

Towards an atomistic understanding of apatite–collagen biomaterials: linking molecular simulation studies of complex-, crystal- and composite-formation to experimental findings

Dirk Zahn · Oliver Hochrein · Agnieszka Kawska ·
Jürgen Brickmann · Rüdiger Kniep

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Abstract The investigation of the atomistic mechanisms of crystal nucleation constitutes a major challenge to both experiment and theory. Understanding the underlying principles of composite materials formation represents an even harder task. For the investigation of the mechanisms of crystal nucleation a profound knowledge of the ion–solvent and the ion–ion interactions in solution is required. Studying biocomposites like fluorapatite–collagen materials, we must furthermore account for the biomolecules and their effect on the growth process. Molecular simulation approaches directly offer atomistic resolution and hence appear particularly suited for detailed mechanistic analyses. However, the computational effort is typically immense and for a long time the investigation of crystal nucleation from atomistic simulations was considered as impossible. We therefore developed special simulation strategies, which allowed to considerably extent the limitations of computational studies in this field. In combination with advanced experimental investigations this provided new insights into the nucleation of biomimetic apatite–gelatin composites and the mechanisms of hierarchical growth at the micro- and mesoscopic scale. Along this line, molecular simulation studies reflect a powerful tool to achieve a profound understanding of the complex growth processes of apatite/collagen composites. Apart

from reviewing related work we outline future directions and discuss the perspectives of simulation studies for the investigation of biomineralization processes in general.

Introduction

The nucleation and growth of crystals is a phenomenon of fundamental interest. This does not only apply to physics, chemistry and materials science, but also to a specific field of bio-sciences and medicine: biominerals reflect a fascinating blend of organic soft matter and inorganic nanocrystals. The resulting composites exhibit properties of both hard and soft matter. Indeed, biominerals combine the toughness of an inorganic material and the flexibility of biological tissues. A profound understanding of these properties requires investigating the interplay of the organic molecules with the ions of the inorganic component. This task is far from trivial. Exploring the atomistic structure of a composite is complicated by non-periodic local constellations of the organic molecules. Crystallographic methods may hence only provide insights in the inorganic, crystalline component of biominerals. Apart from identifying the atomistic structure, understanding the mechanisms leading to these structures reflects an even harder challenge. It requires an atomistic in-situ investigation of ion aggregation, mineralization of the biomolecules and self-organization of the composite material.

Molecular simulation approaches may easily achieve the microscopic resolution needed for such detailed mechanistic analyses. In particular molecular dynamics (MD) simulations appear to be a promising tool for studying biomineral formation. However, the computational effort is immense and special MD techniques are mandatory to

D. Zahn (✉) · O. Hochrein · A. Kawska ·
R. Kniep
Max-Planck-Institut für Chemische Physik fester Stoffe,
Nöthnitzer Str. 40, 01187 Dresden, Germany
e-mail: zahn@cpfs.mpg.de

J. Brickmann
Eduard-Zintl-Institut für Anorganische und Physikalische
Chemie, Technische Universität Darmstadt, Petersenstraße 20,
64287 Darmstadt, Germany
e-mail: brickmann@molcad.de

make simulations reasonable. The present review cannot account for all of such approaches. We instead focus on particularly promising methods, which have proven successful in a series of apatite/gelatin aggregation studies. Therein, the computational investigations are intended to complement experimental work and cross-links are of vital importance. Indeed, we feel that combined efforts from both experiment and theory are needed to tackle the challenging complexity of biomineral formation [1, 2].

Apatite/collagen composites belong to the most abundant biominerals in both humans and animal life forms. The importance of this material as the predominant component of bones and teeth motivated a large number of both experimental and theoretical studies [1]. Despite these efforts, our understanding of biomineral formation is still limited. This particularly applies to phenomena taking place at the atomistic scale. Indeed, only little is known about the interplay of the apatite ions and the collagen proteins. The latter issue reflects a key aspect of the composite as it accounts for crucial characteristics of the biomineral such as composition, morphogenesis and mechanic properties.

