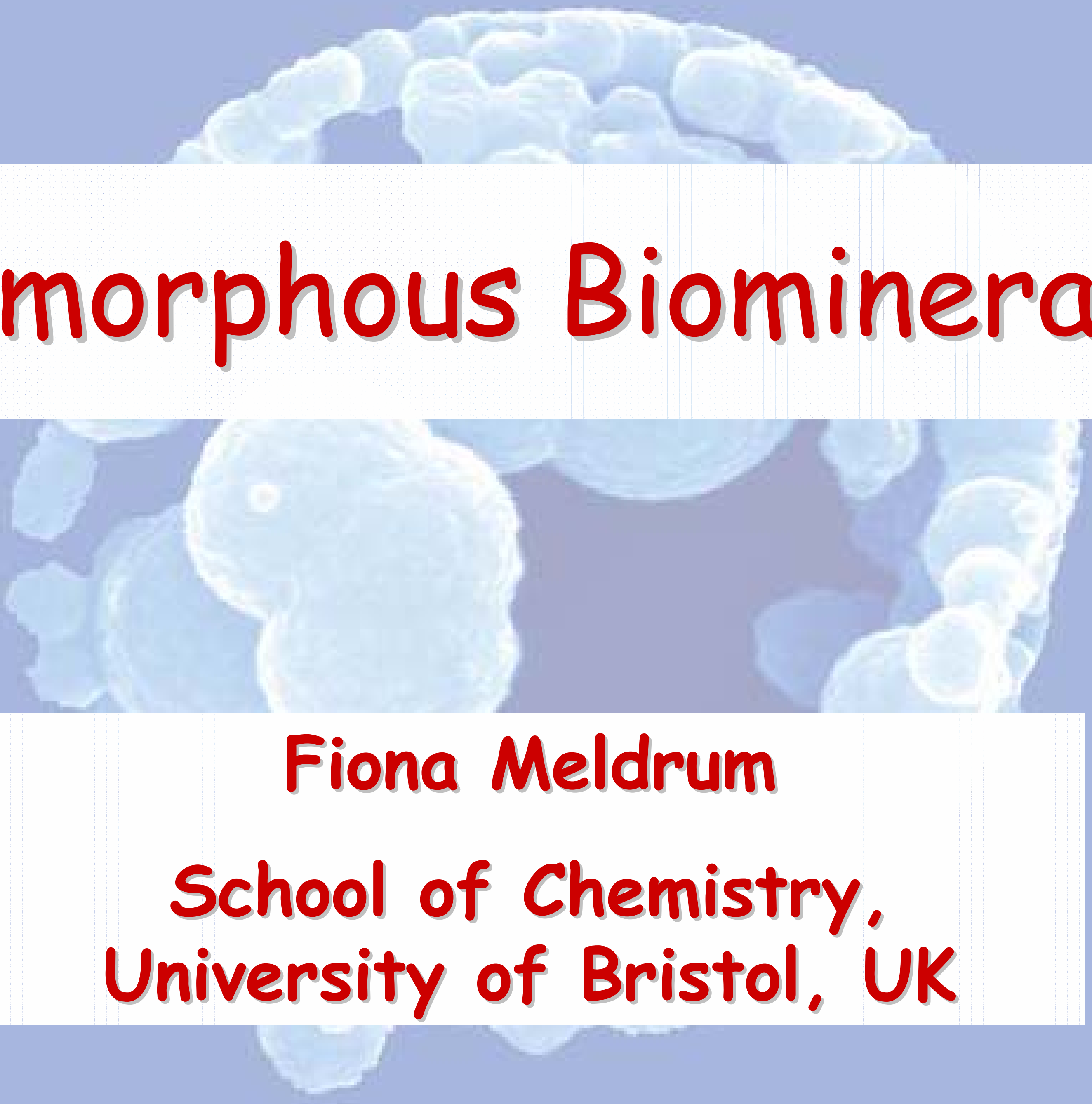


Amorphous Biominerals

Fiona Meldrum

**School of Chemistry,
University of Bristol, UK**

AMORPHOUS MATERIALS

Amorphous biominerals represent ≈ 20 % of known biominerals

Amorphous Calcium Phosphate	Widespread
Amorphous Calcium Carbonate	Sea urchin larvae and regenerating spinal tips, Crab carapaces, Mollusks
Amorphous Iron oxide/ oxyhydroxide phases	Magnetotactic bacteria
Amorphous Iron Phosphates	Chiton teeth, holothurian granules
Amorphous Calcium Fluoride	Gastropod – skin spicules

Compare Properties of Amorphous Materials and Single Crystals ....

Single crystals offer:

- Higher density
- Lower solubility
- Regular bulk and surface structures

Disadvantages include:

- Low resistance to fracture
- The existence of characteristic morphologies

Many benefits of amorphous materials:

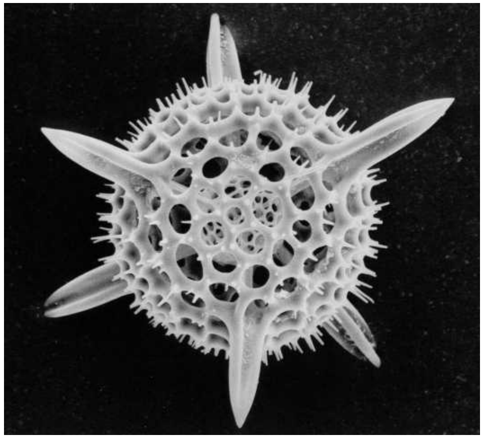
- No preferred growth directions and thus morphology

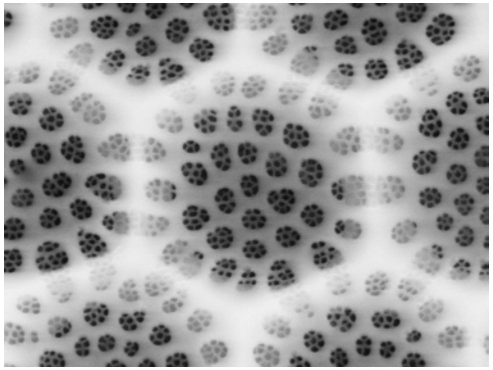
⇒ they can readily be moulded into a desired shape

