interface, including the surface of a droplet, can be sufficient to lead to heterogeneous nucleation, it is not obvious that homogeneous pathways are relevant. Nevertheless, the observation of arrays of spherical particles of $CaCO_3$ may be indicative that it does indeed occur.(70) Direct simulation of homogeneous $CaCO_3$ nucleation is yet to be feasible at the atomistic level. Despite this, there are several pieces of indirect evidence that the first nucleation event occurs via liquid-liquid phase separation to form a dense liquid phase, as discussed previously. Andersson and Stipp(71) have reported preliminary results based on DFT calculations in conjunction with a continuum solvent model, predicting that binodal phase separation occurs, with spinodal decomposition only accessible at > 0.01 M.

The proposed nucleation of calcium carbonate via a dense liquid phase parallels the two-step mechanism proposed by ten Wolde and Frenkel(72) for protein crystals that involves densification prior to the onset of structure. In the context of biomineralization, it is interesting to note the similarity of crystallization pathways between “soft” biomolecules and “hard” minerals.

3.3. Amorphous calcium carbonate

Nucleation of the first solid phase of $CaCO_3$, at least under homogeneous conditions, often yields amorphous calcium carbonate (ACC).(73) This disordered material has a general formula of $CaCO_3.xH_2O$, where x lies between 0 and 1.5, and so is typically a hydrate. Both the thermodynamic stability and structure depend on the water content. Indeed ACC has been shown to exhibit polyamorphism(74) and can exhibit proto-structures that reflect one of crystalline forms of calcium carbonate. Experimentally the local structure of ACC can be characterized using pair distribution functions, which are then mapped to atomistic models using reverse Monte Carlo techniques. Based on this, Goodwin et al(75) suggest that the

structure of ACC contains channels of water. Two explicit structures were published, though both relax significantly when used as the starting point for molecular dynamics.(76) This reflects the need to include an energy function during the reverse Monte Carlo fitting of pair distribution functions, given the limited data available for an amorphous structure. To date there have been numerous works that attempt to simulate the structure of ACC using classical molecular dynamics. Given the slow evolution within an amorphous solid, the final structure is clearly dependent on how it was generated. Despite this, most studies claim reasonable agreement with the experimental local structure.

One of the presumed reasons why ACC forms as the first solid phase is due to kinetics (i.e. it is faster to form a disorder material than an ordered nucleus). However, a notable success of simulation is that it predicted that ACC nanoparticles are actually more stable than those of bulk phase, calcite, below a size of ~4 nm.(65) This parallels other crossovers in phase stability observed when particles enter the nanoscale regime and interfacial effects with solvent become important.(77)

A question that remains as yet unanswered is how ACC transforms to crystalline calcium carbonate. Evidence exists that supports both a direct transformation and dissolution followed by re-precipitation, though the observation from simulations that calcite is not stable as small nanoparticles(78) suggests that direct formation of the crystalline phase may require heterogeneous conditions. An early success of metadynamics simulation in this field was the mapping of the free energy landscape for $CaCO_3$ nanoparticles using Steinhardt order parameters, starting from ACC.(79) Further important insight came from simulations of nanoparticles in contact with an eggshell protein molecule.(80) Here it was shown that a catalytic cycle exists in which ovocleidin-17 binds to ACC, triggering nucleation to calcite, which causes the protein to be released back to solution.

3.4. Crystalline phases

The most stable and best characterized crystalline phases of calcium carbonate are the anhydrous polymorphs calcite and aragonite, with calcite being more stable by about 1 kJ/mol at ambient conditions. While their structure and properties have been extensively studied,(81, 82) computing their thermodynamic stability and possible phase transition pathway are non-trivial tasks, (19) due to their relative enthalpy and free energy having opposite signs and small values that are close to the accuracy of readily applicable *ab initio* methods.

A third anhydrous polymorph, vaterite, is metastable with respect to both aragonite and calcite; however, it is found growing in living organisms. Vaterite is one of the most debated mineral structures(83) due to its disorder and polycrystallinity. Through the application of first-principles methods the complexity of vaterite has been explained.(84, 85) Layers of carbonate anions can be stacked in different sequences with little or no change in stability, giving rise to two main polytypes belonging to the monoclinic C2 and trigonal $P3_221$ space groups. Calculations show that within each of these symmetries, interconversion between multiple structures can occur via rotations of carbonate anions. Raman spectroscopy, ^{43}Ca NMR and X-ray diffraction results are compatible with the two aforementioned structures.(14, 86) Both proposed space groups are chiral, though no experimental technique is at present able to probe this property due to the small crystal size. Recently, it has been shown that, in presence of chiral amino acids, vaterite grows forming chiral toroid superstructures.(87) In the same work, the most stable chiral surface has been simulated in presence of D- and L-aspartate, showing preferential adsorption of the L enantiomer. However, a rigid body/rigid surface model was used and water was not explicitly accounted for.

Two hydrated phases of calcium carbonate are also present in nature. They are unstable unless in very cold or highly saline environments, but often appear as intermediates during the transformation of ACC into calcite or aragonite. Their structure and properties are important

in understanding the water-$CaCO_3$ interaction at a crystalline level, with relevance to ACC and its transformation into either calcite or aragonite. Monohydrocalcite, contains one water molecule per $CaCO_3$ formula unit. Natural samples contain no less than 20% of Mg impurities,(88) mostly concentrated at the surface. It is thought that Mg stabilizes monohydrocalcite by inhibiting its dissolution and re-precipitation as aragonite. Like vaterite, it has a chiral structure,(89) though its chirality has never been investigated. Monohydrocalcite can be mineralized by organisms,(90) though its importance is mostly due to its structure being related to that of hydrated ACC.(91) Ikaite has a formula of $CaCO_3.6H_2O$ with a structure consisting of a network of water molecules in which $CaCO_3$ ion pairs are isolated,(92) making it interesting as a model for a "frozen" supersaturated solution. While it has been observed as an intermediate during the nucleation and growth of calcium carbonate,(93) crystallization by organisms is unknown.

