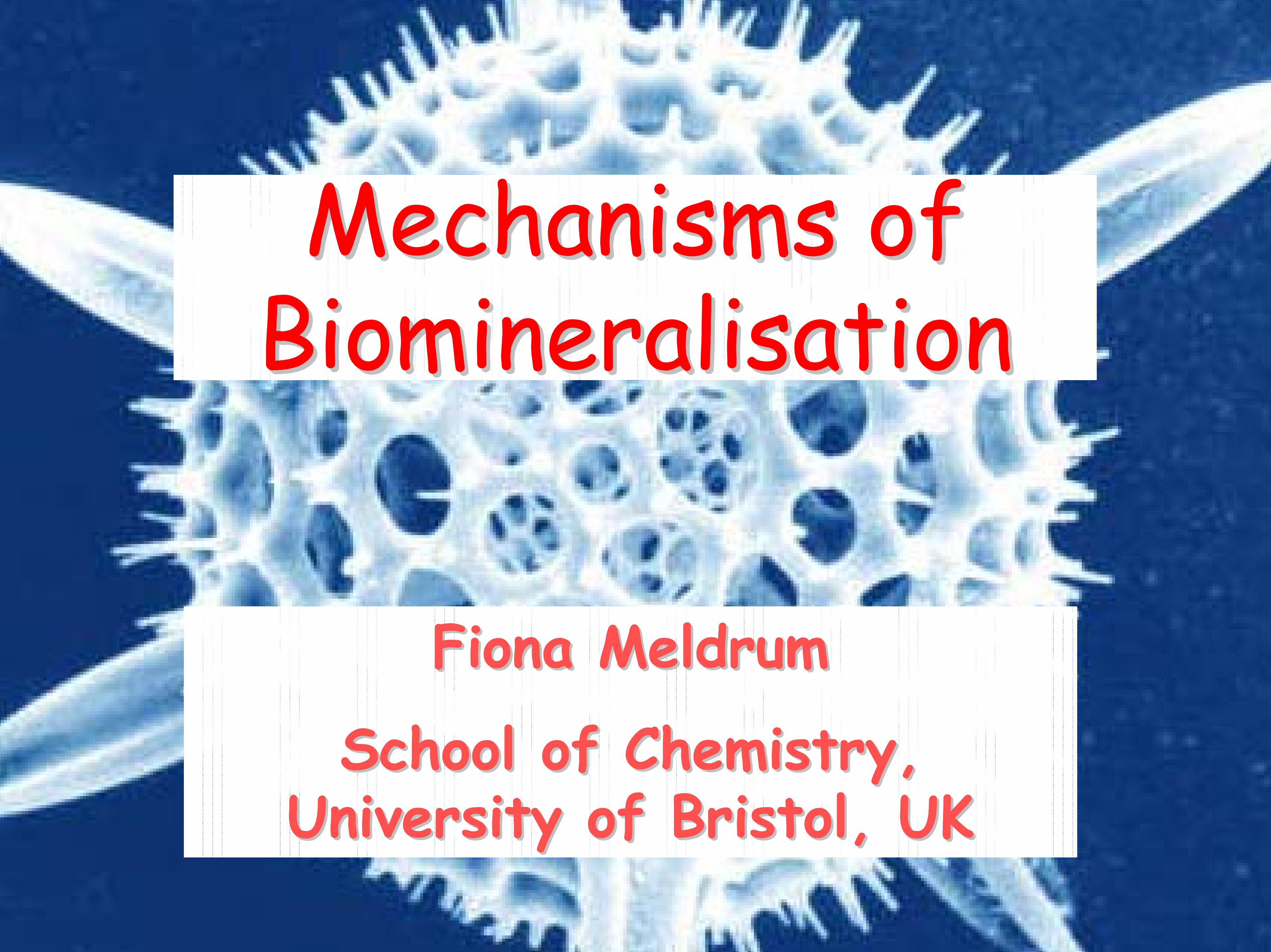


Mechanisms of Biomineralisation

Fiona Meldrum

School of Chemistry,
University of Bristol, UK

Biomineralisation

- Describes processes by which organisms form minerals

- Widespread phenomenon ⇒ over 60 different minerals formed, by members of all 5 kingdoms

Eg. Iron Oxides/Hydroxides:

Magnetite, Fe_3O_4	Bacteria Chitons	Intracellular Teeth	Magnetotaxis Grinding
Goethite α-Fe_2O_3	Limpets	Teeth	Grinding
Lepidocrocite γ-FeOOH	Sponges Chitons	Filaments Teeth	Unknown Grinding
Ferrihydrite $5Fe_2O_3.9H_2O$	Animals/plants Beaver/ rat/ fish	Ferritin Tooth surface	Storage protein Mechanical strength

- 80% of biominerals crystalline, the rest are amorphous

Characteristic Features of Biominerals:

- Display remarkable and unique morphologies

- Are often hierarchically organised on a scale from Angstroms to millimeters.

- Are typically composite materials - intimately associated with organic macromolecules

Show Properties Optimised for Their Function

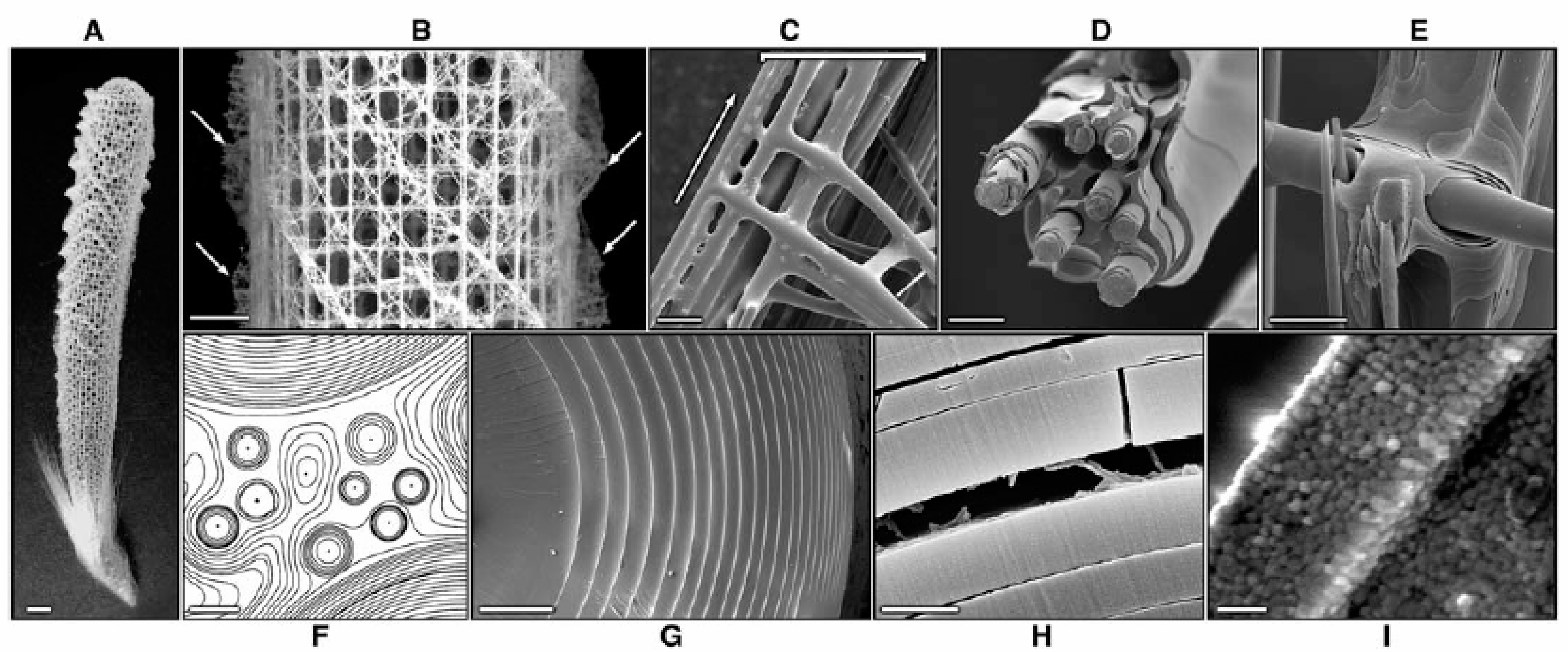
⇒ Skeletal materials show remarkable mechanical properties

⇒ Magnetotactic bacteria exhibit permanent dipole moment

⇒ Ferritin catalyses the formation of a soluble iron oxyhydroxide core within a soluble protein shell

⇒ Single crystal calcite plates in brittle stars can act as lenses

Hierarchical Structures.. Silicaceous Sponge *Euplectella sp.*



Shows seven hierarchical levels ... Outstanding mechanical stability

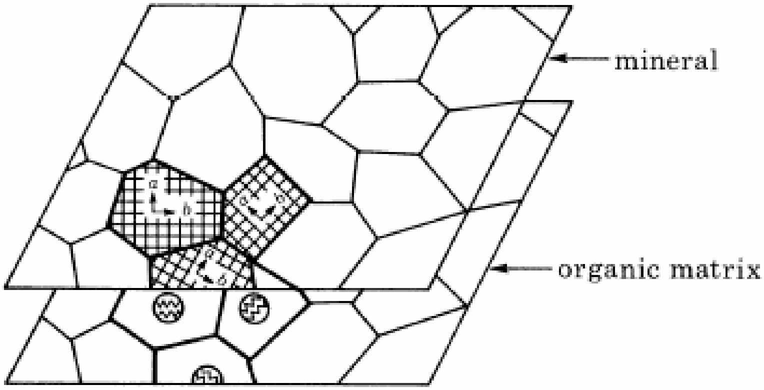
Aizenberg, Weaver, Thanawala, Sundar, Morse, Fratz, *Science*, (2005) 309, 275-278.

Mechanical Properties of Mollusk Shell Nacre

Mollusk shell nacre shows superior mechanical properties – why ?

⇒ Due to structural organisation of nacre

- Comprises stacks of tabular aragonite crystals
- Separated by interlamellar organic sheets



c-axis oriented perpendicular to shell surface, organisation of *a*- and *b*- axes depends on organism

Mechanical Properties of Mollusk Shell Nacre

Nacre has a very low organic content (≈ 1%), yet is superior to most other composite ceramics in stiffness, strength and toughness

⇒ 3000 times more resistant to fracture than a single crystal of pure aragonite

Due to structural organisation of inorganic phase, and the nature of the organic sheets

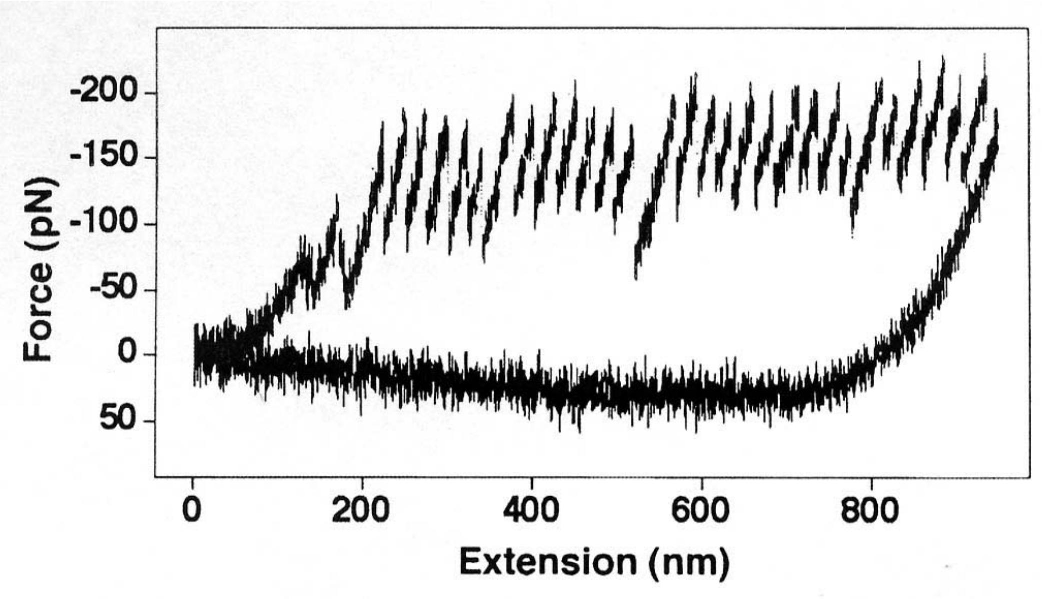
⇒ When a crack propagates through nacre it passes around the platelets by a tortuous path, causing the plates to spring apart, and extending the organic sheets

⇒ The organic “adhesive” between the plates appears to be key in the fracture resistance of this material.

AFM Investigation into Mechanical Properties of Nacre

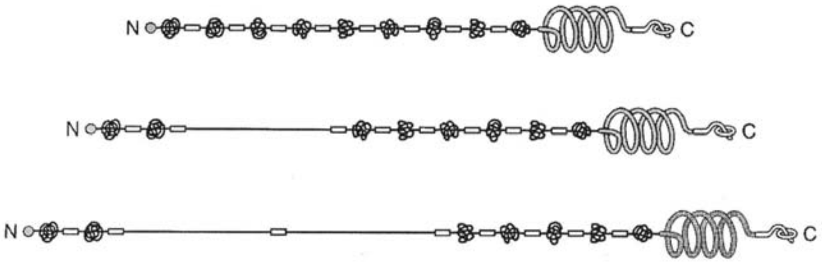
- The organic molecules located between the aragonite plates were stretched using an AFM tip

- The force required to stretch the fibres is measured as a function of the extension

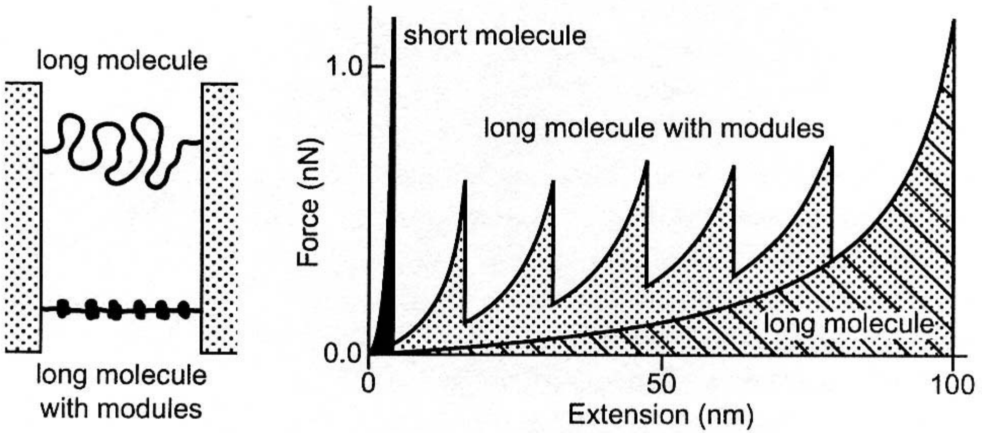


Shows a SAWTOOTH profile

Behaviour can be explained by the organic molecules containing folded domains

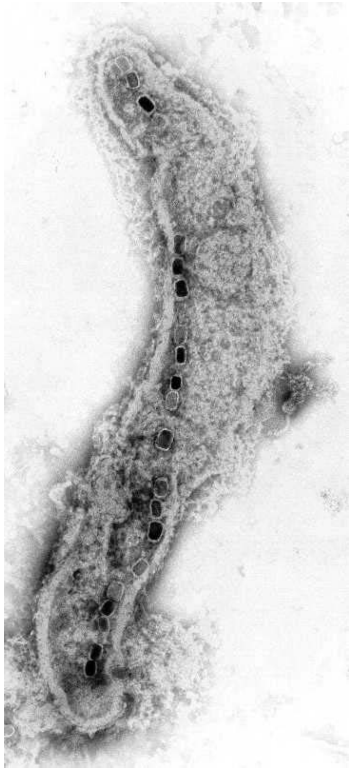


- When the fibres are pulled, the folded domains are extended
- The force required to unfold these areas is less that the force required to break the fibre

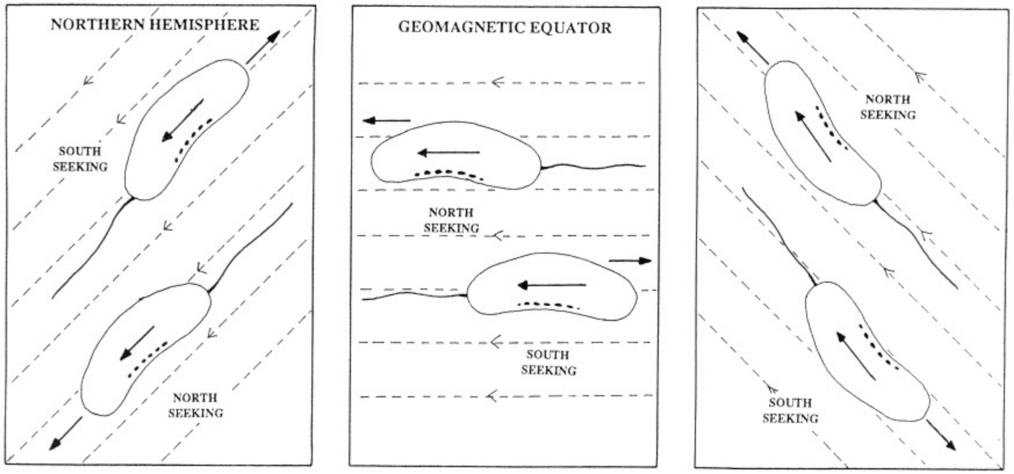


Smith et al, *Nature*, (1999), 399, 761-763;
BL Smith, *Prog. Biophys. Molec Biol.* (2000) 74, 93-113.

