Biomineralization: The Process, the Good, and the Bad

Bertini *et al.* Chapter 6

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1. Insolubility and Biology

Much of what we have talked about has focused on the importance of solubility for bioavailability and function. Insolubility is also significant for biological survival.

1. Insolubility and Biology

The insoluble solid-state structures that we can see on organisms serve the functions of:

- "Skeletal" frameworks
- Defense
- Ingestion

There is a large component in the interior that serve:

- Similar functions
- Storage
- Orientation

2. Biomineralization

The biological process that yields the inorganic-based solid state structures of life.

For bioinorganic chemistry, the interest in biomineralization stems from exploring the dynamics of biology directing inorganic chemistry.

a. Understand the structure and composition of the biominerals

b. Understand the function of the biominerals

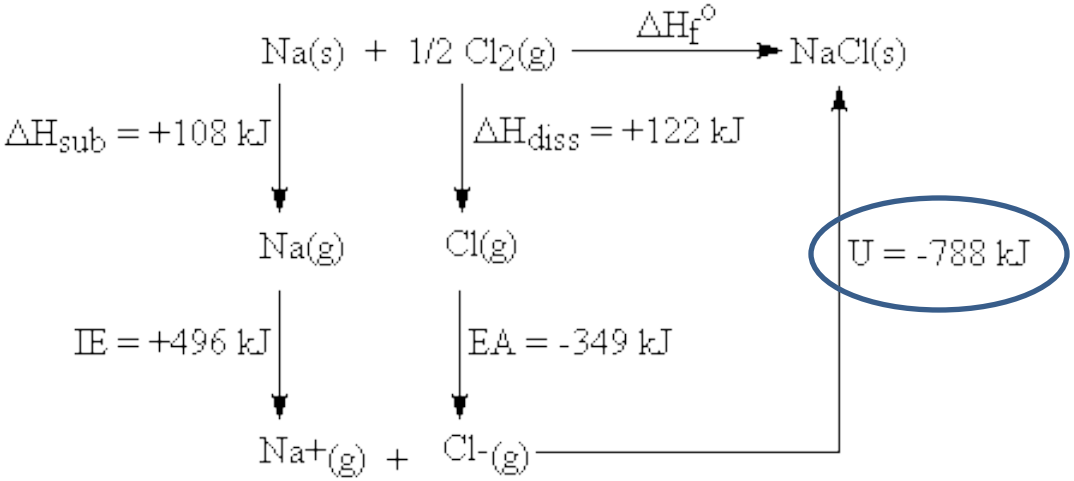
c. Elucidate the mechanisms of formation

- Explore the factors involved in long range coordination chemistry requiring a collection of inorganic atoms and ligands on an organic surface/matrix regulated by biomolecules

3. Biomineralization is not simply crystallization

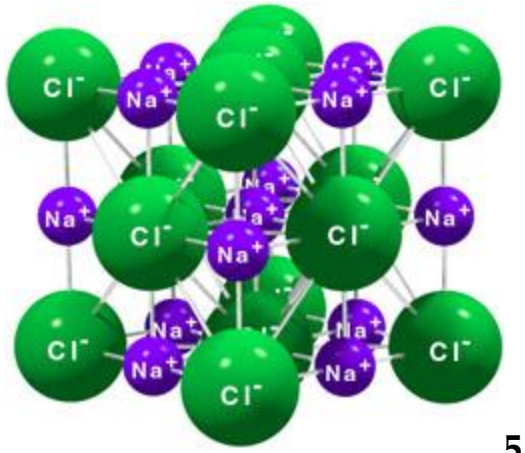
As with crystallization of big and small molecules in different solvents, the process of biomineralization is one that concerns relative solubility and a precipitation event.

Recalling the Born-Haber cycle, crystallization in a lattice is dictated by the release of energy known as the lattice energy (U):



$\Delta H_f^\circ = \Delta H_{sub} + IE + \Delta H_{diss} + EA + U$

$\Delta H_f^\circ = 108 + 496 + 122 - 349 - 788 = -411$ kJ/mole



3. Biomineralization is not simply Crystallization

Biomineralization is not always crystallization in ideal lattices or even lattices with imperfections. In many examples, the structures are of an amorphous type and their formation do not simply involve the atoms that constitute the final mineral.

