

Biom mineralization & Biominerals

“It’s a hard life”

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Fierro

Literature Review

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Outline

- Introduction & History
- Basic Biomineralization Principles
 - Saturation, Nucleation, Growth, & Organic Matrix
- Biominerals & Biomineralization Models
 - Calcium Carbonate
 - Shells: Nacre
 - Silica

Sponge Spicule



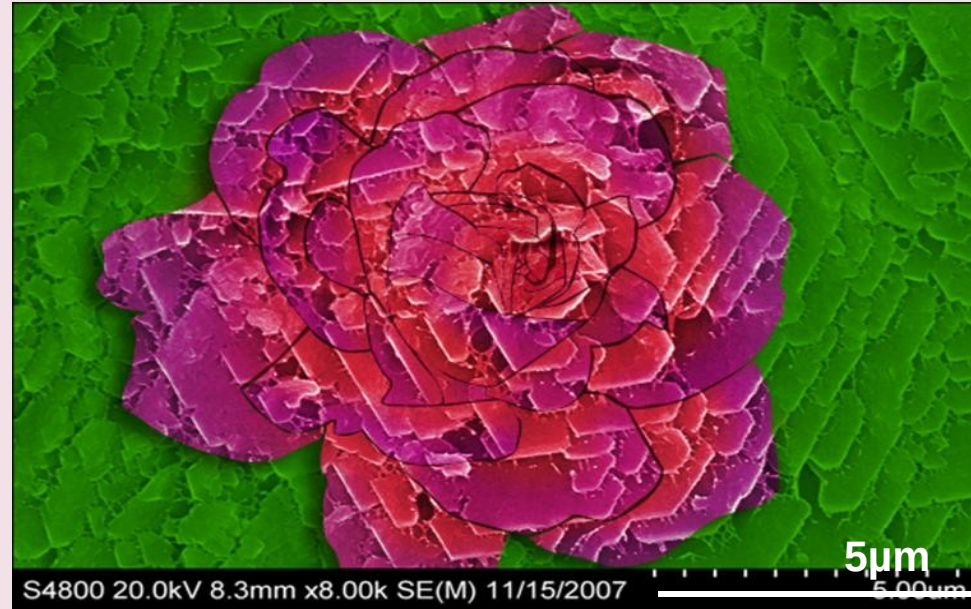
Bleached Coral



Introduction & History of Biomineralization

Introduction: The Process

- Biomineralization is the process by which living form and influence the precipitation of minerals.
- No single or ‘grand’ mechanism.
- Combination of efforts from cells, producing organic and inorganic



- Crystals from the inner shell layer of the Eastern oyster onto a metal implant.
- sheets converge forming a “rosette” structure.
- Organic matrix appears like “glue.”

Image credit: <http://www.scienceasart.org>

Introduction: Why mineralize?

Evolution of Biomineralization has provided organisms with a strong building material.

- Minerals are stiff and brittle (& cheap energy wise)
- Organic materials are soft and pliable

Functions include:

- ❖ Strength & Integrity
- ❖ Protection.
- ❖ Mobility
- ❖ Storage - Biominerals are ion reservoir for cellular functions.
- ❖ Cutting and grinding
- ❖ Buoyancy
- ❖ Optical, magnetic and gravity sensing



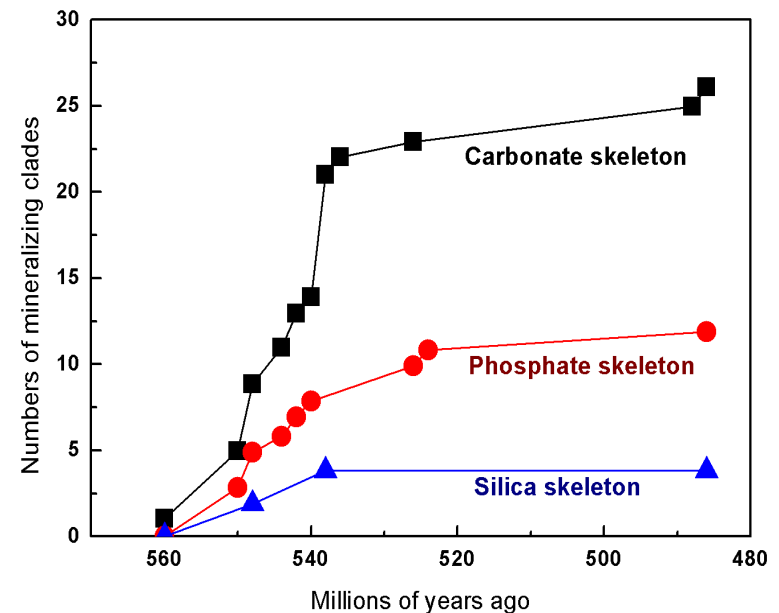
History of biominerals

First biomineralization evidence from microbial stromatolites - 3500M years ago

Not controlled deposition of inorganic solids

560M years ago - organisms from different phyla evolved the ability to form different minerals.

To date there are 64+

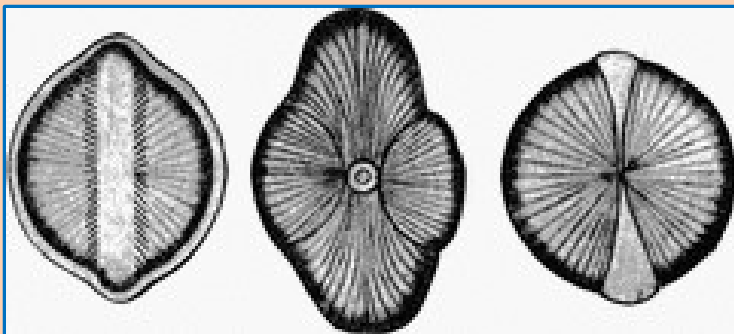


Runnegar and Bengtson (1992)

History of the field

First books on biomineralization:

- **D'Arcy Thompson (1917) on Growth and Form**
- **W. J. Schmidt (1924) Die Bausteine Des Tierkörpers Im Polarisierten Lichte**
- **A. P. Vinogradov (1953) the Elementary Chemical Composition of Marine Organisms**



CaCO₃ structures

changed from “calcification”
The picture is taken from the book of D'Arcy Thompson who discusses mineral growth in the presence of proteins.

- Saturation, Nucleation, Growth, & Organic Matrix

Basic Biomineralization Principles

Basic biomineralization principles

“coccosphere”



- Composites composed of organic and inorganic compounds
- The products are:
 - Created and maintained during life.
 - Upon death they may retain some of the original characteristics.

Crystallization of Calcium Carbonate Beneath Insoluble Monolayers

3

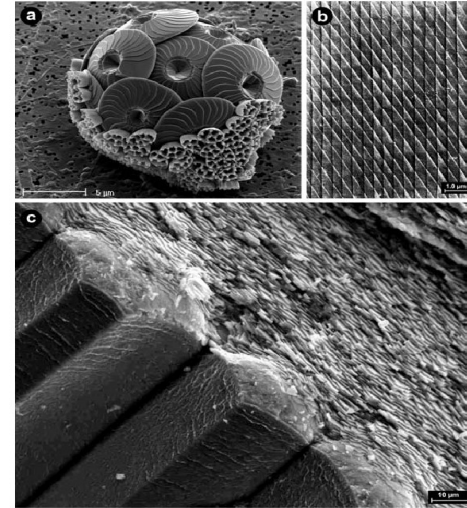


Fig. 1 Examples of biologically produced highly oriented calcareous structures exemplifying the astonishing degree of sophistication with which certain organisms can control the growth of inorganic crystals. **a** SEM of the calcareous test (“coccosphere”) produced by a coccolithophorid, a unicellular marine photosynthetic alga. Note the interlocked arrangement of minute calcite plates that cover the cell. Shown here is a combination coccosphere with both hetero- and holococcoliths (*Calcidiscus leptoporus* & *Syracolithus quadriperforatus*). Combination coccospheres are thought to represent a transitional state between heterococcolithophorid and holococcolithophorid phases of the life cycle [2]. (Micrograph courtesy of J. Young, Natural History Museum, London and M. Geisen, AWI Bremerhaven). **b** Freshly fractured shell of a Devonian brachiopod (*Pholidostrophia nacreata*) showing a crisscrossed pattern of calcite crystals that acts as a natural optical diffraction grating. This pattern gives rise to a nacreous luster and pearly iridescence that is unusual for calcite [3]. (Single-stage platinum-carbon replica. Approximately 20000 \times . TEM photo courtesy of K.M. Towe, Smithsonian Institution). **c** SEM of a fractured shell of *Anodonta cygnea* showing the transition from the prismatic to the nacreous layer (prisms bottom, nacre top). Note that in this species both morphologically distinct layers consist of aragonite, whereas in most mollusc shells the prisms are normally made of calcite. (Micrograph courtesy of F. Marin, Université de Bourgogne)

shell of a Devonian brachiopod



shell of *Anodonta cygnea*; transition from prismatic to nacreous layer

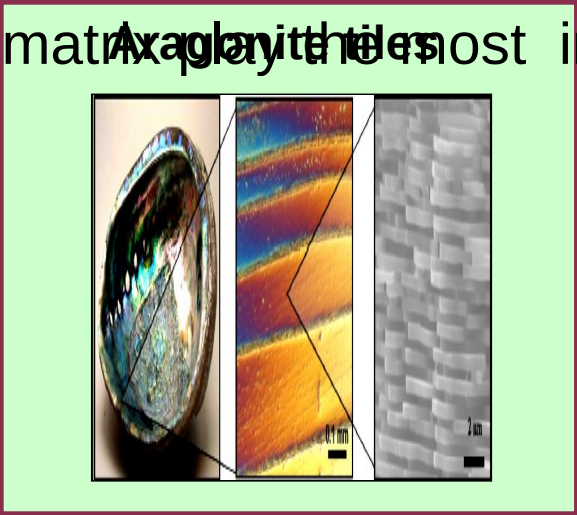


Calcareous structures showing the degree of control on the growth of inorganic crystals

Basic biomineralization principles

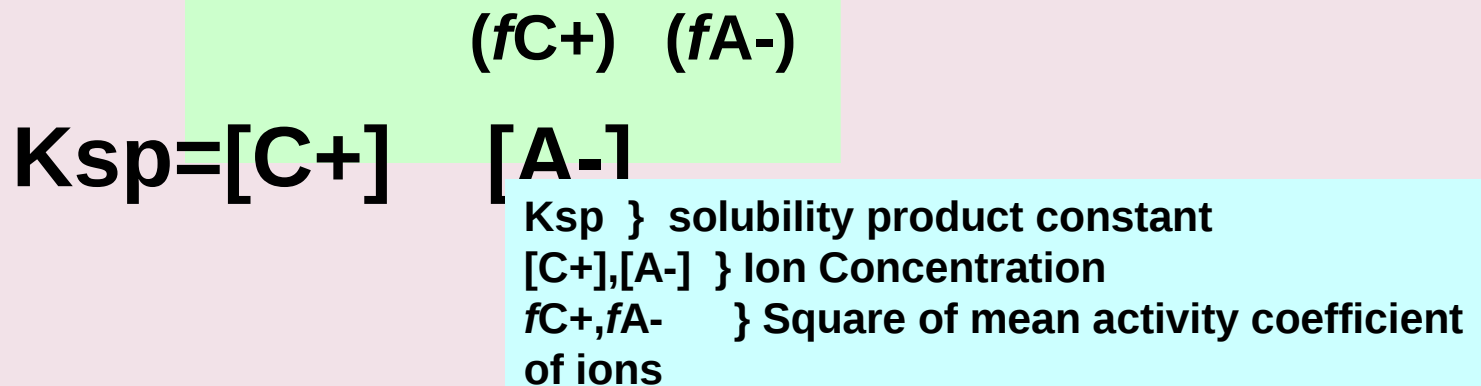
- Starts with an amorphous mineral phase
- The main processes are nucleation and crystal growth; they depend on:
 - Level of supersaturation of the medium
 - Molecular interactions between biomineral and organic macromolecules

Organic matrix plays the most important role in biomineralization



Fluid Saturation

- Crystals only form from solutions with relevant ions if concentrations exceeds solubility product constant.
- Thermodynamic product of all the activities of the ions in a solution in equilibrium with a pure solid.