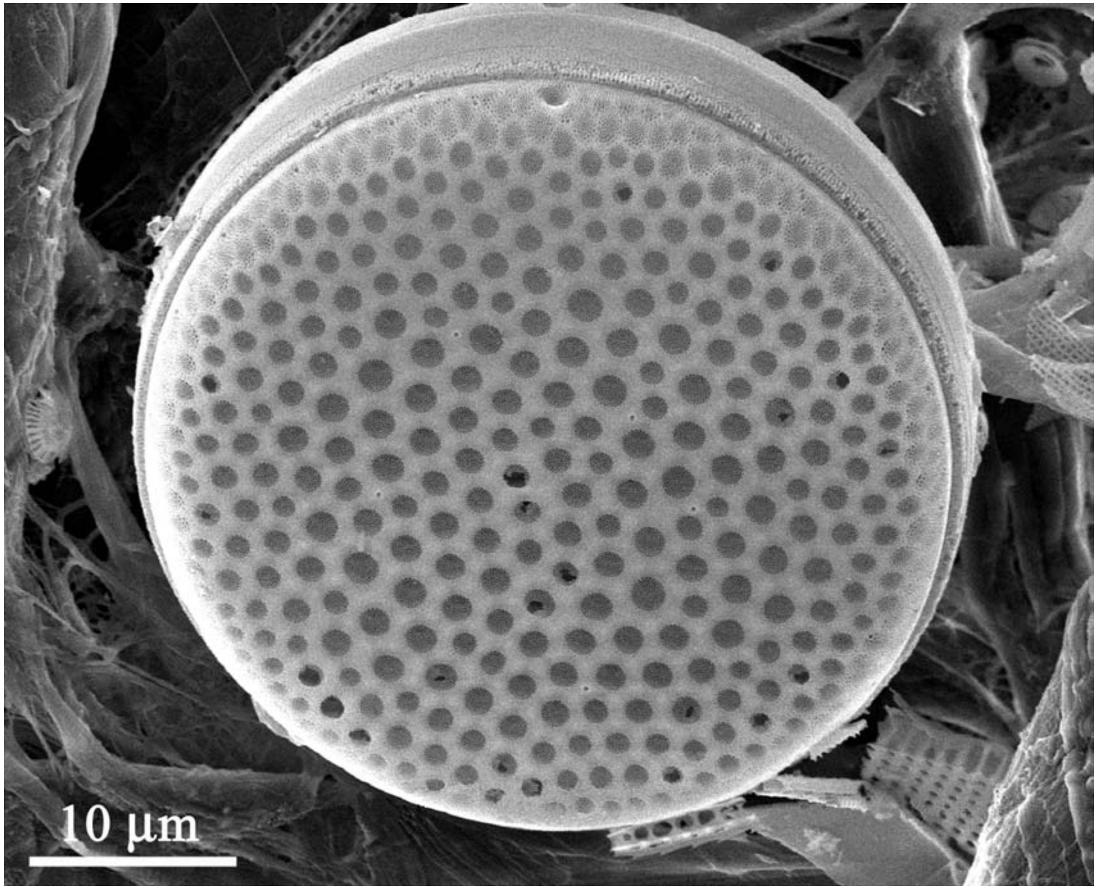
- No fracture planes ⇒ much less brittle than crystalline materials

Amorphous Silica

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







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
- Stabilised by ions such as Mg^{2+}, phosphate, polyelectrolytes

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

What Role does ACC Play in Nature ?

- a temporary storage site
- an intermediate in production of crystalline phases
- morphological control ?
- for skeletal strengthening purposes

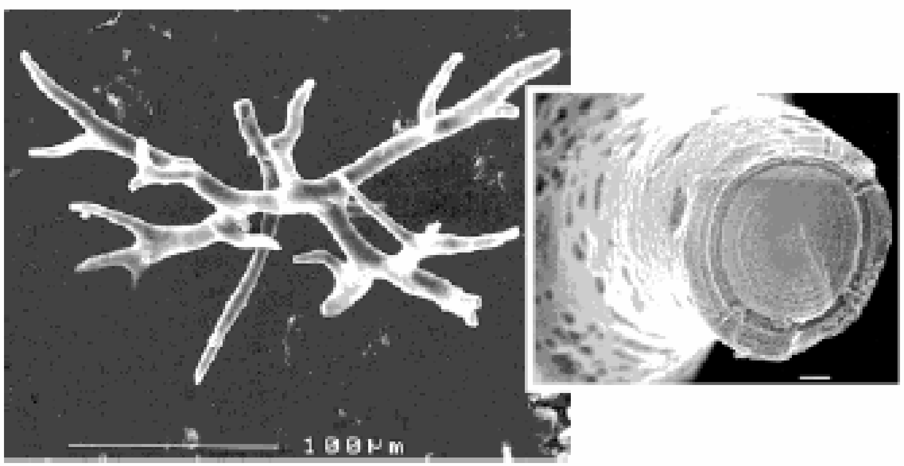
How is this achieved ?

There is considerable evidence that this is achieved using specific macromolecules designed for this purpose, together with ions such as Mg^{2+} and phosphate

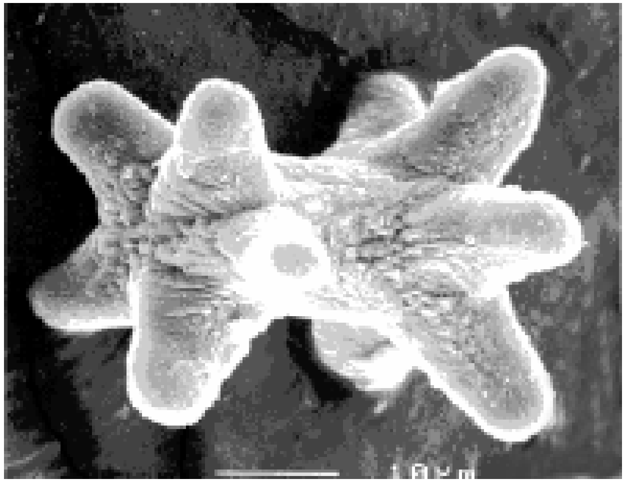
Addadi, Raz, Weiner *Adv. Mater*. (2003) 15(12), 959-970

ACC Biomineral – Ascidian Spicules

Examine role of macromolecules occluded within ACC and calcite of spicules of the ascidian *Pyura pachydermatina*



Body (antler) spicule



Tunic (dogbone) spicules

Antler Spicules ⇒ Comprise stable ACC

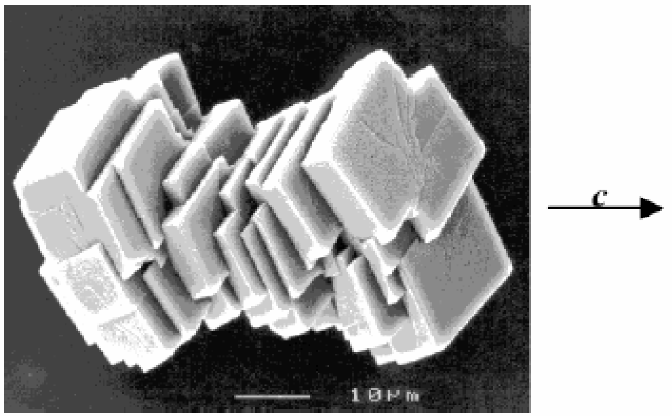
Dogbone Spicules ⇒ Comprise ACC + Calcite

Aizenberg, Lambert, Weiner, Addadi, *J. Am. Chem. Soc.* (2002), 124(1), 32-39.