Atomistic simulation approaches

Atomistic simulations approaches may be divided in two categories depending on the use of quantum or classical mechanics. The latter concept relies on empirical potential energy terms, which may account for hydrogen-bonding and non-bonding interactions with reasonable accuracy. However, the appropriate theoretical treatment of bond breaking and formation usually requires quantum approaches. In contrast to classical force fields, quantum chemical calculations are considerably more demanding with respect to computational resources. The desire to combine the advantages of both classes of methods has motivated the formulation of mixed quantum/classical schemes. These approaches are based on reducing the computationally very demanding quantum calculations on the most relevant degrees of freedom, while describing the remaining modes at much lower costs by classical mechanics. Implemented in an appropriate way, quantum/classical schemes may preserve much of the accuracy obtained from quantum chemistry. On the other hand, the benefits from transferring the efficiency of classical force fields to large parts of the simulation model may easily save orders of magnitudes of the computational costs.

Apatite simulation studies

The importance of apatite has motivated a manifold of atomistic simulation studies. Early theoretical works on bulk apatite crystals date back up to about 10 years ago and

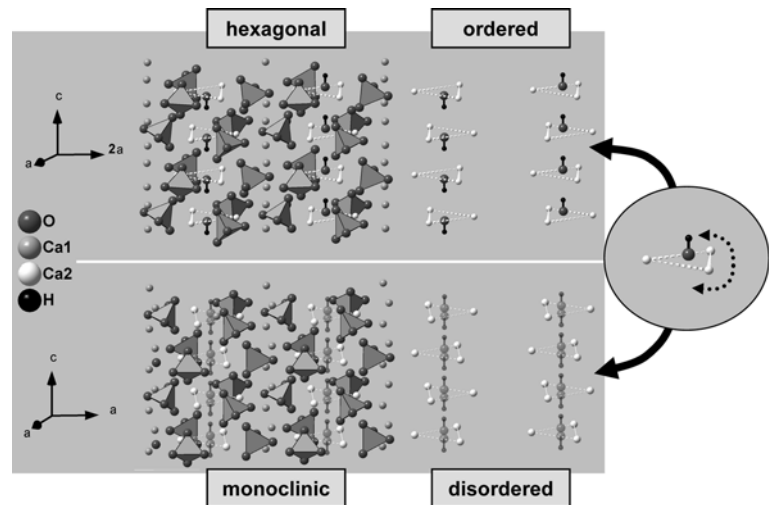
are based on quantum mechanical calculations, exclusively. Lacking reliable empirical force-fields, ab-initio approaches were chosen to investigate bulk crystal structures and the local arrangement of defects in apatites.

Many of the variety of apatite species may be written as an isomorphous $\text{Ca}_{10}(\text{PO}_4)_6\text{X}_2$ series with $\text{X} = \text{OH}^-$, F^- , Cl^- , or Br^- . Therein the ions X are embedded in channels formed by staggered triangles of calcium ions. While sufficiently small ionic species $\text{X} = \text{F}^-$ are located in the center of the Ca-triangles, larger ions were found off-center along the [001] direction [3]. In hydroxyapatite, the most relevant apatite for human hard materials, the OH^- ions may be located either above or below the Ca-triangles. From both crystallographic structure refinements and ab-initio structure relaxation studies, the distance between the hydroxide oxygen atom and the center of the Ca-triangle was found as about 0.2 Å [4, 5]. In the stable configurations, the positively charged hydrogen atom of the hydroxide ion is pointing away from the nearest Ca-triangle. Transformation of the OH^- ordering therefore requires not only crossing the embedding Ca-triangle by dislocation of the oxygen atom by 0.4 Å, but also an orientation inversion of the complex anion.

Depending on the preparation conditions pure hydroxyapatite exhibits either monoclinic (space group $P2_1/b$; $a = 9.4214(8)$ Å, $b = 2a$, $c = 6.8814(7)$ Å, $\beta = 120.00(8)^\circ$, $Z = 4$) [6] (Fig. 1, top) or hexagonal (space group $P6_3/m$; $a = b = 9.432$ Å, $c = 6.881$ Å, $Z = 2$) [5] symmetry (Fig. 1, bottom). The latter modification corresponds to the high temperature phase, which can be obtained from the monoclinic modification via an order \rightarrow disorder transition affecting the orientation of the hydroxide ions, only. By means of optical birefringence experiments [7], X-ray and difference scanning calorimetry measurements this transformation was observed at temperatures above about 200 °C [8]. In principle, two different models may account for the (average) hydroxide ion orientation (occupation of 0.5 for each crystallographic position) in the high temperature phase: the mirror plane perpendicular to the c -axis of the space group $P6_3/m$ may be associated to (i) a “disordered column” model in which the orientation of the hydroxide ions is inverted at random sites within the channels or (ii) an “ordered column” model in which all OH^- ions in a single row point in the same direction, while the orientation of each specific column with respect to the collective of all rows is randomly distributed [5].

