

# Atomistic modeling of apatite-collagen composites from molecular dynamics simulations extended to hyperspace

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**Abstract** The preparation of atomistic models of apatite-collagen composite mimicking enamel at length scales in the range of 1–10 nanometers is outlined. This bio-composite is characterized by a peculiar interplay of the collagen triplehelix and the apatite crystal structure. Structural coherence is however only obtained after drastic rearrangements, namely the depletion of protein-protein hydrogen bonds and the incorporation of calcium triangles which are stabilized by salt-bridges with the collagen molecule. Starting from an isolated collagen triple helix and a single-crystalline apatite structure, a composite model is obtained by gradually merging the two components *via* an additional (hyperspace) coordinate. This approach allows smooth structural relaxation of both components whilst avoiding singularities in potential energy due to atomic overlap.

**Keywords** Biominerals · Hybrid materials · Molecular dynamics simulations

## Introduction

Crystal nucleation and growth, its structure and habitus as well as the resulting materials properties are of fundamental

interest in physics, chemistry and materials science, but also in a specific discipline of biology - the investigation of biominerals. While nucleation processes and materials properties are well characterized at the macroscopic and mesoscopic scale by a wealth of experimental evidence, this does not apply to the microscopic scale, which is much more difficult to access from the experiment. For understanding mechanisms at the atomic level of detail computer simulations have proven to be a very powerful tool. Molecular dynamics simulations may easily achieve the atomistic resolution and hence appear particularly suited for detailed mechanistic investigations.

For complex materials, there are immense obstacles to these else wise very appealing perspectives of atomistic simulations. On the one hand, information of the atomic structure is typically not accessible at full detail. Setting up simulation models from intuition is a tricky task – and may severely bias the reliability of the results. On the other hand, detailed structures may be obtained by exploring the nucleation process itself, but this requires tackling the time-length scale problem inherent to molecular dynamics simulations of crystal nucleation [1].

Here, we focus on collagen-hydroxyapatite composites of about 2 weight-% collagen content mimicking the predominant part of human enamel. The importance of this material lead to a large number of experimental and theoretical studies [2–4]. In particular, we investigated the mechanisms of aggregate formation and growth-control by collagen fibers at the atomistic scale [5, 6]. From this we identified triangles of calcium ions coordinating a fluoride ion in/near its center as a peculiar motif of the apatite crystal structure which formation is promoted by ion association to the biomolecule. Moreover, the collagen triple-helices also induce orientation control to these motifs giving rise to the alignment of the triplehelices along the c-axis of the apatite crystal [2, 6] (Scheme 1).

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The aim of the present work is to outline the design of scale-up models of bio(mimetic) composites, based on building-rules that are derived from earlier investigations of the nucleation of small aggregates and its interplay with the organic component. While studies of ion-by-ion association to collagen are limited to the very early stage of composite growth, *i.e.*, aggregates of a few hundred ions, scale-up modeling can pave the way to much larger atomistic models. The latter shall mimic the bulk composite at length scales up to tens of nanometers without losing the atomistic level of detail.

## Theory

The key difficulty of preparing an atomistic model of a collagen-apatite composite is related to the structural relaxation of both components upon collagen incorporation into an apatite crystal. While the atomic structures of both, isolated collagen triple-helices and the apatite crystal are well-known, our information about the structure of collagen incorporated into apatite is much less detailed. Recent molecular simulation studies revealed that collagen rearranges in order best incorporate calcium ions [7]. During the nucleation of apatite-collagen composites protein-protein hydrogen bonds are broken and replaced by even stronger electrostatic interactions, namely salt-bridges stabilizing  $\text{Ca}_3\text{X}$  ( $\text{X} = \text{F}^-, \text{OH}^-$ ) motifs [6]. Thus, apatite-collagen biominerals represent a prominent example for composites which atomistic structure shows important differences from that of the isolated components.