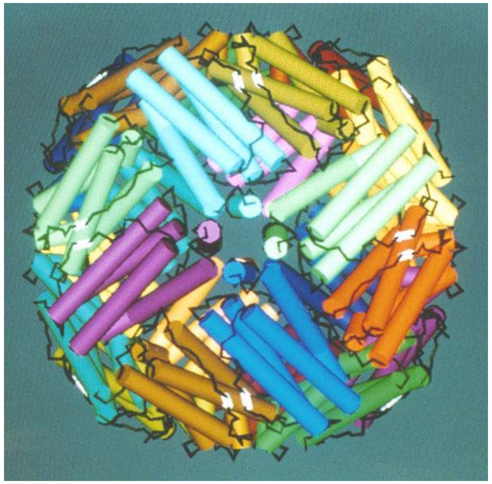
Magnetotactic Bacteria

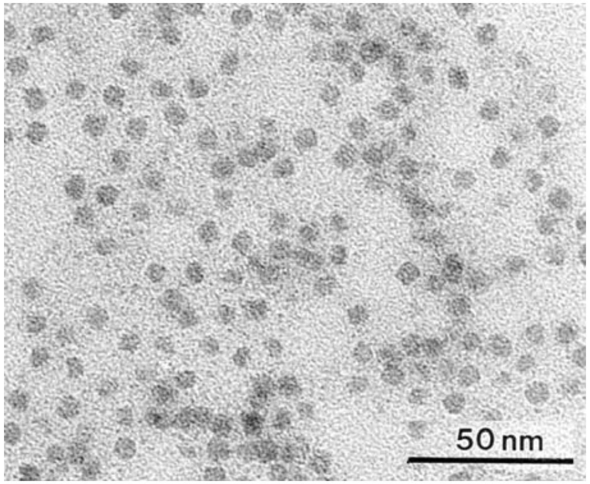


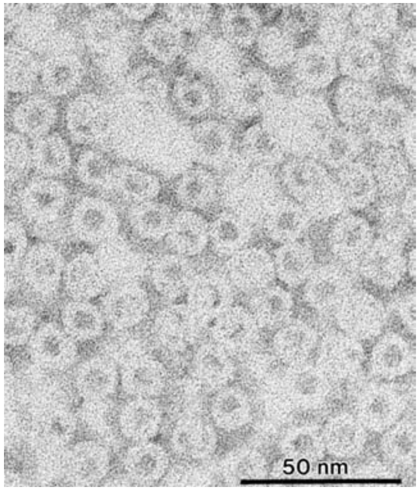
- Single crystals of magnetite are used by bacteria, algae and many animals for navigation
- In magnetotactic bacteria, single domain crystals of magnetite (Fe_3O_4) are aligned along the bacterium
- The crystals are elongated to optimise the magnetic dipole

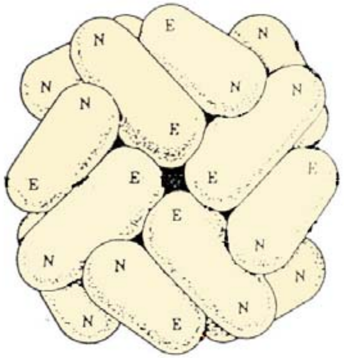


Structure and Function of Ferritin









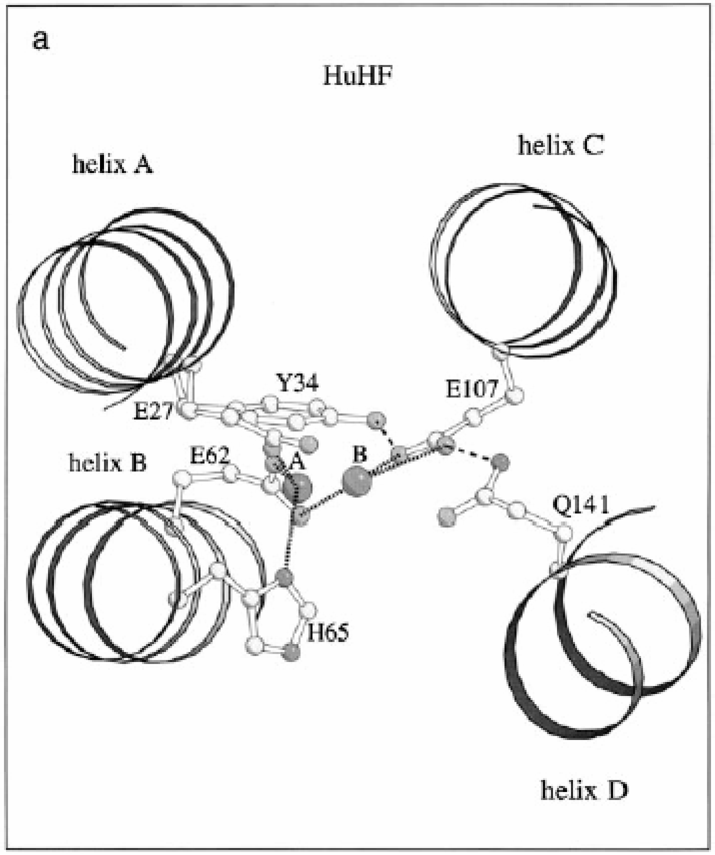
- Large storage capacity for iron ⇒ up to 4500 Fe atoms
- The core is ferrihydrite, 5 $Fe_2O_3.9H_2O$ ≈ 8 nm in diameter ⇒ a poorly crystalline mineral
- The spherical protein shell is 12 nm in diameter and is formed from 24 protein subunits, of types H-chain and L-chain

Ferritin behaves catalytically towards the oxidation of Fe(II) and nucleation of iron oxide due to two key sites:

⇒ a ferroxidase centre located in the intrahelical area of H-chain subunits

⇒ a nucleation site comprising either four Glu residues on the cavity surface (L chain) or two Glu residues (H chain)

These sites appear to act cooperatively in affecting the kinetics of iron oxide deposition within the protein.

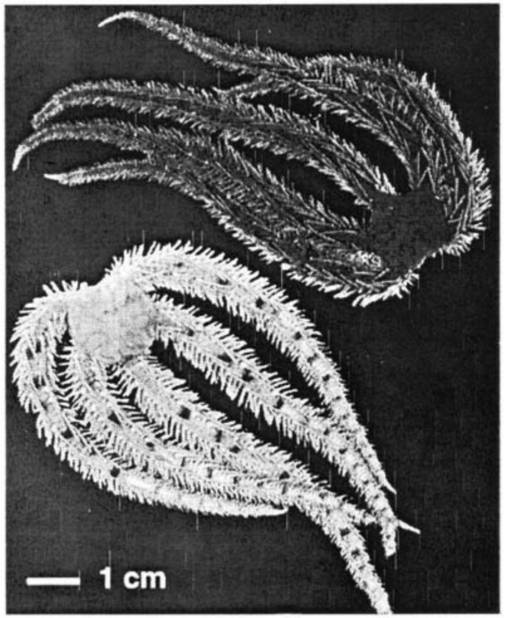


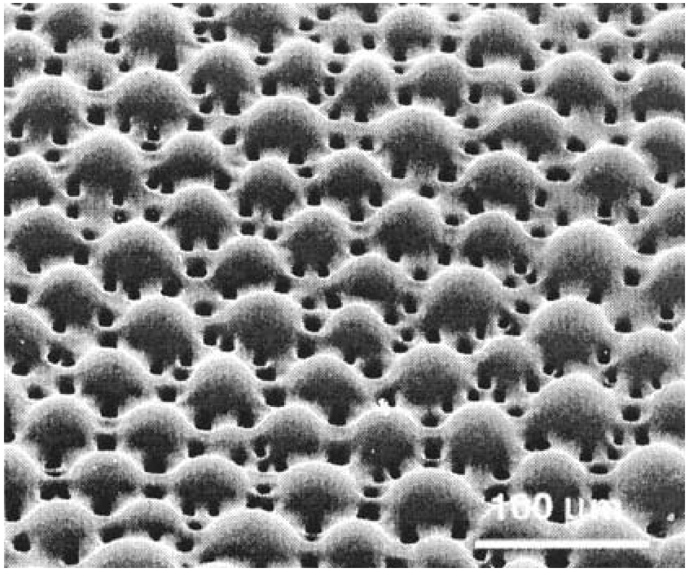
Oxidation site binds 2 Fe^{3+} ions

Chasteen and Harrison *J. Struct. Biol.* 126, 182-194 (1999)

Using the Optical Properties of Calcite

Certain species of brittle stars are light sensitive – why ?

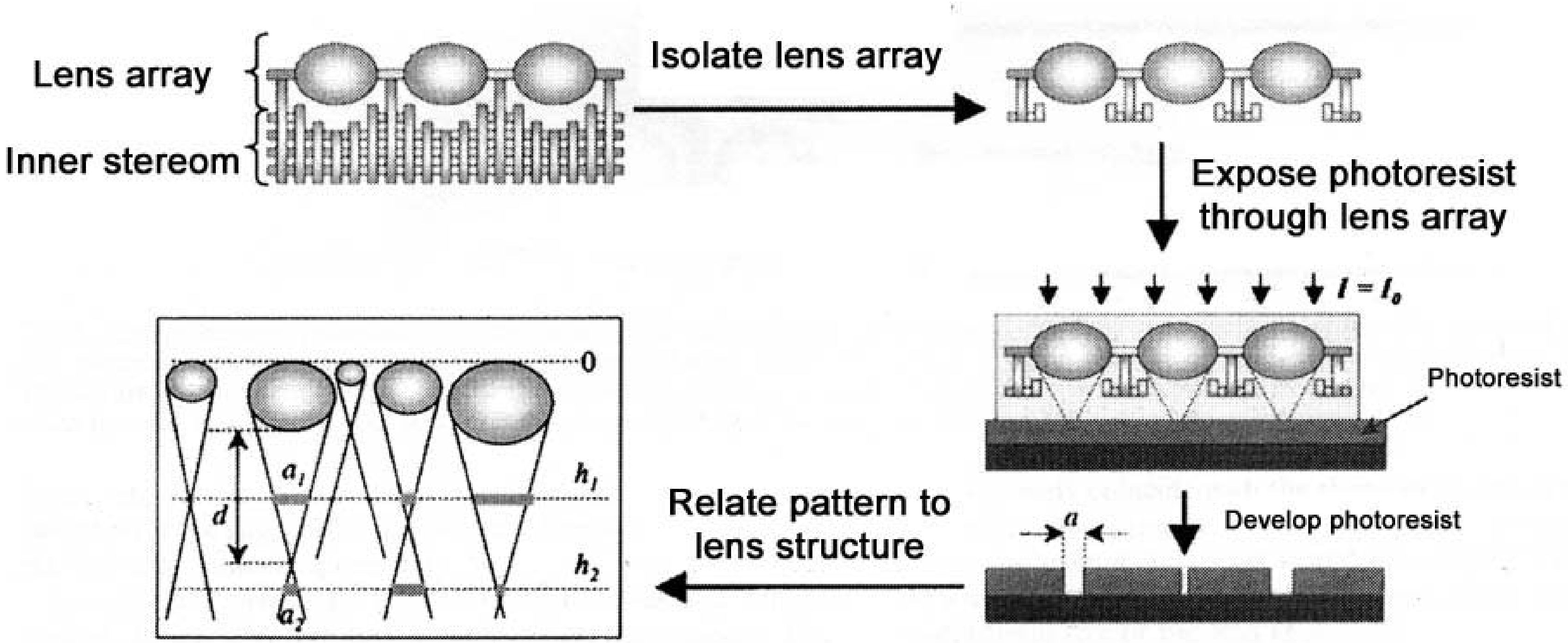




Looking at the structure of the skeletal elements of the brittle star, certain parts **showed a unique structure**

Aizenberg and Hendler, *J. Mater. Chem* 2004, 14, 2066-2072.

Testing the Optical Behaviour…



These structures behave as an array of micro-lenses, significantly enhancing the light intensity, and directing it within the animal

Bio-Inspired Crystal Growth

Synthesis of many "advanced materials" require high temperatures and pressures

In comparison, biology operates under ambient conditions

⇒ achieves a degree of control over mineralisation that is (as yet!) difficult to reproduce synthetically

Use of ambient conditions is very attractive:

- Can employ **heat-sensitive starting materials**
- Can prepare **heat-sensitive products**
- Cheaper

Ultimate goal of synthetic crystal growth must be to achieve the degree of control exhibited by nature

⇒ Many lessons can be learned from biology

Biomineralisation Processes

Biologically Induced Mineralisation

Adventitious mineralisation due to interactions between metabolic processes and environment

⇒ No control over size, morphology, structure and organisation

eg. mineralisation on bacteria cell walls

Biologically Controlled Mineralisation

Highly regulated process that has evolved to produce minerals with specific structures and functions. Characterised by:

- Uniform particle sizes
- Complex morphologies
- Well-defined structures and organisation
- Higher order assembly into hierarchical structures

$CaCO_3$ Polymorphism

- Precipitates in 5 crystalline structures, and one amorphous form.

- Crystalline anhydrous polymorphs:

Calcite ⇒ most stable at RTP

Aragonite ⇒ slightly less stable at RTP than calcite

Vaterite ⇒ Less stable and quite rare

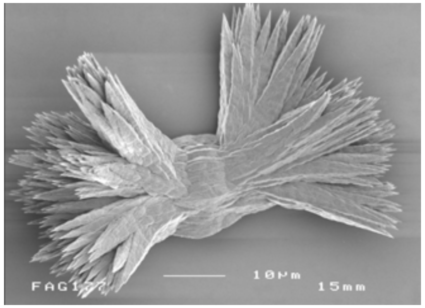
- Crystalline hydrous polymorphs

$CaCO_3$ Monohydrate

$CaCO_3$ Hexahydrate

Amorphous Calcium Carbonate (ACC) has recently also been recognised as an important biogenic mineral

Crystal Structures



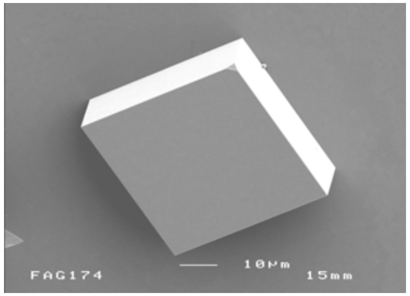
Aragonite and calcite have similar crystal structures:

Calcite ⇒ rhombohedral (hexagonal) unit cell

Aragonite ⇒ orthorhombic

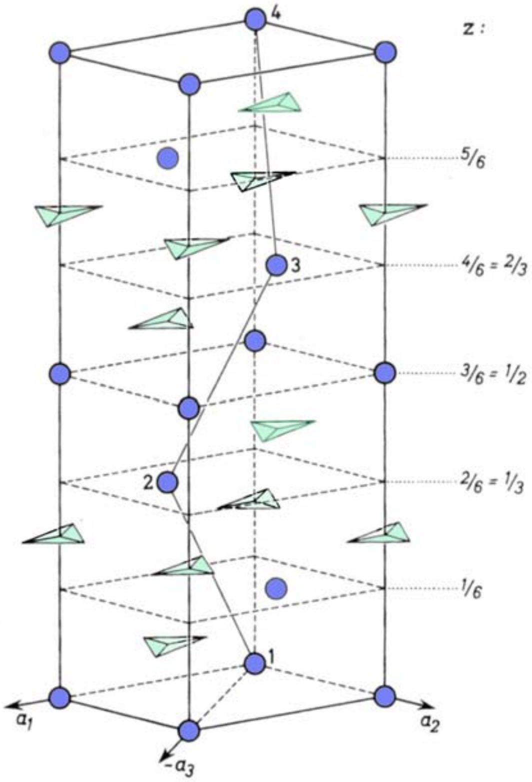
- Interactions are optimised in aragonite, giving better packing ⇒ tends to form as needles as crystal growth is preferred along the *c* axis

- Aragonite is more resistant to fracture as it has no cleavage planes. However crystals are small, needle-shaped and tend to form spherulitic clusters.