Both models are indistinguishable by X-ray or neutron scattering experiments [9] and so far the only evidence which picture accounts for the hexagonal modification of hydroxyapatite was obtained from theory [4, 10, 11]. De Leeuw performed a series of quantum mechanical structure optimization studies based on various predefined OH^-

Fig. 1 Monoclinic and hexagonal structure of hydroxyapatite. While the monoclinic \rightarrow hexagonal transition occurs at 200 °C, the hexagonal modification is metastable at lower temperature



arrangements and energy minimization to local minima [4]. This approach is typically used for low-temperature structure determinations from ab-initio calculations. Indeed, the energy minimization corresponds to zero Kelvin. Simulation setups of non-zero temperature require Monte-Carlo or molecular dynamics approaches which are computationally much more demanding. This motivated the development of empirical potential energy functions for modeling apatites at favorable costs [12, 13]. On the basis of such force-fields we performed molecular dynamics simulations of the temperature-induced monoclinic \rightarrow hexagonal transition [10]. The results of the latter study and the structure optimization calculations of de Leeuw are quite controversial. While de Leeuw predicted concerted inversions of ordered columns [4], independent flipping events of single hydroxide ions were observed from the molecular dynamics simulations [10].

Quantum chemical approaches allow very precise treatment of the atomic interactions. At first sight one might therefore tend to favor the predictions made on the basis of ab-initio calculations. However, in quantum calculations limited computational resources typically imply considerable model simplifications whose effect may outbalance the benefits compared to empirical force-fields. Indeed, in the present case modeling a temperature-induced phase transition at zero Kelvin reflects such an oversimplification. Exploring inversion events of single hydroxide ions on the basis of free energy calculations at room temperature, Hauptmann observed a barrier of 87 kJ mol⁻¹ (S. Hauptmann, Private communication). At higher temperature, the lattice expands and the edges of the Ca-triangles are more susceptible to elongations. This reduces the hydroxide-flipping barrier to 50 kJ mol⁻¹ at 200 °C, i.e., the temperature at which the monoclinic \rightarrow hexagonal transition takes place [10]. Moreover, the changes in potential energy related to a single OH⁻ inversion event in monoclinic hydroxyapatite reduces from 42, 23 kJ mol⁻¹ to

less than 10 kJ mol⁻¹ at 0, 300, and 573 K, respectively [4, 10] (S. Hauptmann, Private communication).

Apatites grown in biologic systems typically contain a manifold of defects (carbonates, water, etc.) [9]. Atomistic simulations offer very detailed insights into the arrangement of defects and local structure changes arising from impurity incorporation. Most of the defect studies related to apatites were dedicated to hydroxyapatite. A central motivation for this is given by the fact that the inorganic component of bones is represented by non-stoichiometric (Ca-deficient) hydroxyapatite [9] whose impurities are believed to be relevant for biological functions [14].

The general perspectives of atomistic simulations to non-stoichiometric apatite studies may be very nicely illustrated by the work of Astala and Stott [15]. Focusing on carbonate incorporation in hydroxyapatite, these authors investigated both hydroxide and phosphate substitution. To avoid net charges, replacing OH⁻ or PO₄³⁻ by CO₃²⁻ ions requires further changes in the system and Schottky defects and protons (associated to hydroxide or phosphate ions) accompany carbonate incorporation. Astala and Stott prepared a series of model systems corresponding to a variety of possible defect arrangements [15]. Each of the simulation models was then optimized by relaxation to a local minimum in the potential energy landscape. From this an energetic scoring was obtained which may be related to the occurrence of specific defect arrangements.