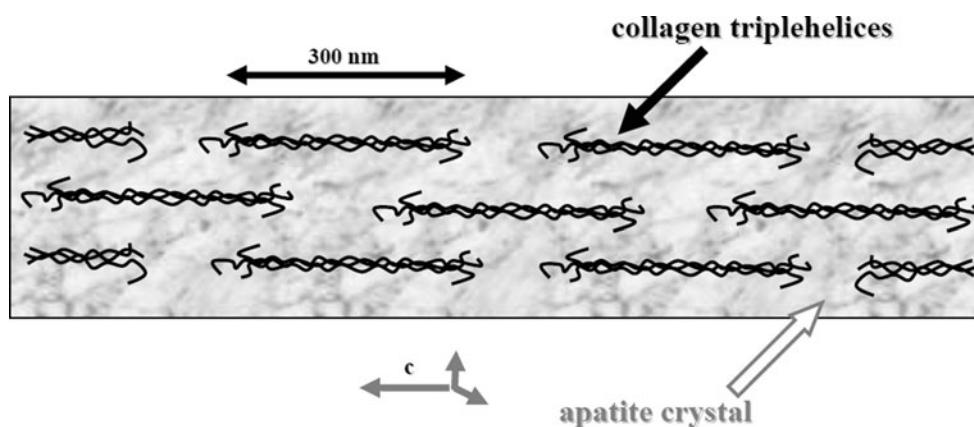
This situation is somewhat similar to the often observed difference in protein structure when comparing its configurations in vacuum and in aqueous solution. Yet, the common procedure for preparing an atomistic model of a protein (or other solute) in solution is based on the isolated systems, *i.e.* the protein structure after relaxation in the vapor state and an

equilibrated solvent box. When introducing the solute, overlapping water molecules are simply cut. The resulting system then needs structural relaxation to adopt to the solvent-solute interactions. In a solution, this may easily be accomplished by constant-temperature, constant-pressure molecular dynamics simulations.

Transferring this to molecule incorporation into a solid (here: collagen into apatite) is in principle possible, yet the structural relaxation now becomes a critical issue. Unlike in a liquid solvent, in the embedding solid ion/molecule diffusion is drastically slower and typically elusive to conventional molecular dynamics simulations. Moreover, the removal of charged moieties should be balanced in order to maintain charge neutrality.

Here, we tackle these problems by using a combination of the approach outlined above and a method for gradually introducing solute-solvent interactions by model extension to hyper-space coordinates. The latter procedure was originally invented by Roux and coworkers for performing free energy calculations *via* thermodynamic integration in 4-dimensional space [8]. Therein, the extra dimension is used to first ‘separate’ solute and solvent, and then gradually merge both systems by reducing the ‘displacement’ along the 4th dimension. Hence, there are two physically meaningful states, *i.e.* displacement along the 4th dimension  $\eta$  equal to zero (the solvated solute) and  $\eta$  being larger than the cutoff-distance  $r_{\text{cut}}$  of evaluating intermolecular forces. The latter state corresponds to two separate simulation systems (solute and solvent fully separated). Within the interval  $0 < \eta < r_{\text{cut}}$  the solute-solvent interactions are artificially flattened as the underlying atomic distances  $r_{ij}$  are calculated from:

$$r_{ij} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2 + (\eta_i - \eta_j)^2} \quad (1)$$



**Scheme 1** Illustration of biomimetic apatite-collagen composites of about 2 weight-% collagen. The collagen triplehelices are arranged parallel to the *c*-axis of the apatite crystal. To restraint system

complexity, in the present study a single-crystalline apatite model is used. Based on experimental evidence, a more sophisticated model should consider a nano-mosaic structure of apatite crystallites [3]

whilst

$$\eta_i = \begin{cases} 0 & \text{for solvent atoms} \\ \eta & \text{for solute atoms} \end{cases}$$

and

$$F_{ij} = \frac{q_i q_j}{4\pi\epsilon_0} \cdot \left(\frac{1}{r_{ij}}\right)^2 + 12A_{ij} \cdot \left(\frac{1}{r_{ij}}\right)^{13} - 6B_{ij} \cdot \left(\frac{1}{r_{ij}}\right)^7 \quad (2)$$

Here  $q_i$ ,  $q_j$ ,  $A_{ij}$  and  $B_{ij}$  denote the partial charges and the van-der-Waals parameters, respectively. The forces between atoms  $i$  and  $j$  are functions of  $1/r_{ij}$  and would become infinite for atoms which overlap in 3-dimensional space. The extension to 4-dimensional space is a mathematical trick to avoid such singularities and to flatten the interaction potentials in a controlled manner.