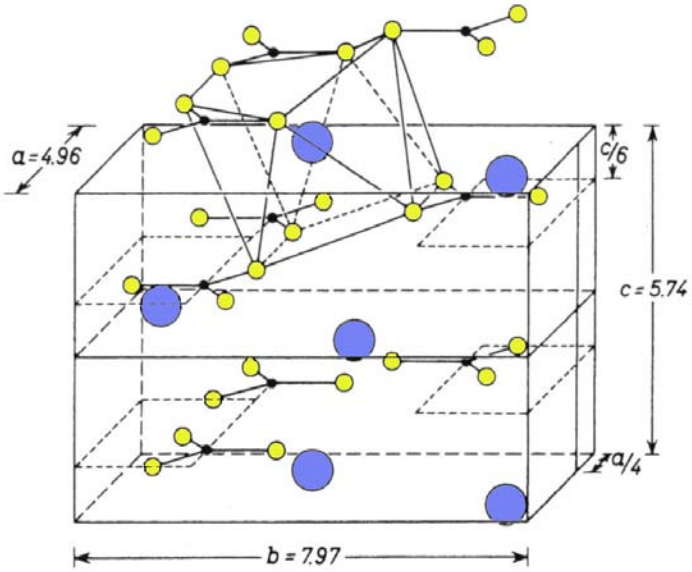


- Synthetic calcite grows as rhombohedra exhibiting planar {10.4} faces.

- Calcite forms large crystals but fractures readily.



Calcite



Aragonite

- Both have alternating layers of Ca^{2+} and CO$_3^{2-}$ ions perpendicular to the *c*-axis (the *ab* plane).

- The Ca ions occupy very similar lattice positions in the *ab* plane

- The carbonate ions lie with their molecular planes parallel to the *ab* layer.

- In aragonite, some of the carbonate ions are raised in the *c* direction to form 2 layers separated by 0.96 Å, and their orientations in the two layers are different ⇒ main difference between the two structures

Control Mechanisms ⇒ Organic Macromolecules

Strategies to control mineralisation rely on organic molecules:

- Confining a space
- Forming an organic matrix framework
- Controlling ion input
- Constructing a nucleation site
- Controlling crystal orientation and growth
- Terminating crystal growth

Can categorise organic macromolecules as:

- Insoluble matrix molecules, or
- Soluble “control” macromolecules

Framework Macromolecules

- Controlled crystal growth in organisms occurs within confined spaces

⇒ Fabricated from framework macromolecules

⇒ Usually relatively hydrophobic, are often cross-linked and provide a structured matrix

eg. collagen in bone, chitin in crustaceans and mollusks

- Organic framework often further functionalised with soluble macromolecules

Growth of crystals within a RIGID COMPARTMENT is an important factor in controlling CRYSTAL MORPHOLOGIES

Control Macromolecules

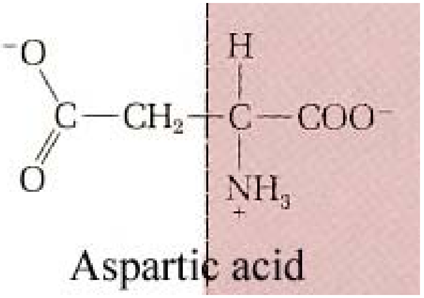
Can control crystal growth ⇒ Bound to a solid substrate

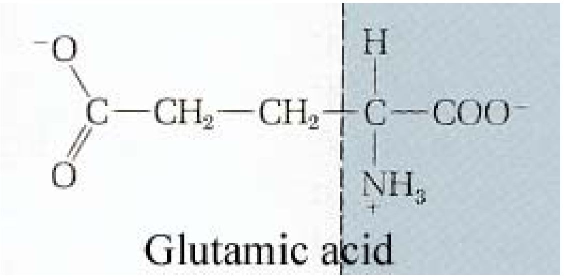
⇒ As soluble additives

Located within minerals, extracted by dissolution of mineral

Control macromolecules from $CaCO_3$

Typically rich in aspartic acid and glutamic acid, frequently contain bound polysaccharides





Anionic groups ⇒ can interact with cations in solution eg. Ca^{2+}

⇒ can interact with the surfaces of crystals

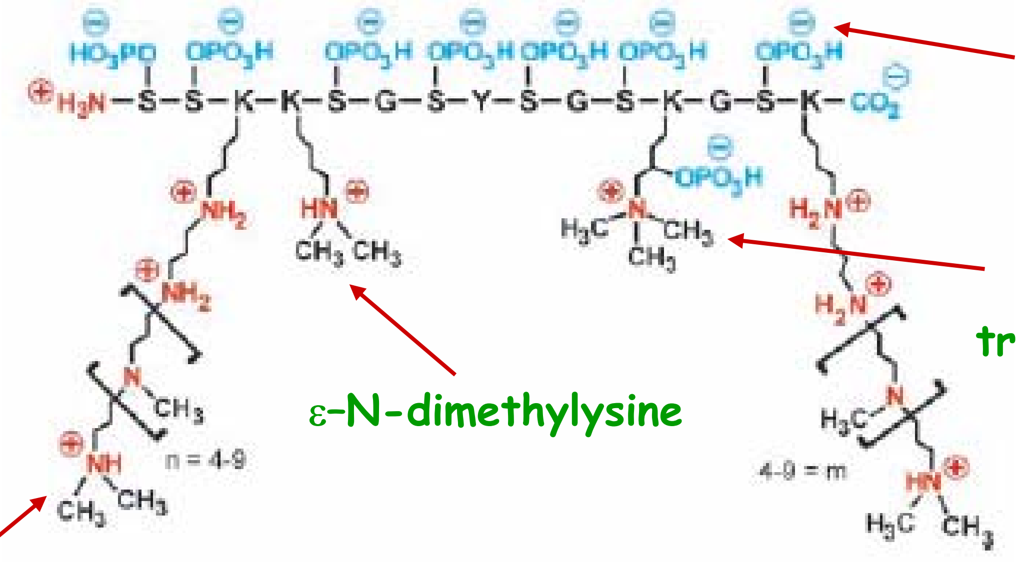
Control Macromolecules from Diatom Silica

Dissolution of silica with HF or NH_4F yields:

⇒ Set of low molecular mass proteins: SILAFINS

⇒ Large quantities long chain polyamides

Silaffins-1A and -1B isolated from *Cylindrotheca fusiformis* are polycationic and contain repeated pairs of lysine residues



serine groups are phosphorylated

phosphorylated ε–N-trimethyl–δ–hydroxylysine

ε–N-dimethylysine

long-chain polyamines comprising 6 to 11 units of N-methyl-propylamine

Structure of natSil-1A

Kroger, Lorenz, Brunner, Sumper, *Science*, 2002, 298, 584-586

A second silaffin protein, termed silaffin-2 has also been extracted from *C. fusiformis*

⇒ POLYANIONIC and also bears unusual amino acid modifications

Role of Silaffins and Long Chain Amines

- NatSil-1A and the long-chain polyamines are extremely active in promoting silica precipitation *in vitro*

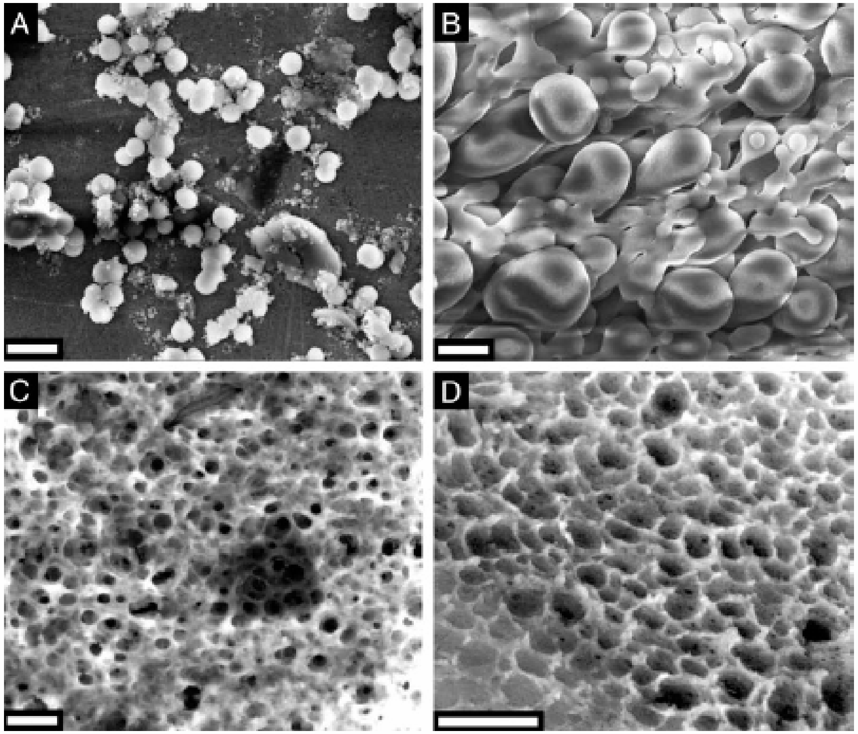
- NatSil-2 alone does not precipitate silica from a silicic acid solution *in vitro*, but is active in combination with long-chain polyamines

⇒ rapidly precipitates silica under conditions where neither of these organic components would do so alone

NatSil-2 may regulate the silica precipitation behaviour of long-chain polyamines and natSil-1A and may be active in silica morphogenesis.

Low
natSil-1A /natSil-2

High
natSil-1A /natSil-2



Intermediate
natSil-1A /natSil-2

Intermediate
natSil-1A /natSil-2

- Large interconnected spherical or pear-shaped silica particles were produced with low or high natSil-1A/natSil-2 ratios,

- Intermediate ratios yielded porous silica blocks permeated with 0.1-1.0 μm pores.

- Assembly of the organic phase in the presence of polysilicic acid may provide a template for the ultimate form of the mineral phase

Poulsen, Sumper and Kroger PNAS, 2003, 100(21), 12075-12080

Crystal Structure

Organisms actively select mineral phases ⇒ frequently produce minerals highly undersaturated or unstable in environment

Can be achieved through:

- Presence of ion-specific pumps and channels
- Control of composition and pH of solution
- Interaction with organic matrix and soluble organic macromolecules

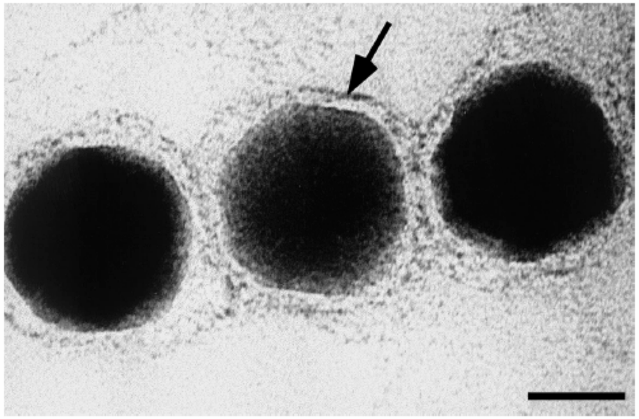
eg. formation of ferrihydrite core, $5Fe_2O_3.9H_2O$ in ferritin

⇒ In absence of protein, lepidocrocite is precipitated under identical conditions

eg. formation of magnetite particles, Fe_3O_4 in magnetotactic bacteria

Magnetite Precipitation in Magnetotactic Bacteria

- Magnetite crystals are formed within separate vesicles termed magnetosomes



- The magnetosome membrane contains at least one major protein which appears common to all strains.

⇒ this protein may function in the accumulation of iron, nucleation of the iron oxides and in redox and pH control

- Magnetosome vesicles form prior to magnetite formation

Schueler and Frankel, *Appl Microbiol Biotechnol* (1999) 52: 464-473

- Mössbauer and HRTEM studies have suggested the magnetite crystals may not precipitate directly

⇒ form via low-density hydrous Fe(III) oxide and ferrihydrite precursors.

Proposed that:

1) Fe(III) is taken up by the cell, where it is reduced to Fe(II)

2) Subsequent re-oxidation within the magnetosome yields a low-density hydrous Fe(III) oxide which is dehydrated to form crystalline ferrihydrite.

3) Finally, partial reduction and dehydration yields magnetite

Recent experimental evidence has indicated that in *M. gryphiswaldense*, Fe(III) is taken up and rapidly converted into Fe_3O_4 in the absence of a precursor phase.

Calcite/ Aragonite Polymorphism

No examples of transformation between calcite and aragonite after precipitation ⇒ selection must occur at nucleation

Good evidence that soluble macromolecules are involved in the selection of calcite or aragonite

Control of $CaCO_3$ Polymorphism by Soluble Mollusk-Shell Proteins

- $CaCO_3$ precipitated on abalone nucleating protein sheet

- Proteins extracted from aragonitic, or calcitic layer of the shell

- Spherulitic calcite crystals were produced in the presence of the calcite-derived proteins

- Aragonite crystals with needle morphologies and oriented in the plane of the nucleating protein layer were produced in the presence of the aragonite-derived proteins

- Mixture of aragonitic and calcitic proteins induced precipitation of flat, polycrystalline plates of aragonite, oriented on (001) faces

⇒ comprised oriented stacks of crystals, similar to molluscan nacre

Calcification in Abalone Shell Nacre..

⇒ Nucleating protein sheet controlled the orientation of the calcite primer layer

⇒ Insoluble proteins of the matrix define the plate thicknesses

⇒ Soluble proteins are then active in controlling further aspects of shell growth such as crystal polymorph and morphology

Belcher, Wu, Christensen, Hansma, Stucky, Morse, *Nature,* (1996), 381, 56-58

Polymorph Selection on Artificial Molluscan Matrix

The organic matrix in mollusc nacre mimicked using layers of β-chitin and silk fibroin and soluble macromolecules extracted either from the calcitic or aragonitic layers of mollusc shells

⇒ Calcite precipitated inside the chitin when calcitic macromolecules were used

⇒ Aragonite precipitated when the macromolecules had been extracted from an aragonite layer

The specificity of these macromolecules was only achieved with the complete substrate assembly.

Falini, Albeck, Weiner, Addadi, Science, 1996, 271, 67-69

- Polymorph selectivity appears to rely on both specialised macromolecules and a controlled microenvironment.

- The β-chitin framework has a porous layered structure, allowing diffusion of ions and macromolecules into the structure

- The silk is essential for the adsorption of the macromolecules

- The macromolecules promoting calcite nucleation are strongly polyanionic and more strongly acidic than aragonite-inducing ones

⇒ May provide a strong binding site for Ca^{2+} ions, creating a high local supersaturation

Levi, Albeck, Brack, Weiner, Addadi, *Chem. Eur. J.* (1998), 4(3), 389-396.

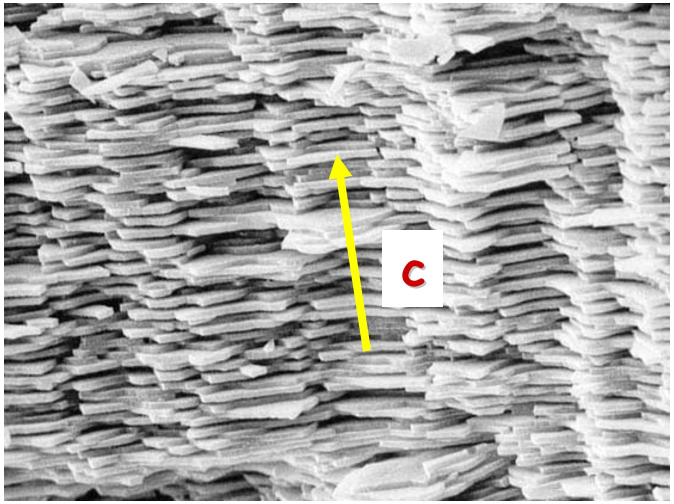
Crystal Orientation

There are many examples of oriented crystals in nature

⇒ Control over orientation must occur at nucleation, on a substrate with structural order (or maybe not !)