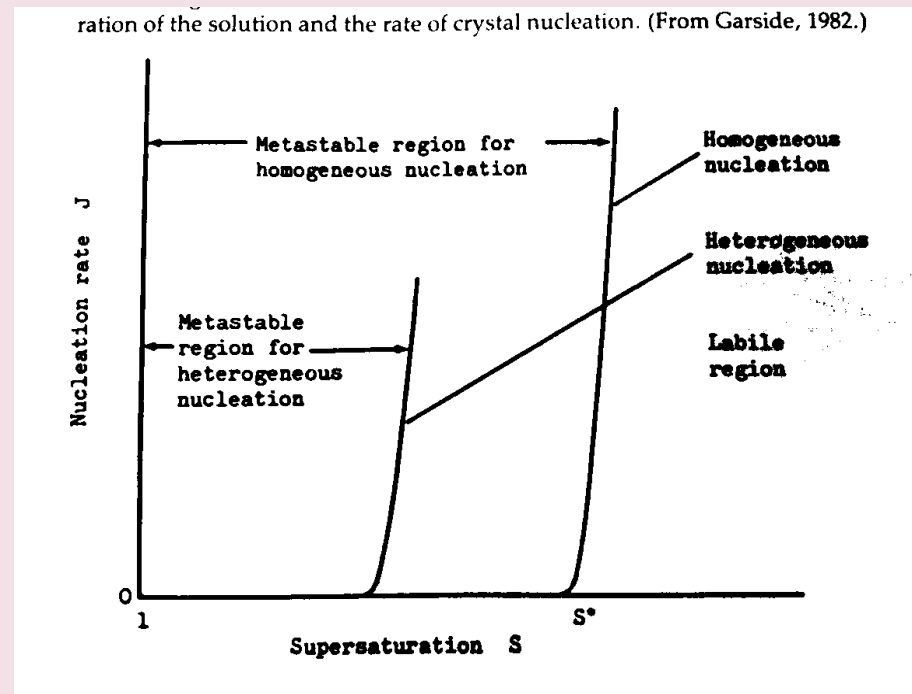


- Product constant is specific for particular arrangement of ions.
 - Calcite $4.7 \times 10^{-9} \text{ Kmol}^2 \text{m}^{-3}$

Nucleation: Level Of Saturation

The number of nuclei formed within a volume is a function of level of saturation.

- Below S^* - Nucleation rate is low
- At a range where nucleation is possible the solution is metastable
- Above S^* (or by seeding) - rate is increased



Crystal Nucleation

When the expenditure of the interfacial energy (ΔG_i) is balanced by the energy released in the formation of bonds in the aggregate (ΔG_{vol}) a stable nucleus is attained.

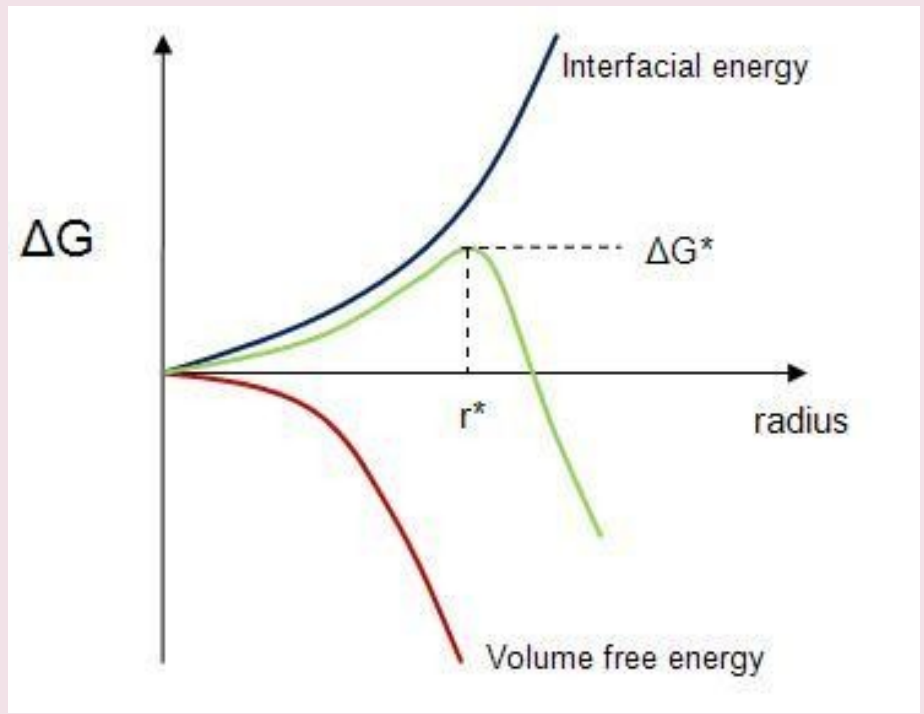
$$\Delta G = \Delta G_i + \Delta G_{vol}$$

Free Energy Change

Energy loss due to surface tension

Negative free energy released by bond formation

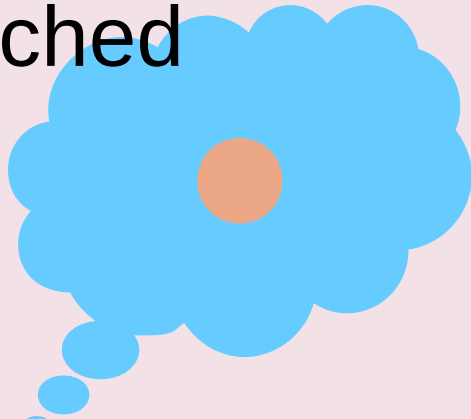
ΔG^* free energy of nucleus at maximum



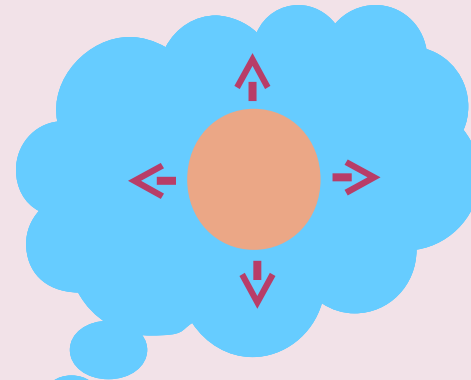
Free energy of nucleation as a function of cluster size (Image credit: wikipedia –JuliyaK)

Nucleation: Homogeneous

- As concentration increases ions associate into small unstable clusters that dissociate if they don't reach a critical size, $r=r^*$
- It costs energy to add molecules until r^* is reached



Energy released by formation is too small so cluster dissociates



Cluster growth is no longer limited by nucleation but by reaction kinetics and diffusion.

Critical Size ~ 10-1000 ions

Nucleation: Biological Systems

- In biological systems, the site of mineral deposition is isolated from the environment.
- Size of that site must limit diffusion into/out of the system.
- Ion supply (or removal) occurs by two

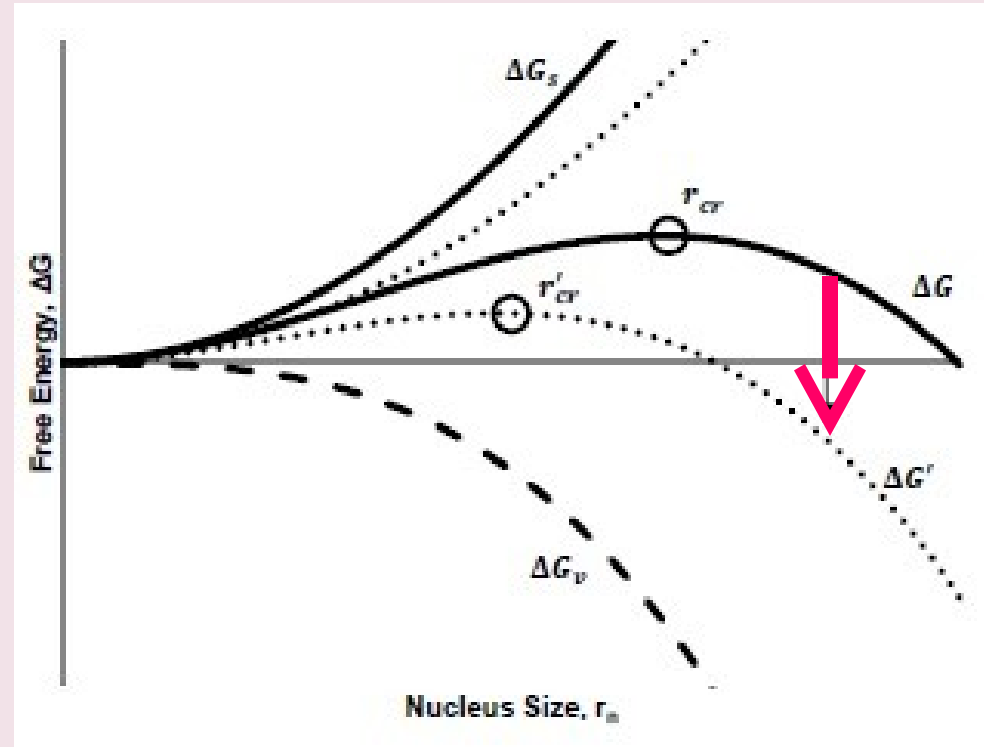
means:
The effect of the organic substrate is to lower the activation energy of nucleation by lowering the interfacial energy.

- Active pumping associated with organelles near the sites of mineralization.



Heterogeneous Nucleation

- In biominerals other molecules, ions, external bodies and surfaces are always present.
- Mineral is deposited on existing surfaces.
- Process can continue at lower saturation levels.



Primed values indicate parameters associated with a lowered free energy barrier due to heterogeneous nucleation

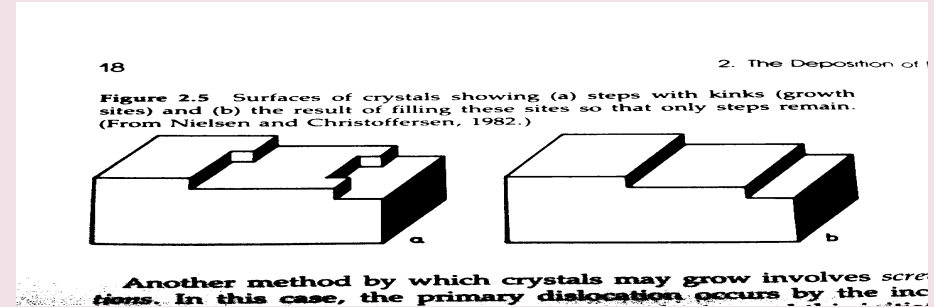
(image credit: Porter (2010))

Growth: [1] 'Kinks'

□ Growth occurs by addition of ions to the crystals.

□ Addition of ions is not consistent - dislocations & steps form.

□ Minute crystals form on a smooth face, edge, step— or—



Surfaces of crystals showing (a) steps with kinks (growth sites) and (b) the result of filling these sites so that only steps remain.

(image credit: Nielsen and Christoffersen, 1982)

Growth: [2] 'Screw Dislocation'

□ Primary dislocation occurs by inclusion of foreign ions or mismatches in surface lattice which initiates a step.

□ Growth takes a spiral course – leads to a growth

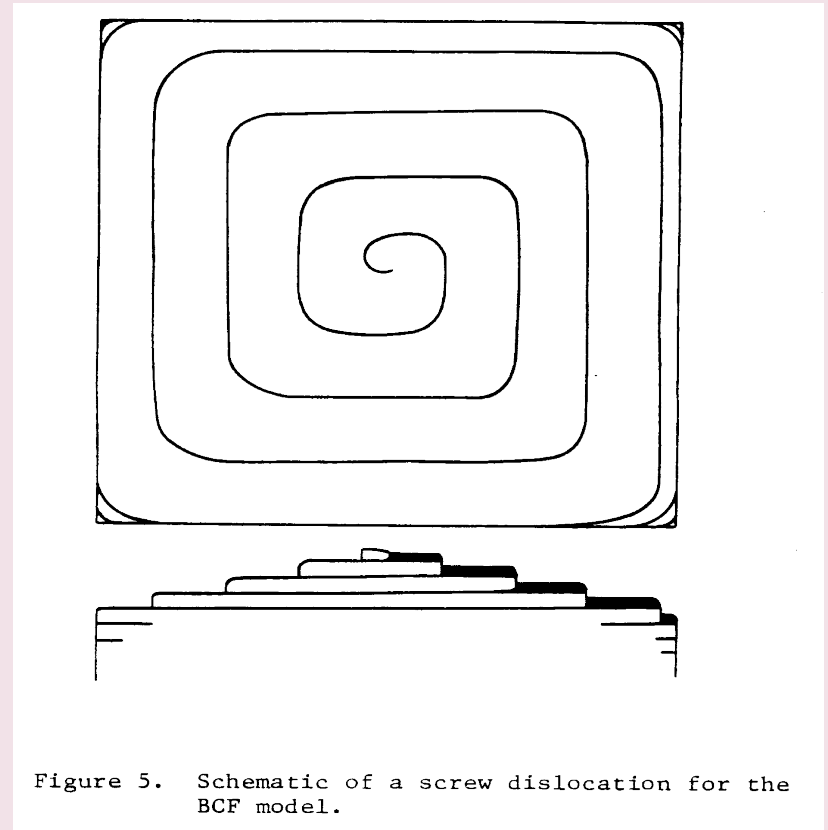


Figure 5. Schematic of a screw dislocation for the BCF model.