The main challenges in modeling these two hydrated phases are the lack of experimental data to compare against and the nature of their structure and fundamental interactions. The ability of DFT to model hydrated calcium carbonates has been assessed,(19) finding that van der Waals interactions are important, and that including empirical dispersion, either through existing or refitted C_6 coefficients, improves the results, though only for monohydrocalcite. Chaka and Felmi performed a full thermodynamic characterization of hydrated magnesium carbonate phases using DFT.(20) Despite good results, the data corresponding to the highly-hydrated phases are likely to be affected by similar errors as reported for ikaite.

3.4.1. Influence of biomolecules on crystal structure and polymorphism

The inhibiting, retarding and templating effects that organic molecules can have on calcium carbonate nucleation will often influence polymorph selection. For example, it was shown that addition of different organics to a supersaturated solution containing pre-

nucleation clusters of both proto-vaterite ACC (pv-ACC) and proto-calcite ACC (pc-ACC)(94) preferentially stabilizes one type of cluster over the other leading to a preferred polymorph.(42) Biogenic calcium carbonate crystals can also exhibit different behavior to their abiotic equivalents, and recently diffraction techniques were able to identify lattice distortions due to the inclusion of organics in the solid. A review of this topic is available elsewhere.(95)

To date, bulk incorporation of organics in calcium carbonate has rarely been tackled using simulation. Recently, Kim et al.(96) combined experimental data with the simulated inclusion of the glycine zwitterion and aspartate dianion into calcite. Although molecular dynamics was used, the low mobility of the incorporated organics at 300 K means that the final configuration is likely to be determined by the initial choice of ions removed to accommodate the defect. Occlusion energies were computed for the two species, leading to approximate slopes of 2,000 and 4,000 kJ/mol per mol% for glycine and aspartate, respectively. Given these high penalties for incorporation, it suggests that occlusion of organics into calcite is only likely at low concentrations and via kinetic trapping at high supersaturation (i.e. by overgrowth of an adsorbed molecule).

An alternative pathway to incorporation of organics in biominerals is via formation of composites, which nature exploits to create materials with enhanced mechanical properties. For example, aragonite combines with ~5% of organics, consisting of proteins and polysaccharides, to form a layered structure in nacre, as found in the iridescent lining of mother of pearl.(97) Although finite element simulations have been used to model the mechanical properties of this system, there has been little atomistic work so far. An early attempt by Ghosh et al(98) used steered MD to examine the mechanical behavior of a fragment of the protein Lustrin A in a droplet of water with an unspecified surface of aragonite. Later Xiao et al(99) have used the same approach to pull a polypeptide composed

of 7 aspartate units from the (001) surface of aragonite leading to an estimated rupture force of 60 pN per sidechain. One of the common organic components of biomineral composites, including nacre, is chitin; a polysaccharide derived from glucose with N-acetyl side groups. Qu et al(100) have performed one of the few studies for carbonate minerals of a genuine composite, using non-equilibrium molecular dynamics to simulate the mechanical properties of a layered calcite-chitin system, including the influence of water on the elastic moduli. Extension of this work to aragonite and mixed organic systems remains a challenge for the future, though considerably more work on phosphate biominerals (specifically, collagen-hydroxyapatite composites) has been performed due to the relevance to bone materials. (101)

3.5. Crystal growth

Growth of crystals from stable nuclei is a key step in obtaining the final mineral, together with other processes such as particle aggregation, precipitation / recrystallization and solid state phase transformation. Here a well-developed schematic view of how growth has emerged based on attachment of units to individual crystal facets. Starting from a perfect surface, the initial and often rate-limiting step is the adsorption of sufficient species to reach a critical 2-D nucleus that is able to form the next layer. For materials such as calcite, where this nucleation event is slow, growth can be dominated by screw dislocations. Once nucleation on the surface has occurred then growth involves further addition of species. In the conventional view, largely adopted from *in vacuo* growth, atoms, ions or molecules adsorb on the terraces and then diffuse until they encounter a step or kink site where either 1-D nucleation or growth can occur, respectively.(102) While the overall picture is broadly accepted, there are many things that are unknown, such as the rates of individual processes or whether growth occurs via single species or perhaps pre-formed clusters? Experimentally, atomic force microscopy (AFM) can image growing surfaces *in situ* and even provide overall rates of step propagation. A key role for simulation is to both assist in the interpretation of

high resolution AFM images, and to help make the connection between atomic mechanisms and rates observed macroscopically. Below we address these issues for the prototypical case of the basal surface of calcite.

3.5.1. Surface-Water interface

Before considering growth, first we should understand the structure and dynamics of the interface between water and the mineral surface. Here one of the most studied examples is the (104) basal surface of calcite, which dominates the equilibrium morphology. Because of its stability, and the fact that it tends to be "clean" (i.e. low concentrations of defects and adsorbed species) this makes it an ideal surface for both experimental and computational study.

In 1997 de Leeuw and Parker were the first to use a force field to investigate the calcite-water interface, performing minimization to analyze the structure and energetics of a single water layer on surfaces with different crystallographic orientations.(103, 104) However, a true aqueous interface could only be investigated once molecular dynamics was applied.(49, 105, 106) Here the water density profile as a function of distance from a clean $\{10\bar{1}4\}$ calcite surface shows two relatively sharp peaks at the interface, at about 2.2 and 3.2 Å, and another two less pronounced peaks at larger distances. Water molecules along the first layer (and in the second to a lesser extent) show a markedly reduced mobility both perpendicular and parallel to the interface, as well as a degree of molecular orientation: waters in the first layer have oxygen coordinated to calcium, whereas waters in the second layer sit atop the carbonate ions and form hydrogen bonds with either the first layer or the underlying anion. These structured layers have been observed experimentally by means of surface X-ray scattering(107) and AFM.(108, 109) Fenter et al.(110) have assessed the performance of

different force fields in describing the calcite-water interface leading to the conclusion that the quality of the parametrization has a greater impact than the type of force field.