Apart from carbonates, the most abundant impurities in biologic hydroxyapatite are H⁺ (associated to phosphate or hydroxide ions), fluoride and chloride ions [16]. The incorporation of halide ions is related to hydroxide substitution and directly preserves the total charge of the crystal. From a dentist's point of view, the most important of these defect species is represented by fluoride ions, which are known to protect human tooth enamel from caries. OH⁻ \rightarrow F⁻ ion replacement in bulk hydroxyapatite was investigated in a series of simulation studies employing

similar strategies as described above [4, 11]. On this basis, fluoride defects were found to promote the monoclinic \rightarrow hexagonal transition. Indeed, the preferred fluoride incorporation corresponds to $\text{OH}^- \cdots \text{F}^- \cdots \text{HO}^- \cdots \text{OH}^-$ constellations. This fully compensates the energetic costs for inverting the orientation of a hydroxide ion within the aligned $\text{OH}^- \cdots \text{OH}^- \cdots \text{OH}^- \cdots \text{OH}^-$ columns of monoclinic hydroxyapatite [11].

Ion/water and apatite/water simulation studies

Interface studies

In biologic systems, apatites are formed from aqueous solutions and remain in contact with water during their use as bone or dental materials. The latter issue, i.e., the structure of apatite/water interfaces may be investigated from relatively straightforward molecular simulation approaches. In 2003, we presented a molecular dynamics simulation study of the hydration of (001) surfaces of hydroxyapatite [17]. The investigated surface types were obtained from different (001) cuttings from the ideal crystal and should be considered as limiting cases locally covering the features of slightly rough crystal faces. Unless acidic conditions are applied, the solubility of hydroxyapatite in aqueous solution is extremely low. Indeed, our 0.5 ns molecular dynamics runs reflect a stable apatite/water interface for which a series of structural and dynamical properties could be sampled. Soon after this work a similar molecular dynamics simulation was presented by de Leeuw from which hydroxide ion dissociation was observed [18]. While no comparison of the two studies was given in [18], we feel the striking differences must be discussed before continuing the use of either simulation model.

The two pictures may be easily discriminated from experiment by adding “dry” apatite and pure water. Even pulverized apatite samples with large surface to volume fractions do not alter the pH of the system. This is in clear contradiction to the observation of hydroxide ion release in the simulations of de Leeuw [18] giving rise to the assumption that the underlying empirical interaction model might be inappropriate. However, comparing the de Leeuw force field and the Hauptmann force field [12] used in our simulations [17] we found both models to be very similar. Instead, it seems the different setups of the molecular dynamics simulations caused the lack of concordance. De Leeuw applied identical relaxation times of 0.5 ps to the isothermal–isobaric algorithm. This choice is rather unusual as in most constant pressure—constant temperature molecular dynamics simulations artificial couplings of the respective variables are avoided by assigning different

relaxation times. Decorrelation is typically achieved by setting the barostat relaxation time about ten times larger than the thermostat relaxation time.

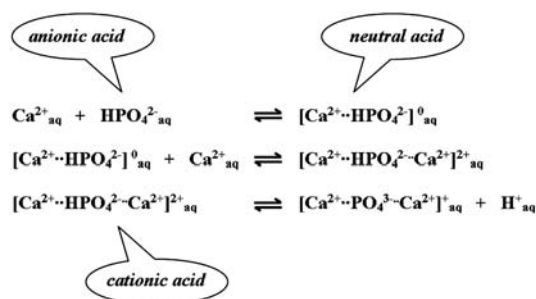
To check the effect of the isothermal–isobaric algorithm on the apatite/water interface reported in [17] we rerun 5 ns sketches of our model system using 0.5 ps/0.5 ps, 0.5 ps/5 ps, and 0.5 ps/125 ps as thermostat and barostat relaxation times, respectively. The larger the thermostat/barostat decoupling, the more reasonably small cell fluctuations were observed. This particularly applies to the cell edge parallel to the *c*-axis of the apatite slab, which varies from -0.5 to $+10\%$ for the 0.5 ps/0.5 ps setup. Applying 0.5 ps/5 ps already reduces the corresponding fluctuations to less than $\pm 1\%$ ($\pm 0.1\%$ for the 0.5 ps/125 ps setup). Increased flexibility, orientation inversion, and release of interfacial hydroxide ions are strongly promoted by simulation cell deformation. In fact, we expect the anomalously large compression/expansion fluctuations of the apatite/water interface study of de Leeuw to account for the observed hydroxide dissociation.