Growth pyramid due to a single screw dislocation

Crystal Growth Modifiers

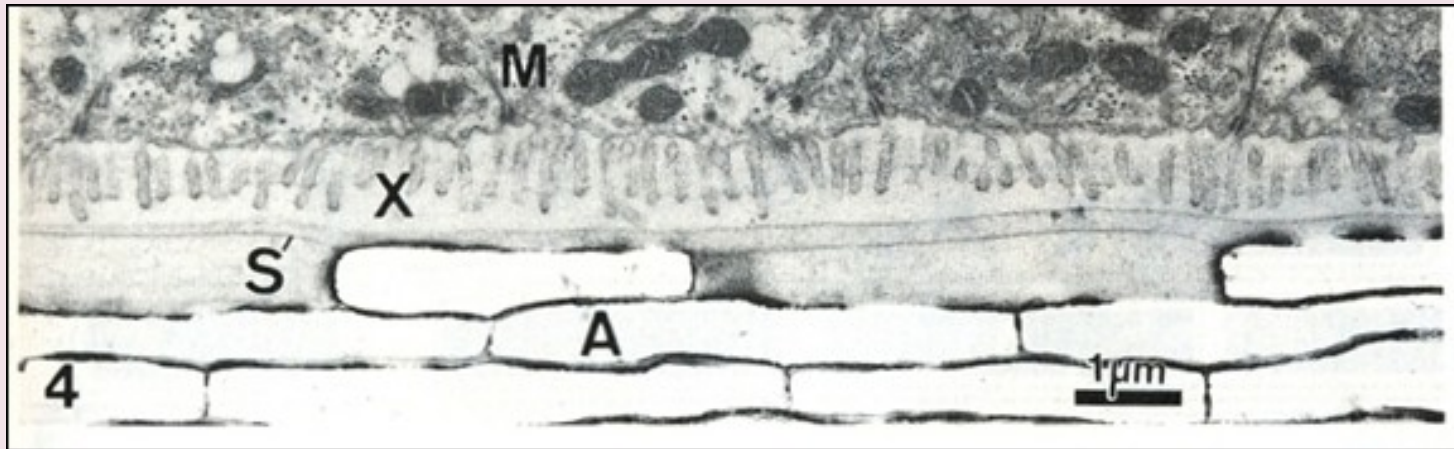
- ▢ Rate Supply of Ions
- ▢ Rate of Diffusion
 - ▢ Adsorption
 - ▢ Integration
 - ▢ Inhibitors

Control of Mineralization: Organic Matrix

Biomaterials have functional structures and shapes.

e.g. curved teeth and light baskets.

- Organic Matrix acts as a mediator of mineralization and as a crystal modifier.



In many invertebrates this organic matrix is secreted by epithelial cells contiguous to the site of mineral deposition

Image credit: Nakahara (1991)

Simkiss and Wilbur (1989)

Control of Mineralization: Organic Matrix

□ Different organic constituents have different functions:

1. Anionic groups: concentrate Ca^{++} on specific sites & induce supersaturation for nucleation.
2. Soluble matrix proteins: inhibit mineral deposition and control mineralization.
3. Matrix proteins: favor growth of particular isomorphs.
4. Soluble matrix proteins that are overgrown by mineral: influence the strength of the crystal.

- Calcium Carbonate, Silica, Hydroxyapatite

Biominerals

Biominerals

Table modified from: *Lowenstam and Weiner (1989)*

Carbonates

Calcite CaCO_3

Mg-Calcite $(\text{Mg}_x\text{Ca}_{1-x})\text{CO}_3$

Aragonite CaCO_3

Vaterite CaCO_3

Monohydrocalcite $\text{CaCO}_3 \cdot \text{H}_2\text{O}$

Protodolomite $\text{CaMg}(\text{CO}_3)_2$

Hydrocerussite $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$

Amorphous Calcium Carbonate $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ or CaCO_3

Phosphates

Octacalcium phosphate $\text{Ca}_8\text{H}_2(\text{PO}_4)_6$

Brushite $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$

Francolite $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$

Carbonated-hydroxyapatite $\text{Ca}_5(\text{PO}_4, \text{CO}_3)_3(\text{OH})$

Whitlockite $\text{Ca}_{18}\text{H}_2(\text{Mg, Fe})_2 + 2(\text{PO}_4)_{14}$

Struvite $\text{Mg}(\text{NH}_4)(\text{PO}_4) \cdot 6\text{H}_2\text{O}$

Vivianite $\text{Fe}^{3+}_2(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$

Amorphous Calcium Phosphate Variable

Amorphous Calcium Pyrophosphate $\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$

Oxides

Magnetite Fe_3O_4

Amorphous limenite $\text{Fe} + 2\text{TiO}_3$

Amorphous Iron Oxide Fe_2O_3

Amorphous Manganese Oxide Mn_3O_4

Sulfates

Gypsum $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$

Barite BaSO_4

Celestite SrSO_4

Jarosite $\text{KFe}_3 + 3(\text{SO}_4)_2(\text{OH})_6$

Hydrated Silica

Amorphous Silica $\text{SiO}_2 \cdot n\text{H}_2\text{O}$

Fluorides

Fluorite CaF_2

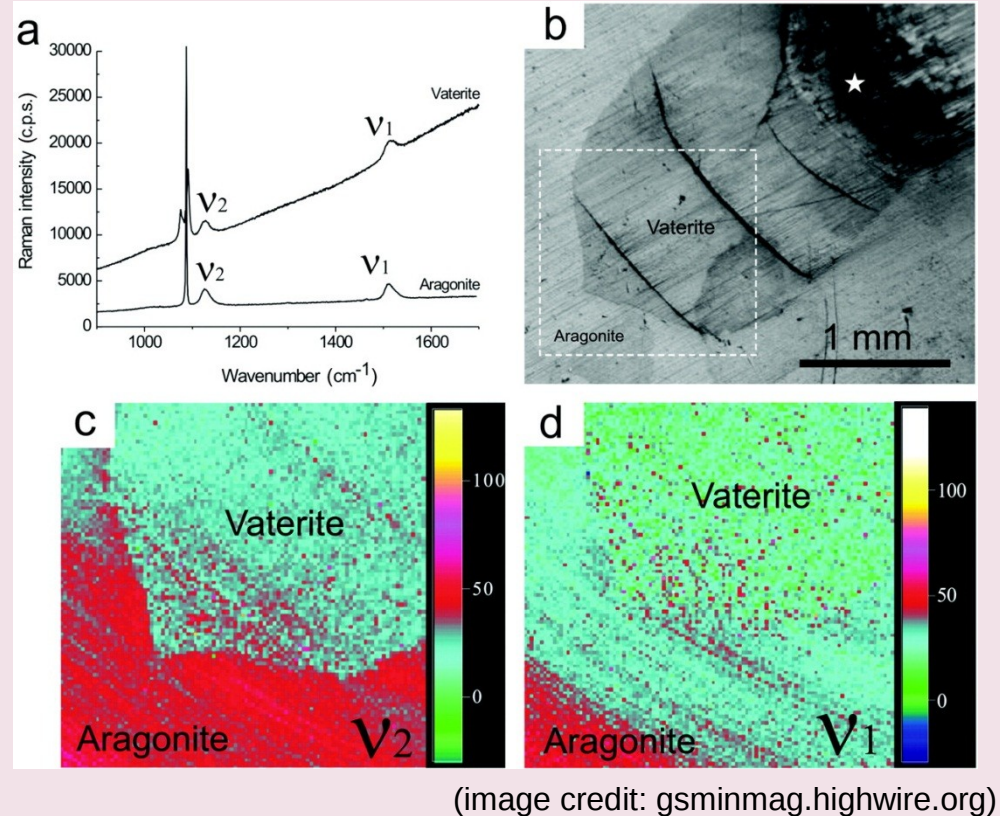
Hieratite K_2SiF_6

- Calcium is exceedingly widespread & the most common constituent in skeletal system
- (e.g. bones and shells)

Calcium Carbonate

Calcium Carbonate: Abundant!

- Calcite – Most stable CaCO_3 at ambient conditions.
- Aragonite – At supersaturated aqueous solutions containing Mg^{2+} at a molar ratio $\text{Mg}/\text{Ca} > 4$ (seawater).
- Vaterite – metastable

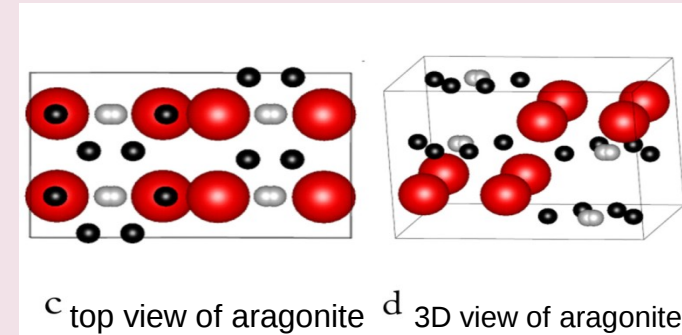
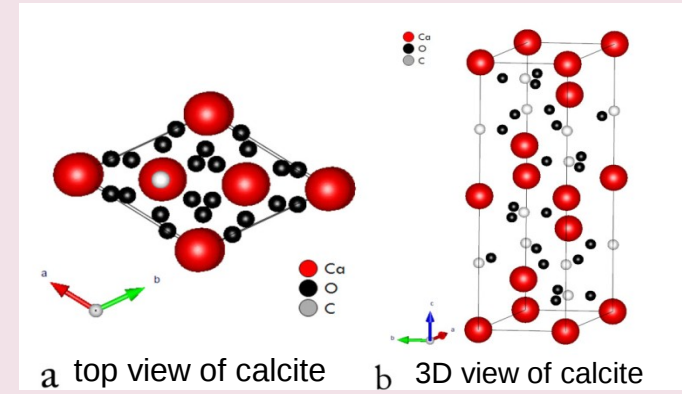


Lowenstam and Weiner (1989)

Fricke and Volkmer (2007)

Calcium Carbonate

- Calcite has a trigonal structure
- Aragonite has the orthorhombic structure.
- Minerals are not isolated in living organisms. - They are connected with organic materials, forming complex hierarchically structured composites.



Comparison of the calcite and aragonite unit cells.

Large spheres depict the calcium ions
 Small darker spheres depict the oxygen ions
 Small lighter spheres show the carbon ions
 (Image: Lopez (2011))

Crystallization of Calcium Carbonate Beneath Insoluble Monolayers: Suitable Models of Mineral-M...

Table 1 Characteristics of the most important CaCO₃ mineral phases

Mineral (formula)	Crystal system (space group)	Spec. density [g/cm ³]	Solubility [-log K _{sp}]	Biologic occurrence	Crystal data ref.
Calcite (CaCO ₃)	Trigonal (R3c)	2.71	8.48	Very common	[6]
Aragonite (CaCO ₃)	Orthorhombic (Pnca)	2.93	8.34	Very common	[7]
Vaterite (CaCO ₃)	Hexagonal (P6 ₃ /mmc)	2.54	7.91	Rare	[8]
Monohydrocalcite (CaCO ₃ · H ₂ O)	Trigonal (P3 ₁ 21)	2.43	7.60	Very rare	[9]
Ikaite (CaCO ₃ · 6H ₂ O)	Monoclinic (C2/c)	1.77	7.12	Unknown	[10]

Data adapted in part from [11]

persaturation the metastable polymorph vaterite precipitates from solution.
 The arrangement of the ions in crystalline CaCO₃ can be described in terms

Calcium Carbonate: Organism Examples

- Mollusc shells
- Foraminifera
- Coccolithophores
- Calcareous Sponge spicules
- Corals
- Echinoderms



Calcium Carbonate: Shells

Shells vary in size and morphology

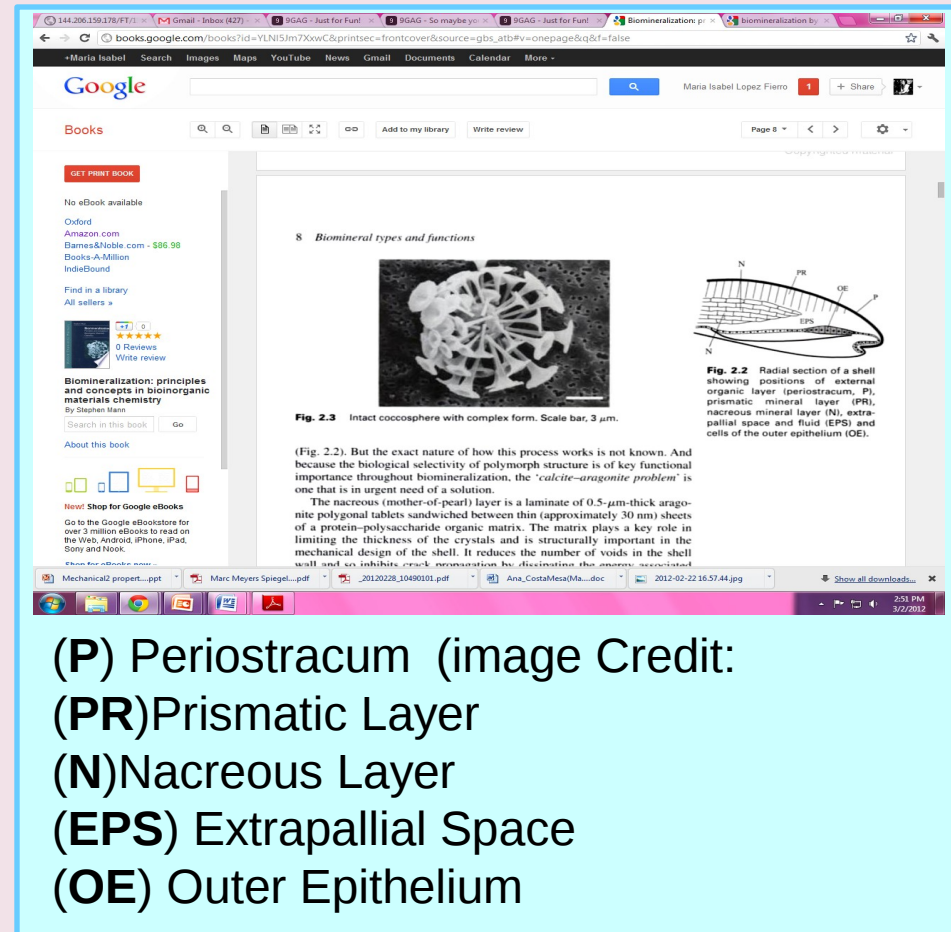
The structure is separated in each part of the shell.