For solute incorporation into a liquid we only need to smoothly change  $\eta$  from  $r_{cut}$  to zero within a constant-temperature, constant-pressure molecular dynamics run. For the introduction of a molecule into a crystal this procedure only allows partial relaxation. Without the removal of ions from the crystal the atomic overlap becomes critical as soon as  $\eta$  get lower than the corresponding van-der-Waals radius. Further reduction of  $\eta$  would lead to strong repulsive forces and eventually the destruction of the crystal model. To avoid this, we suggest the following simulation procedure (Scheme 2):

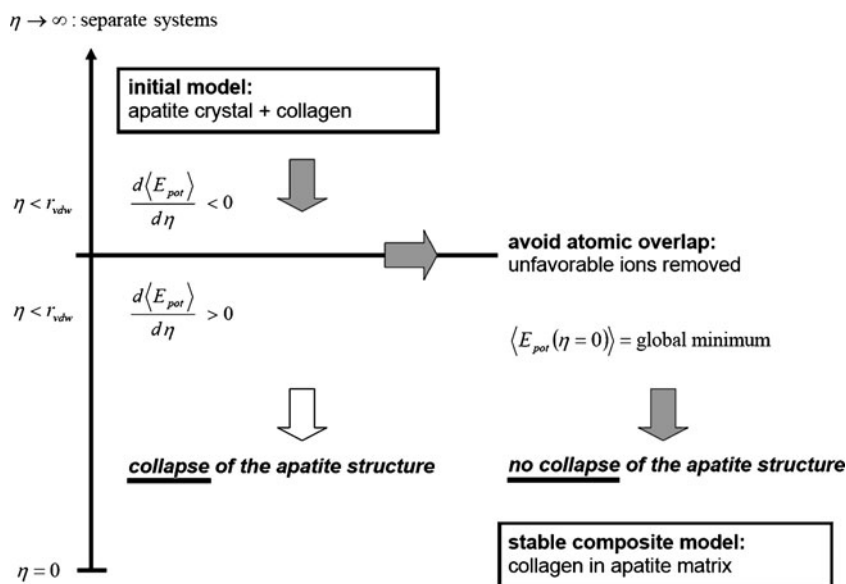
- i) Starting from  $\eta = r_{cut}$  we perform constant-temperature, constant-pressure molecular dynamics run in which  $\eta$  is gradually reduced. The average potential energy is computed as a function of  $\eta$  and the simulation protocol is switched to step ii) as soon as  $E_{pot}(\eta)$  starts to increase.

- ii) Ions overlapping within a distance delimiter  $d$  with the incorporated molecule are removed. Cations and anions are treated differently to maintain charge neutrality of the total system. We use  $d_{cation} = d_{anion} + \Delta d$  with  $|\Delta d|$  always being the smallest value that allows charge neutrality for a given  $d_{anion}$ . The latter is a free parameter which is screened for optimal choices as described in the following.
- iii) The additional coordinate  $\eta$  is then reduced to zero and the potential energy  $E_{pot}(\eta=0)$  is calculated for a given parameter  $d_{anion}$ .
- iv) The above procedure i)-iii) is performed for a series of distance delimiters  $d_{anion}$  typically within  $0 < d_{anion} < r_{vdw}$ . For the resulting series of model systems the incorporation energy  $E_{incorp.} = E_{pot}(\eta = 0) - E_{pot}(\eta = r_{cut})$  is evaluated. Here,  $E_{pot}(\eta=r_{cut})$  refers to the truncated composite from which the collagen molecules were cut (based on the optimized structure for  $\eta=0$ ). The potential energy is taken from a single-point calculation without structural relaxation. Finally, the configuration of maximum incorporation energy is suggested as the most suitable atomistic model of the composite.

### Simulation details

The atomic interactions were mimicked by empirical force-fields as described in ref. [6]. Ewald summation is used for the runs related to the investigation of the final model only. During model preparation, shifted-force potentials with a cut-off radius of 12 Å were used. The molecular dynamics simulations were performed in the constant-temperature,

**Scheme 2** Workflow description of the model preparation. Two separate systems, the apatite crystal and a collagen triplehelix, are linked via a hyperspace coordinate  $\eta$ . By gradually reducing  $\eta$  from infinity (or better the cut-off distance used for evaluating the atomic interactions) collagen-apatite interactions are introduced smoothly. While the extension to hyperspace avoids singularities in potential energy, atomic overlaps are increasingly penalized with decreasing  $\eta$ . To provide system stability and exothermic incorporation energy, unfavorable ions are removed from the apatite model (see also Fig. 1)



constant-pressure ensemble applying room temperature and ambient pressure. A time-step of 1 fs was used.