Dogbone spicules – Appear single crystal in polarised light, XRD

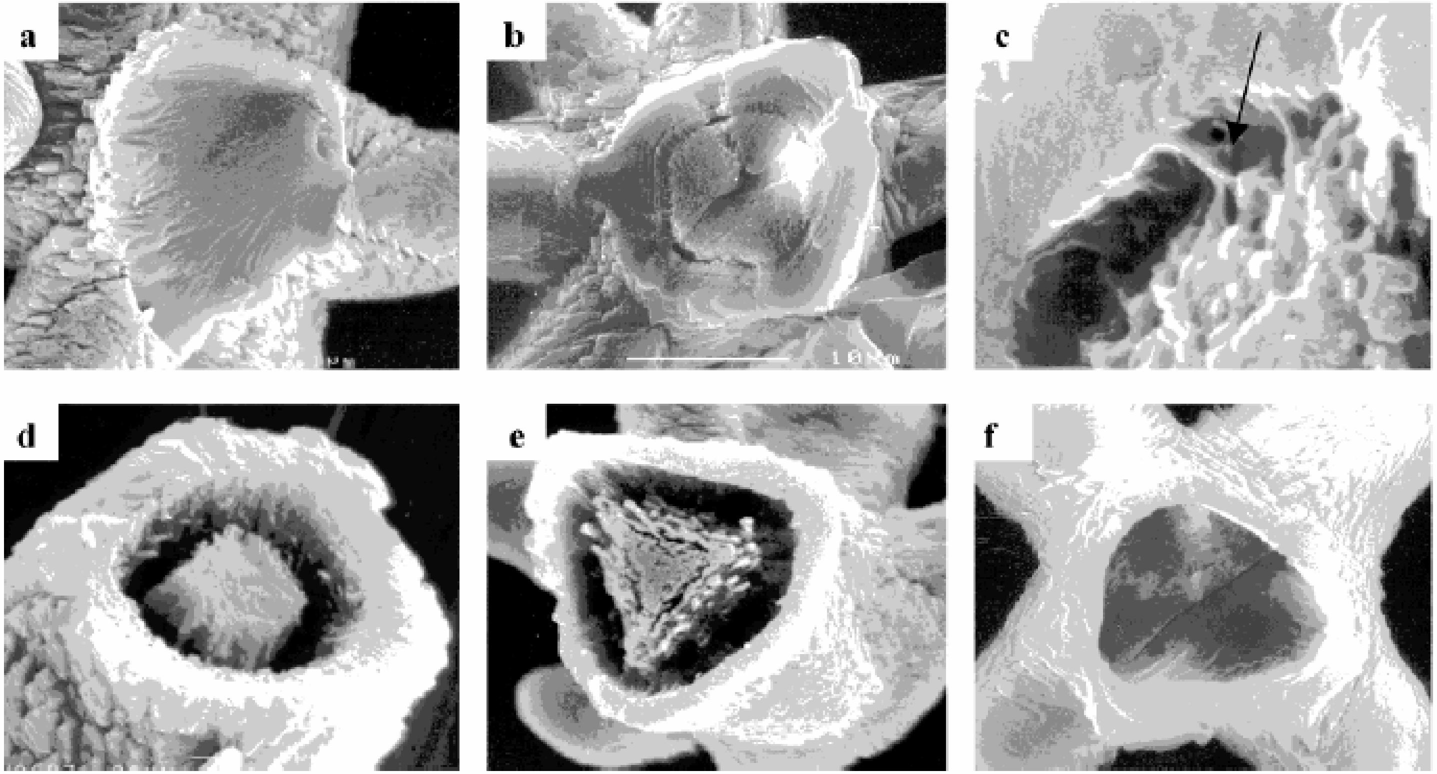
Intensity of XRD not as strong as expected

Epitaxial overgrowth shows the spicule is a polycrystalline aggregate with a preferred *c*-axis orientation and misalignment in the *ab* plane



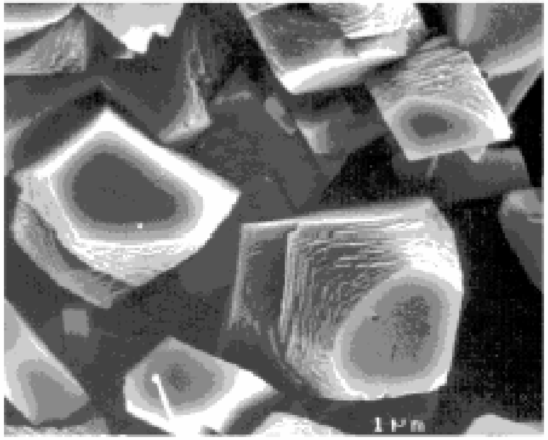
Chemical treatment of dogbone spicules show they comprise **two mineral phases**:

- an ACC core
- a calcitic envelope separated by an organic sheath
- spicule is 15-20% ACC

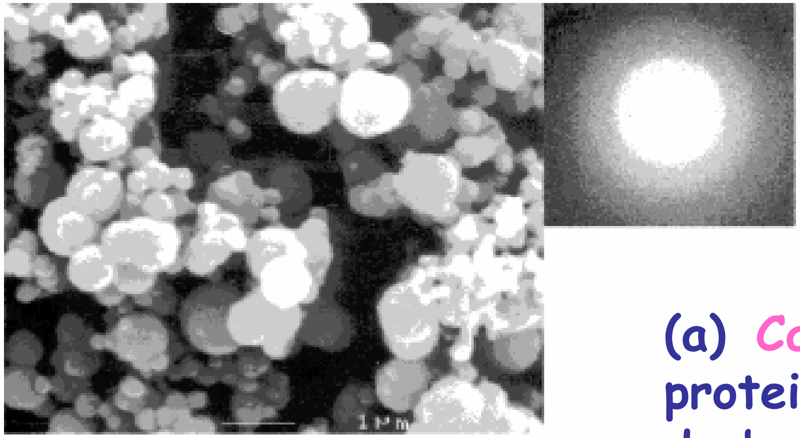


(a) Freshly cut, untreated spicule with a homogeneous cross section.
(b) Slight etching of the inner core in DDW.
(c) High magnification of the phase boundary showing an insoluble organic layer
(d) Selective dissolution of the core material in KOH
(e) Recrystallization of the ACC core into oriented calcite upon heating
(f) Complete removal of the ACC and organic layer in a 2.5% NaOCl solution. Only the calcitic phase remains.

Macromolecules ....



CaCO$_3$ precipitated in the presence of macromolecules extracted from ACC and calcitic phases of the spicules



(a) Calcite crystals grown in presence of proteins from the calcitic layer of dogbone spicules

(b) ACC stabilized by the proteins extracted from antler spicules

Under identical conditions:

- Calcitic proteins promoted formation of calcite,
- Proteins from the ACC antler spicules promoted ACC after inhibiting crystallisation for 1-2 weeks
- Proteins from the dogbone ACC core completely inhibited crystallisation

Macromolecules..