Another relevant property at the interface is the water residence time at surface sites (or its inverse, the exchange rate), providing a measure of mobility. Different studies give values ranging from 40 ps(111) to 300 ps(106) to 2 ns,(38) showing that the computational details can alter this value by up to two orders of magnitude. The latter and most recent value is arguably the most accurate, since the force field was parametrized against thermodynamic quantities and liquid water density/mobility,(50) plus a large simulation box and long runs were used. The latter is particularly important here, as it must be larger than the timescale for exchange events to ensure good statistics. Careful analysis is also required to account for fast librational relaxation, which can lead to incorrect exchange rates. Importantly, the residence time of 2 ns at the surface is one order of magnitude larger than that of water around a calcium ion in solution with the same model.

Recently, MD has been extended to the water structure and dynamics at defective calcite surfaces containing steps and kinks,(38, 111, 112) which are the sites for ion attachment during growth. Two types of steps exist on the $\{10\bar{1}4\}$ calcite surface, known as the obtuse and acute steps. Calcium sites along the steps and at kink sites can bind up to 3 and 4 water molecules, respectively, as opposed to one on the flat surface. Here reported residence times also vary considerably. The most recent study,(38) using a multi-exponential fit, shows that water exhibits substantial variation in residence time at steps from a few hundred ps up to several tens of ns, with quite distinct patterns at the acute vs obtuse steps. Although the values at kink sites are harder to determine, they also appear to be similar.

In addition to the dynamics, there is also a question as to whether water is reactive at surface sites (i.e. can proton transfer occur). Wolthers et al.(112) have applied a charge distribution model for ion adsorption to their molecular dynamics data, and found that the

acidity of calcite surface sites depends on topography, with water at obtuse and acute steps being most and least acidic, respectively, while kink sites are intermediate. However, a QM/COSMO-RS study(113) found the opposite order for the steps, while certain kink sites were sufficiently acidic to be bound to hydroxide.

Periodic quantum mechanical methods have found so far limited application in investigating crystal growth due to their computational cost. Kerisit et al.(105) investigated associative and dissociative adsorption of water molecules on the calcite $\{10\bar{1}4\}$ surface, and found only the former to be favorable, thereby validating the use of non-reactive force fields. Two subsequent studies by Lardge et al.(114, 115) analyzed the effect of different coverages (0-200%) and of surface steps and vacancies on water adsorption; interestingly, water adsorption was never dissociative except for one case, that of an anion vacancy on a clean surface. In none of these studies was it feasible to include bulk liquid water.

A key finding from this body of investigation is the identification of structured layers of water on top of calcite surfaces, with water exchange rates one/two orders of magnitude lower than those of ions in solution. As shown below, these ordered layers have significant implications for adsorption and implies that desolvation of surface sites is likely to be a rate limiting step in the growth of a calcite.

3.5.2. Ion attachment and growth mechanisms

Free energy profiles for adsorption of Ca^{2+} and CO_3^{2-} ions at the calcite $\{10\bar{1}4\}$ surface have been obtained from molecular dynamics using umbrella sampling by both Kerisit et al.(106, 116, 117) and Raiteri et al.(49). These two studies show some quantitative discrepancy for Ca^{2+} adsorption, due to the different models employed. However, the key finding is that Ca^{2+} does not bind to calcite terraces, while CO_3^{2-} only weakly binds with a free energy comparable to ambient thermal energy, primarily in solvent-separated states. These results are consistent with experimental observations of growth via screw dislocations rather than

nucleation on terraces(118). Raiteri et al. have also carried out ion diffusion simulations using metadynamics(49), showing that diffusion is unlikely to occur when species are adsorbed directly onto calcite terraces, in contrast to the conventional view. Instead diffusion of carbonate ions occurs when doubly solvent separated, while calcium migrates via bulk solution.

Very recently, the first free energy landscapes have been computed for ion adsorption on defective calcite surfaces.(119) Based on the proposition that kink nucleation is rate-limiting for calcite growth(120), the adsorption of ions and ion pairs on the acute and obtuse steps of the $\{10\bar{1}4\}$ surface was investigated. A single CO_3^{2-} ion is found to readily adsorb on the step top edges, whereas Ca^{2+} only adsorbs once carbonate is already present (Figure 5). When both ions are adsorbed, they are likely to form a contact ion pair; however, the most stable position for such a pair is not at the growth site (i.e. both ions at the bottom of the step), but instead one ion remains at the top of the step, while the other is sited at the lower corner. This suggests that more than one ion pair needs to be adsorbed to achieve conventional kink nucleation, and that migration of the CO_3^{2-} ion from top edge to growth site is rate-limiting.

One of the key computational points that emerges from the above study is the difficult of accurately determining free energy landscapes for crystal growth in water. Even for the adsorption of a single ion at a step edge the number of collective variables to map begins to become prohibitive. This is because it is necessary to not only map or restrain the position of the ion relative to the step edge (3 Cartesian coordinates), but also to simultaneous accelerate the dynamics of strongly bound water around the growth site (e.g. using the Ca-water coordination number of each relevant ion). Standard techniques, such as metadynamics, become hard to both converge and analyze once the number of collective variables exceeds 3. Therefore obtaining reliable data for adsorption at mineral surface steps and kinks can only be achieved with great care and considerable computational effort, even for simple ions and

molecules. Advanced techniques, such as reconnaissance metadynamics(121) or parallel bias,(122) may provide a means of sampling the large number of collective variables needed in future.