The composition of biominerals:

- Ionic salts and covalent compounds in combination with a complex organic macromolecular matrix consisting of proteins, polysaccharides, and lipids
  - The matrix often serve as the seed for the biominerals
- High lattice energies (namely calcium biominerals)
- Thermodynamic stability and low solubility in biological environments
- Polymorphic structures (same atomic composition but different unit cell arrangement)

Table VI.1.
The Types and Functions of the Main Inorganic Solids Produced by Controlled Biomineralization

Mineral	Formula	Organism/Function
CALCIUM CARBONATE		
Calcite	$CaCO_3$[a]	Algae/exoskeletons Trilobites/eye lens
Aragonite	$CaCO_3$	Fish/gravity device Mollusks/exoskeleton
Vaterite	$CaCO_3$	Ascidians/spicules
Amorphous	$CaCO_3.nH_2O$	Plants/Ca store
CALCIUM PHOSPHATE		
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	Vertebrates/endoskeletons teeth, Ca store
Octacalcium phosphate	$Ca_8H_2(PO_4)_6$	Vertebrates/precursor phase in bone
Amorphous	?	Mussels/Ca store Vertebrates/precursor phases in bone
CALCIUM OXALATE		
Whewellite	$CaC_2O_4.H_2O$	Plants/Ca store
Weddellite	$CaC_2O_4.2H_2O$	Plants/Ca store
GROUP 2A (IIA) METAL SULFATES		
Gypsum	$CaSO_4$	Jellyfish larvae/gravity device
Barite	$BaSO_4$	Algae/gravity device
Celestite	$SrSO_4$	Acantharia/cellular support
SILICON DIOXIDE		
Silica	$SiO_2.nH_2O$	Algae/exoskeletons
IRON OXIDES		
Magnetite	Fe_3O_4	Bacteria/magnetotaxis Chitons/teeth
Geothite	α-FeOOH	Limpets/teeth
Lepidocrocite	γ-FeOOH	Chitons (Mollusca) teeth
Ferrihydrite	$5Fe_2O_3.9H_2O$	Animals and plants Fe storage proteins
IRON SULFIDES		
Greigite	Fe_3S_4	Bacteria/magnetotaxis

[a] Magnesium-substituted calcites are also formed.

4. Types of biominerals

A. Calcium biominerals

Are quite common because Ca^{2+} is abundant in biological fluids and has low K_{sp} values for the anions carbonates, phosphates, pyrophosphates, sulfates, and oxalates.

i. Calcium carbonate biominerals (calciate/aragonite) used for structural support as in seashells
   - Very strong, thin crystals formed by thin sheets of a protein-polysaccharide organic matrix
   - Cracks usually progress in the organic matrix due to strength in the inorganic crystal

4. Types of biominerals

ii. Calcium phosphate minerals (hydroxyapatite, HAP) constitute bone and teeth

- Phosphate (PO_4^{3-}) can be substituted by carbonate (CO_3^{2-}) due to Ca^{2+} deficiency

  How might this happen and why?

- Very important for tooth enamel and bones is a intricate structural design to withstand mechanical stress

- Bone is a bioinorganic mineral

  - Organized mineralization of hydroxyapatite within a fibrous matrix of a structured protein, collagen, and proteoglycans

  - It is said to be a "living mineral" because it experiences growth, dissolution, and remodeling, especially when broken

4. Types of biominerals

B. Iron Oxides

Perform very important functions

i. Orientation

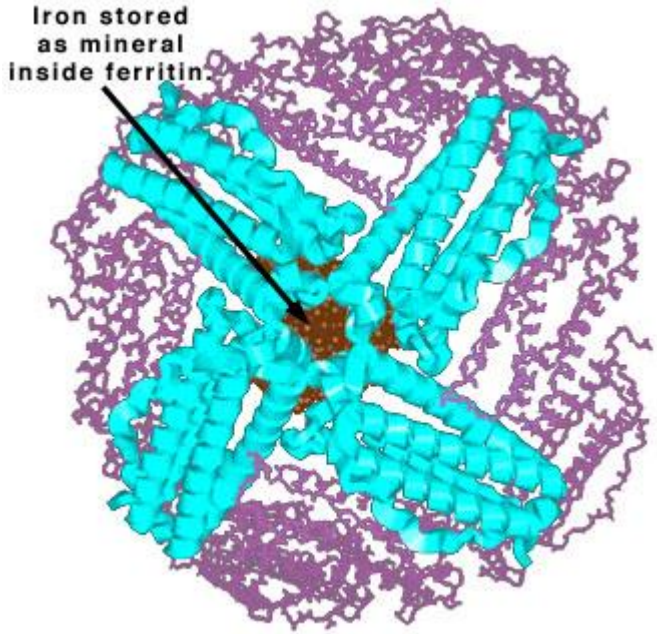
- Magnetotactic bacteria have the mixed-valence compound, magnetite (Fe_3O_4) which orients the bacteria along the Earth's magnetic fields

4. Types of biominerals

ii. Storage

- Ferrihydrate is a biomineral that is basically the product of mixing Fe^{3+} in a sodium hydroxide solution leading to an amorphous solid.