- Macromolecules extracted from calcitic layer of dogbone spicules rich in aspartic acid
- Glycoproteins extracted from ACC core are rich in glutamic acid, hydroxyamino acids (threonine/ serine) and possibly sugars

⇒ very similar to antler and *Clathrina* sponge spicules

Suggests structure of macromolecules directly related to the stabilisation of ACC

Structure of ACC

Synthetic ACC is typically described as $CaCO_3.1.5\ H_2O$

- Biogenic ACC - also hydrated?

ACC is amorphous to XRD - but does it contain any short range order?

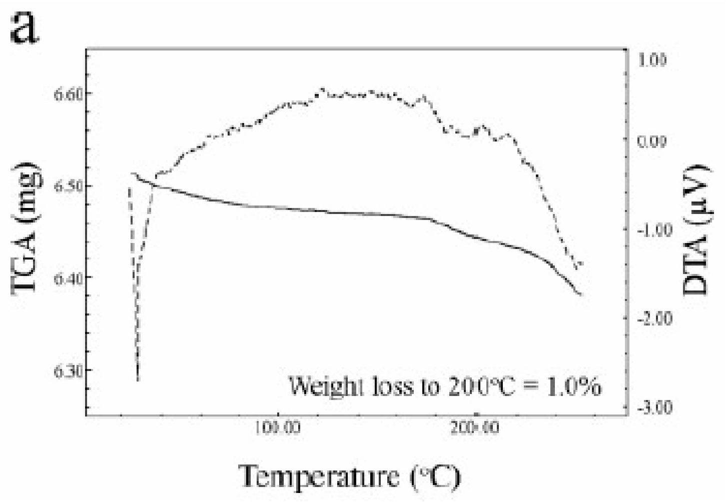
ACC extracted from different sources can vary considerably in stability

- Transient
- Stable - again varies considerably in stability on isolation from organism

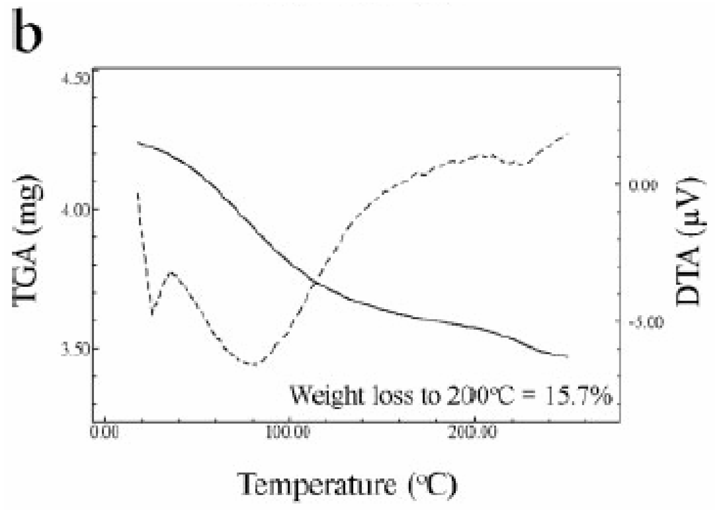
Why these variations?

Look at structure of ACC more closely ...

Hydration of ACC

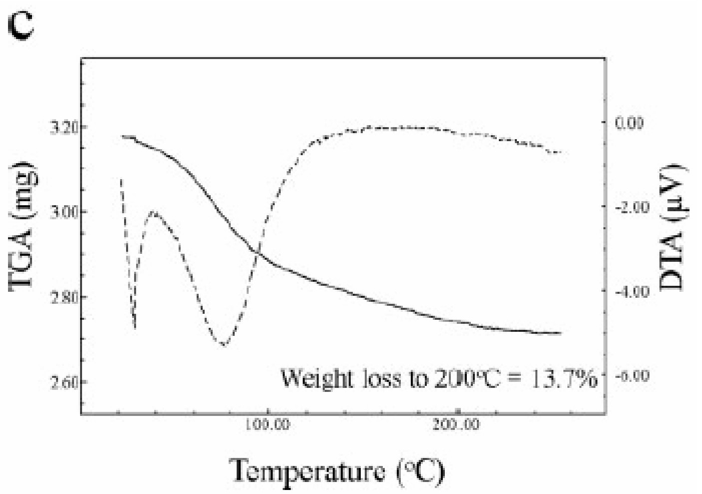


TRANSIENT biogenic ACC appears to be NON-HYDRATED



STABLE biogenic ACC HYDRATED

≈ $CaCO_3.H_2O$



SYNTHETIC ACC HYDRATED

Raz, Hamilton, Wilt, Weiner, Addadi, *Adv. Funct. Mater.* (2003), 13(6), 480-486

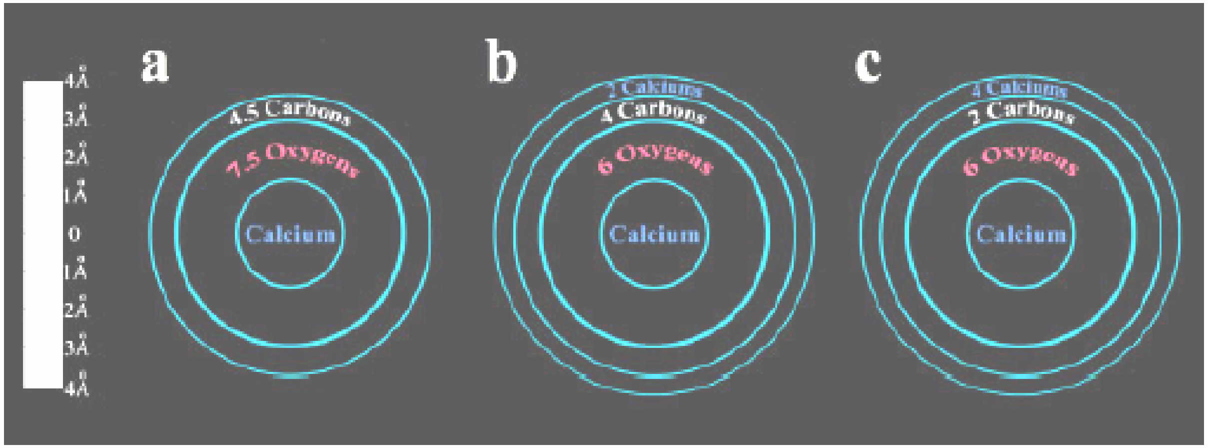
EXAFS Analysis of Local Order

EXAFS used to probe short-range structures of a number of contrasting stable ACC phases:

(a) Body spicules of an Ascidian

(b) Lobster cuticle

(c) Cystoliths from the leaves of a *Ficus* tree

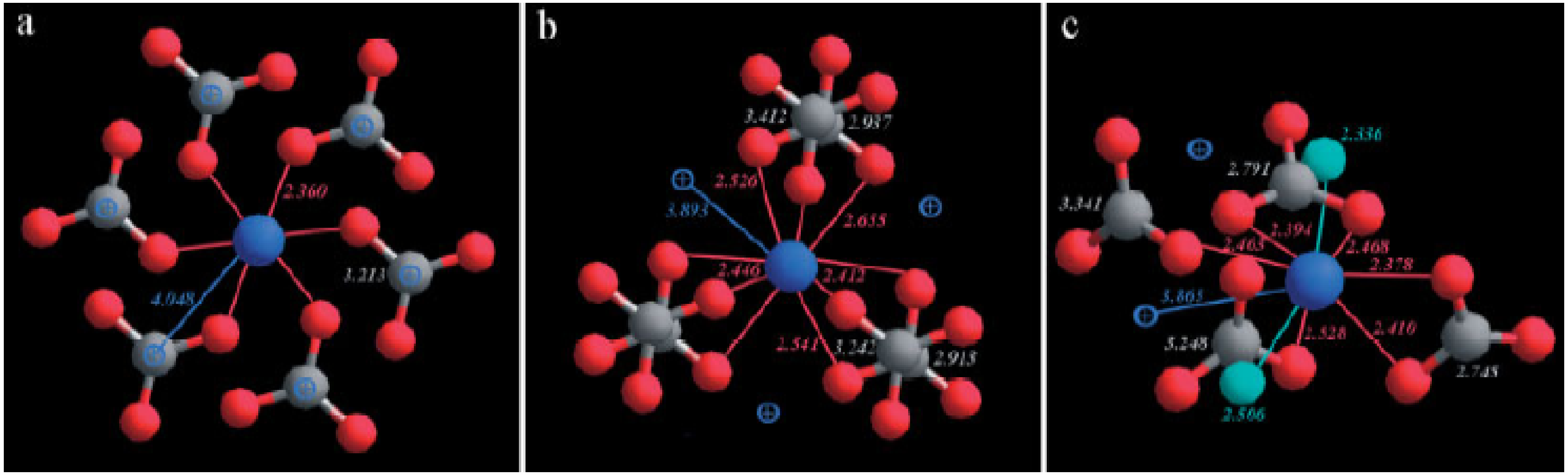


- All structurally most similar to calcium carbonate monohydrate
- Vary in terms of number of coordinating Ca^{2+} ions in first, second and third coordination spheres

More order in ions around the Ca^{2+} ions in the cystoliths

⇒ may provide an ordered centre which nucleates the crystalline phase

⇒ cystoliths unstable when isolated, other ACC biominerals stable



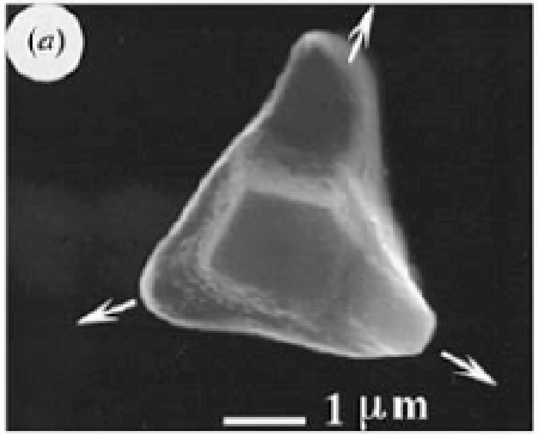
ACC represents a family of phases

Short-range order may act as a "blueprint" for a product crystalline phase

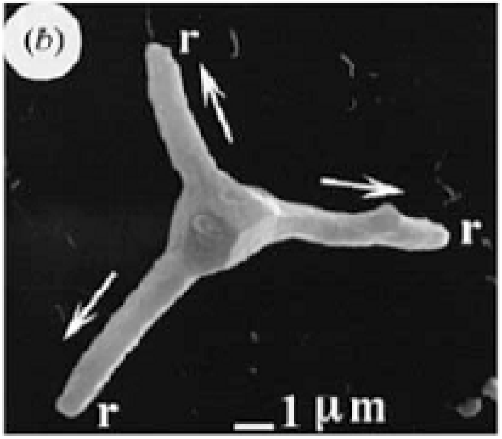
Addadi, Raz, Weiner *Adv. Mater.* (2003) 15(12), 959-970

Role of Transient ACC in Calcification

Formation of sea urchin larval spicules provides first example of ACC acting as a precursor to crystalline calcium carbonate phases



20 h embryo - rhombohedral calcite crystal and three radii starting to grow



Triradiate spicule (25 h embryo)

- Spicule formation starts with deposition of a small calcite rhombohedron ⇒ triradiate spicule

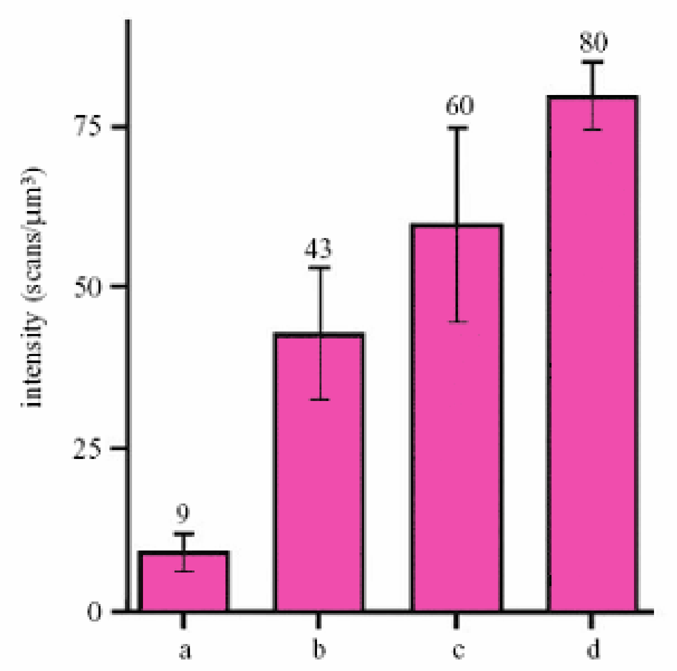
- Growth continues with radial outgrowths along c-axes

Beniash, Aizenberg, Addadi, Weiner, *J. R. Soc. Lond. B* (1997), 264, 461-465.