As a starting point, a  $10 \times 10 \times 12$  super cell of monoclinic hydroxyapatite was prepared using periodic boundary conditions. In analogy to our previous study of apatite aggregation on collagen, the triplehelical backbone is mimicked by a (Gly-Pro-Hyp)<sub>12</sub> model. Radmer et al. [9] and Periskov et al. [10] established this simplified approximant as the most stable sequence occurring in the collagen helical domain that captures all the significant features of its unique characteristics.

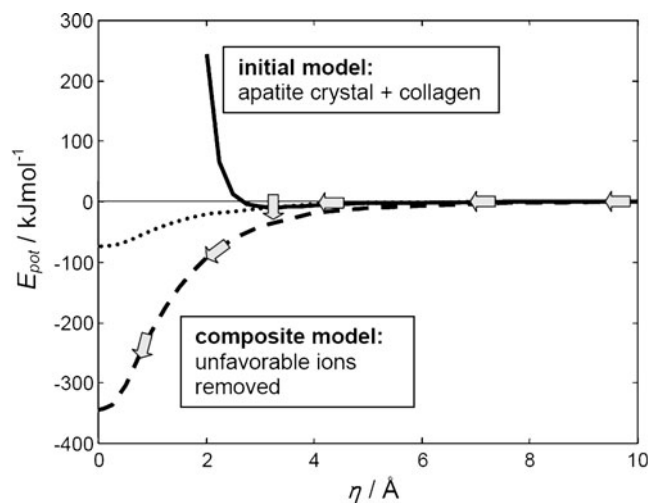
The hyperspace coordinate was initially chosen as 12 Å (the cut-off distance) and then reduced at a rate of 0.1 Å per picosecond. From comparing different rates, this value was found to be sufficiently low to allow structural relaxation of both sub-systems in case that the collagen is oriented approximately parallel to the apatite c-axis prior to starting the molecular dynamics simulations. The distance delimiter  $d_{\text{anion}}$  was scanned in steps of 0.01 Å within the interval 1.0 Å–5.0 Å.

For the evaluation of the bulk moduli of the single crystalline hydroxyapatite and the composite model, we first performed structural relaxation runs at ambient pressure and zero Kelvin. The volume was then varied from 0.99 to 1.01 times the equilibrium value and the corresponding potential energy was interpolated by a harmonic fit in order to assess the bulk modulus as the second derivative.

## Results

A series of apatite-collagen composites were prepared using different distance delimiters  $d_{\text{anion}}$  for cutting ions which overlap with the collagen triple-helix. The potential energy as a function of the hyperspace coordinate is shown for different values of  $d_{\text{anion}}$  (Fig. 1). The solid line corresponds to  $d_{\text{anion}}=0$ , *i.e.*, to merging the collagen molecule and the apatite crystal without cutting ions. The strong repulsive forces imply very unfavorable potential energy levels and eventually cause the destruction of the crystalline order. The optimal composite model is obtained for  $d_{\text{anion}} = 1.8 \text{ \AA}$  ( $\Delta d = +0.5 \text{ \AA}$ ). Much lower, yet exothermic incorporation energies are obtained for larger distance delimiters for cutting overlapping ions. As a limiting case this includes configurations in which the collagen triple-helix is laterally attached to the surface of a large cavity in the apatite crystal.

While collagen adsorption to an apatite surface implies only minor structural changes and comparably low incorporation (or better association) energy, the composite model obtained from our simulation procedure using optimal cutting delimiters ( $d_{\text{anion}} = 1.8 \text{ \AA}$  /  $d_{\text{cation}} = 2.3 \text{ \AA}$ ) exhibits