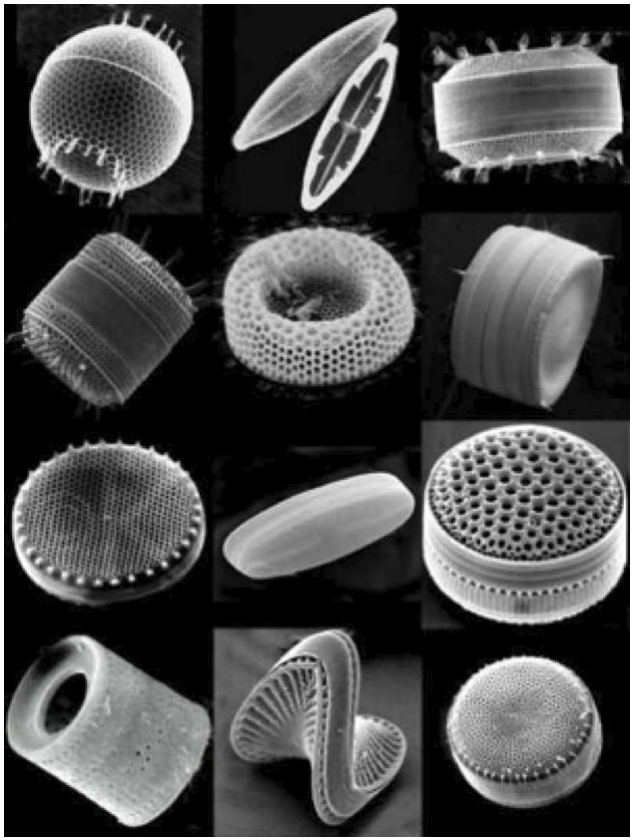
- This mineral is employed by cells to store iron as we have discussed in ferritin, for mobilization purposes when iron levels in the body are low



4. Types of biominerals

C. Silica ($SiO_2 \cdot n\, H_2O$)

Silica minerals are of an amorphous composition with great variability in terms of the Si-O-Si bond angles and the degree of hydroxylation. This results in elaborate mineral shapes featured in unicellular organisms (diatoms and radiolaria).



4. Types of biominerals

D. Sulfides

Sulfide minerals are largely the products of bacteria.

i. Iron sulfide minerals are generated by sulfate-reducing bacteria
   - Some are adventitious products of the reaction of H_2S with a source of Fe^{3+}
   - Ferrimagnetic mineral, greigite (Fe_3S_4)

ii. Cu, Zn, and Pb sulfides are deposited on external walls of bacterial cells

iii. CdS are localized intracellularly.

5. The processes of biomineralization

Biologically Induced versus Biologically Controlled Biomineralization

Both involve a precipitation event due to a shift in concentration.

5. The processes of biomineralization

A. Biological induction

- Formation of inorganic materials as a byproduct of environmental changes due to metabolic activity

$$Ca^{2+}(aq) + 2\ HCO_3^-(aq) \rightleftharpoons CaCO_3\ (s) + CO_2(g) + H_2O$$

The removal of CO_2 by photosynthetic organisms can lead to the formation of $CaCO_3$ due to Le Chatelier's principle.

  - Several algae engage in this intercellular mineralization and their colonies reside in entombed $CaCO_3$ structures
  - A very blatant example of this process is the existence of coral reefs

- Adventitious precipitation along cell surface due to localized concentrations because of the influx/efflux of species