The prismatic layer consists of large calcite crystals

The nacre region is a plate like aragonite crystals

Switching of polymorphs is achieved by the outer epithelium (OE)

OE is separated from the inner shell surface by a space filled with aqueous solution (extrapallial space)



8 Biomineral types and functions

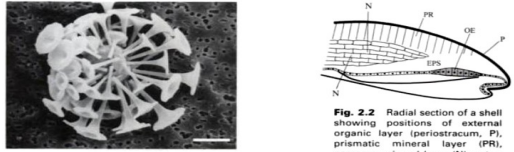


Fig. 2.2 Intact coccosphere with complex form. Scale bar, 3 μ m.

(Fig. 2.2). But the exact nature of how this process works is not known. And because the biological selectivity of polymorph structure is of key functional importance throughout biomineralization, the 'calcite-aragonite problem' is one that is in urgent need of a solution.

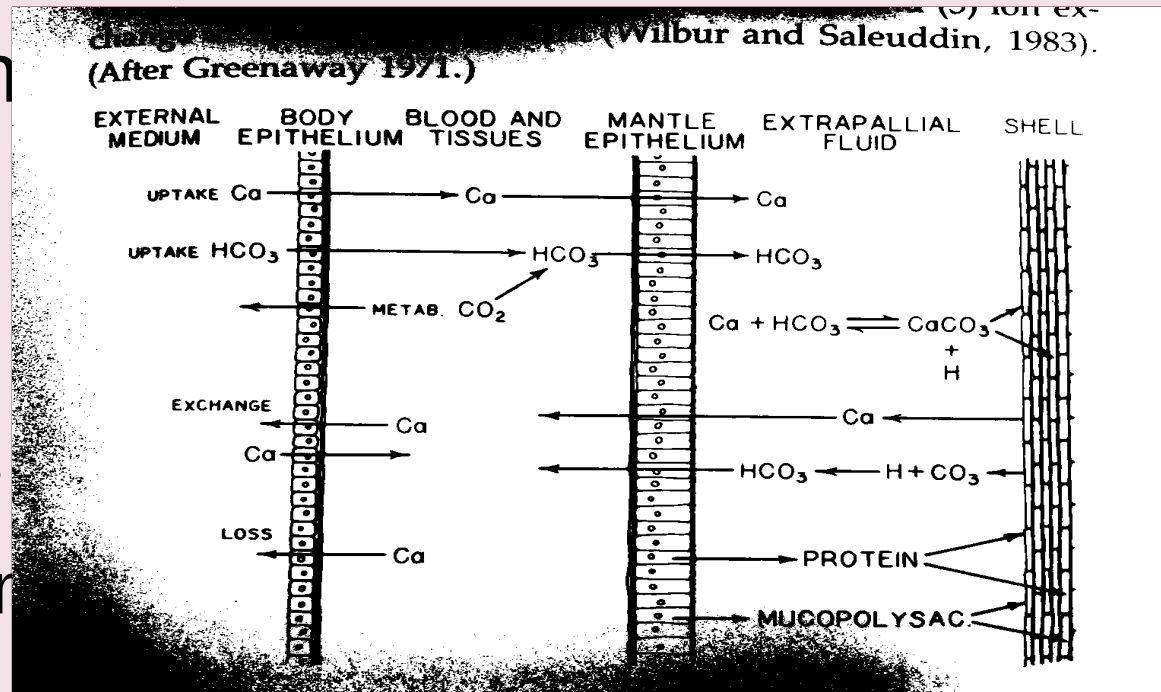
The nacreous (mother-of-pearl) layer is a laminate of 0.5- μ m-thick aragonite polygonal tablets sandwiched between thin (approximately 30 nm) sheets of a protein-polysaccharide organic matrix. The matrix plays a key role in limiting the thickness of the crystals and is structurally important in the mechanical design of the shell. It reduces the number of voids in the shell wall and so inhibits crack penetration by *Ascaris* the enemy associated

(P) Periostracum (image Credit:
(PR) Prismatic Layer
(N) Nacreous Layer
(EPS) Extrapallial Space
(OE) Outer Epithelium

Shells: Calcium Carbonate

Six Compartments

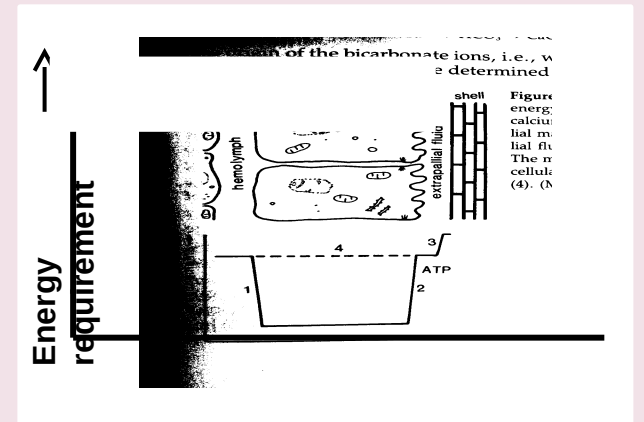
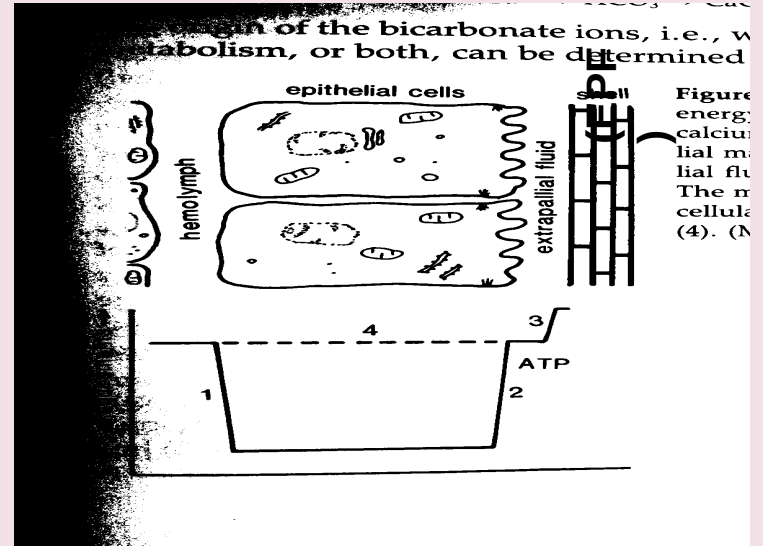
- Outer medium
- Body epithelium
- Blood & Tissues
- Mantle epithelium
- Extrapallial fluid
- Shell



Ion movement
bidirectional

Energy Requirement: From hemolymph to EPF

- Ca^{++} enters epithelial cells from hemolymph (1)
- To pass EPF, energy is supplied by ATP (2)
- Crystal formation requires more energy to raise ion activities above solubility



*Adenosine triphosphate (ATP)

Nacre Formation

- Each structure regardless of complexity is formed directly by a single layer of epithelial cells.
- Cells are involved in movement of minerals ions to the site of deposition and in the secretion of organic matter that will become the matrix of the deposit.

This epithelial layer of the mantle is ideal for experimental studies as it is separated from the mineral it deposits!

Formation induced by Organic Matrix

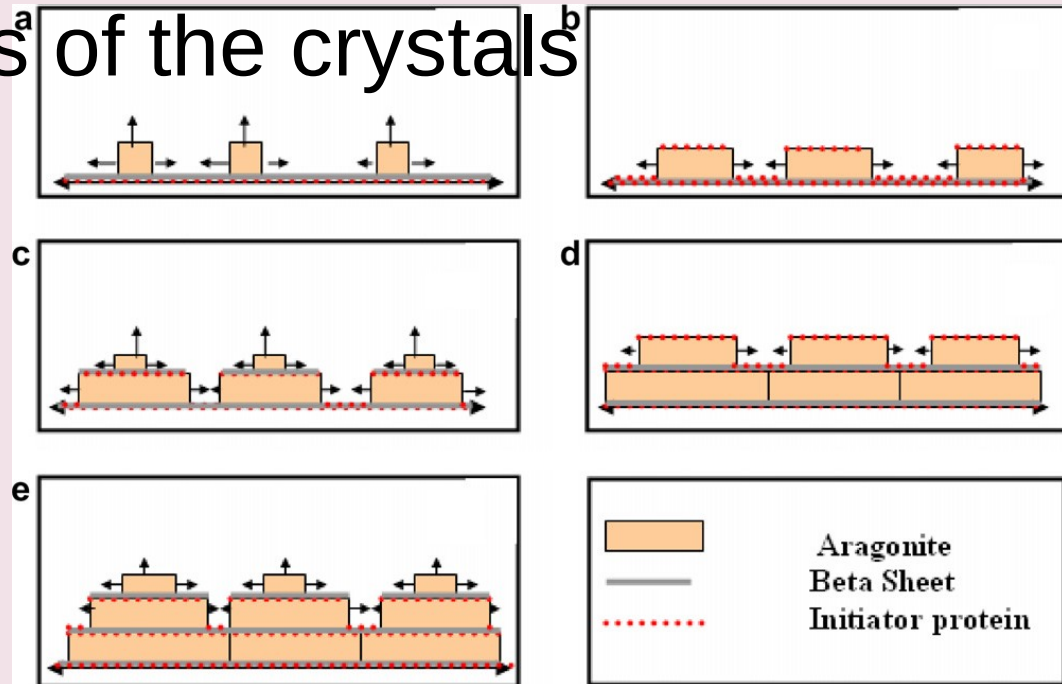
□ Secretion of the sheet covering many stacks of crystals can have an effect in terminating

(a,b) Crystal nucleation & protein deposition causing the arrest of crystallographic growth in the “c” direction;

(c) Second growth spurt after deposition of beta sheet and nucleation;

(d) First aragonite plates are butted together while growth of second layer continues in “a, b” direction;

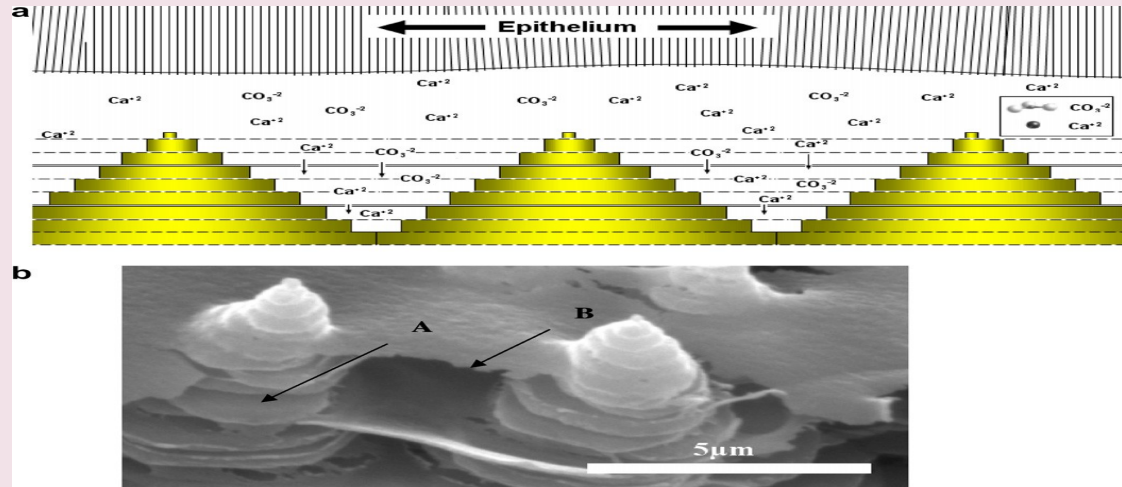
(e) Nucleation of third layer as second layer growth continues in “a” direction



Meyers et al. (2008).