Beniash, Addadi, Weiner *J Struct Biol* (1999), 125, 50-62

XRD Spectra of spicules measured over time

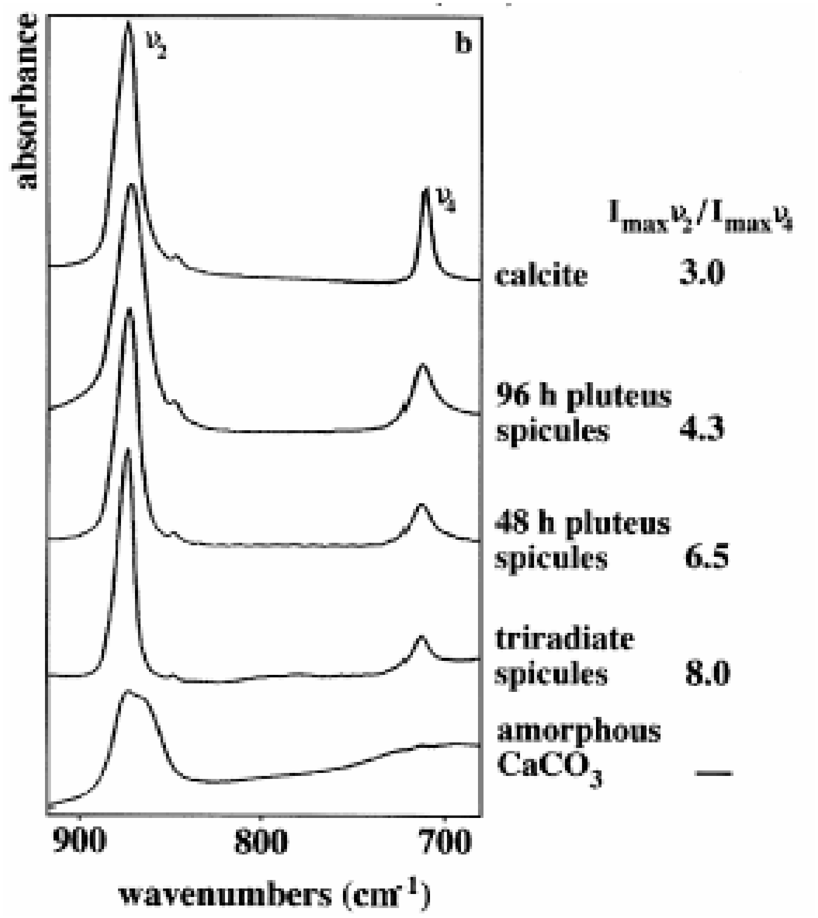
⇒ intensities generally LOWER than expected



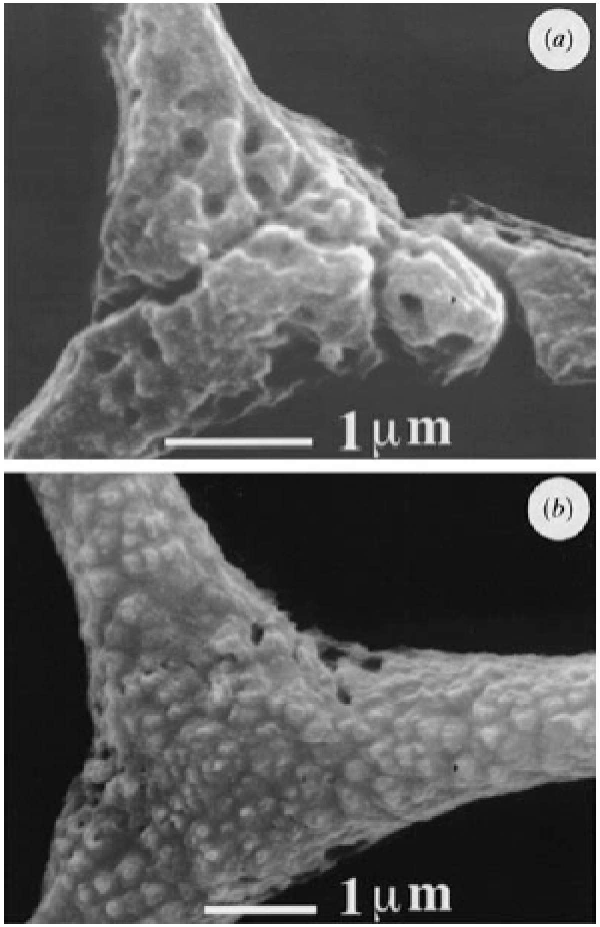
Average intensities of 104 reflections of individual spicules, volumes normalised

(a) 48 h
(b) 96 h pluteus spicules
(c) 96 h pluteus spicules stored dried in a freezer for four months
(d) synthetic calcite crystal

No growth of spicule between (a) and (b) - BUT 5-fold increase in intensity ⇒ Crystallisation of an amorphous phase?



IR spectra showing presence of ACC and crystallisation over time



Surfaces of spicules after etching with (*a*) water and (*b*) 1N KOH.

Mechanism of Sea Urchin Larval Spicule Formation

- Often considered that intravesicular crystal growth occurs in a fluid environment

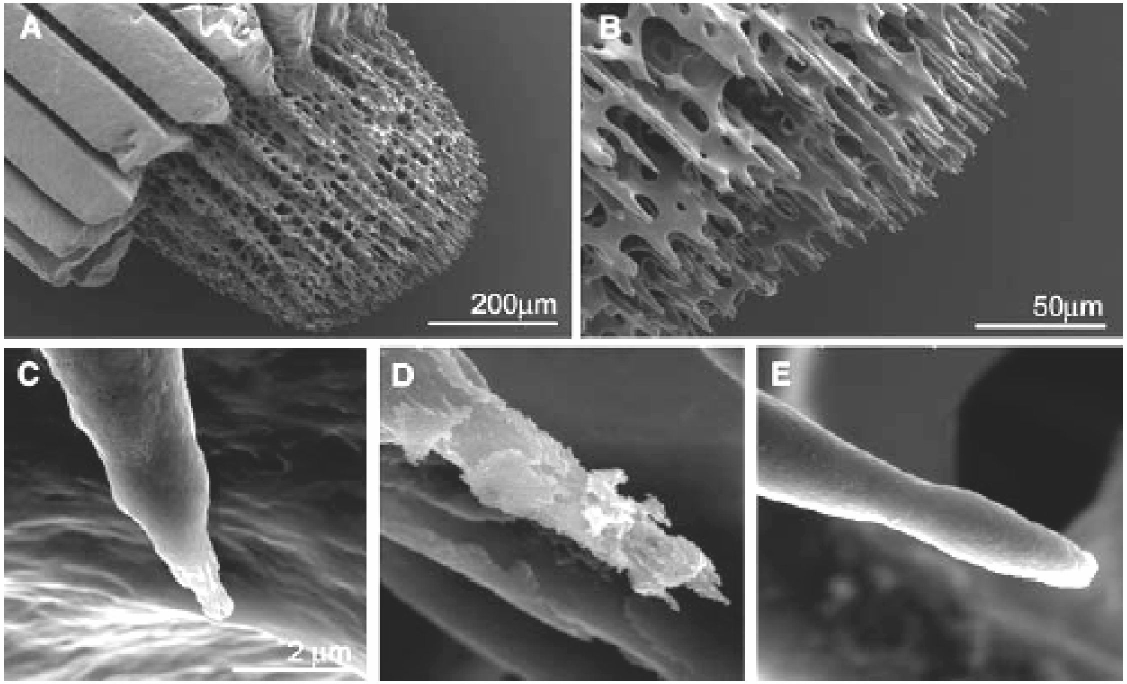
⇒ sea urchin larval spicules were tightly surrounded by the spiculogenic compartment membrane