Aggregation studies

For the investigation of apatite nucleation a profound knowledge of both the ion–water and the ion–ion interactions in solution is required. While there are well-established empirical force-fields for studying calcium, phosphate, hydroxide and fluoride ions in aqueous solution, proton transfer events cannot be assessed without quantum mechanics so far. Apatite formation is known to dramatically depend on the pH [1]. A full account of the ion–ion/ion–solvent interactions hence must include the consideration of proton transfer reactions. At ambient conditions, it is reasonable to assume that these processes only occur in solution and at the apatite/water interfaces. Proton transfer reactions may hence be expected during ion aggregation and crystal growth, but not inside the bulk material.

The crystallization of calcium phosphates from aqueous solution is a process of considerable complexity and the degree of protonation of the phosphate ion can play an important role. Depending on the pH, phosphoric acid exhibits several stages of deprotonation ($\text{p}K_{\text{a}1} = 2.16$, $\text{p}K_{\text{a}2} = 7.21$, $\text{p}K_{\text{a}3} = 12.32$; at 25 °C [19]). At physiologic conditions the fraction of completely deprotonated phosphate ions may hence be expected to be extremely small. Nevertheless, crystallization may yield calcium phosphates (including hydroxyapatite) consisting of PO_4^{3-} ions even under acid conditions. In such cases, the deprotonation of the (di)hydrogenphosphate ion has to occur during crystal growth. We investigated the initial steps of calcium and hydrogenphosphate ion aggregation in aqueous solution by means of quantum/classical molecular mechanics simulations [20]. By combination of studying calcium ion

aggregation to (hydrogen)phosphate ions and investigating hydrogenphosphate deprotonation, we identified the $[\text{Ca}^{2+}\dots\text{PO}_4^{3-}\dots\text{Ca}^{2+}]$ ion triple as the smallest stable aggregate, which may be expected to contain an entirely deprotonated phosphate ion. This example demonstrates the deprotonation of the hydrogenphosphate ions to be promoted by its local environment, i.e., by positively charged calcium ions:



During the initial steps of calciumphosphate nucleation in aqueous solution the acidity of HPO_4^{2-} increases dramatically. By association of one Ca^{2+} ion, the anionic acid HPO_4^{2-} is converted into a $[\text{Ca}^{2+}\dots\text{HPO}_4^{2-}]^0$ complex, which may be interpreted in terms of a neutral acid. While more likely to be deprotonated, the acidity of $[\text{Ca}^{2+}\dots\text{HPO}_4^{2-}]^0$ is still rather weak. Proton transfer becomes likely only after aggregation of a second calcium ion leading to a $[\text{Ca}^{2+}\dots\text{HPO}_4^{2-}\dots\text{Ca}^{2+}]^{2+}$ complex which may be considered as a cationic acid.

For a realistic molecular dynamics study of crystallization processes from solution simulation models corresponding to slightly super-saturated salt solutions are required. In the case of low salt solubility, this implies immense numbers of solvent molecules. The major obstacle to straightforward molecular dynamics simulation of crystal formation from strongly diluted solutions is related to the mainly diffusion controlled character of such systems. In the extreme limit the ion concentration is so low that most of the solutes does not interact with each other and behave as if being in an infinite dilution. In this picture the ions diffuse freely, until some of them happen to get at close distance to each other. Attractive ion–ion interactions may then lead to the formation of aggregates.

The formation of ion pairs and triples as described above was investigated by ignoring ion diffusion and focusing on the association of nearby ions. The related free energy landscape is scanned as a function of a few degrees of freedom and the minimum energy path is used as a model reaction coordinate. In complex systems like ion solutions the real reaction coordinate depends on all degrees of freedom, including the solvent molecules. However, for studying the association of an ion pair, in many cases the ion–ion distance reflects a very suitable model of the

reaction coordinate. Adding further ions, we already have to consider at least two degrees of freedom. Apart from the distance coordinate also the orientation of the aggregate with respect to the newly attached ion must be considered.