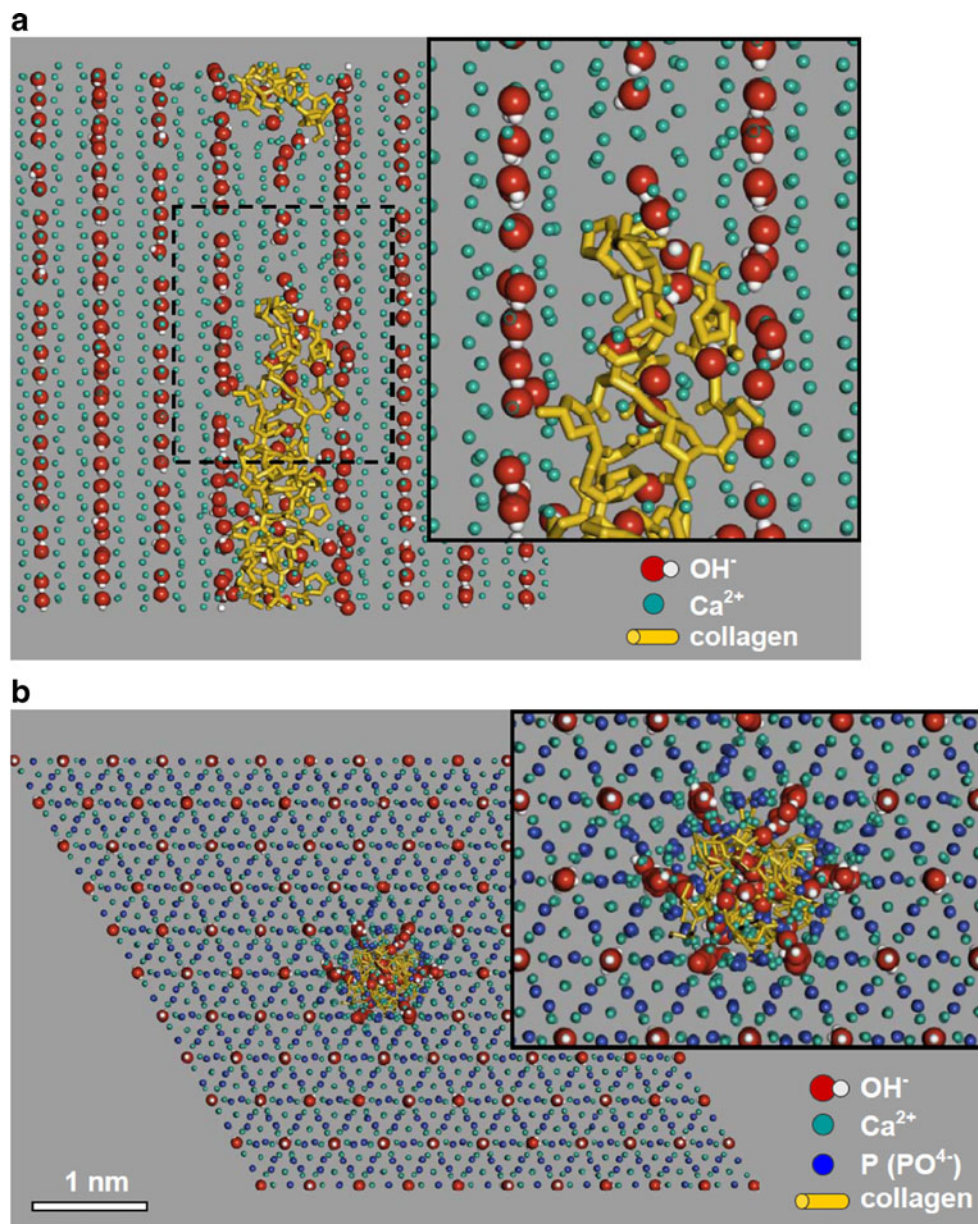
**Fig. 1** Potential energy as a function of the hyperspace coordinate. The solid line corresponds to merging the collagen molecule and a single crystalline apatite model, which implies atomic overlap and destruction of the crystal. By removing apatite ions (whilst maintaining charge neutrality) collagen incorporation becomes favorable, and induces only local structural changes of the apatite crystal. The dashed line reflects the maximum in incorporation energy which is obtained by setting the distance criterion for avoiding atomic overlap to  $d_{\text{anion}} = 1.8 \text{ \AA}$  ( $d_{\text{cation}} = 2.3 \text{ \AA}$ ). As indicated by the dotted curve, for larger cut-off distances the incorporation energy reduces considerably and eventually evolves to the energy of adsorption obtained for placing the collagen triple helix on the surface of an apatite crystal

strong structural changes which reflect an optimized interplay of both components forming the composite as described in the following.