Not only are continuing advances in accurate free energy calculations shedding light on the mechanisms of crystal growth, but it is now feasible to determine rate constants. The use of methods such as forward flux sampling(123) and reactive flux(124) allows determination of reaction rates beyond standard transition state theory (TST). These techniques have been successfully used to compute the rate constants for $Ba^{2+}_{(aq)}$ addition to a step on the surface of barite ($BaSO_4$).(125) Recently, a similar approach has been used to determine the transmission coefficients for ion removal from kink sites on calcite.(126) The values obtained vary between 0.04 and 0.08, demonstrating that the probabilities of reaction are at least an order of magnitude less than those obtained from uncorrected TST.

Although the derivation of the thermodynamics and kinetics of growth events is an important advance, it is necessary to connect this information with experimental observables. Here Monte Carlo simulations have a valuable role to play. By formulating a complete picture of possible growth events, with a tabulated set of probabilities for transitions, a macroscopic picture of the evolution of either a single surface or entire crystal can be achieved.(127) This can be driven by chemical potential differences relative to the saturation state of the solution or even use a full set of rate constants in the case of kinetic Monte Carlo (kMC). Over the last two decades there have been numerous applications of kMC to the calcite basal surface based on fitting rate parameters, (128, 129) though soon it may be possible to predict growth from purely atomistic simulation data. Recently a general model for non-equilibrium growth of macroscopic crystals based on tilings has been proposed and applied to calcite,(130) which now extends the scope beyond the (104) surface.

3.5.3. Interaction with organics

To understand biomineralization it is necessary to determine how organic molecules interact with minerals at different stages of their crystallization, from ions in solution in the pre-nucleation regime, through to surface binding during growth of the bulk phase. Organic additives at the mineral-water interface can have varying effects on growth. For instance, growth can be inhibited through surface absorption, leading to blocking of ion attachment.(131, 132) The concentration, pH and functional groups of the organic will all influence the resulting inhibition.(133) Absorption can be driven though electrostatics, hydrophobicity, or through the ability of the molecule to specifically recognize the crystal surface.(131, 134-136) Conversely, organic molecules can promote calcium carbonate nucleation by acting as a template, or accelerate growth after absorption at steps.(62, 133, 137)

For computer simulation, determining how organics bind to minerals during crystallization represents one of the most challenging tasks since not only does the complexity of the system increase, but also the availability of well-tested force fields diminishes. Most studies to date have mixed established biomolecular force fields, such as AMBER or CHARMM, with ionic mineral force fields.(138) Inorganic-organic interactions have largely been approximated by combination rules in the absence of data for fitting. Beside the issues with the underlying models, there has also been a tendency in some studies to either avoid being quantitative, or to use differences in internal energies averaged over periods of trajectories to estimate the thermodynamics of binding. However, configurational entropy cannot be neglected for species in an aqueous environment, as demonstrated for ion pairing,(60) where entropy often drives association in opposition to the enthalpy. Consequently, it is essential to compute the free energy of binding with proper sampling of all relevant collective variables to get reliable results.

To illustrate how varied results can be for even the simplest cases, consider the case of a

single $Ca^{2+}_{(aq)}$ ion binding to the glycine zwitterion. Here QM cluster calculations with various levels of hydration have yielded binding free energies of between -28.6(139) and -85.3(140) kJ/mol, whilst use of a 3D-RISM solvent model gives -7.1 kJ/mol,(141), closer to experimental estimates. Kahlen et al(141) have carefully examined the prototypical example for a carboxylate group binding to a calcium ion, namely the Ca-acetate ion pair. Here they took a range of variants of the GROMOS and OPLS force fields leading to a scatter in the PMF of almost 50 kJ/mol. This work highlights the perils of using uncalibrated off-the-shelf force fields in this field. However, they showed that refinement of the van der Waals parameters can lead to an improved model, provided there is either experimental or AIMD data for calibration.

One of the existing force fields identified in the study of Kahlen et al as giving reasonable thermodynamics has also been used to study the binding of acetate, aspartate and citrate with a range of species relevant to $CaCO_3$ crystallization, from the ion, through the ion pair, to an ACC nanoparticle and the basal surface of calcite.(30) Here the free energy of binding decreased in this order for each organic molecule, pointing to a preference for association with pre-nucleation species and Ca sites with a low coordination number. Once the calcite basal surface is reached then acetate and aspartate only form solvent-separated complexes, while the triply charged citrate is repelled from the surface. Other work has considered the binding of aspartate at the surfaces of calcite, also finding that direct adsorption did not occur at the (104) facet.(142) However, direct coordination was observed for the (110) surface and at acute, but not obtuse, steps, though only internal energies were reported.

Surveying the increasing number of simulations that address the binding of organics at the basal surface of calcite there is a large variation in the quality of results, some of which propose unlikely adsorption energies beyond normal chemical strengths. Here we highlight

three literature studies that appear to offer plausible free energies of adsorption on the calcite (104) surface, while excluding studies under dry conditions not relevant to biomineralization. Firstly, Freeman and Harding(143) studied both mannose and methanoic acid, obtaining binding free energies of -2.9 and -1.6 kJ/mol, respectively. They went on to further estimate that the protein ovocleidin-17 would have a much larger adsorption free energy of -189 kJ/mol. Secondly, Shen et al(144) have considered the binding of polystyrene sulfonate monomers and oligomers on calcite. For the (104) surface, two binding configurations were identified where the sulfonate group either binds directly to the surface, or more favorably the molecule lies flat, solvent separated from calcite by the ordered water layers. The later configuration gives a binding free energy of approximately -5 kJ/mol. They also considered binding at a polar model for the (001) surface where attachment was much more exothermic (-60 kJ/mol). Finally, Ukrainczyk et al.(136) have used metadynamics to examine the free energy landscape for binding of tartrate to calcite where there are two carboxylate groups and a torsional angle to consider. On the basal surface, tartrate adsorbs on top of the water layers with a free energy of -6.5 kJ/mol. However, the (1-10) surface was also examined leading again to strong binding (-38.5 kJ/mol).