For solute/solvent combinations of very low solubility such as calciumphosphate/water or CaF_2 /water systems we found very stable ion complexes which did not dissolve even during high-temperature annealing runs [20, 21]. Aggregates larger than ion pairs or triples exhibit several adsorption sites. While the energy score of one ion docking process might be more favorable than another, a variety of adsorption sites may lead to stable aggregates. In such systems, crystal growth does not necessarily follow the minimum energy path, but is also controlled by the local availability of ions which is directly connected to ion diffusion in the dilute solution.

As computational costs force us to avoid direct molecular dynamics simulations of slow diffusion processes we developed a simulation scheme which instead mimics ion distribution in solution originating from diffusion [21]. This is accomplished by a Monte-Carlo step in which an ion is placed at a random position in the vicinity of the aggregate. The nearest adsorption site is then guessed from steepest descent energy optimizations. Finally, full relaxation of the total simulation system provides a new aggregate to which further ions may be attached. During this iterative procedure the ions are added one-by-one to the aggregate being formed. The initial aggregate consists of a single ion and each growth step represents the association of a further ion. Despite its appealing simplicity, we wish to point out that this ion-by-ion attachment procedure relies on a series of approximations which should be carefully checked for each specific study of crystal formation from solution.

The example of CaF_2 aggregation from aqueous solutions nicely demonstrates the perspectives of our aggregate growth model. Starting from single ions, we can track crystal nucleation from the very beginning. The early stage of CaF_2 formation is of particular interest because the evolution of small complexes to aggregates counting 50–100 ions reflects a disorder \rightarrow order transition. Indeed, our simulations offer insights into the self-organization process leading to the CaF_2 crystal structure. We identified the formation of regular motifs to nucleate in the inner core of the aggregate (Fig. 2). Adding further ions to the aggregate surface implies structural rearrangements which cause the growth of the ordered domain and eventually leads to crystallite formation.

The limitations of the aggregate growth model may be nicely demonstrated by the example of NaCl aggregation from aqueous solution, i.e., a solute/solvent combination of high solubility. Transition path sampling molecular dynamics studies of this process revealed a competition of

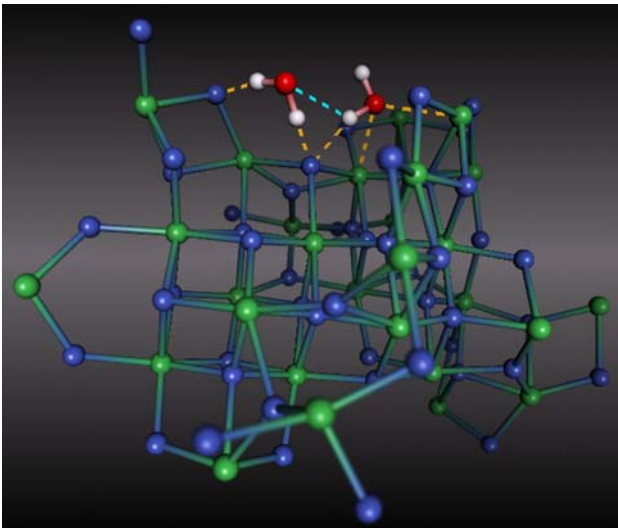


Fig. 2 Snapshot of a $[\text{Ca}_{28}\text{F}_{54}]^{4+}$ aggregate as obtained from our aggregation model reported in [21]. Calcium and Fluoride ions are illustrated in *green* and *blue*, respectively. Only two water molecules of the dilute aqueous solution are shown (*upper part*). The dashed lines indicate ion water (*yellow*) and water–water (*cyan*) interactions which stabilize the aggregate surface and its solvent structure

association and dissociation events, as well as the agglomeration of small aggregates [22]. Crystal growth is hence controlled by the net dynamics of growth/dissolution events. In the aggregate growth scheme described above this competition is expected to be fully biased in favor of association steps only. Dissociation events are assumed to be negligible. In terms of classical nucleation theory this means that even the smallest possible aggregate is considered as a post-critical nucleus. This approximation may only hold for solute/solvent combinations of very low saturation concentration exhibiting purely diffusion controlled aggregate growth. As NaCl solubility in water is rather large, it is not surprising that dissociation events were observed during the aggregate relaxation runs [21]. This is a clear indicator of the invalidity of our aggregate growth scheme for the study of this specific solute/solvent combination.