In the optimized composite model, the collagen triple-helix exhibits a parallel orientation with respect to the c-axis of apatite (Fig. 2). Within the ab-plane, the biomolecule is located next to a row of  $\text{Ca}_3\text{OH}$  motifs which tend to be enwrapped by the triple-helix. This arrangement shows remarkably small distortion of the apatite crystal. Indeed, the latter largely maintains its crystalline order and structural changes are only observed in close proximity of the collagen molecule. Such stable (both in terms of structural coherence and incorporation energy) configurations are however only obtained for model preparation runs in which the biomolecule is directed along the c-axis prior to the incorporation process. Simulation runs for which collagen incorporation into apatite is attempted at orientation which strongly differ from this alignment, failed to provide reasonable composite models (destruction of the apatite crystal). Whilst small misfit angles were found to be corrected, excessive tilting would probably require extremely low incorporation rates  $\eta$  to allow structural relaxation.

Focusing on the optimized apatite-collagen composite model as illustrated in Fig. 2, an additional molecular dynamics run applying ambient pressure and a cycle of gradual heating/cooling was performed. Starting from room temperature, 100 K increments were implemented every 25

**Fig. 2** Relaxed model of the apatite-collagen composite. The collagen triplehelix embeds a large number of triangles of calcium ions in/near which center a hydroxyl ion is located. Hence, this motif of the apatite crystal is largely preserved. Defects and distortions of the apatite crystal are only observed in close proximity of the biomolecule. This hints at the coherent structural interplay of collagen and apatite (after relaxation of both components). In accordance to previous findings, we observed the replacement of protein-protein hydrogen bonds and the incorporation of calcium triangles which are stabilized by salt-bridges with the collagen molecule. **(a)** viewing direction along [010], **(b)** viewing direction along [001]

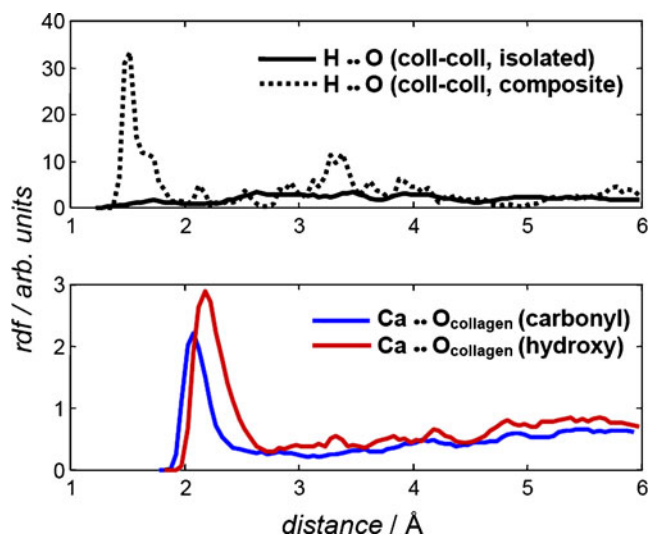


ps. Upon heating up to 1000 K and subsequent cooling to 0 K at the same rate, the composite model underwent only minor structural changes hinting at the stability of the interplay of both components.

A striking signature of this interplay is the dissociation of intramolecular hydrogen bonds of the biomolecule and their replacement by salt bridges which stabilize  $\text{Ca}_3\text{OH}$  motifs. Qualitatively, this was anticipated from our earlier investigations of  $\text{Ca}_3\text{F}$  motif incorporation into collagen based on (fluorapatite) aggregate growth simulations [6]. Here, we can provide sufficient statistics to perform a quantitative analysis. Figure 3 shows the radial distribution functions (rdf) of the  $\text{H} \cdots \text{O}$  distances within the collagen triplehelix. For the isolated triple helix, integration of the rdf of the hydrogen atoms – carbonyl oxygen atoms