⇒ IDEAS OF EPITAXIAL GROWTH

Mollusk shell nacre ⇒ much studied system



Abalone nacre

⇒ comprises stacks of aligned tabular aragonite crystals which are separated by organic sheets

All species c-axis perpendicular to shell surface

Structure of Nacre

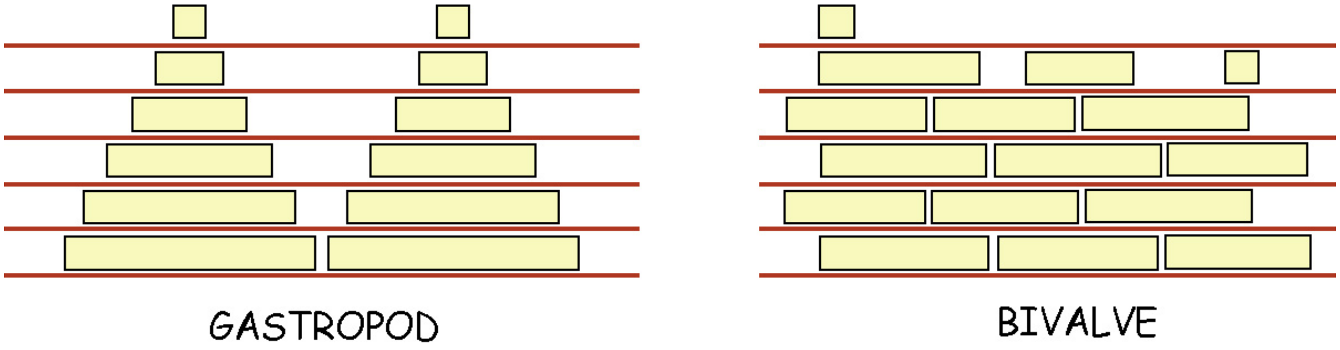
Mollusk shell is characteristically layered, and is composed of calcite, aragonite or both

⇒ When both, the polymorphs are separated into different layers

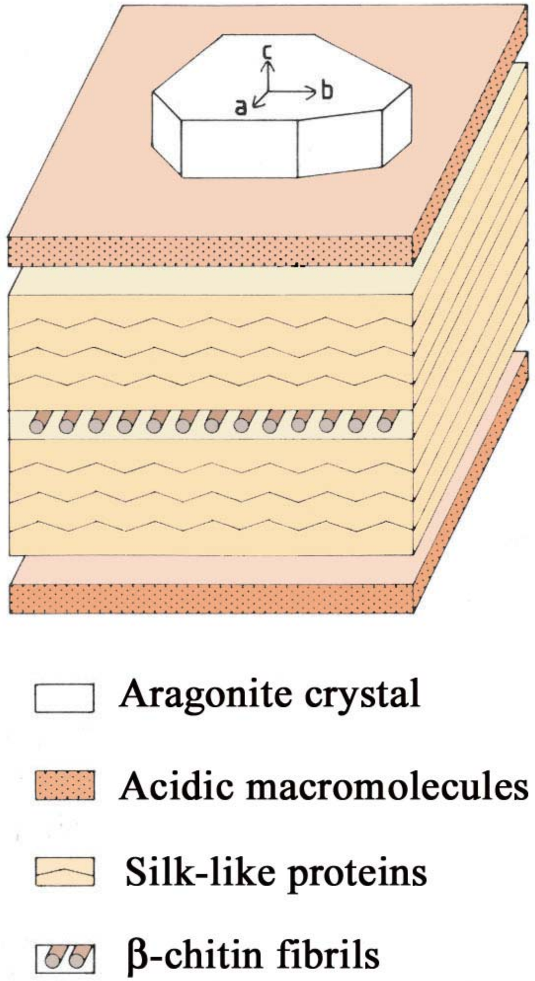
Mollusks form wide range of shell structures

- Gastropod nacre ⇒ no preferred alignment of a- and b- axes within layer. Aragonite crystals in “stack of coins” structure

- Bivalve nacre ⇒ typically good/ excellent alignment of a- and b- axes over large area. Has “brick wall” structure



Control over Nucleation in Mollusk Shell Nacre (Bivalve) - Old Model



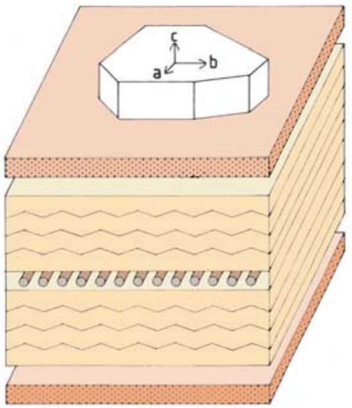
- Aragonite crystals nucleate at specific sites on a pre-deposited matrix

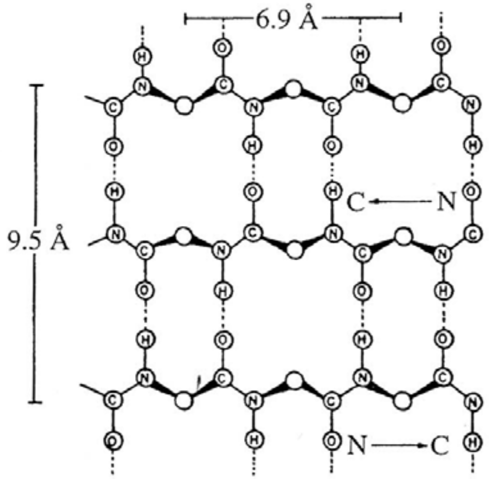
- The organic matrix comprises thin layers of highly ordered β-chitin, sandwiched between two thicker layers of silk fibroin-like proteins in a β-sheet conformation, onto which acidic macromolecules are adsorbed.

- The chitin polymers and the protein-polypeptide chains are orthogonally aligned and are aligned with the *a* and *b* axes of the aragonite tablets respectively.

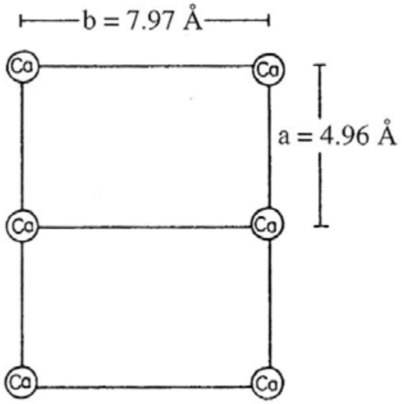
Weiner and Traub, *Phil. Trans. R. Soc. Lond. B*, 1984, 304, 425-434.

Epitaxial Growth

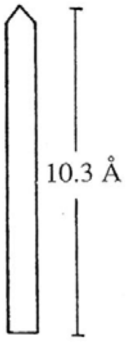




Antiparallel β-sheet of silk-like protein



a,b plane of aragonite



β-chitin fibre direction

- **Binding of Ca^{2+} ions to the oriented proteins could mimic the arrangement of ions in the *ab* face of the growing aragonite crystal**

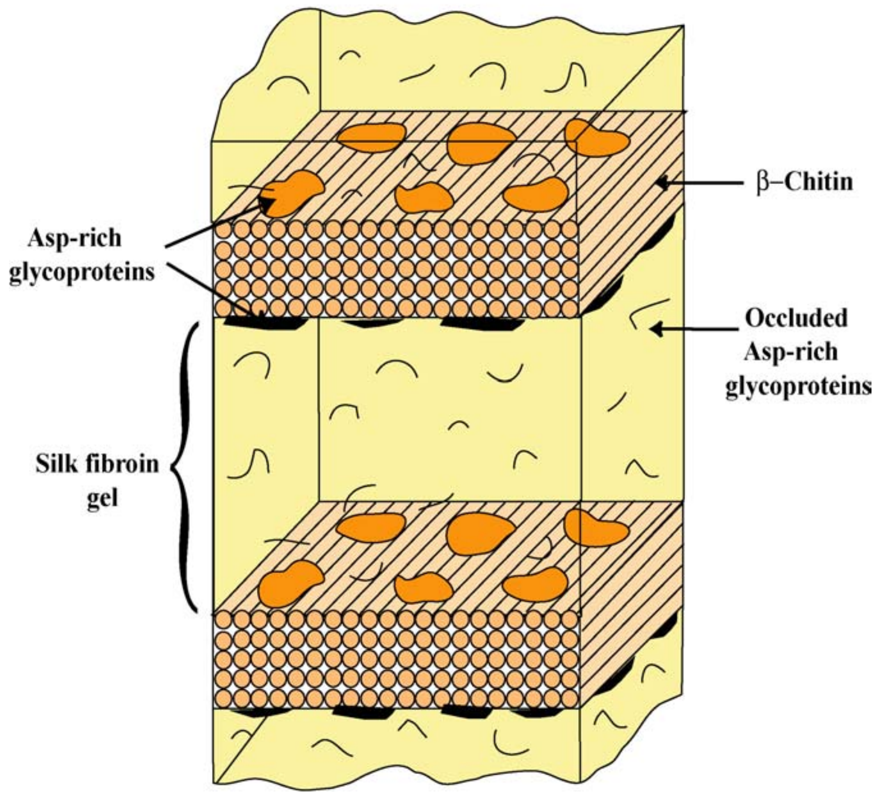
⇒ **causes the crystal to nucleate from the *ab* face**

This structural model was principally developed from TEM and X-ray diffraction analyses of dried samples

Weiner and Traub, *FEBS Letts*, 1980, 111, 311-316.

New Model

Cryo-TEM of mollusk nacreous layer in the hydrated state has suggested an alternative model



- The silk may be in the form of a hydrated gel, located between, rather than within the sheets of chitin fibrils

- The acidic macromolecules may be situated in localised areas on the surfaces of the chitin layers which act as nucleation sites for the aragonite crystals, as well as within the silk gel

⇒ Contradicts many of the previous ideas on oriented crystal growth in this organism

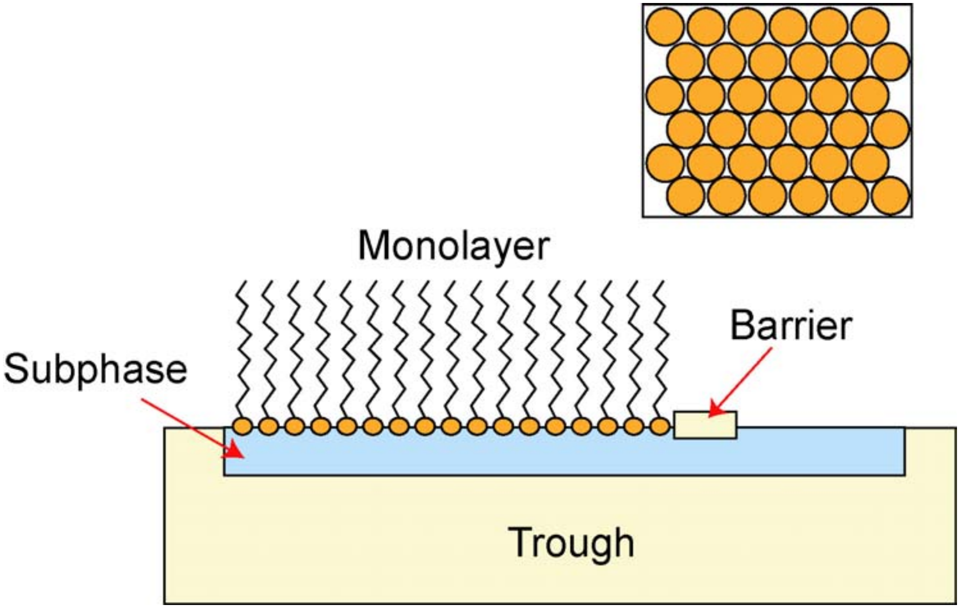
Levi-Kalisman et al *J Struc. Biol.* 2001, 135, 8-17.

Epitaxial Growth – an Outdated Theory ?

It is difficult to study oriented crystal growth directly in biological systems (very complicated!)

⇒ Use a MODEL system

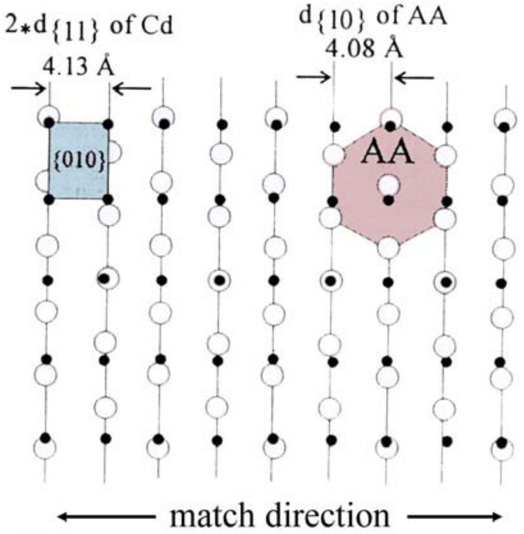
An excellent model system in which to study crystallization at an organised organic interface is provided by Langmuir monolayers

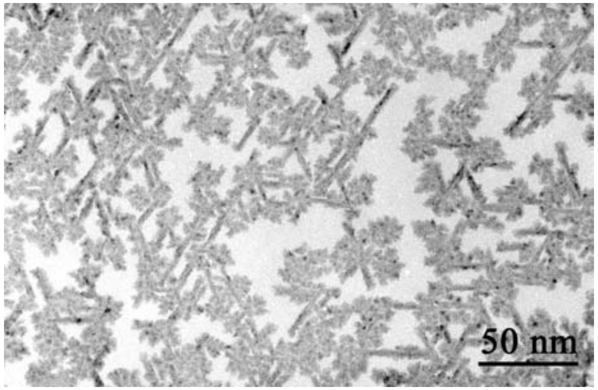


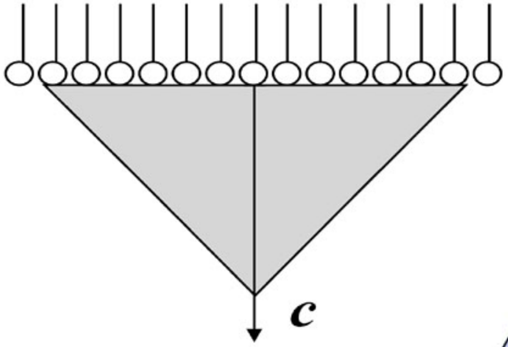
In has been suggested that the orientation and crystal polymorph are directed by the:

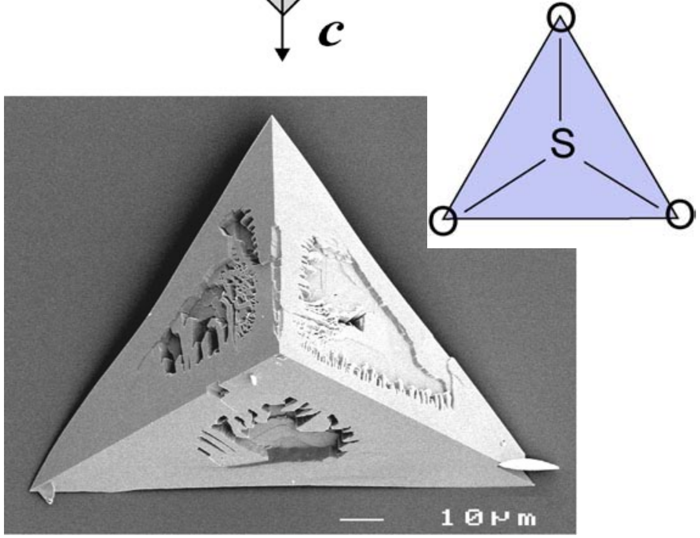
- Spacing and geometry of the surfactant headgroups
- Stereochemistry of the monolayer headgroups

CdS









$CaCO_3$

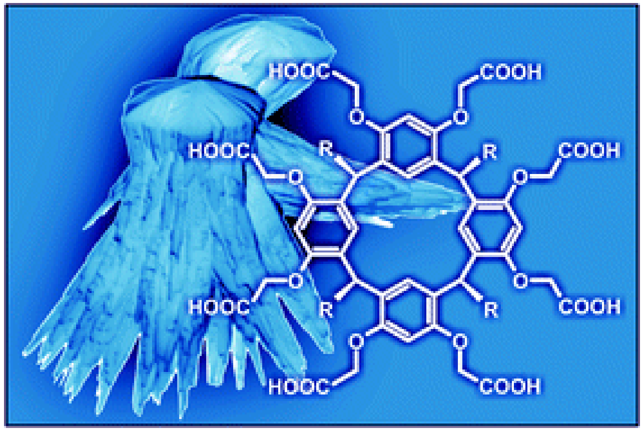
However ….