Our aggregate growth model should be considered as complementary to other approaches to crystal formation such as transition path sampling and free energy methods combined with classical nucleation theory. The latter methods are suited for systems of large ion concentration (most applications are actually related to crystallization from the melt, i.e., at 100% ion concentration). While these approaches become increasingly inefficient for solutions of low ion concentration, our method is dedicated to the study of the aggregation of compounds exhibiting very low solubility constants. As nature chose particularly insoluble (in water) compounds as biominerals, we consider our approach as well-suited for aggregation studies in biomimetic model systems.

Apatite/collagen/water systems

Setting our focus to apatite/collagen composites we can clearly identify which interactions may be modeled by empirical force fields and for which a quantum description is inevitable. In bulk apatite the arrangement of the ions is dominated by the Coulomb interactions and repulsive forces, which prevent atomic overlaps. This interplay can be described by empirical force fields at reasonable accuracy [12, 13, 23]. A similar situation is represented by the soft matter part of the composite, i.e., the collagen proteins. The latter may be modeled by force field packages designed for biopolymers like Amber, Charmm, etc. Along this line, Buehler performed comprehensive model studies of the mechanical properties of collagen triple helices and assemblies thereof [24, 25]. Apart from quantitative agreement with experimental data this work provides new insights to collagen deformation and fracture behavior and reflects a nice example how model simulations may complement experimental findings to extend our mechanistic understanding.

However, this only holds for the bulk phases. In the previous section we discussed proton transfer reactions occurring during phosphate aggregation from aqueous solution. Similarly, also the collagen protein could be subject to (de)protonation events promoted by ion association. While the most appropriate account of such processes is surely represented by quantum chemistry methods, the complexity of collagen/ion/water systems motivates the choice of computationally less expensive approaches. In the two case studies described in the following ion aggregation is explored by considering different protonation states in parallel classical mechanics calculations. Within the mainframe of the force fields, each specific protonation state is fixed and bond breaking/formation reactions cannot be assessed. However, by comparison of several possibilities—like HPO_4^{2-} and PO_4^{3-} ion aggregation to collagen—one can at least obtain some insights into the importance of proton transfer reactions.

The association of single calcium, phosphate and fluoride ions to a collagen protein reflects the very initial step of fluorapatite nucleation in a collagen matrix. As an entire matrix of fiber proteins cannot be tackled within atomistic simulations, it is reasonable to focus on specific aspects of the collagen matrix. The tails of the collagen fibers are expected to be relatively flexible (compared to the inner part of the triple-helices) and were therefore suggested as particularly suitable for associating ions and accommodating apatite aggregates [1]. Indeed, the charged groups located in the ends of the peptide strands were found to be practically freely accessible to ion attachment from aqueous solution [26]. This applies to several side chains and the termini of the peptide backbone. The variety of putative

adsorption sites in the tails of collagen fibers was systematically investigated from free energy calculations. Each candidate for calcium, phosphate or fluoride ion association was ranked on the basis of the resulting energy profiles. The analysis of protein–ion bonds demonstrated the ability of the collagen tails to ‘trap’ specific ions from the aqueous solution and hence promote local aggregate formation. The most stable complexes were found for calcium association, which is favored by 20–25 kJ mol⁻¹ over the dissolved state [26]. The tendency to phosphate and fluoride ion adsorption is less pronounced, however these processes are still exothermic exhibiting formation energies of about 10 kJ mol⁻¹. Other species, like sodium or chloride ions, were found to be unable to form stable bonds to either the teleopeptide model (tails of collagen) [26] or the bulk triple helix [27]. These findings are in excellent agreement with experimental findings. Indeed, sodium or chloride ions are often used as inactive counterparts for biomimetic apatite crystallization from phosphate and calcium solutions, respectively [1]. On the other hand, from atomic force microscopy experiments calcium ions are known to strongly interact with collagen and even account for a stiffening of the biomolecules [28–30].