From these highlighted studies, plus earlier mentioned work,(30) several conclusions can be drawn from the studies to date. It seems that the majority of organics examined only weakly adsorb on the dominant basal surface of calcite in wet conditions, with a free energy per functional group that is similar to ambient thermal energy. Many functional groups fail to penetrate the ordered water layers and instead prefer to adsorb on top of them. For other surfaces that offer more exposed calcium ions then stronger, contact adsorption is feasible with free energies that are an order of magnitude larger (i.e. a few tens of kJ/mol). There is yet to be any real quantum mechanical validation of these force field results since treating a truly wet calcite surface for an appreciable length of time is prohibitive, though we note that

the adsorption free energy for acetate on calcium oxalate dihydrate(17) has been computed using AIMD and the results support the above conclusions.

At the opposite extreme to organic adsorption on mineral surfaces for growth modification is the deposition of inorganic ions from solution onto an organic layer leading to heterogeneous nucleation. Given the length- and time-scale challenges of nucleation, most simulations have approached the latter problem by depositing either pre-formed particles or considering epitaxial matching of mineral facets with the organic substrate. Duffy and Harding modelled the effect of stearic acid monolayers on $CaCO_3$ nucleation and growth.(69, 145) Their simulations revealed that epitaxial matching of the mineral and organic template is not the only factor increasing the nucleation rates of calcium carbonate: The polarity of the interface, pH and surrounding water also influence the interfacial energies and nucleation rate, as well as the morphology of the growing crystals. While studying the interface of alkanethiols and calcium carbonate with metadynamics, Quigley *et al.* also determined the importance of the ionization of the organic monolayer on inducing crystallization.(146) Additionally, they were able to corroborate the experimentally observed nucleation at the $(01\bar{1}2)$ surface after allowing the monolayer to move. This illustrated the ability of the organic film to adjust and match the growing crystal, and demonstrated structural feedback between the mineral and organic phases at the interface.

4. Conclusions and Perspectives

Although the above is just a cursory summary of the literature for simulation of the biomineralization of calcium carbonate alone, let alone other minerals, it should be apparent that there is considerable work still needed to build up a comprehensive atomistic view of such systems. For example, there has been almost no systematic and careful work that has determine the binding free energies of organics to key growth features, such as steps and

kinks; yet these are the main sites for surface growth. Here we have focused on calcium carbonate, but we note that similar debates regarding pre-nucleation clusters and crystallization pathways exist for phosphate systems,(63) creating a similar imperative for simulation studies.

Two main technical barriers can be identified for the field of biomineral simulation. Firstly, until AIMD becomes sufficiently affordable to tackle systems of the size and timescale currently treatable by classical MD, there is a need for thermodynamically accurate, well-tested force fields for organic-mineral systems. Use of simple combination rules appears to be unreliable and therefore generation of data followed by explicit fitting is required. Use of more sophisticated models, such as those that incorporate on-site polarization, may prove more transferable. Secondly, there is also a need for advances in techniques for accelerated dynamics to ensure reliable sampling of the free energy surface where large numbers of collective variables are needed. Given that both the dynamics of bound water and conformational changes of biomolecules can be slow on an MD timescale, numerous collective variables are required unless prior assumptions are made as to the pathway for crystallization. Tackling homogeneous nucleation of biominerals also remains a grand challenge, given the low concentrations at saturation. This may necessitate the use of hybrid atomistic/coarse-grained approaches to overcome the time- and length-scale difficulties. Despite the tremendous challenges identified, the prospects for simulation of biomineralization are looking promising given the rapid progress that has already been made in the last decade.

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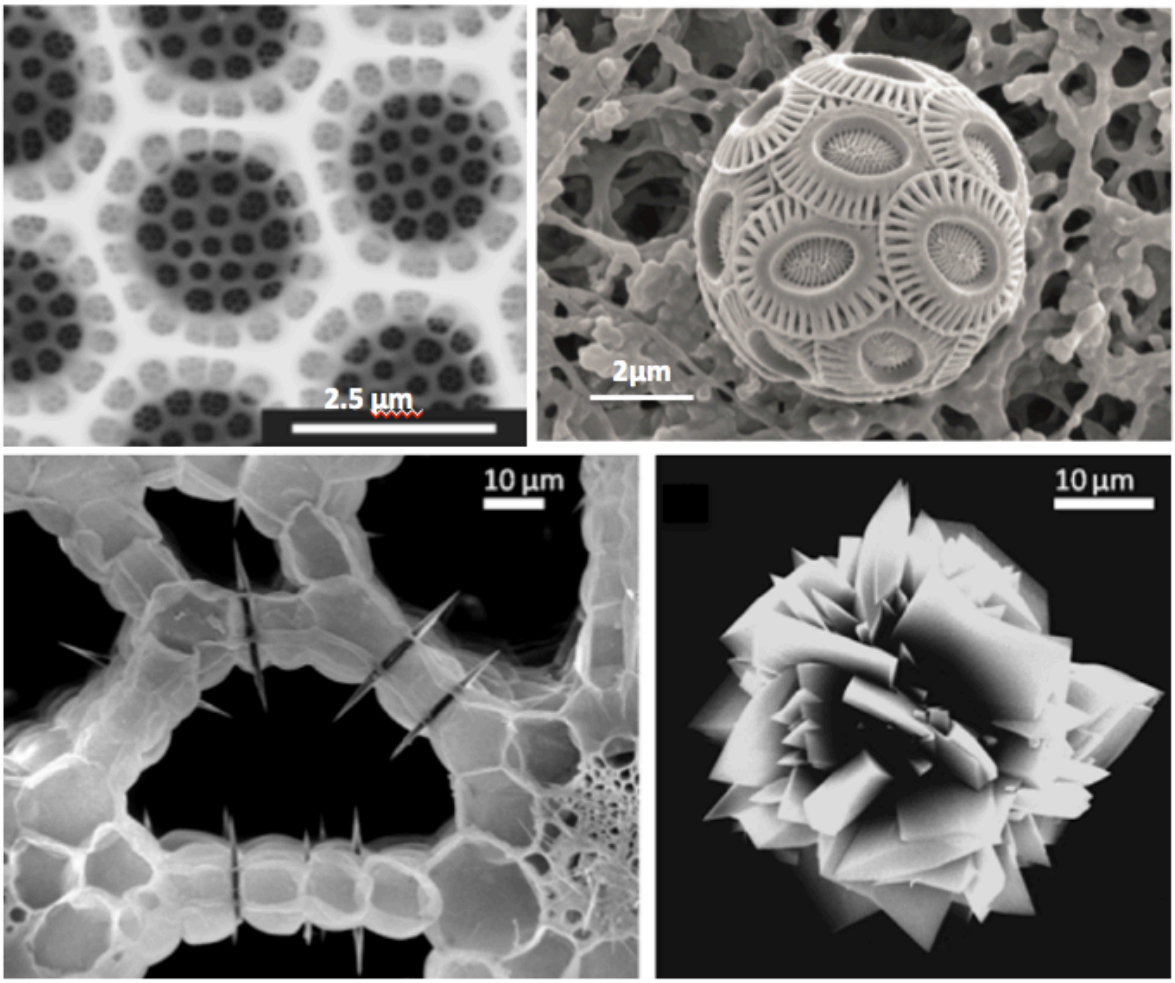