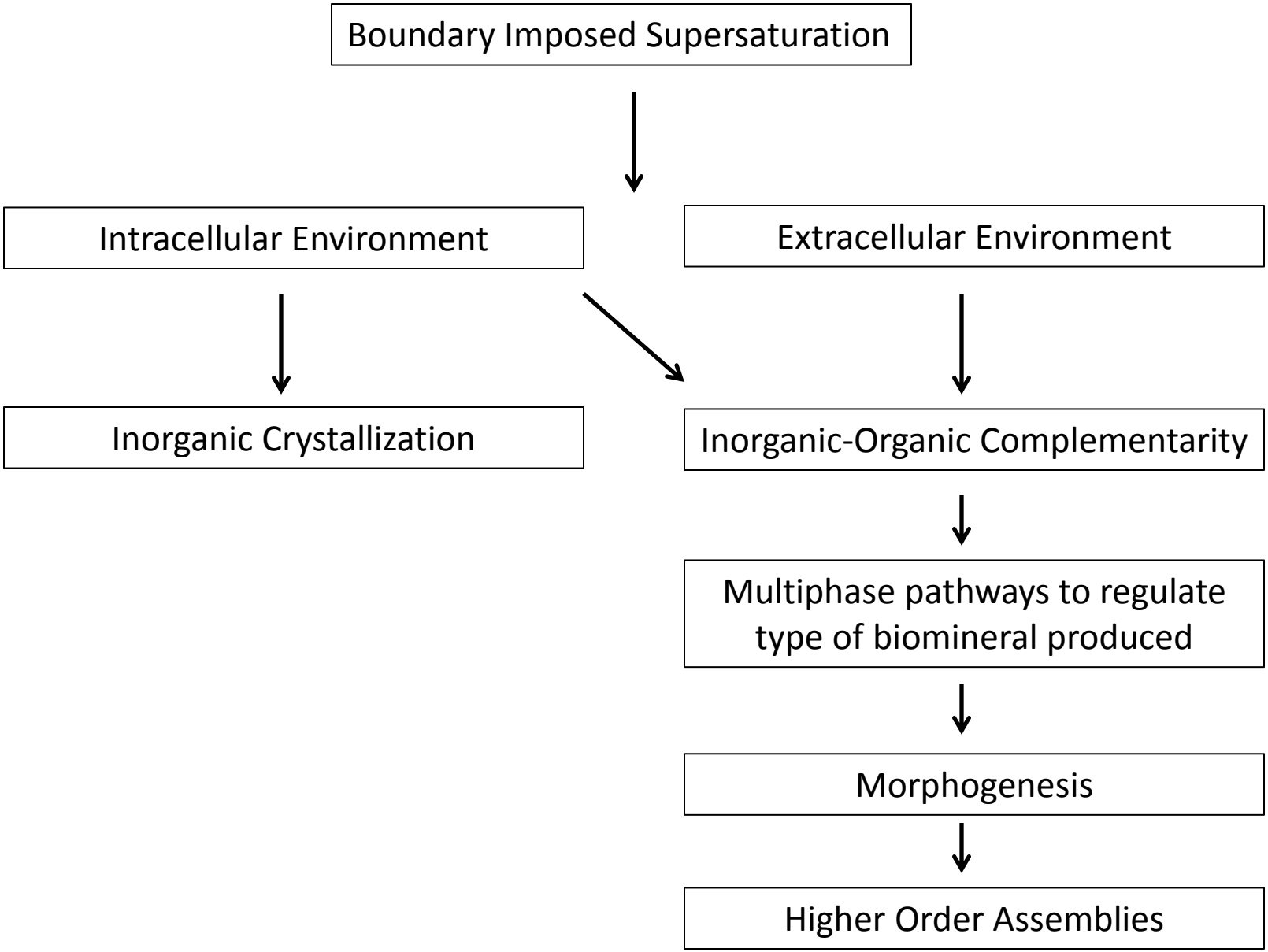
  - Minerals look like inorganic precipitates generated in lab

5. The processes of biomineralization

B. Biological control

Biomineralization can be a highly regulated process because the materials produced have a very specific function and require well-defined composition, shape, size, and arrangement.

- The process can occur in both the intra- and extracellular environment.
- Involves the regulation of the physicochemical properties of solubility, supersaturation, nucleation, and crystal growth.


Boundary Imposed Supersaturation
Intracellular Environment
Extracellular Environment
Inorganic Crystallization
Inorganic-Organic Complementarity
Multiphase pathways to regulate type of biomineral produced
Morphogenesis
Higher Order Assemblies

i. Boundary-organized biomineralization

Localized concentrations are key to the precipitation event and these are achieved by precise compartmentalization to generate **supersaturation**. The boundaries help to control size, volume, and shape of the biominerals.

- Intracellular mineralization

Occurs within membrane-enclosed compartments (vesicles) that strictly control transport of metal ions and molecules. Biominerals produced do not have to remain inside the cells.

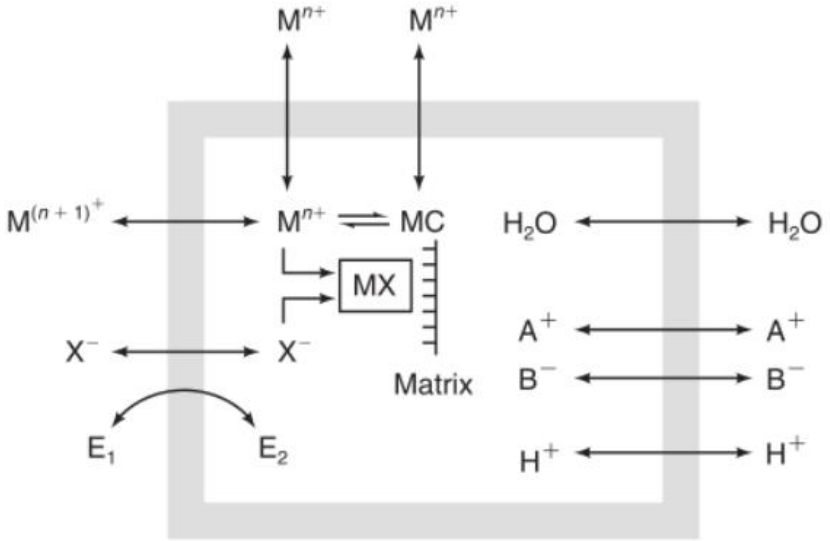


Fig. VI.5.
Generalized strategies for controlling supersaturation in biomineralization. The mechanisms can be either direct (membrane pumps, complexation, and enzymatic regulation) or indirect (H_2O, H^+, and ion fluxes).