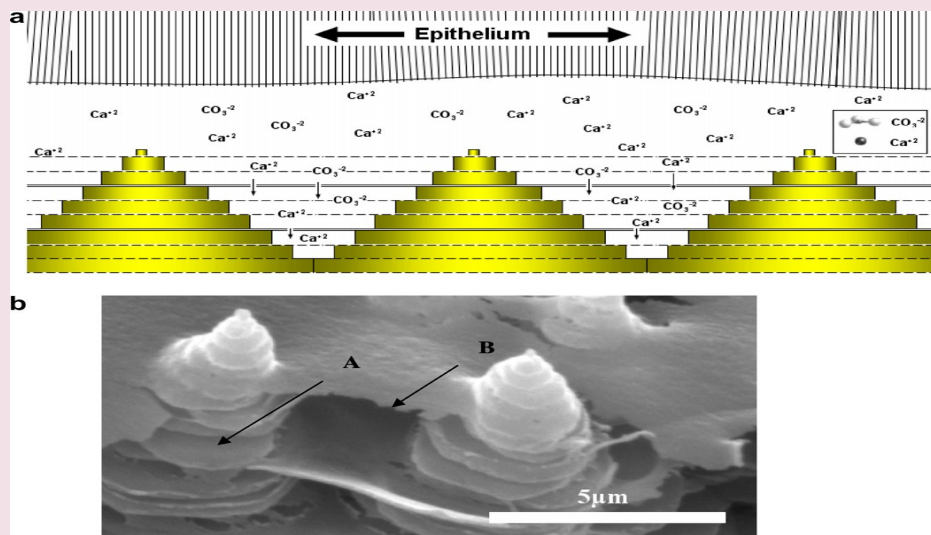
Nacre: Formation

Growth Of Nacreous Tiles by Terraced Cone Mechanism

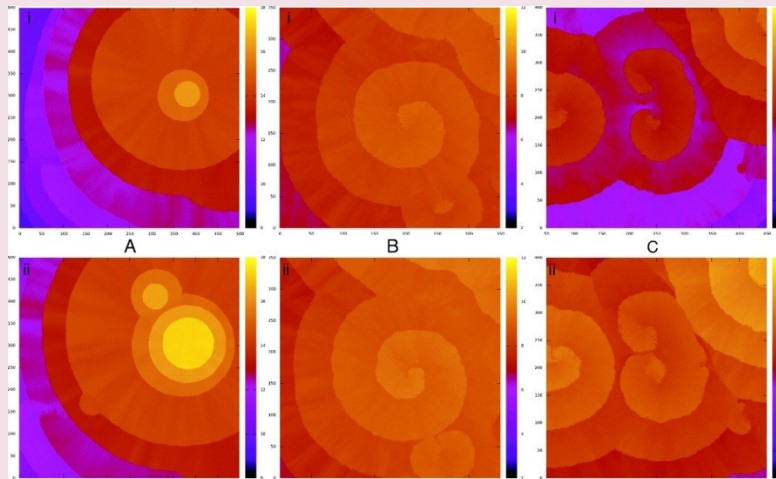
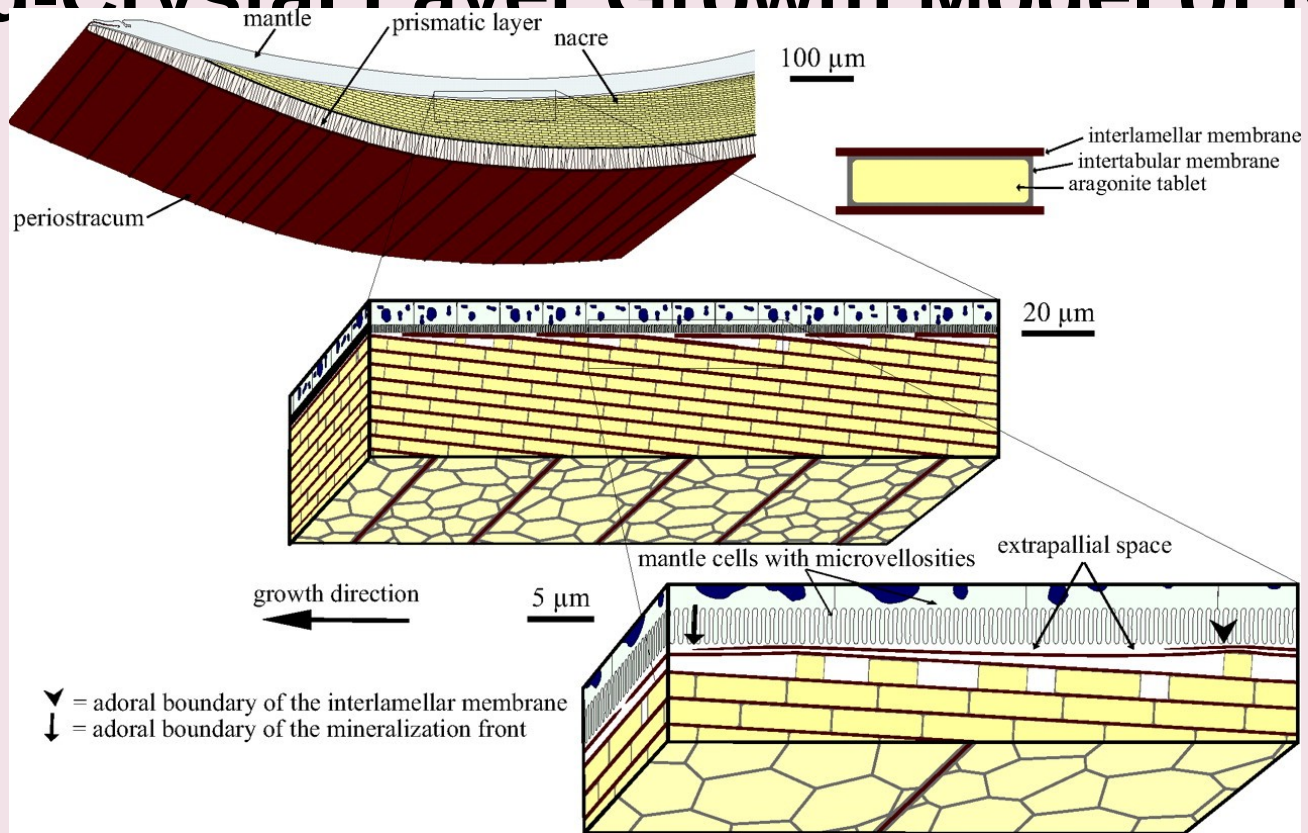


Schematic of growth mechanism showing intercalation of mineral and organic layers

SEM of arrested growth showing partially grown tiles (arrow A) and organic layer (arrow B).

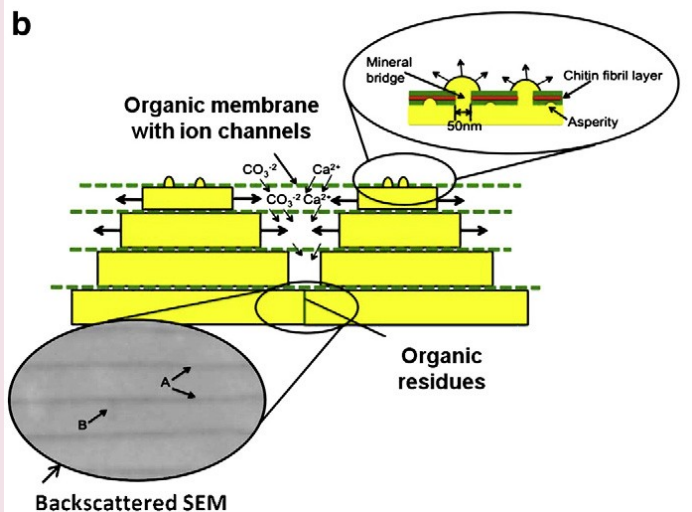
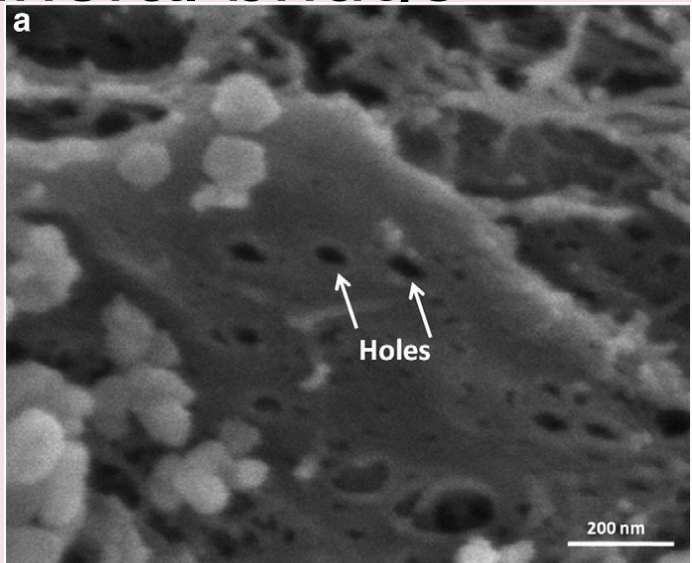