- Many examples where monolayers with entirely different structures induce nucleation from IDENTICAL crystal faces

- Studies of $CaCO_3$ precipitation under monolayers of derivatised calixarenes has suggested that oriented nucleation may be controlled by:

NON-SPECIFIC ELECTROSTATIC EFFECTS

- Results suggest that it is the density of Ca^{2+} ions associated with the monolayer which defines the polymorph and nucleation face

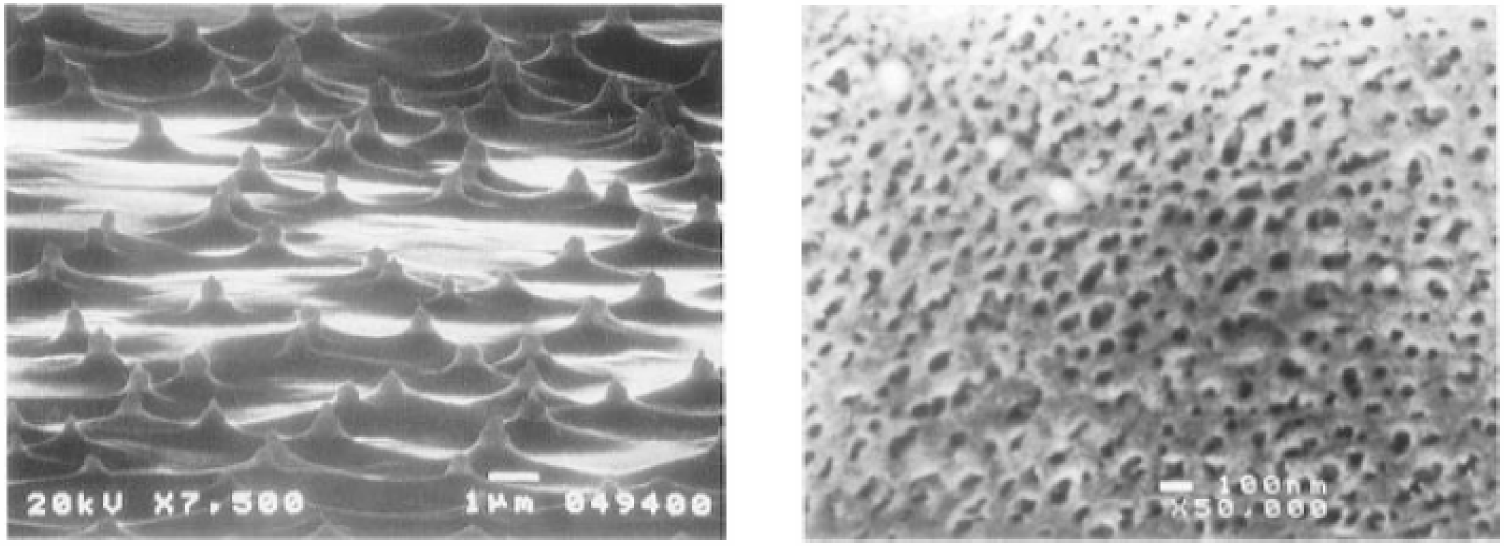


Volkmer et al *J. Mat. Chem*. 2004, 14, 2249-2259.

Orientation Control in Abalone Nacre

Examination of growth surface of abalone nacre suggests that successive layers form via MINERAL BRIDGES

⇒ Bridges grow through pores in interlamellar organic sheets

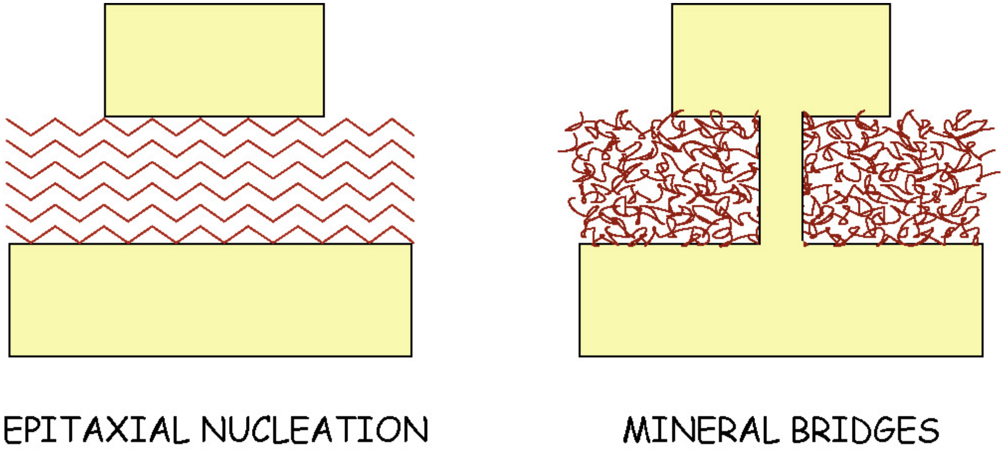


Growth of oriented stacks of aragonite crystals is due to mineral bridges between aragonite tablets

Schaeffer et al. *Chem. Mater.* 1997, *9,* 1731-1740

Mechanism:

- Specialised sheet of protein governs initial nucleation of oriented calcite crystals
- Secretion of 2 different families of proteins induces a switch from calcite to aragonite production
- The soluble proteins direct polymorph selection and orientation



Further control of orientation over many layers occurs via mineral bridges ⇒ stacks of crystals effectively SINGLE CRYSTALS

Crystal Morphologies

- One of most striking feature of many biominerals ⇒ remarkable morphologies.

- Many of these unusual morphologies occur when the biomineral is amorphous ⇒ no preferred morphology

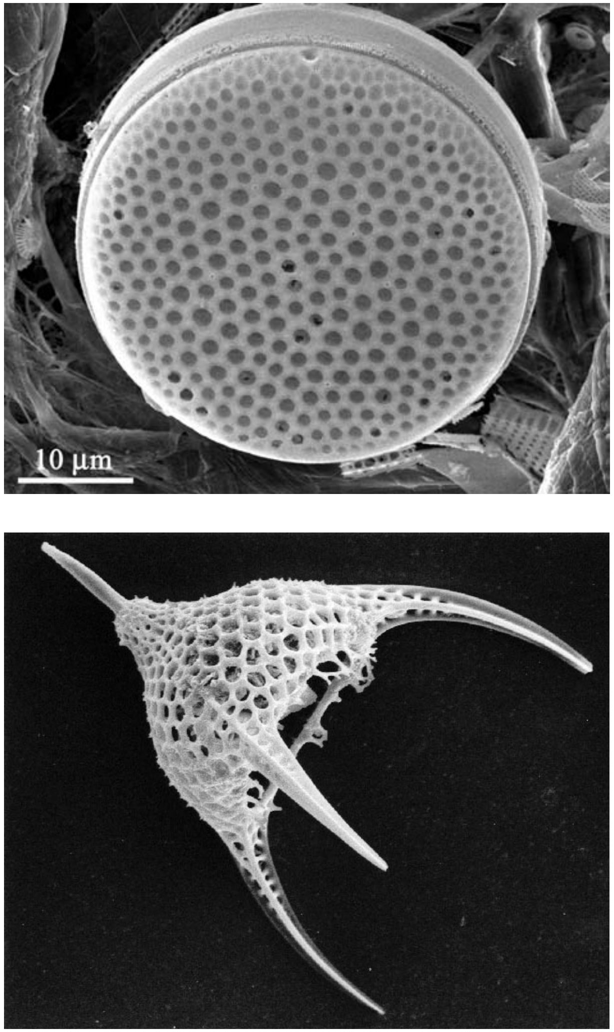
- Organisms can also produce single crystals with complex shapes and curved surfaces

⇒ Have developed mechanisms which override the basic growth form of a crystal

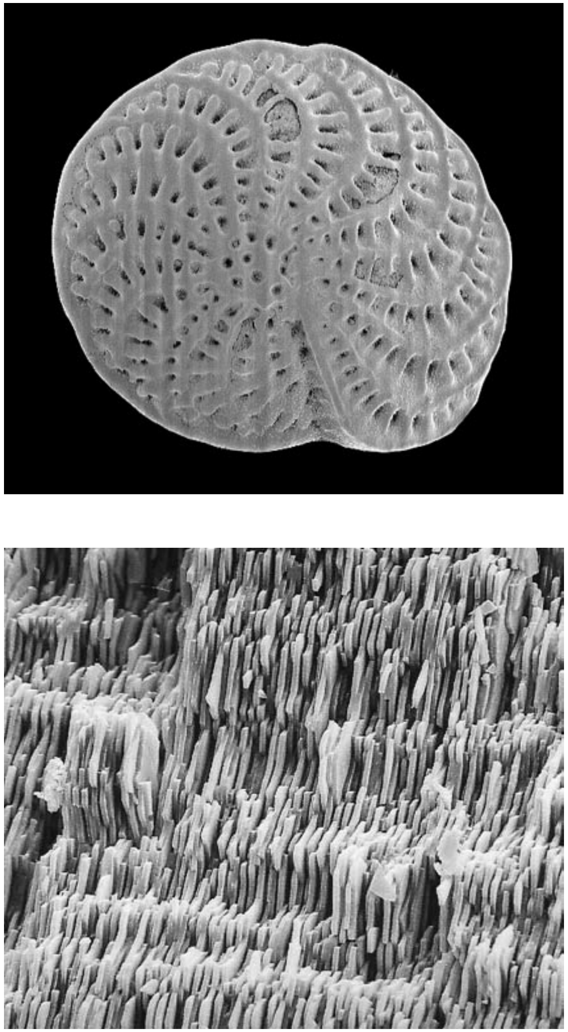
⇒ Produce crystals whose overall morphologies often bear no relationship to the symmetry of the crystal lattice

- Many biominerals with complex morphologies are polycrystalline structures – can be oriented or non-oriented

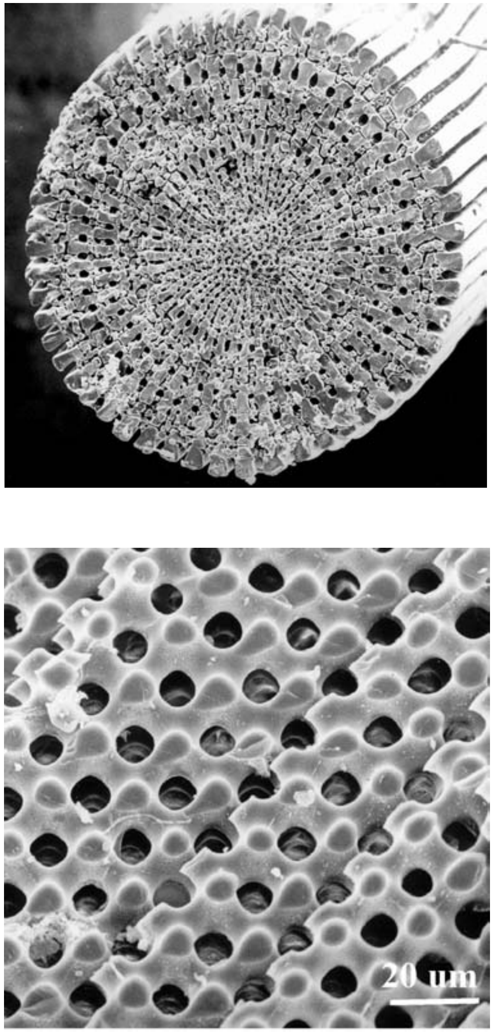
Biomineral Structures



AMORPHOUS



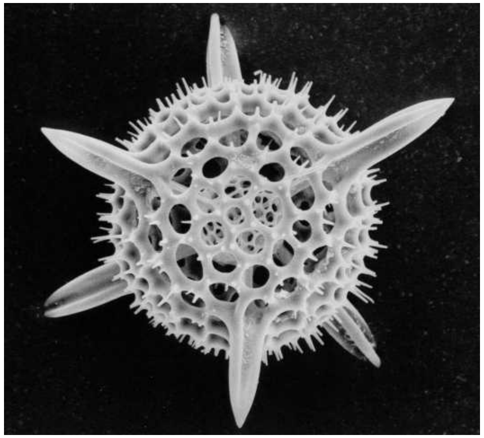
POLYCRYSTALLINE

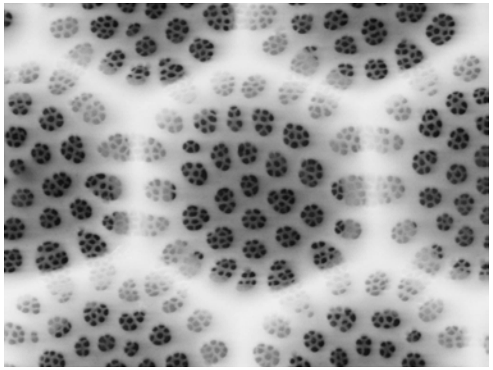


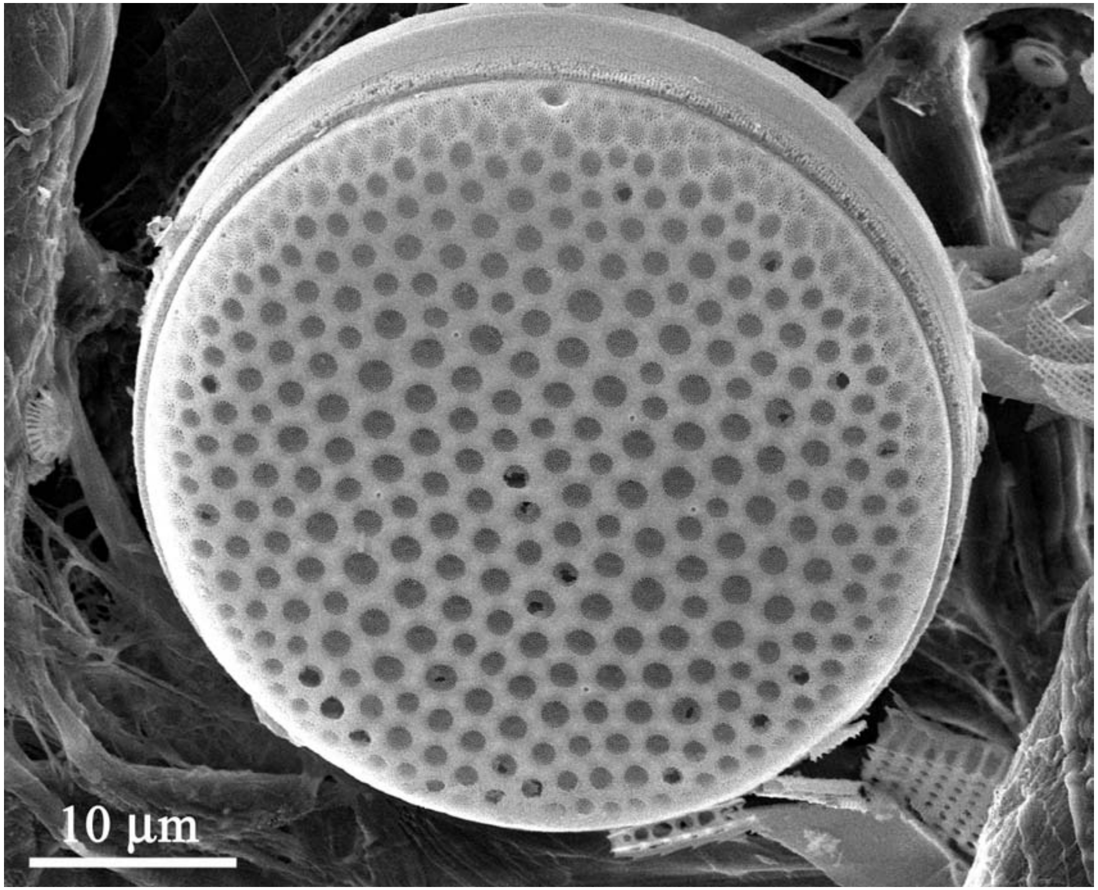
SINGLE CRYSTAL

Amorphous Silica

Amorphous silica is very abundant - the polymeric structure allows it to be moulded into unusual structures eg. diatoms

