

## Stereochemical recognition revisited: A step-specific model for shape control

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Living organisms produce crystal structures that exhibit morphologies differing dramatically from the simple shapes obtained during laboratory growth from pure solutions. Analyses of these biomineral structures typically reveal the presence of both inorganic and macromolecular species, with the latter containing a large fraction of acidic residues.

In the past, the paradigm of "stereochemical recognition" has been invoked to explain these observations. According to this model, stereochemical matching of macromolecules to the crystal lattice of otherwise unexpressed faces leads to their stabilization, presumably by lowering their surface energies, thereby generating a new crystal shape.

Here we review results from *in situ* AFM investigations of a number of crystal-impurity systems that argue for a change in this paradigm. The systems studied included: 1) calcite plus inorganic metal ions, simple amino acids, a series of poly-aspartates and dipeptides, and complete proteins from marine organisms, and 2) calcium oxalate plus citrate, a small organic modifier, and osteopontin, a naturally occurring protein.

For all systems, we find that the resulting growth kinetics and crystal habit are defined by modifications to atomic steps on existing crystal faces and that the degree of modification on a given face is step-specific. Moreover, changes in macroscopic crystal shape mimic the modifications seen at the atomic level. While the exact mechanism of growth modification differs in each system, the feature these systems have in common is that the important molecular-scale interaction that drives modification is between the impurity and a specific set of steps on the existing crystal faces. Molecular models that calculate the modifier-crystal interaction, energy support these conclusions in that 1) they predict that the binding energies are greatest at the step edges, and 2) they give relative values in accordance with the step-specificity observed in the experiments.

These results argue for a shift in the paradigm of stereochemical recognition away from stabilization of new facets through matching to their atomic planes, towards a model recognizing the paramount role of steps. In particular, the inherently non-planar nature of steps provides an environment in which multiple bonding accommodations provide a high degree of coordination and, consequently, a large binding energy for non-planar growth modifiers.

## Dynamic simulations of polypeptide networks to form Ca-carbonate seed crystals

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Over the years, there have been a number of attempts to form synthetic organic templates that mimic dynamic processes at the interface between organic matter and mineral surfaces. One approach has been to isolate the templating matrix from mineralized tissues and examine the growth of calcium salts in the presence of this matrix. Other experiments have focused on synthetic (bio-)organic templates, such as polymers, macromolecular complexes, phospholipid vesicles,  $\beta$ -pleated polyamino acids entrapped in gelatin self-assembled monolayers on gold substrates, and Langmuir films. In the case of Langmuir monolayers, the amphiphilic molecules can be designed in such a way that they act as artificial two-dimensional nuclei for the promotion of crystal nucleation. Such films have been used as templates to direct the crystal nucleation and growth of calcium carbonate. For example, Buijnsters et al. (2001) used Langmuir films of amide-containing phospholipids in the presence of calcium ions to form well-defined two-dimensional domains at the air-water interface.

This is the starting point of our molecular dynamics simulations. After deriving a pure-core potential set for fast molecular dynamics simulations, we have created different two-dimensional networks of amide-containing phospholipids that serve as templates for Ca carbonate seed formation. We can vary the distance and structural arrangement of the functional groups to control adsorption and seed formation. The molecular dynamics runs in these calculations contain water with different concentrations of  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  ions.

We have chosen a slightly different approach for polypeptide chains as template formers. Hybrids of two and three-dimensional networks of these chains with varying connectivities (chemically and structurally) were used to simulate interfaces for early seed formation. Charged (mostly negatively) functional groups on the networks allow polar carbonate surfaces to be exposed at the interface whereas in air or water (without a template), typically the non-polar surface such as (104) is the most stable one.

The ultimate goal of this project is to provide systematic insight into template and, thus, seed formation control from a theoretical point of view. Ultimately, we want to understand which carbonate will form with which surface at the interface depending on the template provided.

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## Brachiopod shell biomineralization: Structural and chemical characteristics

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Magnesian calcites of biogenic origin play an important role in geochemical processes such as diagenesis, calcification, cement formation/dissolution and seawater chemistry. These hard tissues are advanced materials which can be addressed as multi-scaled (from nanoscale to macroscale) biological composites (inorganic matter and biopolymers), where inorganic processes (e.g. crystallisation of calcite and thus shell formation) occur through biological mediation.