Figure 1 Examples of different shapes and textures of functional hard tissues. Top line, left to right: HR-SEM image of a valve from *C. radiatus* (silica, from "M. A. Sumper, Phase Separation Model for the Nanopatterning of Diatom Biosilica. Science 2002, 295, 2430". Reprinted with permission from AAAS); SEM image of coccolith *Emiliana huxleyi* (calcium carbonate, from © The Trustees of the Natural History Museum, London). Bottom line: calcium oxalate styloids from *Eichhornia* sp. and spherical druse from *Peperomia* sp. leaf (kindly provided by Harry Hornet, Jr.).

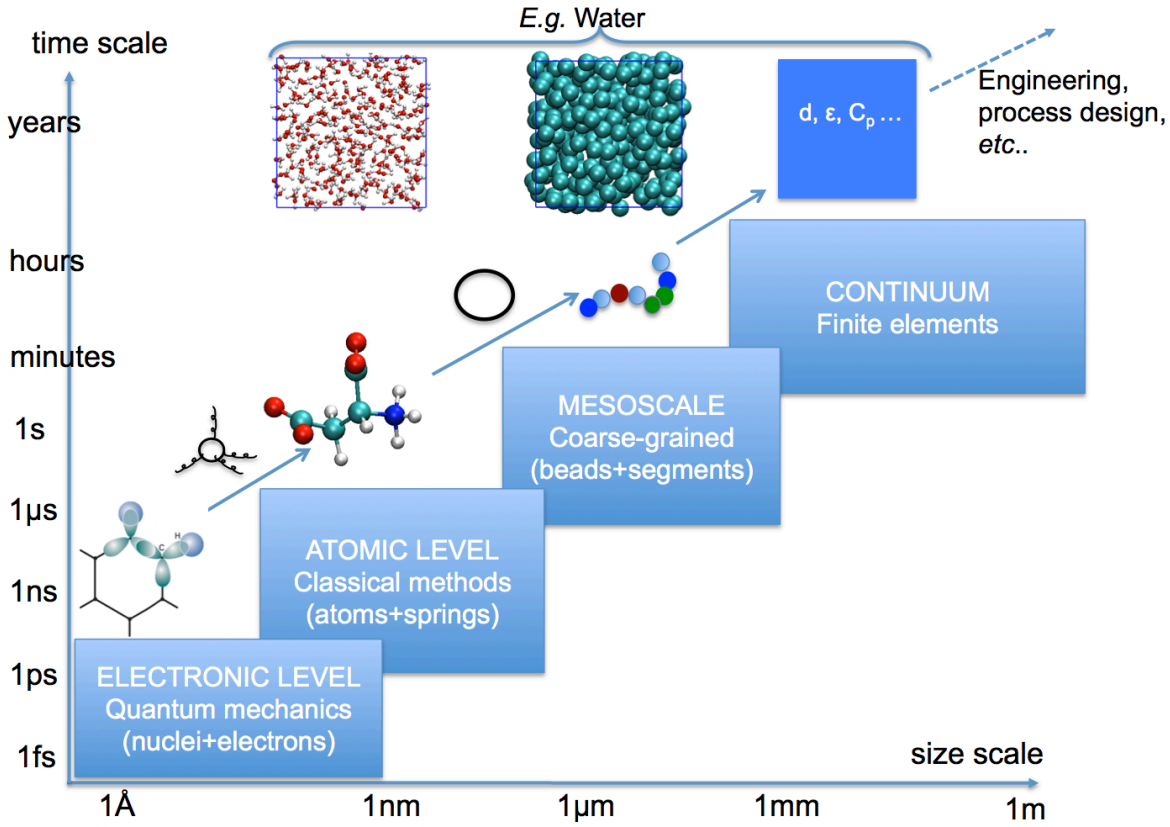
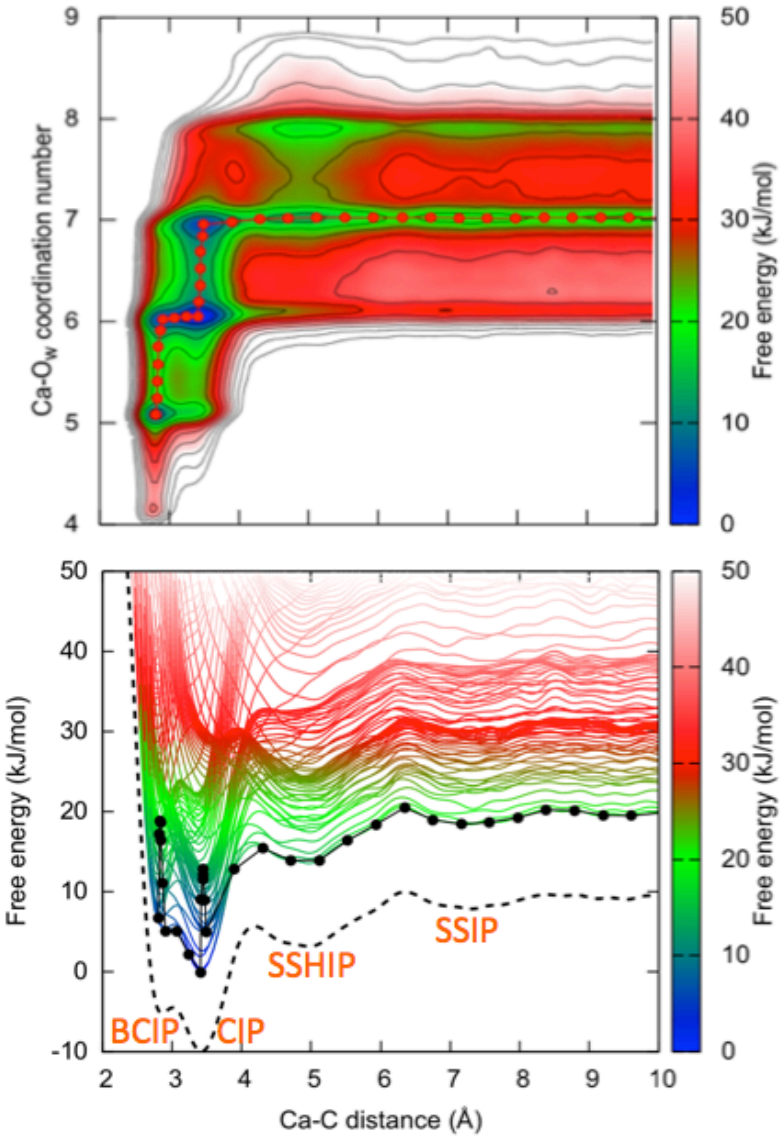