Liquid-Crystal Layer Growth Model of Nacre



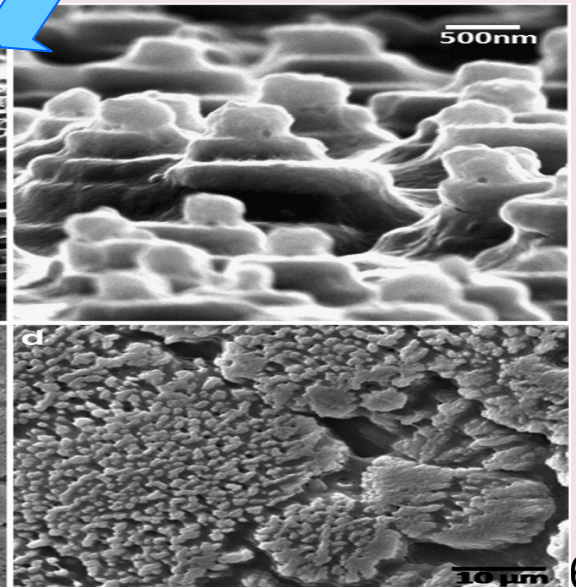
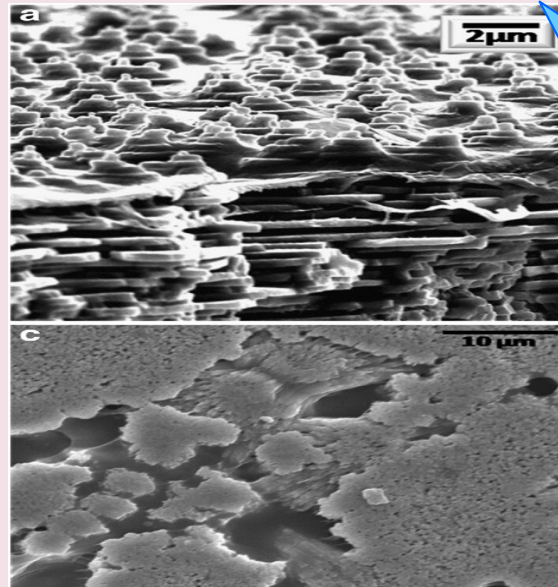
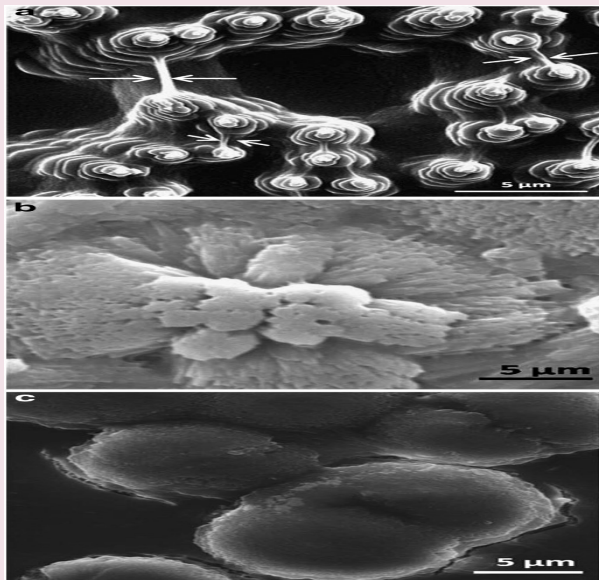
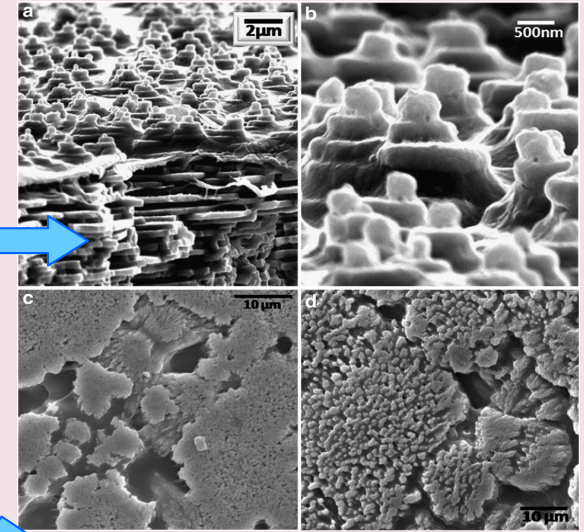
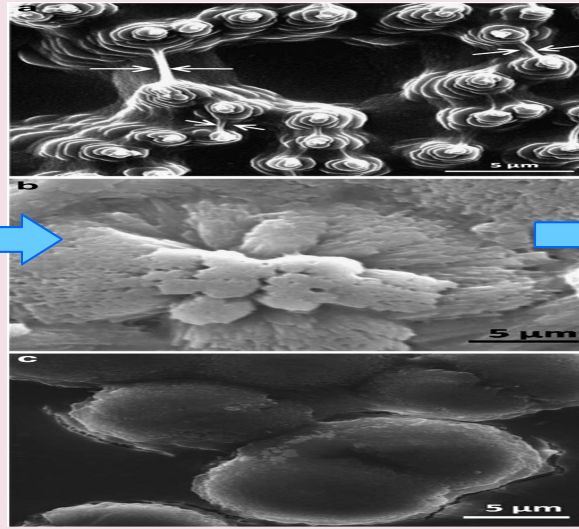
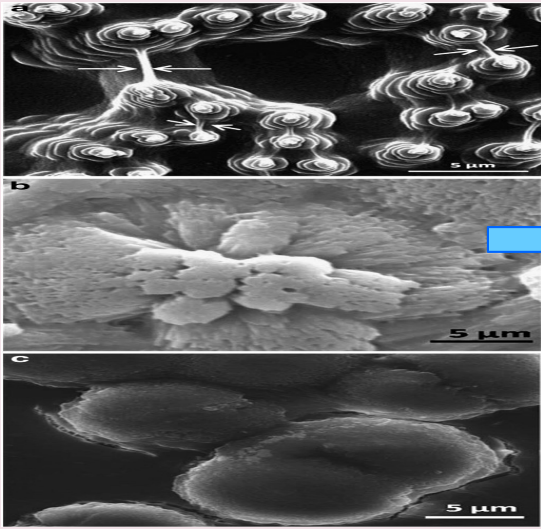
Formation Through nacre Mineral bridge

- Holes in the matrix exist between mineral layers
- Nucleation event for a given stack of crystals occurs once.



This model has been supported further by observations of 3-D coherence between crystals in a given stack using electron diffraction

Growth of Nacre



Silica

Silica: distinctive!

- Amorphous – Odd for biominerals as it is less stable (more soluble).
- Lack of crystallinity



- Opal is common!
 - Common in single celled organisms
 - Small bodies within multicellular tissues (sponges)
 - Small = stronger
- Fracture planes are missing, it can be mol *Diatomos* means “cut in half” *DISS* of strength.

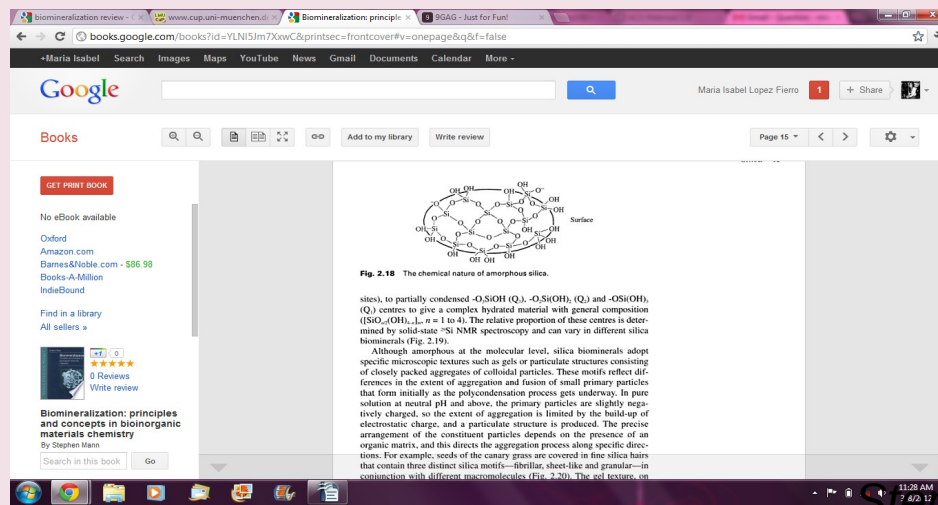
Silica Formation

- Deposition of silica is different from ionic minerals
 - At a neutral pH the soluble form is silicic acid
 - At $>1\text{mM}$ the acid undergoes polycondensation reactions to produce amorphous gels or colloidal particles.



Silica Formation

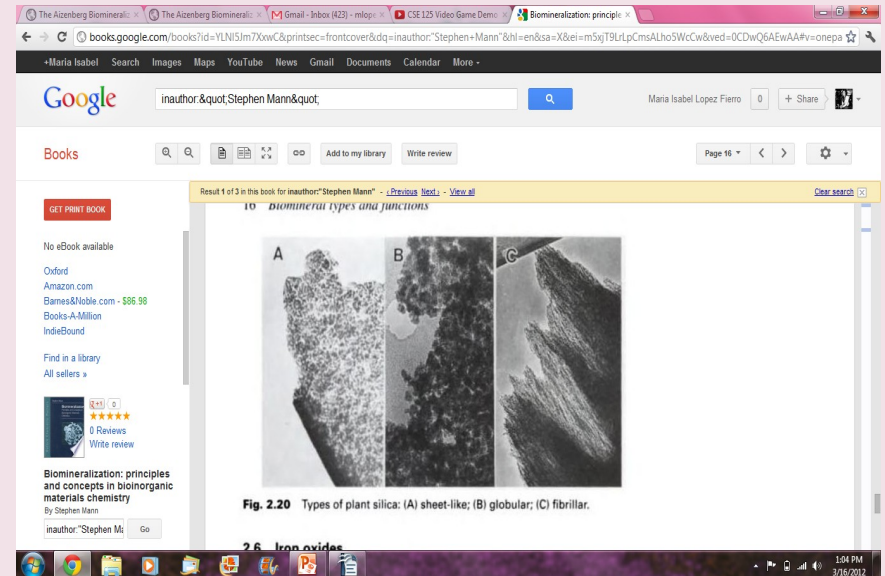
- Amorphous silica's structure varies based on the polymeric network of the randomly arranged siloxane centers and various layers of hydroxylation.
- These range from condensed to partially condensed centers giving a complex material



The screenshot shows a Google Books page for the book "Biominerization: principles and concepts in bioinorganic materials chemistry" by Stephen Mann. The page displays a chemical structure of amorphous silica, labeled as Fig. 2.18. The structure is a complex, interconnected network of silicon (Si) and oxygen (O) atoms, with various hydroxyl (OH) groups attached to the silicon atoms. The structure is shown as a cross-section of a surface, with the label "Surface" on the right. Below the structure, the caption reads: "Fig. 2.18 The chemical nature of amorphous silica." The text below the caption describes the chemical nature of amorphous silica, mentioning siloxane (Si-O-Si) and silanol (Si-OH) centers, and the general composition $(\text{SiO}_2)_n$, where $n = 1$ to 4 . It also discusses the relative proportion of these centers determined by solid-state ^{29}Si NMR spectroscopy and how they vary in different silica biomaterials. The text further explains that although amorphous at the molecular level, silica biomaterials adopt specific microscopic textures such as gels or particulate structures consisting of closely packed aggregates of colloidal particles. These motifs reflect differences in the extent of aggregation and fusion of small primary particles that form initially as the polycondensation process gets underway. In pure solution at neutral pH and above, the primary particles are slightly negatively charged, so the extent of aggregation is limited by the build-up of electrostatic charge, and a particulate structure is produced. The precise arrangement of the constituent particles depends on the presence of an organic matrix, and this directs the aggregation process along specific directions. For example, seeds of the canary grass are covered in fine silica hairs that contain three distinct silica motifs—fibrillar, sheet-like and granular—in conjunction with different macromolecules (Fig. 2.20). The gel texture on

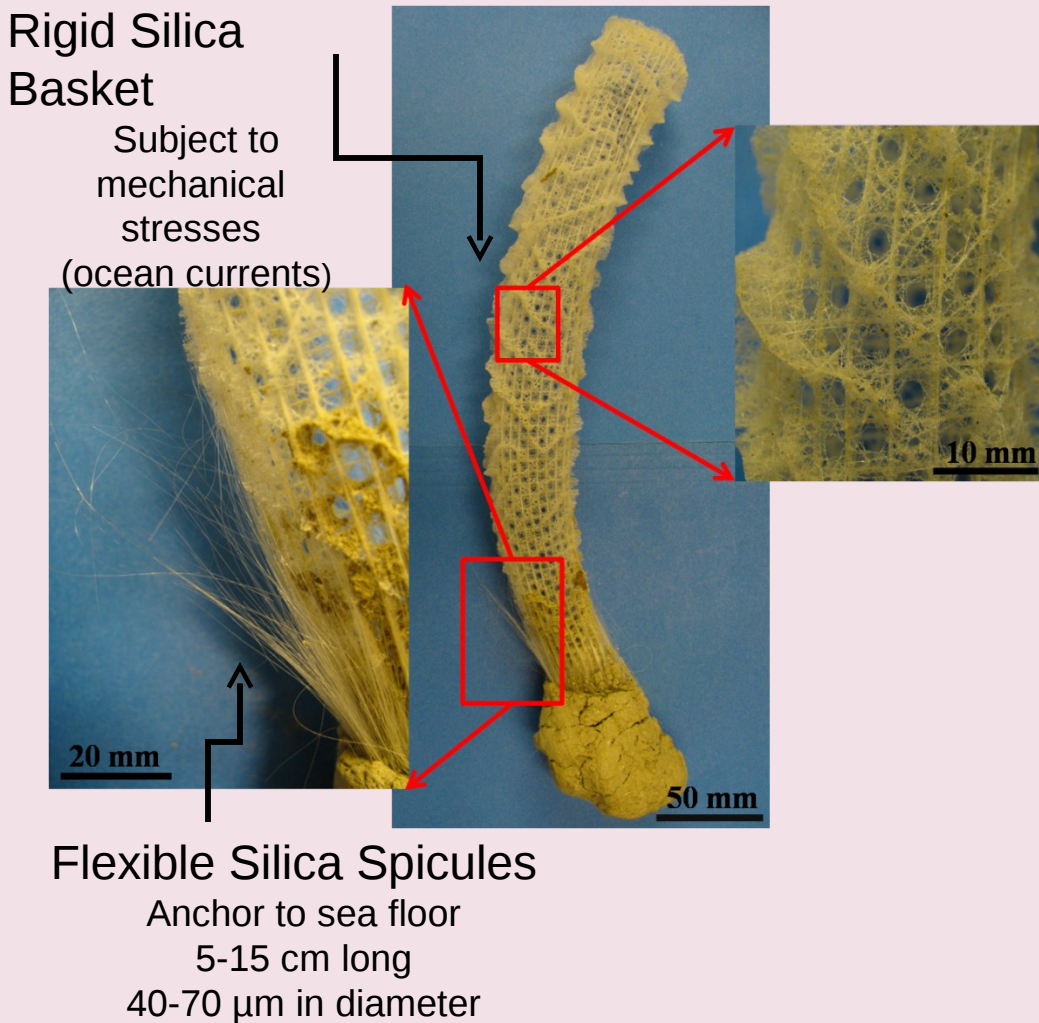
Silica Formation

Microscopic features exist due to the differences in aggregation of particles that formed during the polycondensation process.