i. Boundary-organized biomineralization

a. Control ion concentrations

b. Transport of metal complex into a membrane followed by destabilization of the complex

c. Shifts in equilibria favoring precipitation

d. Influencing solubility via changes in ionic strength

e. Influencing solubility via control of water levels

f. Changes in pH to control soluble and insoluble speciation

i. Boundary-organized biomineralization

- Extracellular mineralization (organic matrix-mediated biomineralization)

The mineralization compartments are produced within a biopolymeric structure formed from insoluble organic macromolecules (i.e. proteins, polysaccharides)

a. The organic matrix is specifically synthesized under genetic regulation, before and sometimes during the biomineralization
   - For extended structures (i.e. bone), insoluble polymeric structures are constructed before inorganic precipitation occurs.

b. The size/shape of the biominerals are controlled by the empty space defined by the insoluble matrix.
   - In bone development, calcium phosphate nucleates in nanospaces organized within a supramolecular assembly of collagen fibrils.

c. Supersaturation is achieved by ion transport from neighboring cells or careful dissolution of precursor mineral materials on the surface of the matrix that then react and mineralize.

ii. Matrix-mediated nucleation and complementarity

The organic matrix not only provides a template for nucleation of crystals and localized supersaturation. It helps to align the biomineralization to favor a particular arrangement of the biomineral.

There is molecular recognition between the inorganic-organic interface:

a. Complementarity of charge, structure, and stereochemistry

b. Lowering of activation energy for nucleation of the inorganic layer that best complements the organic layer

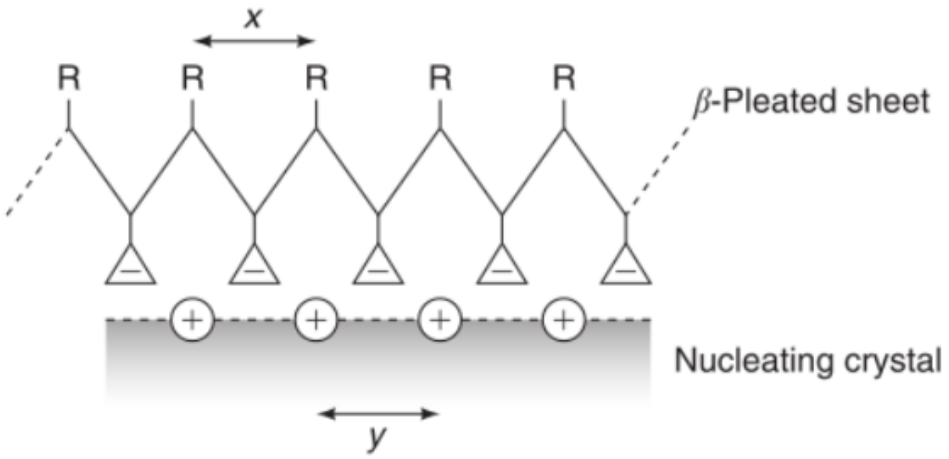


Fig. VI.8.
Geometric matching (epitaxy) in biomineralization. Cation–cation distances in one specific crystal face and polymorph structure are commensurate with the spacing of periodic-binding sites on the organic surface.

iii. Accelerators/Inhibitors Regulate Polymorph Development

In the biomineralization process, several of the polymorph forms of a specific mineral grow depending on the presence and concentrations of precursors and even of other polymorphs (and intermediates) as shown in the figure (Fig. VI.9) below.