- ACC granules are involved in spicule formation

Either transferred across the membrane to form the growing spicule, OR serve as storage sites, dissolve prior to transfer

- Transformation of ACC to calcite occurs over time to give a single crystal of calcite with triradiate form

ACC also observed as a precursor to aragonite in mollusk larval shells, and in regenerating sea urchin spine tips



(A) Five-day-old regenerated spine growing on the original broken spine.
(B) High mag view of the tip of the new growth and newly formed microspines.
(C) One microspine formed after 4 days of regeneration, observed fresh.
(D) Four-day-old microspine, etched in water while fresh.
(E) Four-day-old microspine, etched in water 1 month after regeneration.

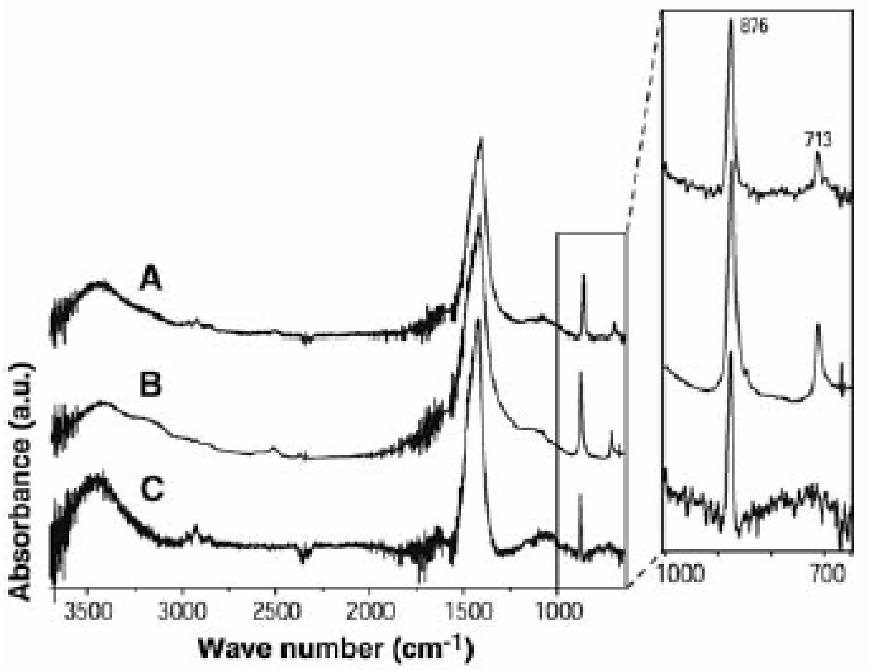
Politi, Arad, Klein, Weiner, Addadi, *Science*, (2004), 306, 1161-1164.

ACC confirmed by FTIR analysis of particles removed from fresh regenerated spines

A) Freshly removed particles ⇒ mixture of ACC and calcite

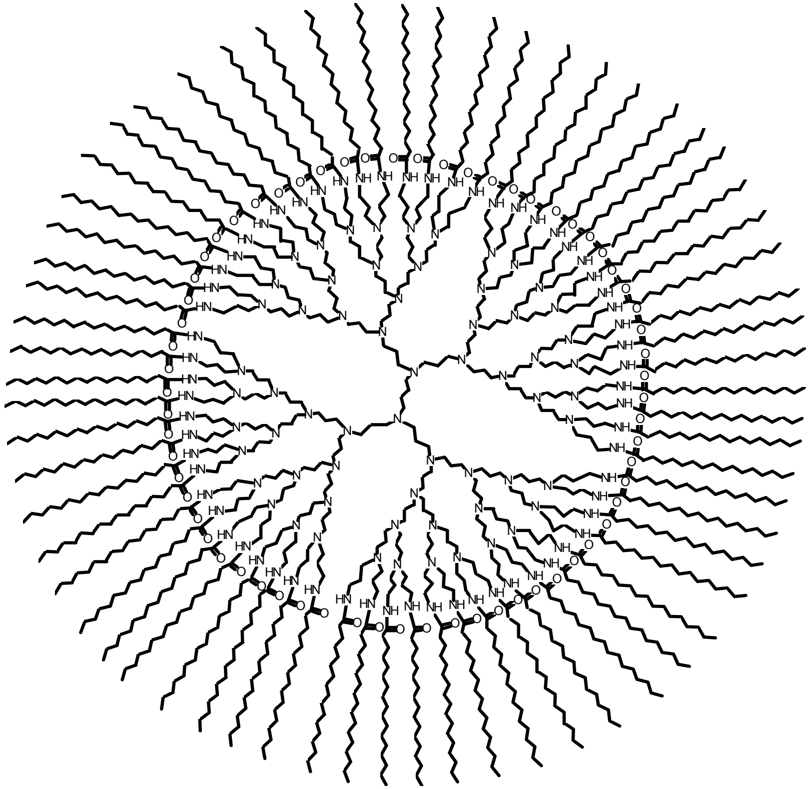
B) Material removed from the mature part of the spines ⇒ calcite

C) Spectrum of the particles in (A), after subtraction of the spectrum of calcite from the old part of the spine ⇒ ACC



Adult spines also regenerate into single crystal calcite via an ACC precursor phase ⇒ mechanism not restricted to larvae

Amphiphilic Dendrimers: Polymorph Selection



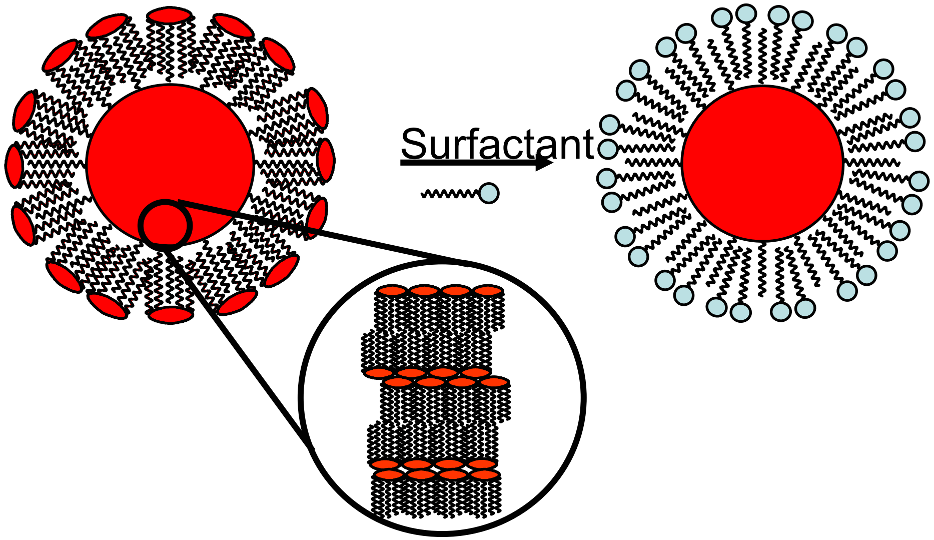
- Poly(propylene imine) dendrimers were modified with long aliphatic chains.