An example of this is seeds of the canary grass covered in fine silica hairs with 3 different morphologies

sponge spicule



- Siliceous spicules consist of hydrated silica in a layer arrangement around an axis filament'
- Each silica rod is composed of a central pure silica core.

Sponge Spicule

Formed intracellularly in sclerocyte within an organelle bounded by a membrane

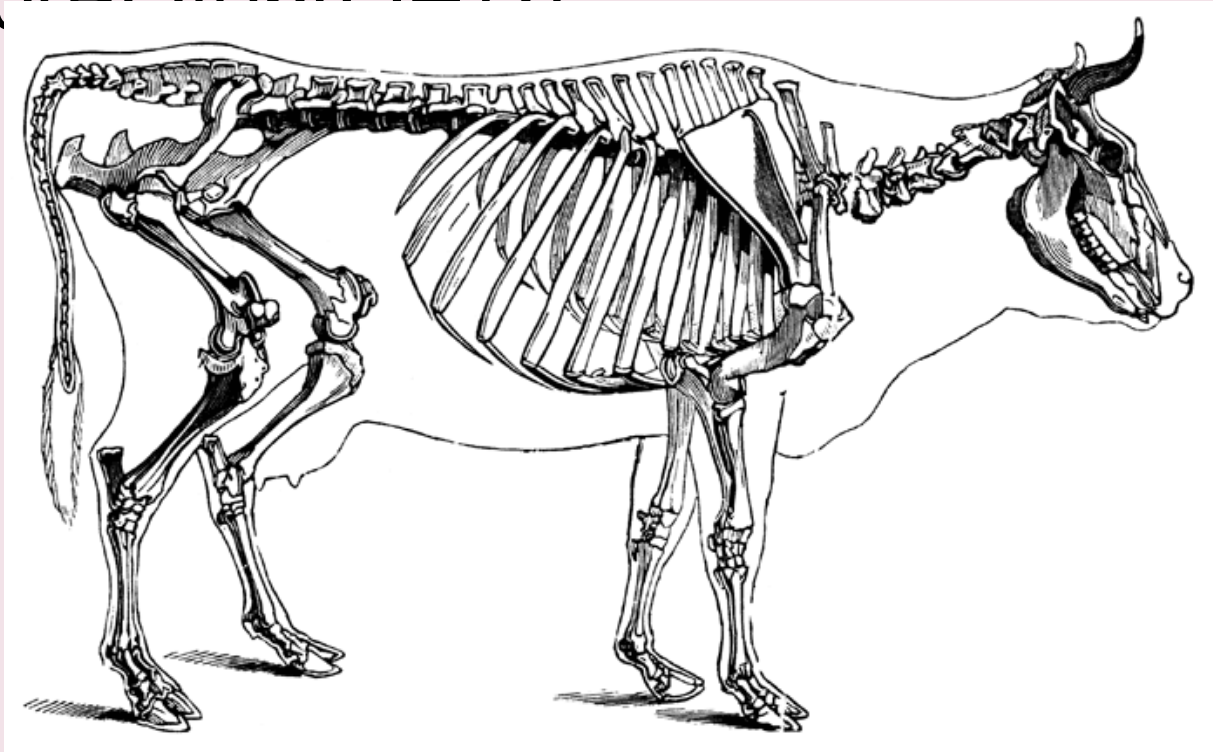
- 1. Axial filament formation
- 2. Mineralization gives it shape
- 3. Punctures sclerocyte
- 4. Spicule is moved to definite position



Hydroxyapatite

Hydroxyapatite

- The mineral that forms bones and teeth of vertebrates is hydroxyapatite
 $[Ca_{10}(PO_4)_6(OH)_2]$



S. G. Goodrich, *The Animal Kingdom Illustrated*

Hydroxyapatite

- Pure state:
Monoclinic structure
with Ca/P ratio of
10/6
- In most substituted
forms: hexagonal
structure in where
molar ratio changes

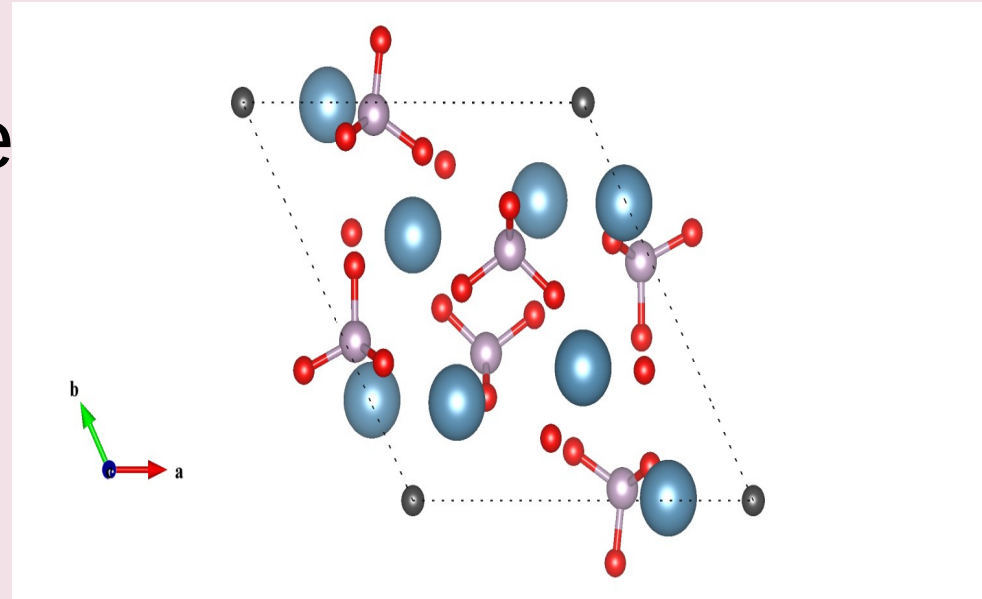
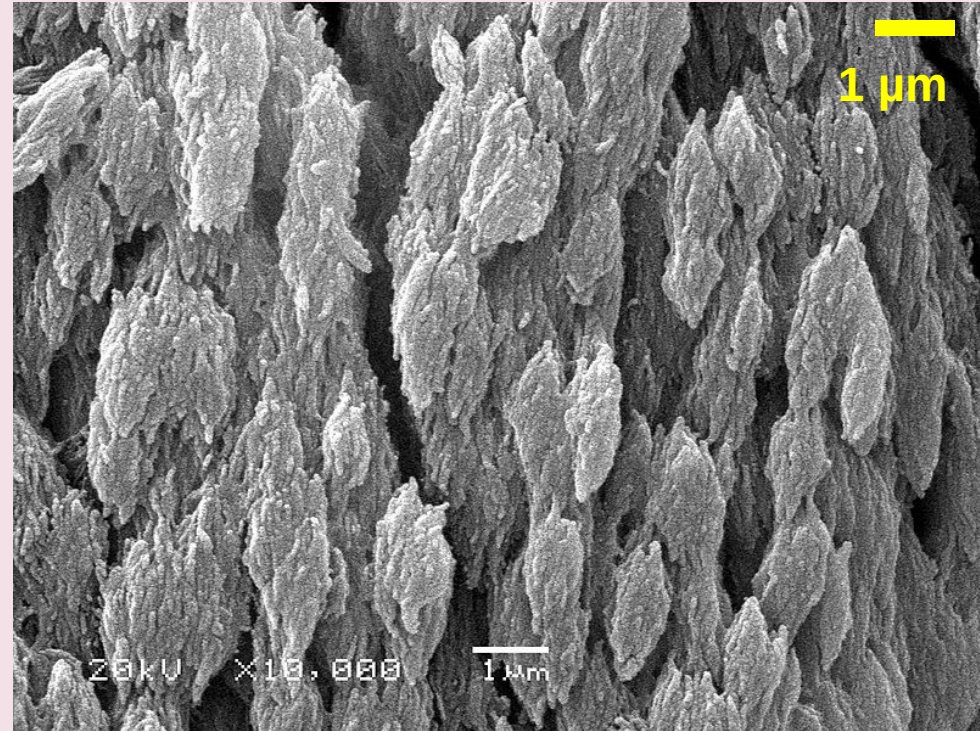


Image Credit: Michael Porter

Bone Mineral

- Hard to determine solubility constant and understand formation:
 - Ion substitution affects solubility
 - Various Ca/P ratios exist

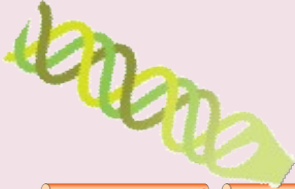


(image credit: Bertazzo S, wikipedia.com)
SEM deproteinized bone - cranium rat)

Bone Matrix

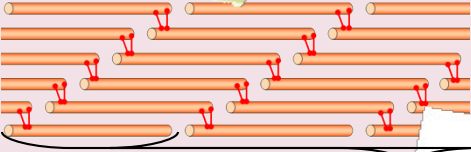
Collagen molecule

1.5 nm



HA crystals

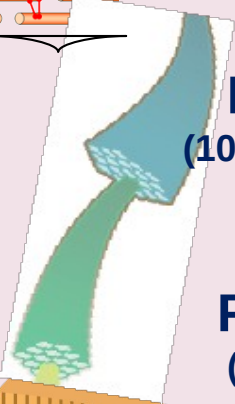
(40x4) nm



300 nm

Fibrils

(100-200 nm)

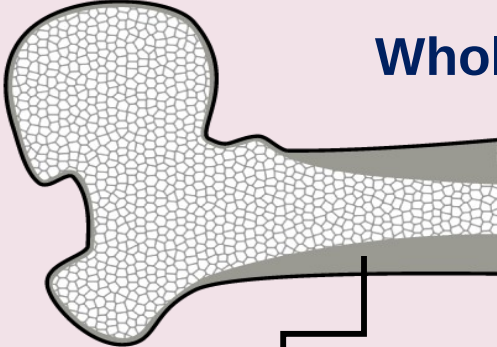
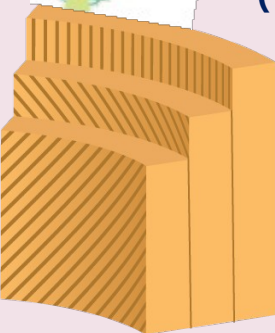


Fibers

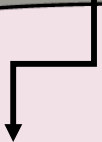
(~ 1 μm)

Lamella

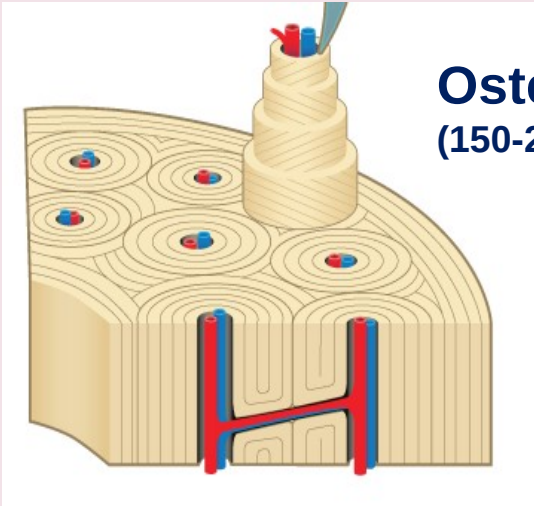
(5-10 μm)



Whole bone



Cortical bone



Osteon

(150-250 μm)

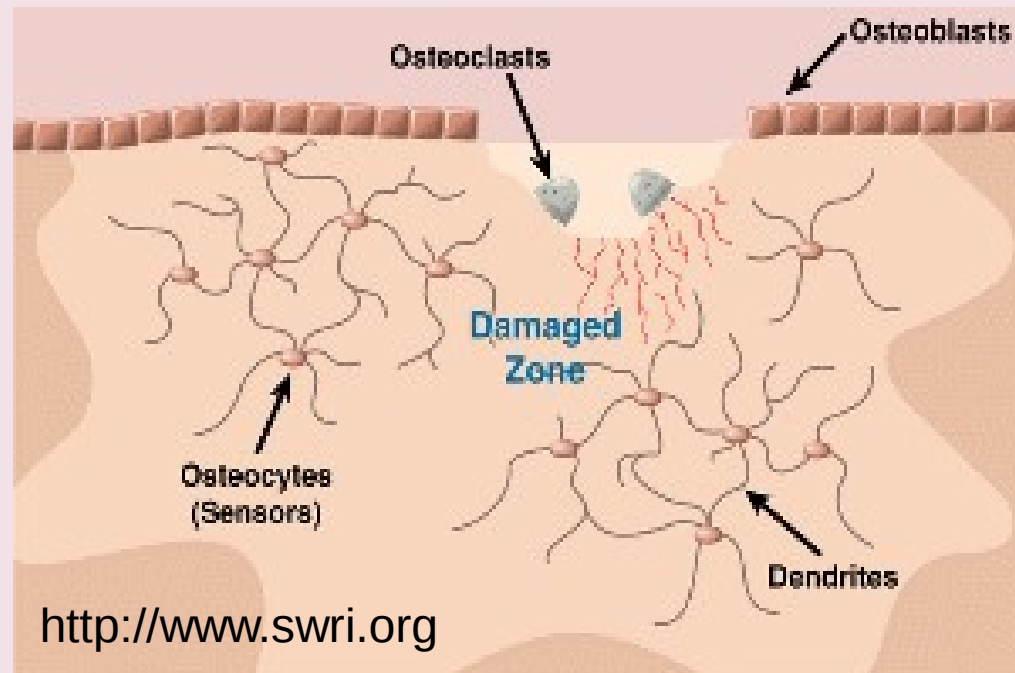
Bone: Cellular interactions

□ 3 Main Cells associated

- Osteoblasts (formation)
- Osteocytes (maintaining)
- Osteoclasts (resorption)