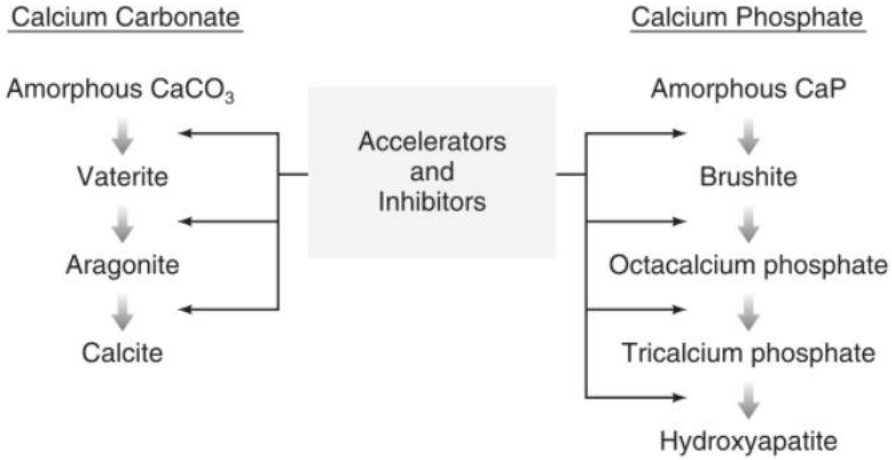


Fig. VI.9. Control of polymorph structure in biomineralization by controlled release of molecules that act as accelerators or inhibitors of crystal growth and phase transformation.

Biological fluids contain numerous components that can interfere with the growth of biominerals and the multiphase pathway for the development of different polymorphs.

iii. Accelerators/Inhibitors Regulate Polymorph Development

- Extraneous ions and molecules can tip the equilibrium between supersaturation and solubility and can inhibit nucleation by binding to surface sites of the organic matrix
- The presence of certain molecules (i.e. soluble macromolecule, metabolites) may inhibit certain polymorphs from forming while favoring crystallization of others
  - These inhibitors can be attenuated by the presence of enzymes, which these inhibitors are substrates of
- Amorphous granules seeded with high levels of inorganic and organic components of a certain phase are known to localize at the organic matrix
  - They temporarily dissolve to produce a saturation pulse and initiate the phase-transformation pathway by encouraging crystallization of the biomineral in comparable phase

iv. Morphogenesis

The elaborate shapes that biominerals assume come from the patterned organic assemblies of vesicles and frameworks derived from biosynthetic pathways. These become part of the biomineralized core. There are two routes in the morphogenesis process, both involving the organic layer as the directing layer:

1. The inorganic mineral basically adopts the pattern produced by the organic matrix like a "cast produced in a mold"

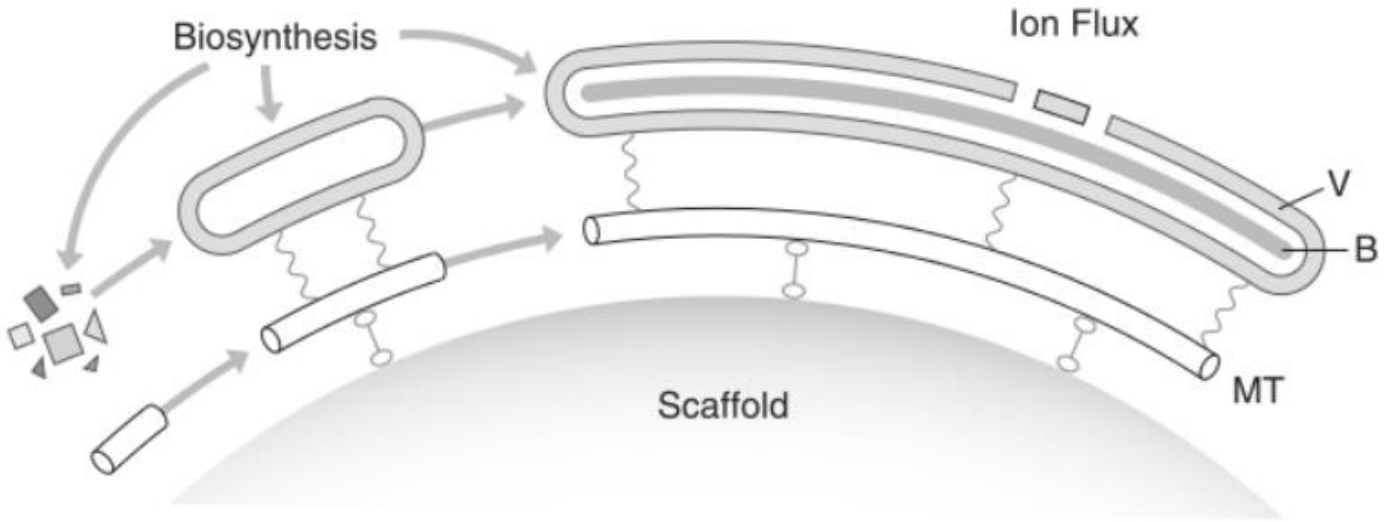


Fig. VI.10.
Cell walls, intracellular organelles, and cellular assemblages can act as scaffolds for the assembly of microtubules (MT), which in turn are used as directing agents for the patterning of vesicles (V) involved in biomineralization (B).