We have investigated the ultrastructure and chemical composition of the modern calcitic brachiopods *Megerlia truncata* and *Terebratalia transversa* with SEM, EBSD, microhardness indentation and LA-ICP-MS. The outer, primary shell layer can be regarded as a nanocrystalline thin film that forms a hard protective coating around the inner, much softer secondary shell layer that can be expressed as an organic/inorganic fibre composite. While the hardness of the nanocrystalline layer gives a mechanical protection against abrasives, the fibre composite material provides elasticity to the structure. The fibrous, curved growth of the secondary shell layer crystals occurs in arbitrary directions perpendicular to the <0 0 1> triad symmetry direction of calcite and is most likely obtained by simple confinement to a protein sheath. The curvature of the fibres is caused by rearrangements of the secreting cell array during growth, whereby the existing crystal lattice is not distorted. Thus a biologically mediated calcite crystallization is a purposeful process and seems to be significantly different to the inorganic crystallization of calcite. A strong decrease in microhardness is accompanied by distinct change in chemical composition from the primary or the outermost part of the secondary layer towards the innermost portion of the secondary shell layer. Thus, our measurements show that chemical and structural inhomogeneity occurs even in modern brachiopods.

## Amorphous oligomer nucleation and aggregation mechanism for biomineralization

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Among the distinguishing features of biominerals are their unique morphologies compared to their inorganically grown counterparts. The involvement of organic macromolecules is widely acknowledged, usually, in a template-directed mechanism. Epitaxial matching or stereochemical control is sought between the functional groups on the macromolecule and atom positions or crystal dimensions in the mineral, and may operate in the case of biogenic CaCO<sub>3</sub> growth [1]. Similar epitaxy may be more difficult to find in other biominerals such as hydroxyapatite in bones and teeth, and the biogenic amorphous silica of diatoms, sponges and radiolaria.

We present, here, an alternative mechanism for biomineralization. First, specific functional groups locally promote nucleation of amorphous oligomers. Multiple functional groups along the macromolecule then promote aggregation of the oligomers to form a solid phase that may, subsequently, undergo phase transformation to the final form if the ultimate biomineral is crystalline (e.g. bone-apatite).

We have examined biomimetic amorphous silica precipitation. We used <sup>29</sup>Si NMR to follow the kinetics of monoamine- and polyamine-catalyzed organosilicate hydrolysis and polymerization, and SEM to examine the amorphous silica morphologies produced. The amines represent the active portions of silica-precipitating enzymes. Results suggest that monoamines and polyamines promote organosilicate hydrolysis and polymerization via a S<sub>N</sub>2 nucleophilic mechanism, where a hypervalent silicon reactive intermediate may exist [2, 3]. Subsequent silica oligomer aggregation is promoted by cooperative, steric effects of neighboring amine functionalities on the polyamines [2]. Aggregation is promoted by macromolecular conformation, without a strict requirement for epitaxial matching.

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## Dinoflagellate toxins stimulate coral calcification and cause bleaching

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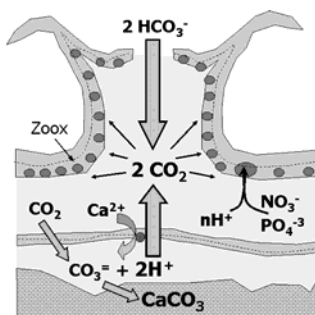
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*Symbiodinium*, the dinoflagellate symbiont of reef corals, makes potent activators of cellular calcium influx called zooxanthellatoxins. Scenarios describe how algal toxins may stimulate coral calcification and promote algal invasion and exit from the host.

The coral's calcifying ectoderm appears particularly susceptible to zooxanthellatoxins because of its abundant calcium transporters. Ectodermal cells compensate for the enhanced calcium influx by pumping calcium toward the skeleton, in exchange for protons. That causes calcification. The coral secretes the protons from calcification into its internal coelenteron cavity. That promotes photosynthesis and nutrient acquisition, by converting bicarbonate to carbon dioxide, and by stimulating mechanisms such as proton-nutrient co-transport. Toxin production schedules likely contribute to faster coral calcification during the daytime and during nutrient shortages.

Zooxanthellae may also use toxins to modulate host cell phagocytosis and/or exocytosis, assisting their entry into or exit from host corals. Dinoflagellates produce more of some toxins when nutrient shortages retard their progression through the G1 phase of the cell cycle, which can last for months in nutrient deficient corals. High toxin levels may then induce the coral to evict the algae, or cause infected endodermal cells to lose adhesion and slough away. Either way, algal toxins help the algae to disperse to new hosts with better access to nutrients. Nutrient shortages correlate with high sea surface temperatures, contributing to the correlations between temperature and coral bleaching.

Such putative inter-species uses for dinoflagellate toxins may have evolved from internal uses involving calcium modulation of chromatin conformation and activity.