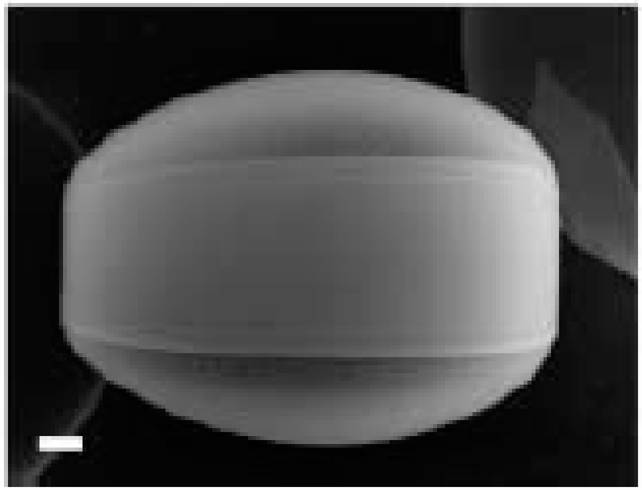




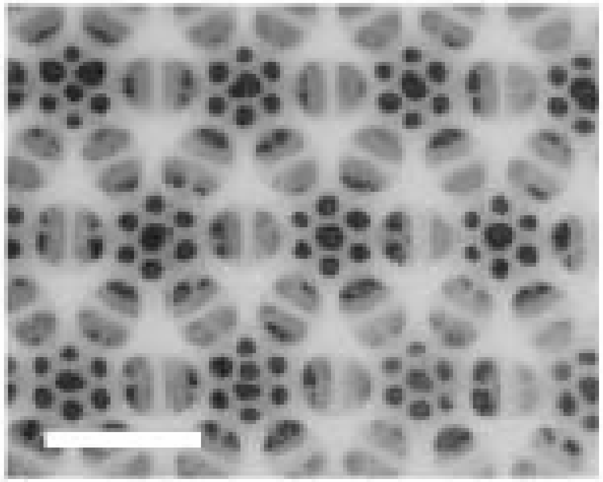


Biosilica Morphogenesis in Diatoms

SILAFFINS and LONG-CHAIN POLYAMINES induce formation of silica nanospheres from silicic acid in vitro



Cell wall of *Coscinodiscus granii*
SB=20 mm



Nanoscale architecture
SB=1 mm

Coscinodiscus ⇒ structure comprises a hierarchy of heaxagonal silica structures

M. Sumper, *Angew. Chem. Int. Ed.* 2004, 43, 2251 –2254

Principal organic component of *Coscinodiscus* shells polyamines

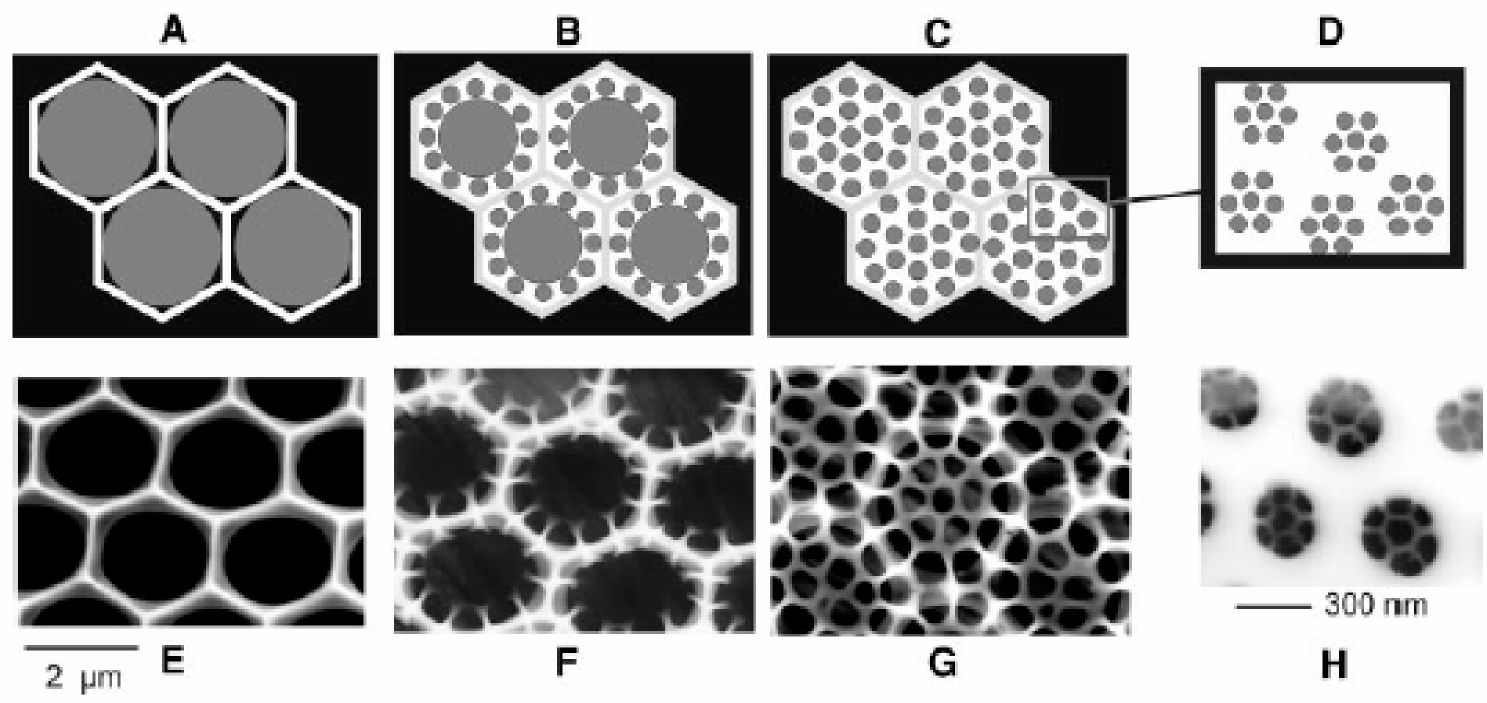
⇒ Model of pattern formation built on PHASE SEPARATION OF POLYAMINES

- Phase separation occurs within SDV to form microdroplets of polyamines

- Close-packed arrangement of microdroplets ⇒ hexagonal monolayer of droplets

- Aqueous interface between polyamine droplets contains silicic acid derivatives ⇒ promotes silica formation, catalysed by polyamine surfaces

Repetition on smaller length scales gives intricate patterning of diatom frustrules



(A) Monolayer of polyamine-containing droplets in close-packed arrangement within the SDV guides silica deposition

(B and C) Consecutive segregations of smaller (about 300 nm) droplets open new routes for silica precipitation.

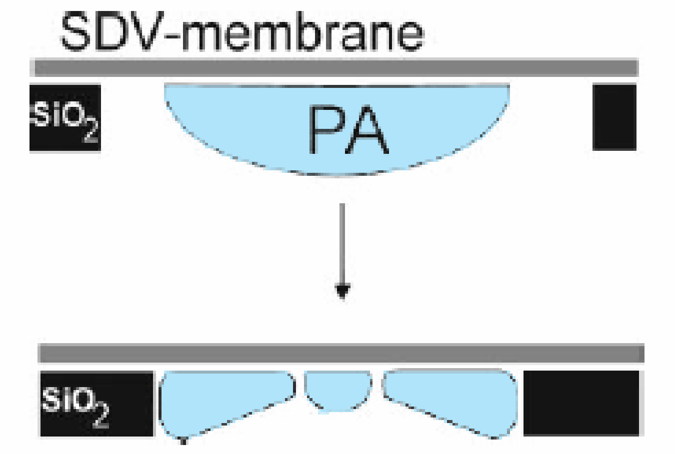
(D) Dispersion of 300-nm droplets into 50-nm droplets guides the final stage of silica deposition. Silica precipitation occurs only within the water phase (white areas). The repeated phase separations produce a hierarchy of self-similar patterns. (E to H)

M. Sumper, *Science*, 2002, 295, 2430-2433.

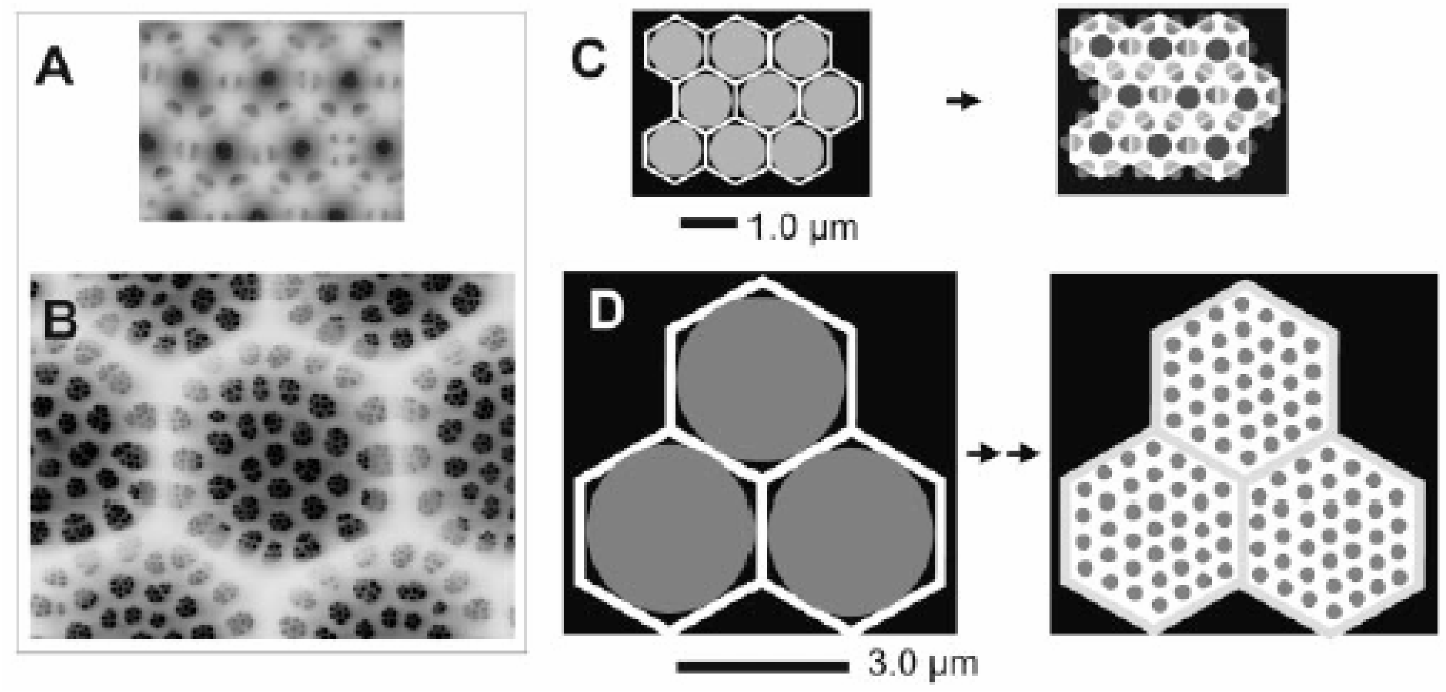
Dispersion of droplets into smaller and smaller units may arise from:

- Consumption of polyamines during precipitation
- Creation of negatively charged silica surfaces with high affinities for positively charged polyamine-containing surfaces

⇒ Should favour surface expansion by fragmentation of the polyamine-containing surface



Mechanism also supported by species-specific patterns...



- Initial size of microdroplets limited by wall-to-wall distance of areolae: 1 mm in *C. granii* and about 3 mm in *C. asteromphalus*

- The number and arrangement of secondary droplets formed are then governed by the wall-to-wall distance of the areola

⇒ Short distance in *C. granii* interferes with segregation of secondary drops – appear to be positioned across areola wall

Amorphous Calcium Carbonate (ACC)

- ACC can be prepared synthetically by mixing high concentrations of Ca^{2+} and CO_3^{2-} ions
- It is hydrated and is described as ≈ $CaCO_3.1.5\ H_2O$
- It is very unstable and rapidly crystallises

ACC is also observed in biology...

TRANSIENT ACC

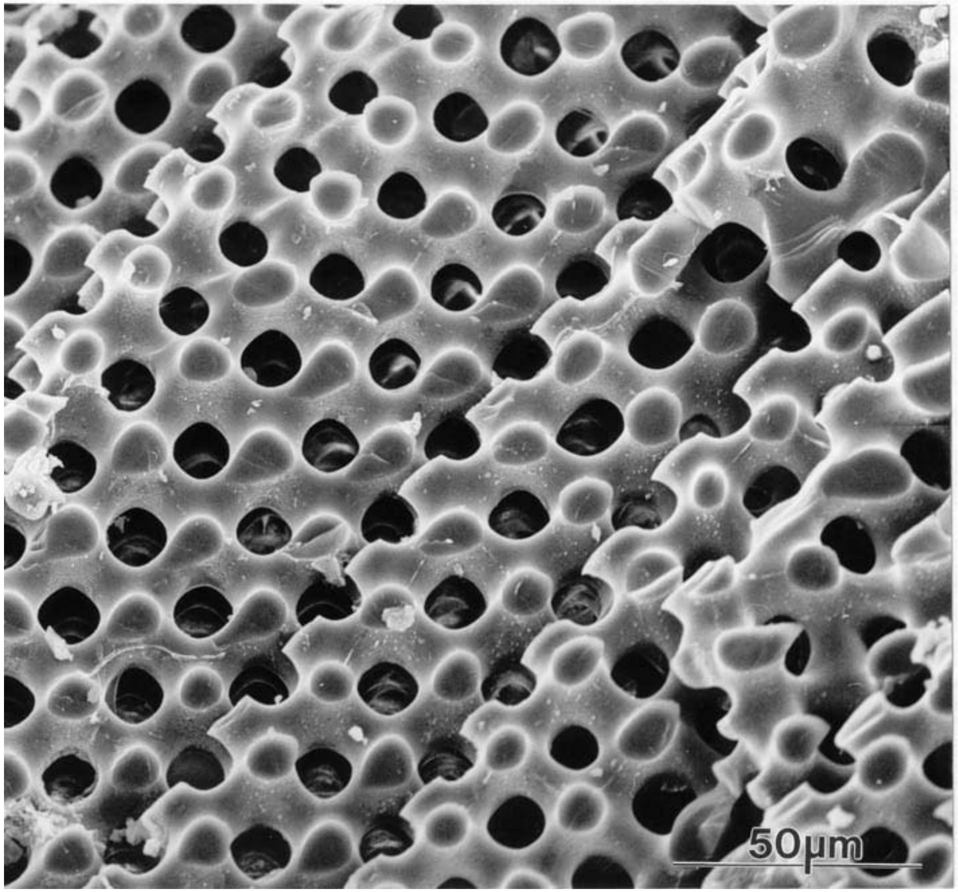
Can be short lived ⇒ acts as a precursor to a crystalline phase

STABLE ACC

Biogenic ACC can also be stable for long periods of time

⇒ organisms must actively stabilise it

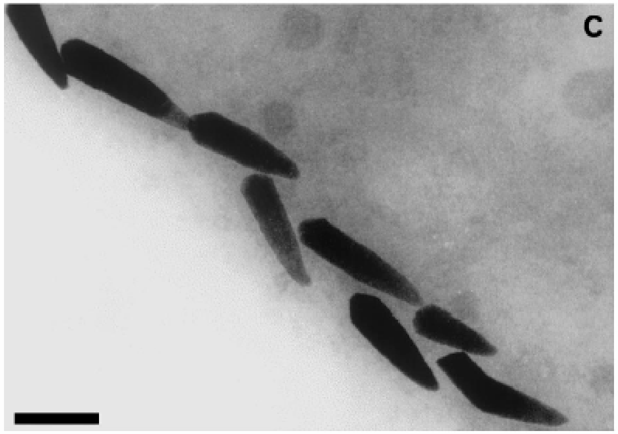
Morphological Control of Single Crystals



Structure of Skeletal Plates of Sea Urchin

Mechanisms:

- Changes in the activity or positioning of ion pumps and channels during mineralisation ⇒ may lead to crystal growth in preferred directions



Single crystals of magnetite in a magnetotactic bacterium

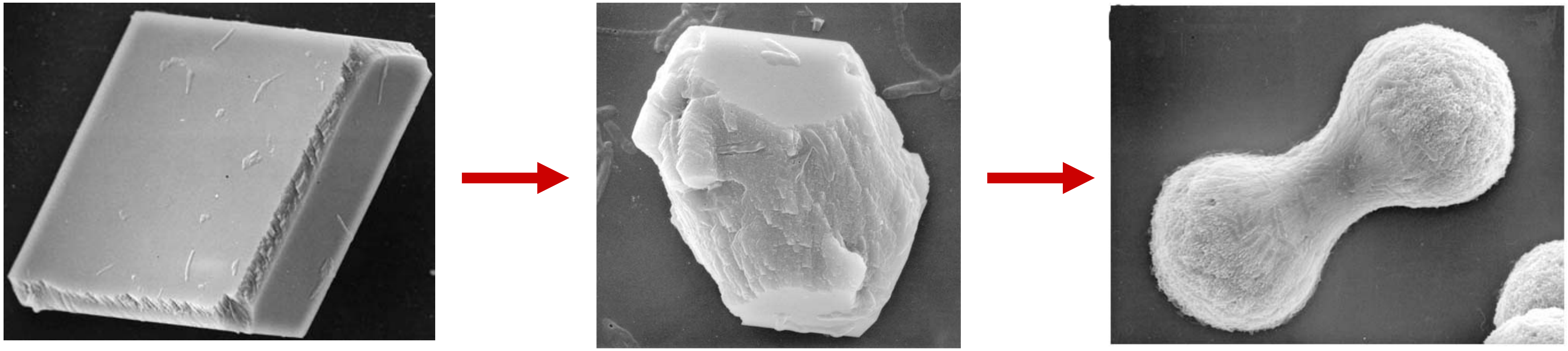
⇒ equilibrium morphology cubic

- Interaction of growing crystals with soluble additives, and which are occluded within many biominerals, can also produce subtle changes in morphology

- More dramatic changes in morphology are imposed by the physical form of the compartment in which mineralisation occurs.