Figure 2 Schematic representation of computational methods classification, according to their level of approximation and to the time and size scale that they can access.


Ca-O$_w$ coordination number
Free energy (kJ/mol)
Free energy (kJ/mol)
Ca-C distance (Å)
BCIP
CIP
SSHIP
SSIP

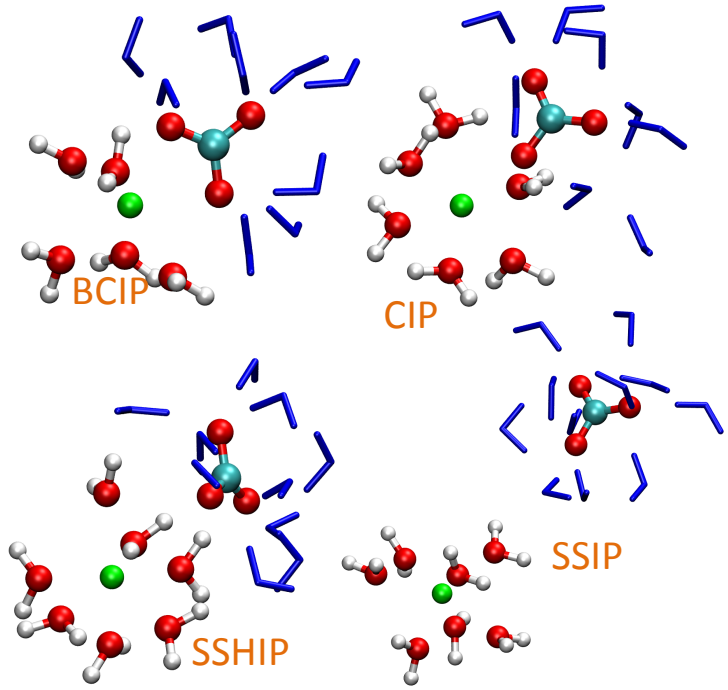

BCIP
CIP
SSIP
SSHIP

Figure 3 $CaCO_3$ ion pair formation. Top: Free energy landscape as a function of Ca-C distance and Ca-O_w coordination number, where O_w is the oxygen of water. The minimum energy path is indicated with red dots. Centre: Projection of the top figure along the Ca-C distance. Four distinct minima are present, corresponding to the bidentate contact ion pair (BCIP), the contact ion pair (CIP), the solvent shared ion pair (SSHIP, *i.e.* the ions share part of their solvation sphere), and the solvent separated ion pair (SSIP). Bottom: The four aforementioned states are depicted. Calcium is represented in green, the carbonate ion is represented in cyan (C) and red (O) ball and sticks, the first water coordination shell is represented with blue sticks for carbonate and with red (O) and white (H) sticks for calcium.