## Interaction of mineral surfaces with oligopeptides as organic templates: An insight into biomineralization

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Calcite is a rhombohedral polymorph of calcium carbonate ( $\text{CaCO}_3$ ). The lowest energy faces of pure calcite are the {104} family of faces. The mineral(calcite) deposition on the exoskeleton of the organism can be related to its DNA pattern, and hence, to the protein sequence that forms the hard body exoskeleton. As a precursor of biomineralization, we are studying the interaction of oligomers/polypeptide chains on (104) calcite surfaces. Our main objective is to find suitable orientations of amino-acid residues in these peptide chains, where these peptide chains align themselves parallel to the calcite surface or parallel to a step on these surfaces. Various sequences of small-chain (3-aminoacid) peptide residues on both polar and non-polar surface steps on calcite (104) faces have been studied. The residue with the highest adsorption energy of the ones that we studied is Phe-leu-lys<sup>+</sup> with total adsorption energy of -1.071 eV (non-polar calcite surface step) or -0.3571 eV/residue. The pH during the calculations is assumed to be high such that the carboxylic groups are completely deprotonated. For the interaction of a 12-amino acid long peptide chain in alkaline conditions with polar steps, we calculate an adsorption energy of -0.09824 eV/amino acid residue and -0.1978 eV/amino acid residue when the peptide residue is neutral (acidic condition and non-polar step). Peptide residues that contain negatively charged amino acids (high pH, alkaline condition) are more stable along the  $\text{Ca}^{2+}$  polar step edge. The 12-amino acid long peptide chain with alternating glycine and alanine shows better parallel alignment.

Studies of mineral surface interactions with one-dimensional and two-dimensional peptides indicate the dependence of structural matching and parallel alignment as principle criterion for inorganic nucleation on organic template. In order to understand the nucleating mechanism of inorganic mineral surfaces on organic templates during biomineralization, we are studying the growth and nucleation of calcium carbonates on three-dimensional peptide-chain networks.

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## Polyaspartate as a stereochemical switch for controlling the growth and morphology of calcite

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The controlled synthesis of biominerals involves active constraints on growth processes that result in functional structures beyond what is possible by passive growth in inorganic systems. Using proteins as nucleation templates or growth modifiers, studies are largely aimed at understanding the crystal phase that forms, along with the resulting local and macroscopic structures. As a result, numerous studies show that proteins regulate the type of CaCO<sub>3</sub> polymorphs generated, as well as their morphologies and orientation. Effectiveness of these proteins can be linked to their peptide sequences and structures. However, the mechanisms by which primary and secondary protein structure characteristics affect crystal growth are only beginning to be understood.

Polyaspartate domains are a dominant feature of proteins associated with biogenic carbonates. These biomolecules are implicated in modifying crystal morphology through specific interactions with step edges. In this study we find that polyaspartates, Asp<sub>n</sub>, introduced to solutions during calcite growth exhibit binding selection between obtuse and acute steps that is chain-length dependent. Shorter Asp<sub>1</sub> and Asp<sub>2</sub> favour interactions with acute steps while Asp<sub>4,5,6</sub> favour the obtuse. This crossover between Asp<sub>2</sub> and Asp<sub>4</sub> is expressed in differential roughening and rounding of the acute and obtuse steps as evidenced by *in situ* force microscopy images.

Using semi-empirical quantum mechanical modeling of polyaspartate-calcite binding energies, we independently determine that the crossover occurs at n=2 and is caused by a switch in the dehydration energy penalty of binding a given polyaspartate to acute versus to obtuse steps depending on its length. Step velocity measurements demonstrate that the linear increase in binding energy with aspartate chain length produces an exponential decrease in the aspartate concentration that gives complete growth inhibition. Using a step adsorption model, from fitted data, we find that complete inhibition of step growth is achieved for a fixed fractional coverage of step edges by all Asp<sub>n</sub> studied here. Step rounding due to favourable changes in step interaction energies with Asp<sub>n</sub> generates growth in new directions, enabling changes in hillock morphology. These findings suggest a mechanism by which primary structure in peptides may directly control crystal shape and point to role of solvation in controlling interaction energies.