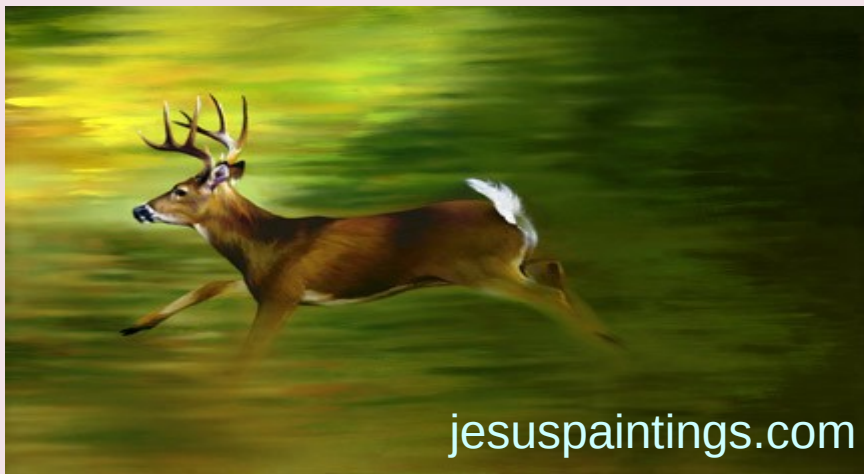
□ Application of pressure stimulates

Though as a “living mineral” because it undergoes continual growth, dissolution & remodeling



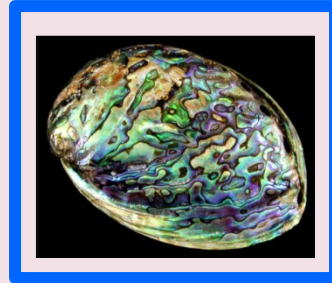
Bone

- By controlling the different levels of mineral content you can control the stiffness (young's modulus).
- E.g. a fast moving animal (deer) requires a highly elastic bone (less mineralized – 50 weight %)

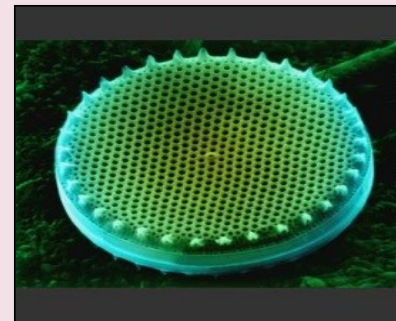
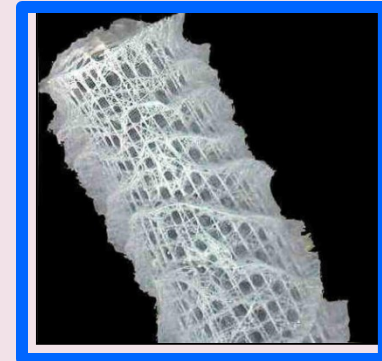


Summary

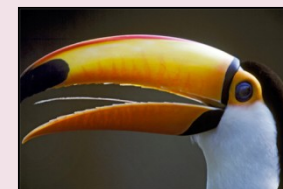
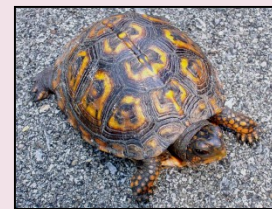
calcium carbonate
(CaCO_3) + chitin +
protein



amorphous
silica (SiO_2) (H_2O)_n



calcium phosphate
hydroxyapatite
($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) + collagen



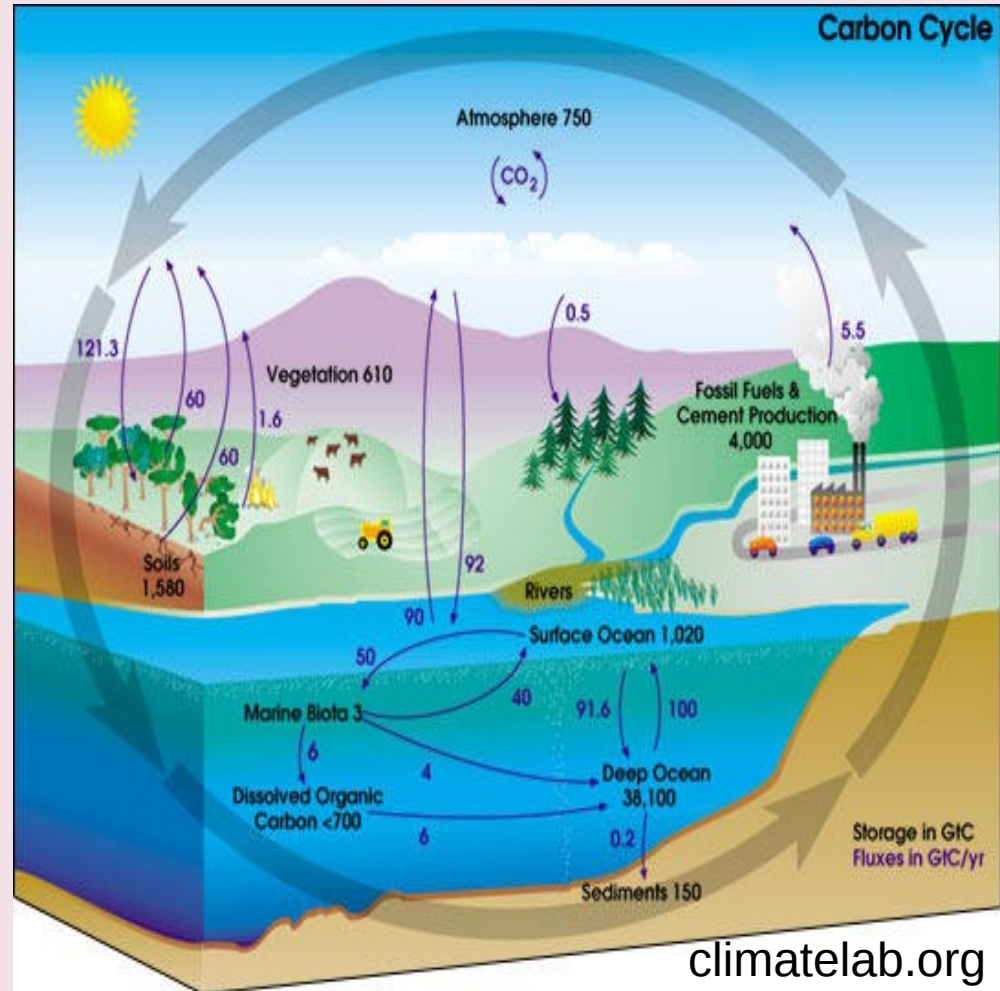
□ Social Impact

Why do we need to understand
biomineralization?

Social Impact: Ocean Acidification

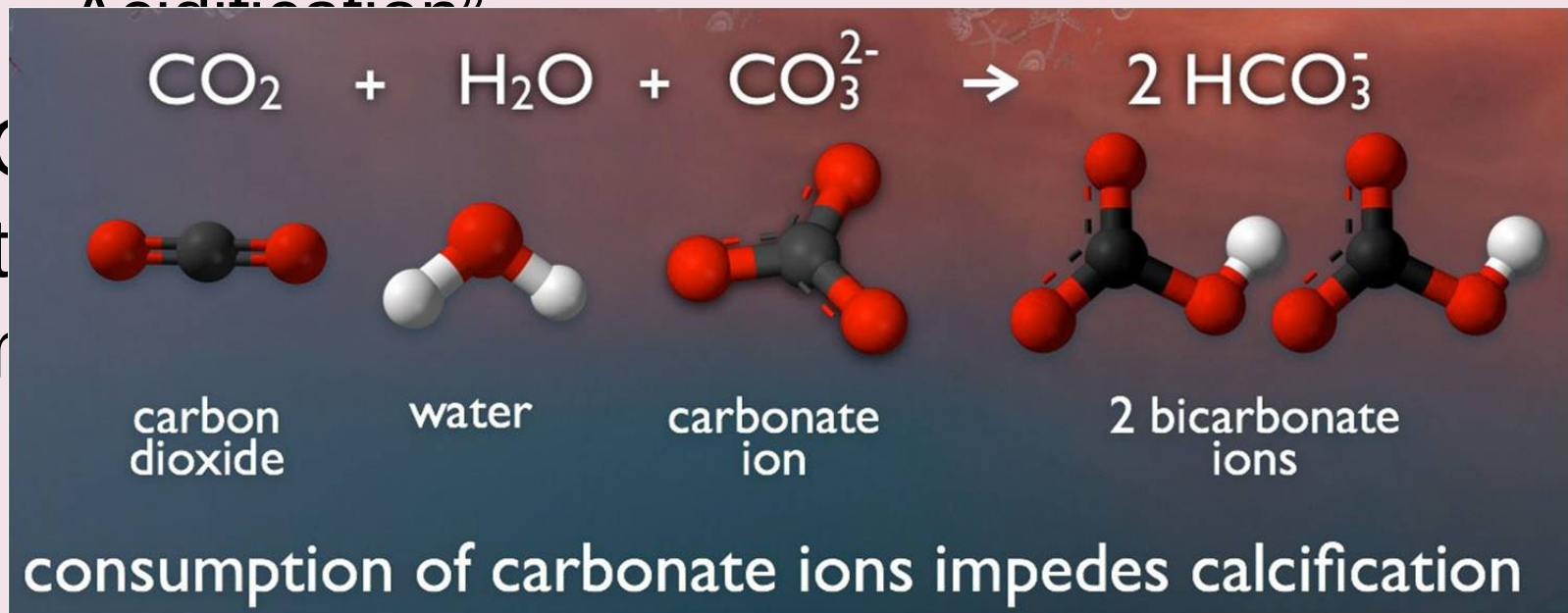
Changes in seawater chemistry are occurring in the ocean.

Industrial and agricultural activities is increasing amount of CO₂ in the atmosphere.



Social Impact: Ocean ACidification

- When CO₂ is absorbed by seawater
 - Carbonate ion concentration and saturation states are lowered
 - pH level in ocean is reduced – “Ocean Acidification”



Social Impact: Ocean Acidification

□ The pH of surface ocean waters has fallen by 0.1 pH units

□ ~ a 30% increase in acidity.

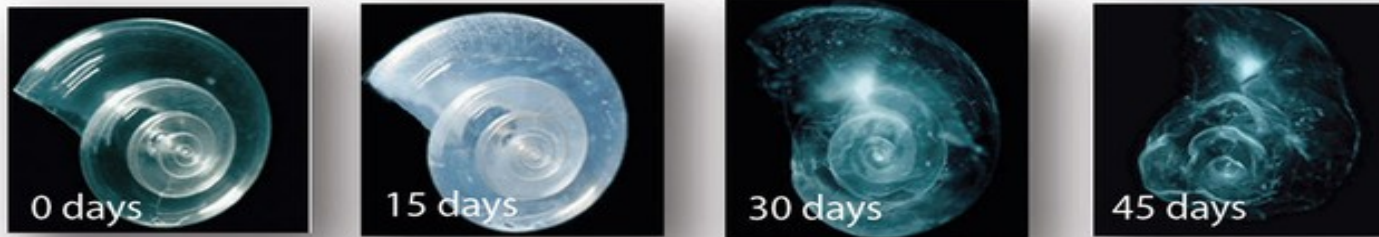
□ Many organisms are at risk!

□ Remember that by threatening our shelled

friend

□ The

ou



National Geographic Images.

photos below show what happens to a pteropod's shell when placed in sea water with pH and carbonate levels projected for the year 2100. The shell slowly dissolves after 45 days.

Summary & Conclusions

- Biomineralization is the process by which living organisms form and influence the precipitation of minerals.

- No 'grand' mechanism.

- Saturation, Nucleation Growth & Influence of Organic Matrix .

- Many Biominerals:

- Calcium Carbonate – Abundant!

- Silica – Distinctive!

- Hydroxyapatite – Living!



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