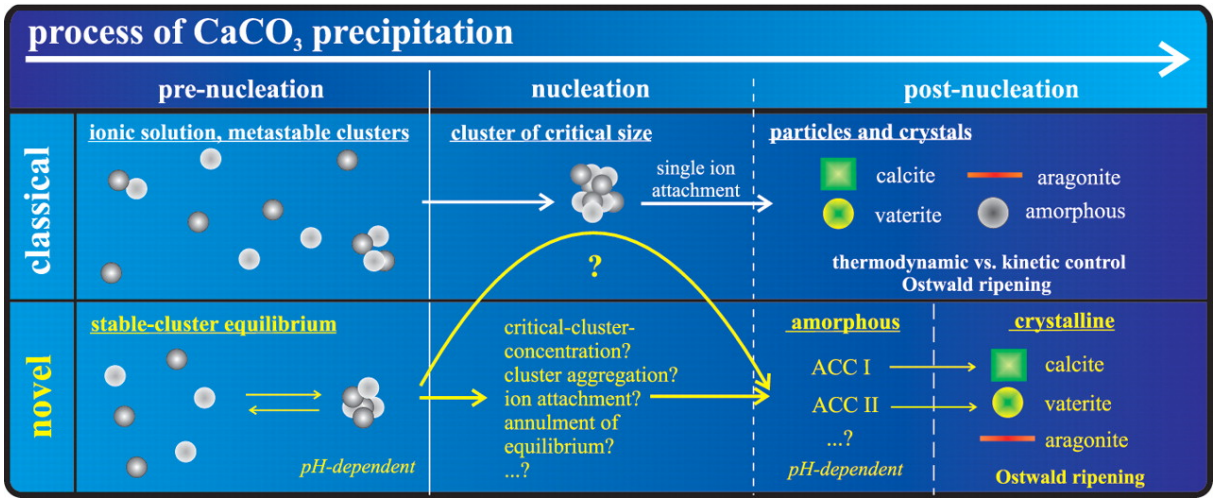


Figure 4 Schematic representation of the classical and alternative views of mineral nucleation and growth. From "D. Gebauer, A. Völkel, H. Cölfen, Stable Prenucleation Calcium Carbonate Clusters. Science 2008, 322, 1819". Reprinted with permission from AAAS.

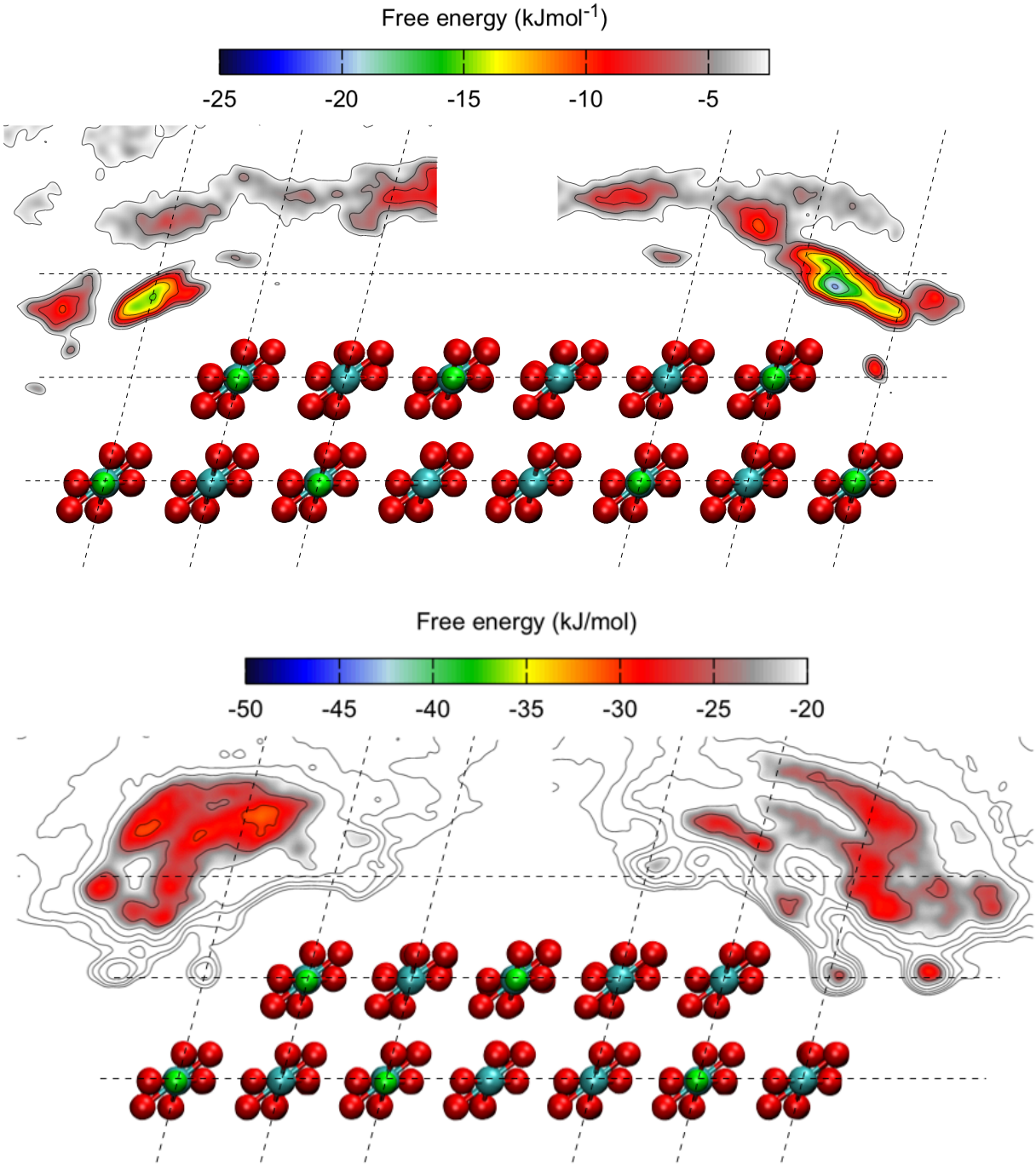


Figure 5 Free energy iso-surfaces for ion adsorption at the obtuse (left) and acute (right) calcite steps on a {10$\bar{1}$4} surface. Top: adsorption of a CO_3^{2-} ion. Bottom: adsorption of a Ca^{2+} ion when a CO_3^{2-} unit is already adsorbed at the step top edge. The calcite structure is shown with calcium, carbon and oxygen in green, blue and red, respectively. Dashed lines represent lattice planes; where these lines cross represents the position of ions that match the underlying bulk structure. From "De La Pierre et al. 2017 Angew. Chem. Int. Ed. 56, 8464". Reprinted with permission from Wiley and Sons.