iv. Morphogenesis

2. Inorganic mineralization and vesicle shaping proceed in concert, with the inorganic layer forming steps behind the organic layer. After some time, the vesicle is no longer confined to an associated biological structure in the cell and more exotic structures can start to develop

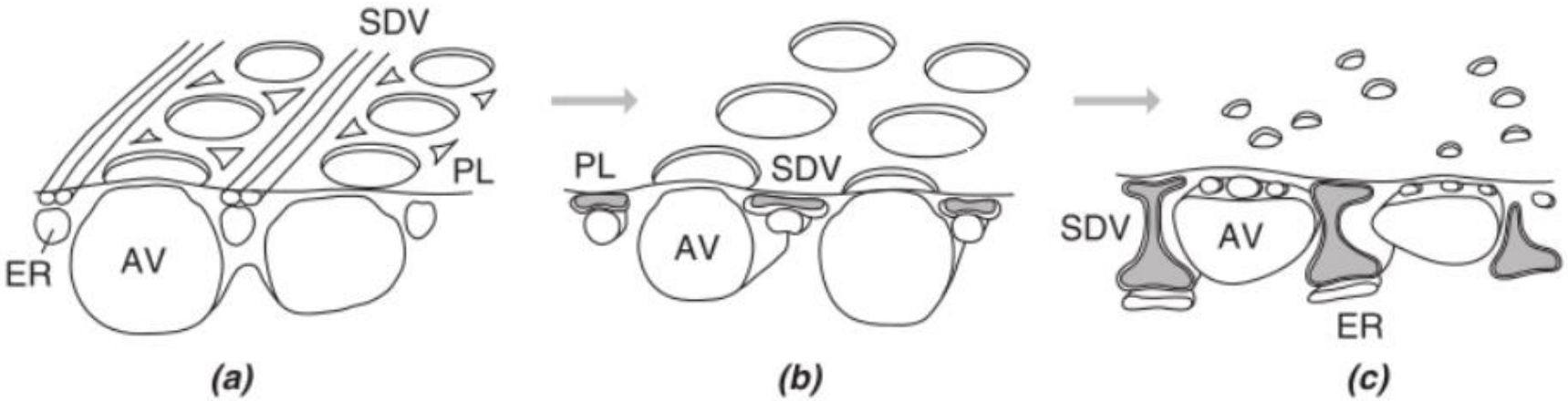


Fig. VI.11.
Illustration of the key stages in the formation of the siliceous diatom exoskeleton. (*a*) Silica deposition vesicles (SDV) are preorganized with microtubules around the boundary spaces of large areolar vesicles (AV) attached to the plasmalemma (PL). (*b*) The SDVs are mineralized with amorphous silica to give a patterned porous wall. (*c*) The mineralized wall is thickened by extension of each SDV in association with the endoplasmic reticulum (ER). In some diatoms, detachment and retraction of the areolar vesicles from the plasmalemma results in infiltration with new SDVs and further mineralization of the pore spaces.

v. Higher order assemblies

The higher order assemblies of many biomineral structures in micro- or macroscopically organized architecture come from the constructional mechanisms involved in cellular processing.

- These constructions can occur within or outside the cellular space or on the cell surface by sequential or concerted processes.