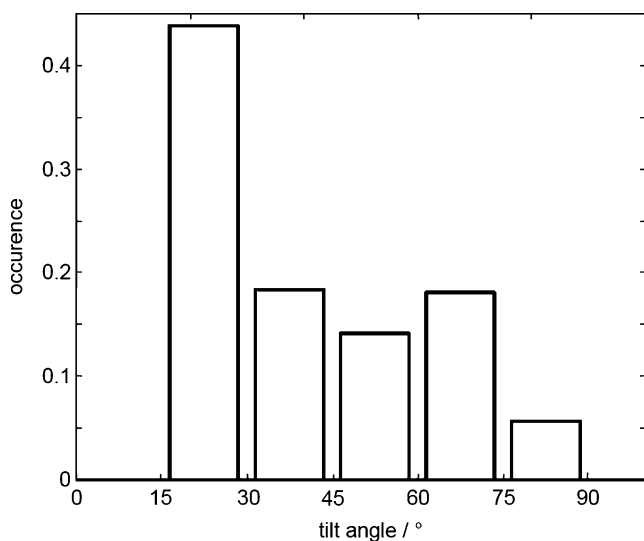
indicate a coordination number of 0.8, *i.e.*, in average about one hydrogen bond per carbonyl group. These are almost entirely depleted in the course of apatite-collagen composite formation. On the other hand,  $\text{Ca}^{2+} \cdots \text{O}$  bridges are formed leading to a coordination of the incorporated calcium ions by about 3 oxygen atoms. Integration of the  $\text{Ca}^{2+} \cdots \text{O}$  radial distribution functions yields 1.1 and 1.7 contacts to carbonyl oxygen atoms and to the hydroxyl group of the Hyp residues, respectively. The Hyp residues hence change from hydrogen bond donors to acceptors of  $\text{Ca}^{2+} \cdots \text{O}$  bridges. This is implemented by local rearrangement of the Hyp side-chain, and does not compromise the overall triplehelical structure of the collagen molecule.

For the  $\text{Ca}_3\text{OH}$  motifs embedded in the collagen triple helix an occurrence distribution of the tilt angle of the



**Fig. 3** Upper panel: radial distribution functions of the H...O distances related to hydrogen bonds within the collagen triplehelix. For the isolated triple helix, integration of the rdf of the hydrogen atoms - carbonyl oxygen atoms indicate a coordination number of 0.8. These hydrogen bonds are almost entirely depleted in the course of apatite-collagen composite formation. Lower panel: the hydrogen bonds are replaced by  $\text{Ca}^{2+}$ ...O bridges leading to a coordination of the incorporated calcium ions by about 3 oxygen atoms (1.1 contacts to carbonyl oxygen atoms and 1.7 to the hydroxyl group of the Hyp residues) of the collagen molecule

normal vectors of the calcium triangles with respect to the c-axis of the apatite crystal was performed (Fig. 4). From this a tendency in favor of parallel alignment may be deduced which again is in reasonable agreement to our



**Fig. 4** Occurrence distribution of the tilt angle (with respect to the c-axis of apatite) of the normal vectors of  $\text{Ca}_3\text{OH}$  triangles embedded in the collagen triple helix. The tilt angles in the single crystalline matrix arising from thermal fluctuations take a maximum of a few degrees, only

previous simulation study related to the growth of aggregates counting a few hundred ions [6].

Collagen incorporation into apatite is attributed to a series of unique materials properties, including hierarchical growth mechanisms and reduced brittleness [1]. The latter aspect may be illustrated from calculating the bulk moduli of pure apatite and the composite model, respectively (Table 1). Comparison with the experimental shows qualitative agreement only. Surely, our single-crystalline simulation models cannot provide quantitative agreement with the much more complex hydroxyapatite polycrystals and enamel biominerals, respectively. From a qualitative point of view the mechanism of hardness reduction of apatite by collagen incorporation is nevertheless demonstrated.

## Conclusions

While our simulation model surely cannot account for the whole complexity of enamel, we nevertheless consider the presented simulation procedure as a reasonable approach to the preparation of nanometer scale apatite-collagen composite models. Indeed, our atomistic models mimic important aspects of enamel at length scales in the range of 1–10 nanometers. On length scales of 1 nm and below, we demonstrated the modeling of the peculiar interplay of the collagen triplehelix and the apatite crystal structure. This includes orientation alignment of both components and details of the atomistic structure such as the depletion of protein-protein hydrogen bonds and the incorporation of calcium triangles which are stabilized by salt-bridges to the collagen molecule. On the other hand, the presented composite model accounts for dimensions of about  $5 \times 5 \times 8 \text{ nm}^3$  and may hence serve as a starting point to exploring materials properties of the bulk composite by means of atomistic simulations.

**Table 1** Comparison of the bulk moduli calculated from our simulation models with the available experimental data [11]. While the single-crystalline simulation models cannot provide quantitative agreement with the much more complex hydroxyapatite polycrystals and enamel samples, qualitatively the mechanism of hardness reduction of apatite by collagen incorporation is nevertheless demonstrated

	$B_0$ / GPa (simulation model)	$B_0$ / GPa (experiment, ref. [11])
HAP	101.2 (single crystal)	88.0 (synthetic polycrystal)
HAP-collagen composite	86.6	63.1 (bovine enamel)
relative change	–14%	–28%

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