Effect of Additives on Crystal Growth

- Additives can interact with specific crystal faces during crystal growth
- These faces are then stabilised and grow slowly ⇒ they become the large faces in the product crystal
- The final crystal morphology therefore differs from that of a crystal grown without additives



During this process additives become incorporated within crystals

⇒ can modify mechanical properties

Soluble Additives in Biomineralisation

- Soluble macromolecules extracted from $CaCO_3$ biominerals can adsorb to specific calcite crystal faces during precipitation

⇒ modifies crystal morphologies

- The macromolecules may possess structural motifs that match the atomic arrangement on one set of crystal planes, causing the additive to interact with and stabilise these faces

- Following adsorption to specific crystal faces, the macromolecules become overgrown and are occluded within the crystals

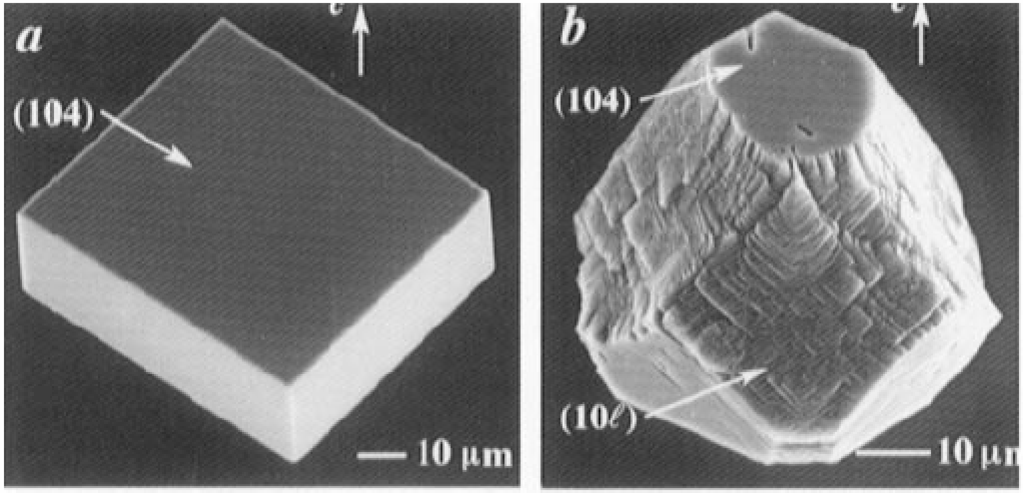
- The occluded macromolecules are unique to a particular biomineral

Macromolecules Extracted from Sea Urchin Spines...

Whole assembly of macromolecules

- Interact with planes approx parallel to the *c*-axis
- Rough faces, not well-defined, produced
- Indexed as {10*l*} where l ~ -1.5

Control crystal



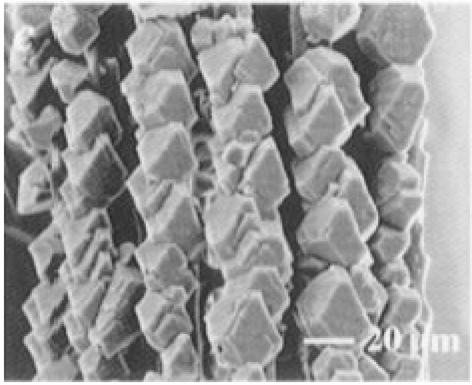
Grown with total assembly of macromolecules

Aizenberg, Hanson, Koetzle, Weiner, Addadi *J. Am. Chem. Soc.*, 1997, 119(5), 881-886.

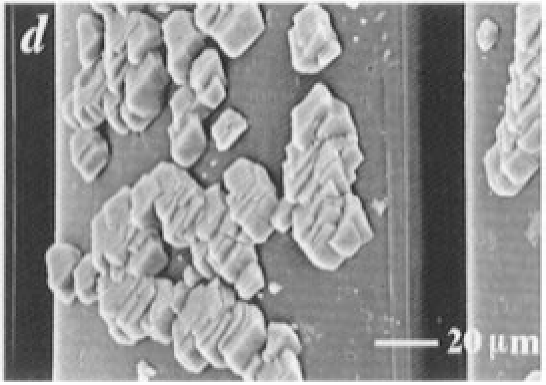
Macromolecules further investigated to identify which moieties are involved in interaction with growing crystals

Chemically and enzymatically treated to yield three fractions:

- The polysaccharides were removed yielding deglycosylated protein
- Isolated polysaccharide chains
- Densely glycosylated peptide cores were produced, comprising short peptides with attached polysaccharides



Epitaxial growth on young spine with released macromols



Epitaxial growth on mature spine with released macromols

Crystals grown in presence of macromolecules released on mild etching of biomineral

Polysaccharide fraction – interact non-specifically with a subset of planes oriented ~ parallel to *c*-axis ⇒ rough faces produced

Deglycosylated proteins - interact specifically to produce large faces in addition to {104} faces. Many analysed as {-203}.

Glycosylated peptides - interact specifically to produce large faces in addition to {104} faces. New faces close to {010}

- Non specific interaction of isolated polysaccharides may result from loss of conformation on isolation.

- Calcite crystals grown epitaxially in presence of proteins released under mild conditions show same faces as produced by entire assembly of proteins, but better defined

Polysaccharides may enable fine-tuning of interactions

Albeck, Weiner, Addadi, *Chem. Eur. J.* 1996, 2(3), 278-284.

Control of Morphology through Binding to Calcite Surface Steps

AFM study of the growth of the calcite (10.4) face in the presence of the chiral molecules D-aspartic acid and L-aspartic acid showed that interaction with the crystal steps was asymmetric.

- Growth hillock shows 2 acute and 2 obtuse steps to the cleavage plane

- Addition of glycine – 2 acute steps become curved, 2 obtuse unaffected

- Chiral D-Asp and L-Asp: growth hillocks mirror images of each other

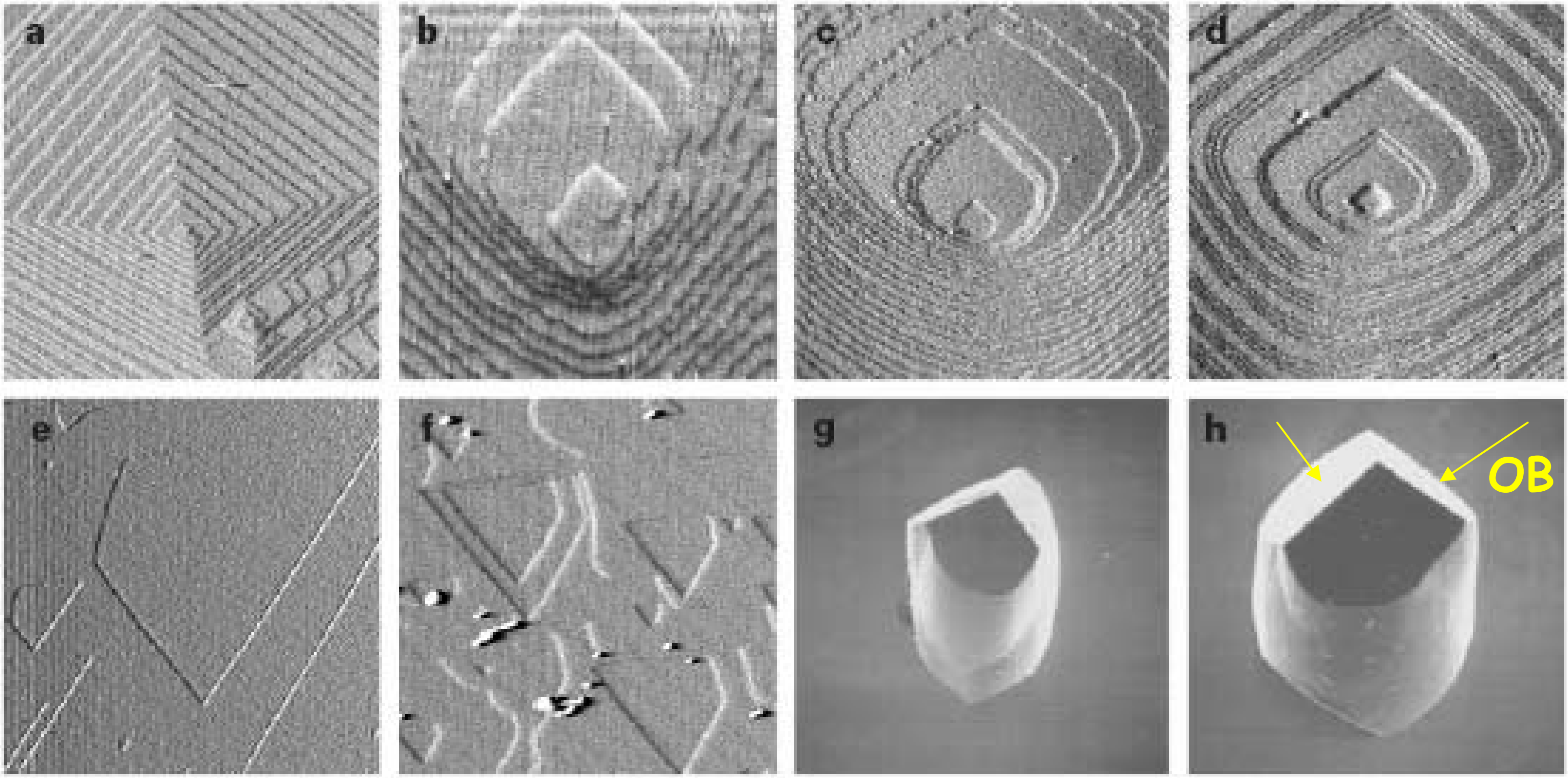
Orme et al, *Nature*, 2001, 411(6839), 775-779.

A pure calcite growth hillock

Glycine -achiral amino acid

L-Asp

D-Asp

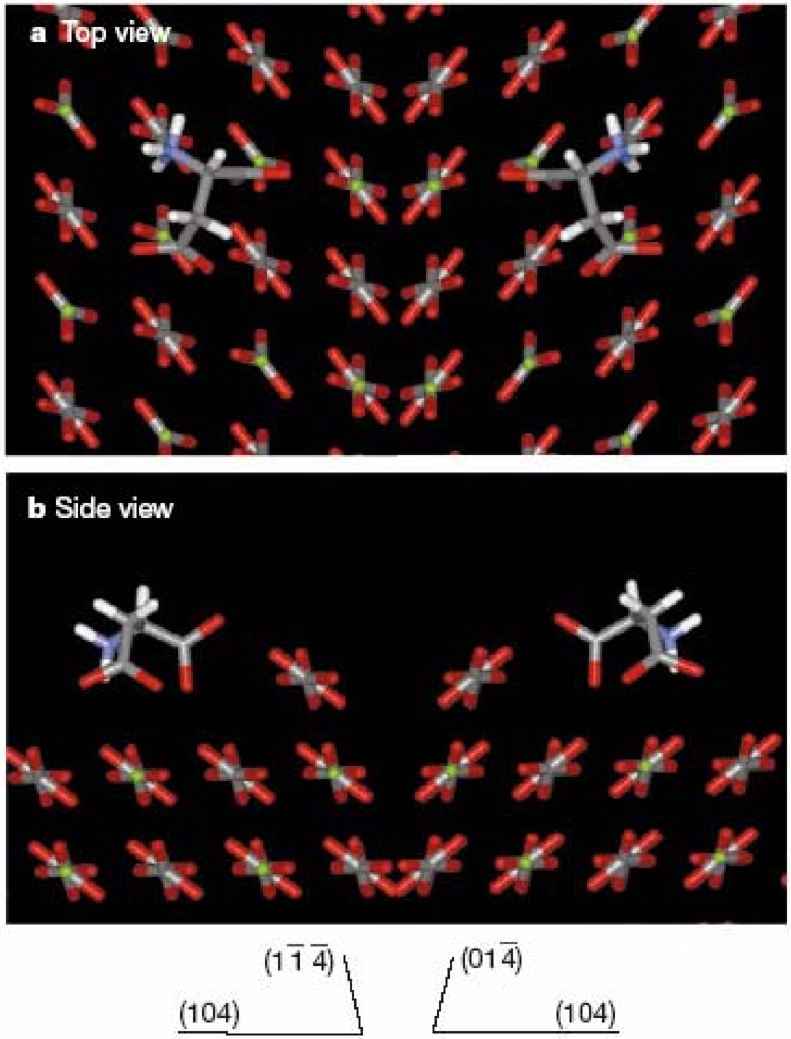


Dissolution pits with L-Asp

Dissolution pits with D-Asp

001 oriented calcite grown with L-Asp

001 oriented calcite grown with D-Asp



D-Asp binds to the (01–4) riser such that one of its negatively charged carboxyl groups completes the coordination of calcium ions, while the positively charged (NH_3^+) group remains in registry with the positive ions at the surface.

The carboxyl group of does not closely match the carbonate groups on the other actute step

Geometry of binding for Asp adsorbed on (104) steps of calcite. L-Asp (left) and D-Asp (right) binding to acute steps of calcite. Red=O, Blue=N, Green =Ca

The specific amino acid enantiomers therefore bind to the step edges that offer the best geometric and chemical fit.

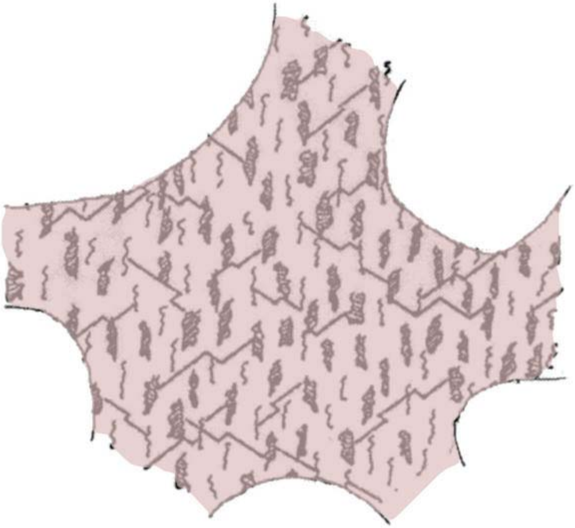
This changes the step-edge free energies, which in turn results in macroscopic crystal shape modifications.

Suggested that organic additives influence crystal morphologies by binding to the surface-step edges rather than single crystal faces.

⇒ Crystal morphologies depend on stereochemical recognition and the effects of binding on the interfacial energies of the growing crystal

Texture of Biominerals

How can large molecules such as proteins be incorporated within a single crystal ?