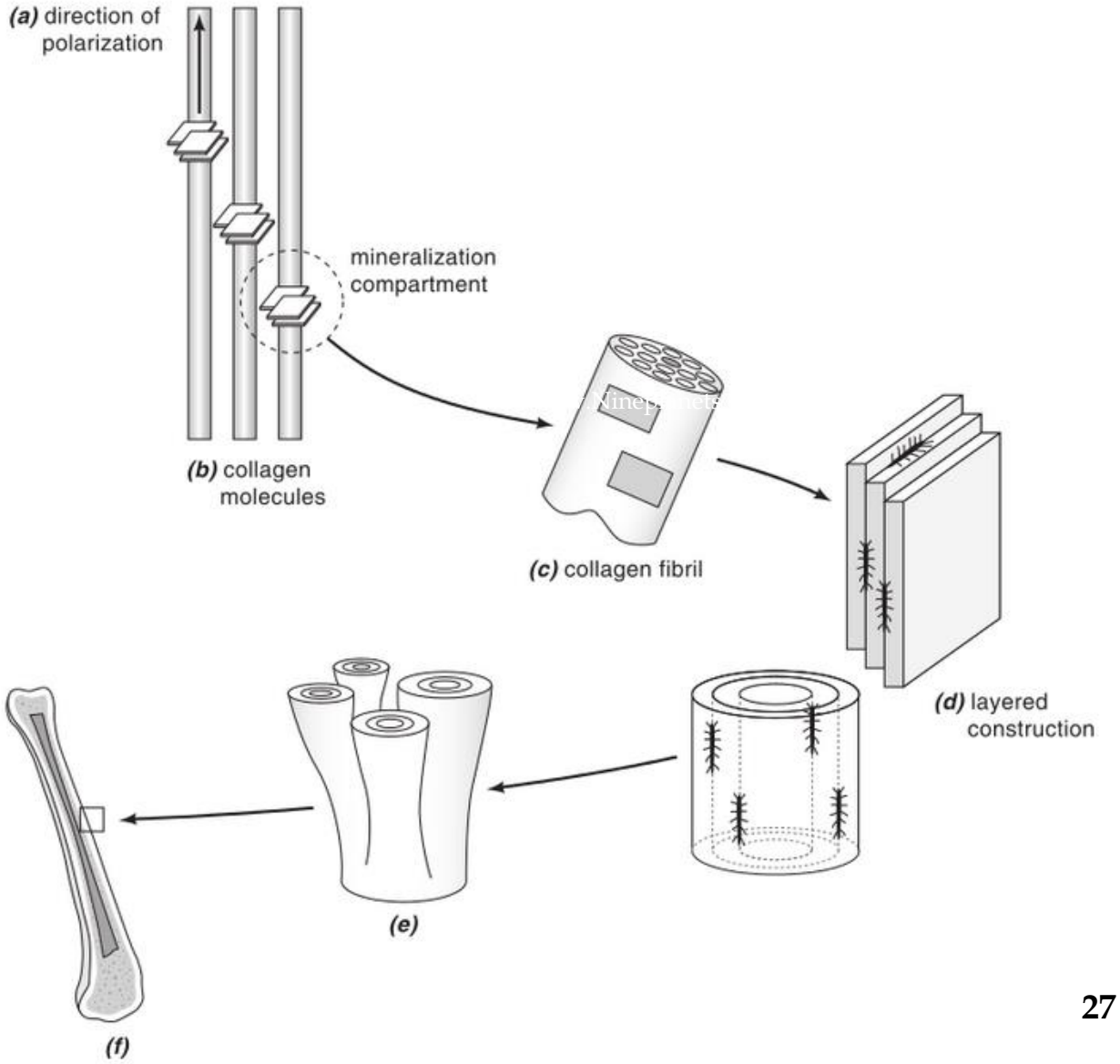
The macroscopic architecture of bones consist of a structural hierarchy:

a. The collagen molecules are arranged in parallel leading to an extended extracellular matrix

b. These collagen molecules are then transformed into a supramolecular structure with the help of specialized cells such as osteoblasts (bone formation) and osteoclasts (bone resorption)
   - The collagen fibrils are organized into sheets that are then layered into the osteon form
   - The osteons are arranged into various microstates (i.e. woven bone)
   - Cellular differentiation then dictates the macroscopic shape of the whole bone influenced by gravitational and mechanical force fields

The hierarchical structure of bone.

Fig. VI.12.
The hierarchical structure of bone. [Reprinted with permission from VCH-Wiley Publishers.]



6. Fossilization

6. Fossilization

-Joseph Castro's *How Do Fossils Form?* (www.livescience.com)

When organisms die they usually decay completely, unless the right conditions exist for preservation of remains as fossils.

1. Whole-body fossils
   - A very rare process that involves freezing, drying, and encasement (in tar or resin)
   - Fossilization as the organism was when living

6. Fossilization

2. Non-whole-body fossils

A. Carbonization

- Heat and pressure from burial in sediment causes tissues of organisms to release hydrogen and oxygen, and leave behind carbon
- A carbon impression of the dead organism

B. Permineralization

- After the soft tissues decays and the hard parts of the bodies remain, water covers the hard parts. Minerals in the water will fill into the free spaces of the hard parts and will crystallize (very much in a biomineralization process). These crystallized minerals harden the remains and make them encased in the sedimentary rock.

6. Fossilization

C. Replacement

- Imagine the permineralization process, but the hard parts of the organism dissolve. Therefore, the minerals from the groundwater replace the minerals from the hard parts.

- Fossils basically form from molds and casts and you are left with:

➢ External mold: Impression of the organism's exterior in the rock

➢ Internal mold: Minerals filling out a shell or skull of an organism.

7. The Bad of Biomineralization

When the mechanisms that regulate biomineralization are not under homeostatic control, biomineralized products can develop in excess.

Kidney stones are an example of this imbalance and typically stems from poor hydration or excess intake of salts.

7. The Bad of Biomineralization

Sometimes biomineralization leads to undesired side effects.

- The formation of tonsil stones (tonsilliths) is a major example of this. These are the calcification product of food debris caught in the pores of the tonsils. The formation of these stones are pronounced in people with excess biofilm population in their throats, which keeps debris caught in the area of the tonsils. They lead to halotosis.