The hardening of the collagen fibers by pretreatment with calcium ions was recently used to control the morphogenesis of biomimetic fluorapatite–gelatin composites [27]. In order to explain the relation of the different growth mechanisms observed from electron microscopy and the ion impregnation prior to the crystallization experiments, we performed a series of Ca²⁺, HPO₄²⁻, and PO₄³⁻ docking simulations to a model triple helix [27]. Therein the collagen fibers were mimicked by (Hyp-Pro-Gly)_n polypeptides (Fig. 3) and the aggregate growth scheme [21] described in the previous section was used for efficient sampling of possible ion–protein complexes. Along this line, calcium ions were found to be incorporated inside the triple helix. Though the local structure of the protein must be changed, calcium association does not alter the overall shape of the triple helix which may be described as a linear coil. On the other hand, phosphate ions (irrespective of the protonation state) were found to bind laterally to the collagen model. The protein–phosphate ion interactions may involve 1–3 hydrogen bonds. Binding via a single protein–phosphate contact does not affect the shape of the triple helix. However, this picture changes dramatically when more than one hydrogen bond are involved. In the latter case we observed biomolecule deformation induced by partial ‘wrapping’ of the protein around the phosphate ion. By association of multiple phosphate ions, the fiber is bent several times with no preference for either direction. Impregnation of the collagen molecules by phosphate ions hence implies a considerable structural manifold, whereas calcium impregnation fixates the linear alignment of the

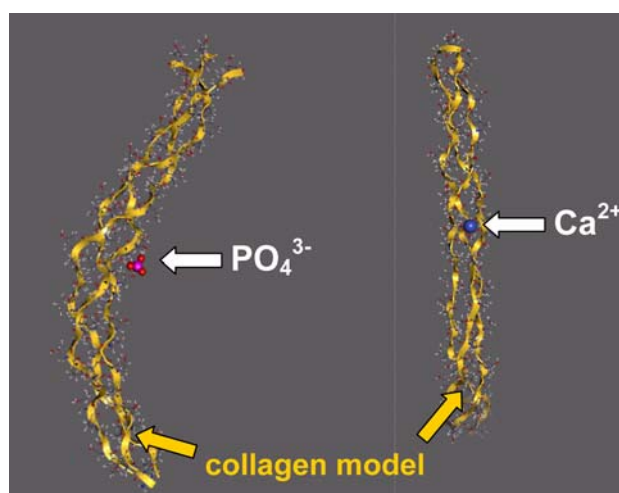


Fig. 3 $3 \times (\text{Hyp-Pro-Gly})_n$ polypeptide model mimicking a collagen triple helix. The backbone of each fiber strand is illustrated by a yellow ribbon. *Left*: association of a phosphate ion with binds laterally and causes a deformation of the biomolecule. *Right*: incorporation of a calcium ion leading to a stiffening of the polypeptide. The water molecules of the solvent are omitted for better visibility

triple helices. On the basis of atomistic simulations we can hence identify different collagen pre-structuring effects induced by calcium and phosphate ion association and rationalized the different composite morphogeneses observed from experiment [27].

Conclusion

It is clearly a long way to go from single ion association to apatite/collagen composite formation, and our work should be considered as a first step on this journey. In yet ongoing efforts, we explore the formation of larger aggregates using the same methods as described in this review. However, with the computational resources available today, atomistic simulations can only focus on fundamental aspects of biomineralization. Our studies of ion aggregation in water and in collagen/water systems may illustrate the perspectives of such simulations.

While special methods of atomistic simulations allow the investigation of nanocrystal aggregation from solution [21], insights at the meso- or macroscopic scale require different approaches. Atomistic models mimicking a mesoscale system would comprise of an overwhelming number of particles. To avoid this computational collapse, we need strong simplifications whose basis is hard to get. In a recent study of the electric field intrinsic to apatite/collagen composites, we approximated the fiber proteins by a simple dipole model [31]. In this specific case, the required model parameterization could be based on comprehensive experimental data [1]. Nevertheless, we are still

far from a generally applicable mesoscopic model of apatite/collagen materials and complexity reduction by coarse graining of biominerals remains a major task for the future.

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