Schematic diagram of the domain structure of a crystal

Introduction of macromolecules causes defects in a single crystal

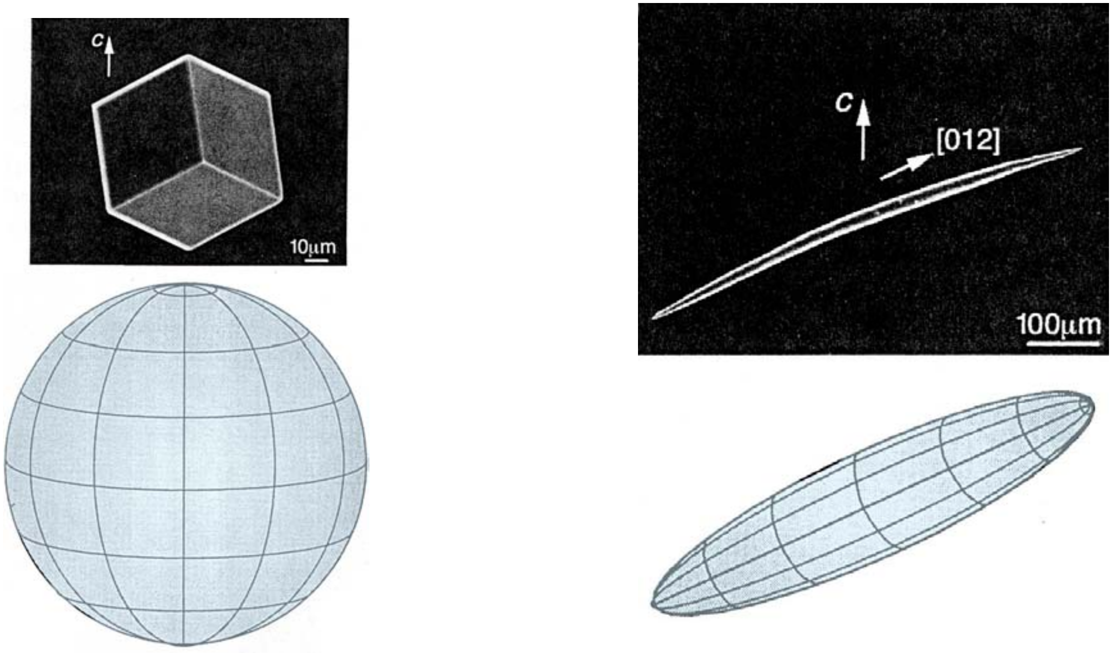
⇒ The coherence length and domain spread in the biological crystals provides information on the location of macromolecules

Coherence length - the size of perfect crystalline domains

Domain spread - the misalignment of the crystalline blocks

Texture of Biominerals

3D mapping of the distribution of defects in the curved monaxon spicule of the sponge Sycon reveals a domain shape resembling the overall morphology



- Pure geological calcite ⇒ CL 800nm and DS 0.003°
- Sponge spicule
  ⇒ plane perpendicular to long axis CL 130 nm and DS 0.07°

Aizenberg et al, *JACS*, 1997, 119, 881; Aizenberg et al, *Chem. Eur. J*, 1995, 1(7), 414.

Strong correlation between the distribution of defects within the spicules, and their macroscopic morphologies

⇒ Defects arise from incorporation of macromolecules

Suggested that the macromolecules play an important role in the modulation of morphologies

HOWEVER....

Soluble additives cannot be responsible for complex morphologies

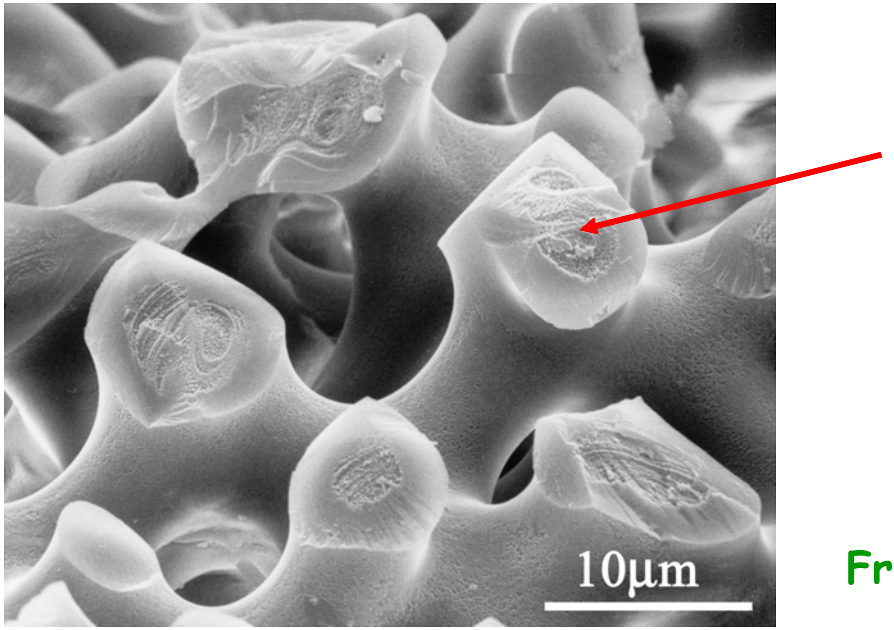
⇒ These must be defined by the shape of the environment in which the crystal forms

⇒ Additives fine tune morphologies?

⇒ Regulate mechanical properties?

Role of Occluded Macromolecules ?

Biominerals ⇒ typically exhibit superior mechanical properties



Conchoidal fracture surface

Fractured sea urchin plate

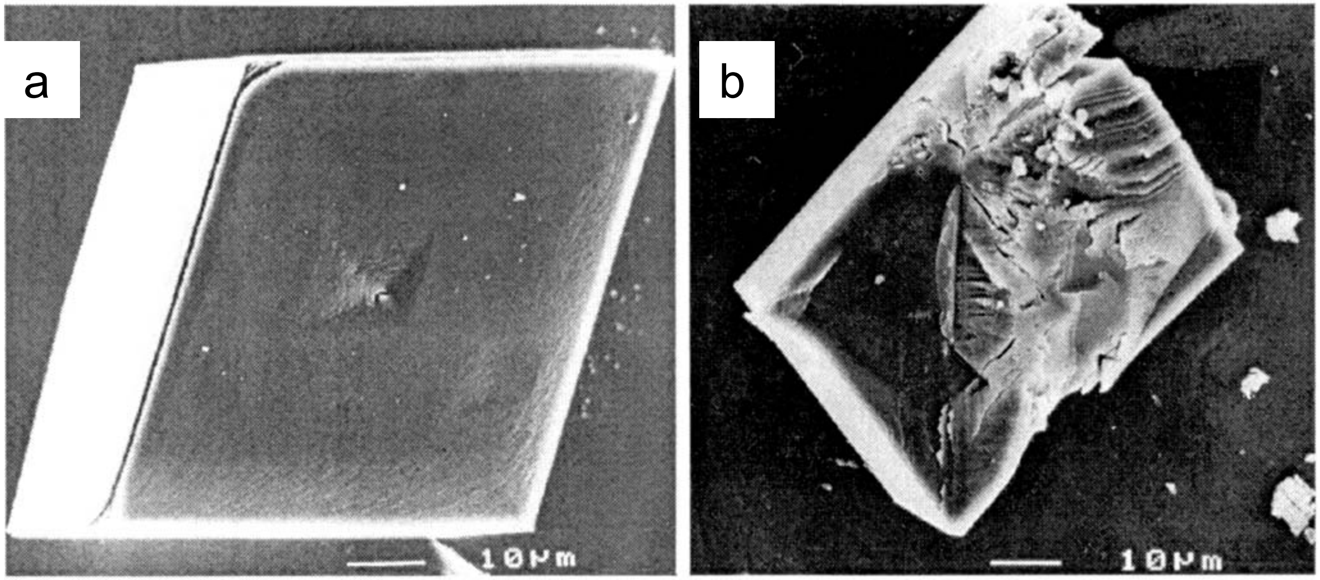
- Sea urchin calcite ~ 0.1 wt% organic macromolecules

⇒ the single crystal plates are effectively a composite material

- Macromolecules interfere with crack propagation along cleavage planes, reinforcing the crystal against fracture

Application of this Strategy to Synthetic Crystals

Calcite crystals grown (a) in the presence of and (b) in the absence of sea urchin proteins



Micro-indentation experiments show that the occluded proteins endow the crystal with far superior fracture resistance

Weiner et al, *Mat. Sci. Eng. C* 2000, 11, 1-8.

Shape Constraint

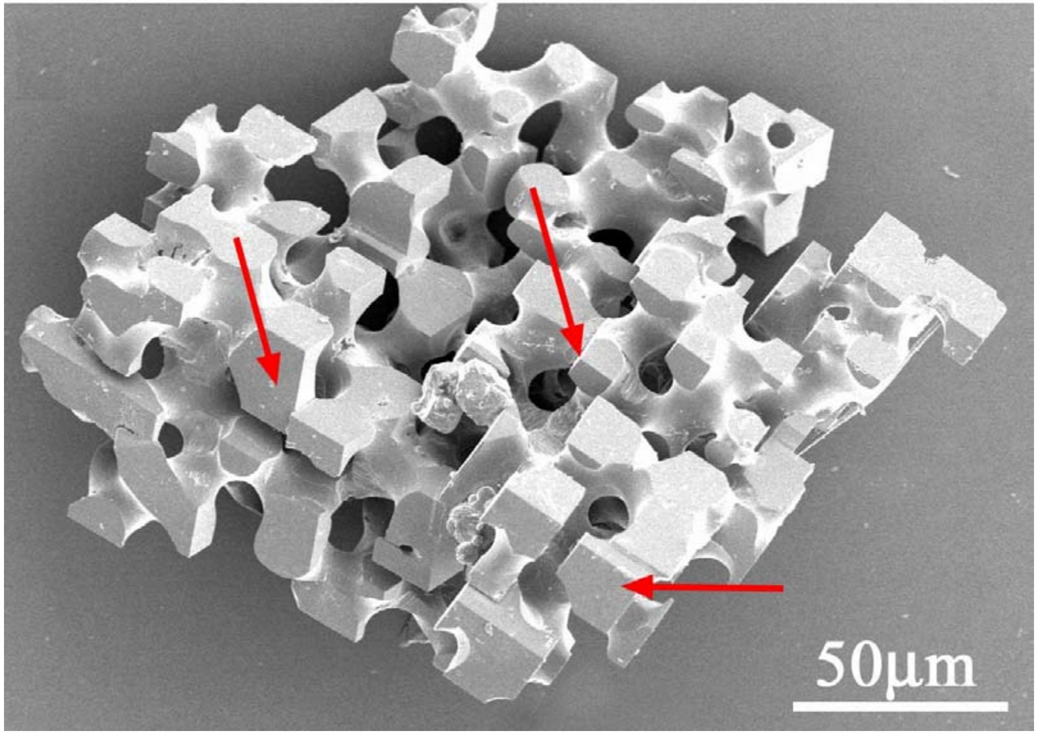
Crystals with unusual morphologies and curved surfaces are typical of growth within vesicles

eg. calcite scales produced by coccoliths
sea urchin larval spicules

- Vesicles have defined shapes, and crystals grow until they impinge upon the vesicle, which effectively acts as a mould
- The size and shape of the vesicle may be altered during the crystal growth process.
- Soft organic membrane imposes a form on a crystal

⇒ interaction of the crystal with the vesicle membrane may stabilise the high energy rounded crystal surfaces?

Control of Single Crystal Morphologies by Growing Crystals within a Rigid Template



$CaCO_3$ crystal grown within a sponge-like polymer membrane

Growth of single crystal within a rigid template ⇒ sufficient to control morphology

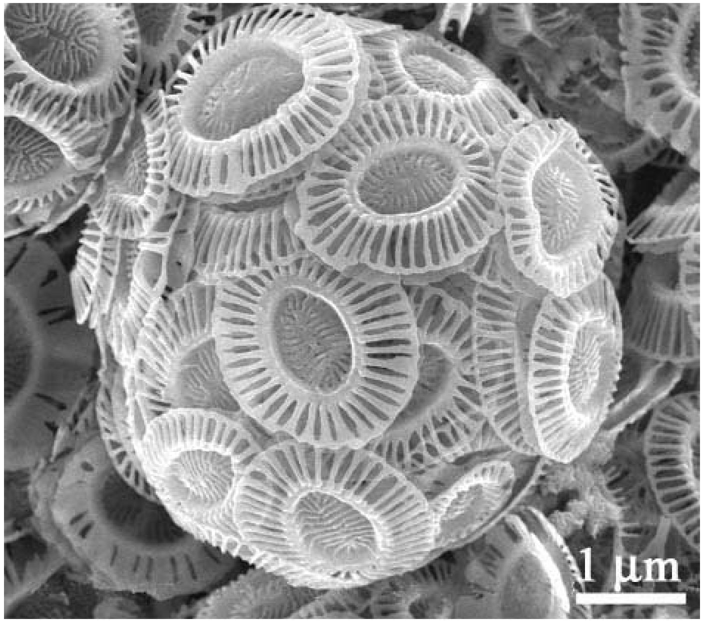
Park R.J. et al *J. Mater. Chem.* 2004, 14, 2291-2296.

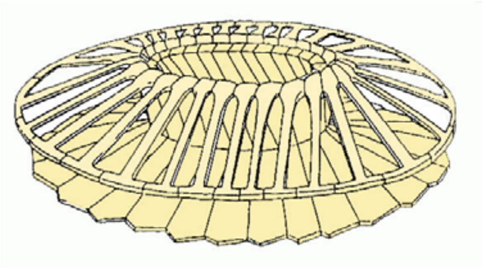
Poly-Crystalline Biominerals

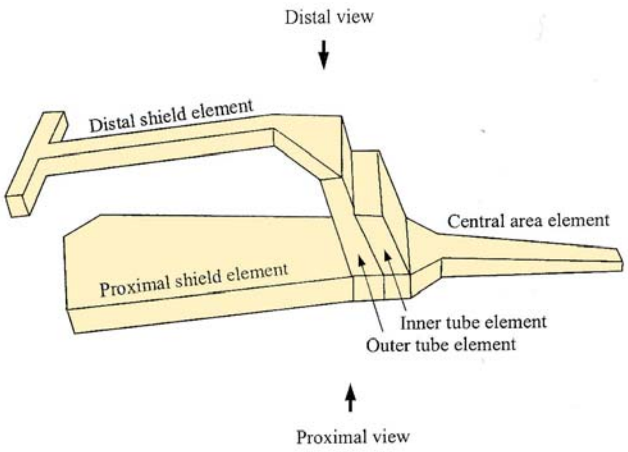
Many biominerals with complex morphologies are polycrystalline
⇒ formed by organised assembly of single crystal components

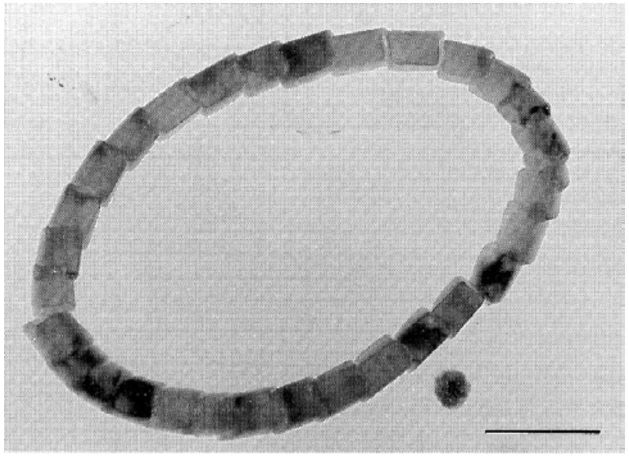
eg. coccosphere of the algae *Emilania huxleyi*

The coccolith scales each comprise about 30-40 units which are organised in a ring to give a double-rimmed structure.









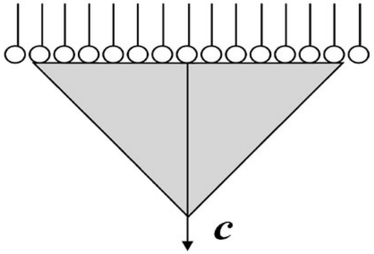
Proto-coccolith ring

- Formation of coccoliths begins with assembly of vesicles along the rim of an organic base plate scale
- Nucleation then occurs within the vesicles to generate a proto-coccolith ring of interlinked calcite crystals

- The crystals initially form as 40nm thick rhombohedral plates which are inclined to the plane of the ring
- The plates grow to a height of 100 nm, and radial outgrowth along the *c*-axis from the top and bottom faces generates a Z-shape.
- These units become inter-linked by selective growth along the inside rim, and further radial growths from the base and top of the element produce the proximal and distal shield elements

S.Mann "Biomineralization", OUP, 2001.

Further Lectures ....



- Crystallisation at Interfaces
- Amorphous Biominerals
- Mineral Morphologies