## Mechanisms of formation of plants bioliths

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Complex research of plants bioliths by physico-chemical, chemical and mineralogical methods let speak about some mechanisms of their formation. The main ones are accumulation processes of chemical elements on biological membranes, which are physiological barriers on migration ways of intercellular plants juice. In addition physico-chemical mechanisms of solution evaporation from surfaces have been established. The most intensive mineralization of plant tissue is connected with many-year evaporation concentration from porous medium to atmosphere through a surface of tree trunk barks and stumps. The objects of our plants biomineralization research are different organs and tissues of pine (*Pinus silvestris*), common birch (*Betula platyphyla*; *B. verrucosa*), Dahurica and Siberian larch (*Larix dahurica*, *L. sibirica*), Siberian fir (*Picea obovata Ledeb*), Siberian fir (*Abies sibirica*), tremble aspen (*Populus tremulus*), goat willow (*Salix carpea*). The main are external loping layers of their trunk bark. These bioobjects are characterized by non-barrier accumulation, linear-proportionally to concentration in rootinhabited zone of some ore chemical elements forming "large" bioliths 50-500 μm suitable for research by general mineralogical methods. Au bioliths concentrations on the main biological membranes, which are separation borders between bark, bast and wood, have been established for all 7 investigated tree families wood plants in Siberia. The most important border is the xylem (sapwood) separation surface, on which there is a rising flow from roots to leaves, and phloems (bast), on which there is a descending flow of solutions. Received data prove that enlargement of Au bioliths in wood and bark consisting of bast and bark is of different mechanisms. In bast and especially in bark Au particles fall out from chelated solutions and grow under conditions of evaporation concentration of microboliths number on the borders: "alburnum – bast" and to a less extent "bast–trunk bark". Adsorption mechanisms of less intensive radial flow, mainly mineral solutions from alburnum to trees ore are apparently to predominante in wood.

## **Biomineralization mechanisms: A novice biomineral enthusiast's perspective**

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### **Biomineralization: A General Model**

Organic mediated growth of mineralized skeletal components by single-celled eukaryotes is a fundamental process that occurs through several steps. Extraction of the chemical constituents for biomineral growth from aqueous solution occurs as ions are pumped through specialized cation pumps to allow specific concentration gradients to develop within the cell (Bhattacharyya and Volcani, 1999). Initial growth is fostered within membrane-bound vesicles that are shaped by cytoskeletal elements into a template for mold for test construction (Leadbeater, 1987). Within the vesicle, pH and ionic composition are controlled and allowed to develop supersaturated conditions for the nucleation and aggregation of mineral material (Vrieling et al, 1999). The walls of the vesicle are composed of polysaccharides and proteins that act as nucleation points for crystal growth. The placement of these compounds along the vesicle walls and the overall form of the vesicle are coded for within the genome of biomineral secreting protists and chromists (Hildebrand et al, 1997). After precipitation of the test, the test is either exocytosed to the exterior of the cell as in coccolithophores and diatoms or remains with the cell as an internal test as exemplified by radiolarians and silicoflagellates.

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## **Effects of temperature and transport conditions on Magnesium contents in calcite**

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The potential for extracting environmental conditions from chemical impurity signatures recorded in fossil calcite samples motivates much current effort in paleoclimatology. However, correlations between trace element contents and physical or chemical conditions are not simple. Often, multiple controls appear to be at work in governing uptake of a given impurity. Reliable interpretations of element-based paleoproxies will require understanding, with some certainty, the growth behaviour of calcite for the relevant conditions. Notably absent from the literature are definite conclusions regarding relationships between temperature, growth rate, and impurity partitioning for Mg in calcite. Studies disagree on the importance of growth rate and only a few bulk precipitation studies have systematically varied temperature.

Another factor not often considered is the rate-controlling mechanism, *i.e.*, whether growth rate is limited by diffusive transport of chemical components to the mineral surface or by surface reactions such as dehydration, adsorption, and formation of lattice bonds. Variations in the dominant mechanism obviously cause variations in growth rate and boundary layer thickness and may thus affect the extent and nature of impurity incorporation during growth. The distinction potentially has important ramifications for the Mg contents of natural calcite samples.

This study links direct measurement of nanoscale effects of temperature, fluid Mg concentration, and near-surface transport conditions on calcite growth rates with high-spatial-resolution analysis of Mg contents in resulting crystals. In contrast to previous experiments on Mg partitioning into calcite, here the layer-growth mechanism was observed *in situ* and step speeds precisely measured with fluid cell atomic force microscopy over a range of temperatures, degrees of supersaturation, and solution Mg concentrations. Data collected from 15° to 30°C yield an activation energy for calcite precipitation of 33 kJ/mol for solutions with [Mg]=5x10<sup>-5</sup> M. Electron microprobe analyses of large hillocks grown at corresponding conditions demonstrate that Mg has a strong preference for incorporation at negative (acute) step edges, rather than at positive (obtuse) edges when growth rate is limited by surface reactions. This preference is reversed when growth is instead limited by diffusion through a boundary layer at the mineral-solution interface. This result shows that transport conditions during mineral growth may be a first-order control on impurity contents and distribution.