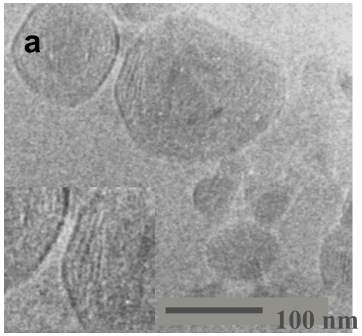
- Single-chain surfactants intercalated into the exposed long chains

Used as growth modifier for $CaCO_3$ precipitation

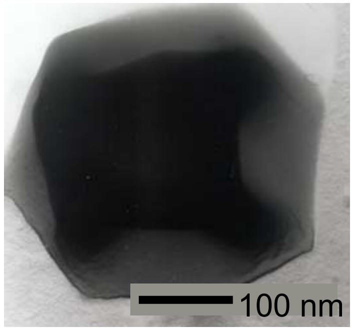
A.P.H.J. Schenning *et al.*, *J. Am. Chem. Soc.*, 118, 8199 (1996)
J.J.J.M. Donners et al. *Chem. Eur J.*, *8* 2561 (2002)

Dendrimer/Surfactant Templates





Dendrimer



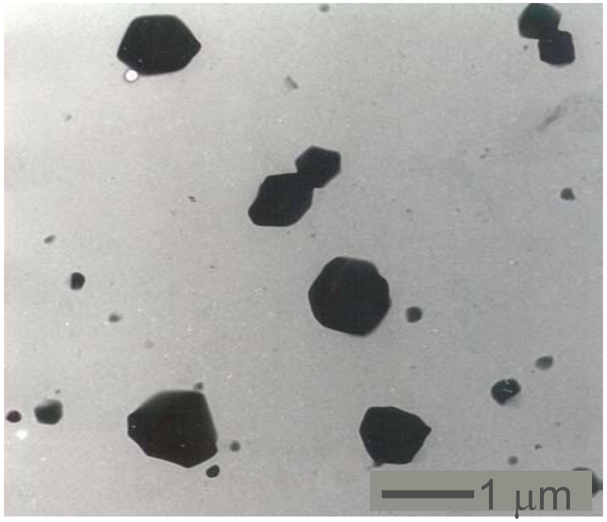
Dendrimer/ODA

Dendrimer/surfactant species form RIGID aggregates in solution ⇒ size and shape depends on surfactant

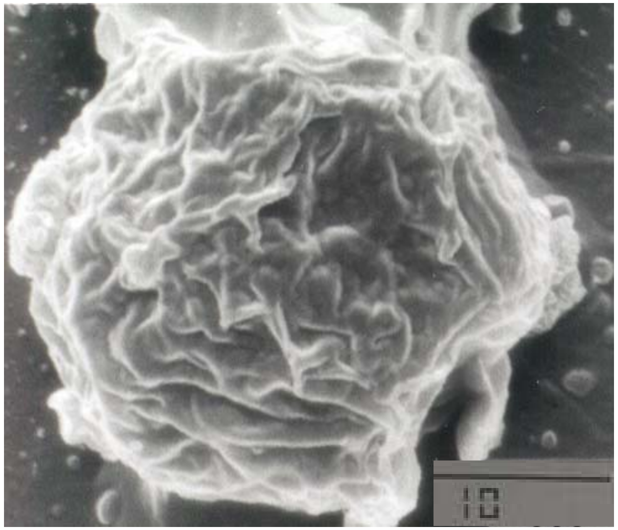
Octadecylamine (ODA) ⇒ polyhedral in shape, 250nm diameter

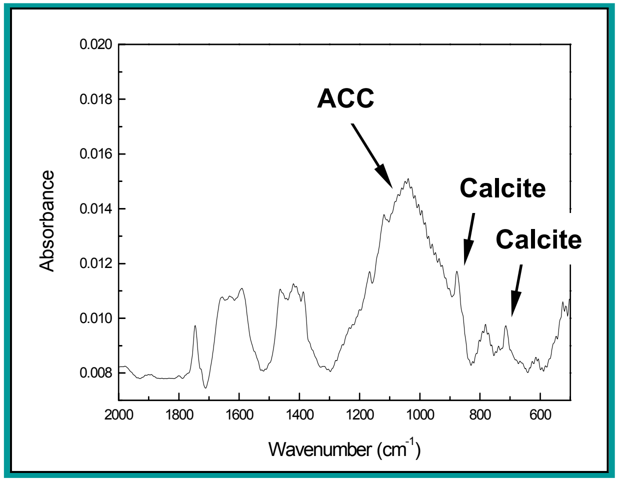
J.J.J.M. Donners et al. *Chem. Commun.* 1937 (2000).

Mineralisation of Octadecylamine/ Dendrimer Aggregates



15 mins - *ACC*





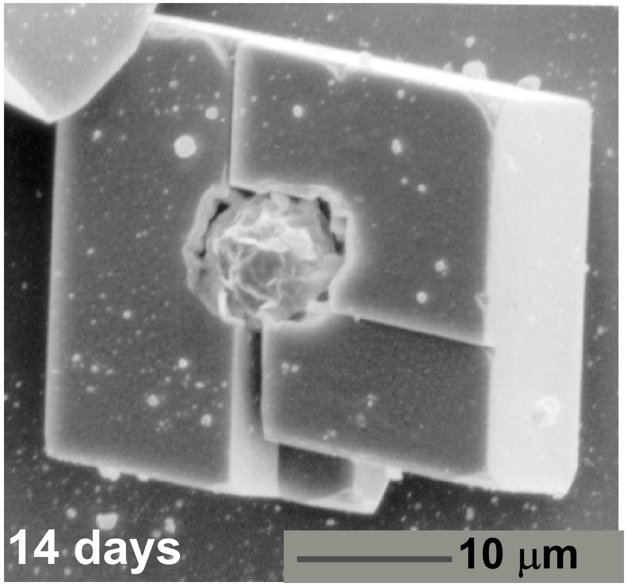
15 mins – calcified aggregates retained polyhedral shape

1 day - *ACC*

J.J.J.M. Donners et al. *Chem. Eur J., 8* 2561 (2002)

Continued incubation ⇒

Observe rhombohedral calcite crystals associated with ACC particles

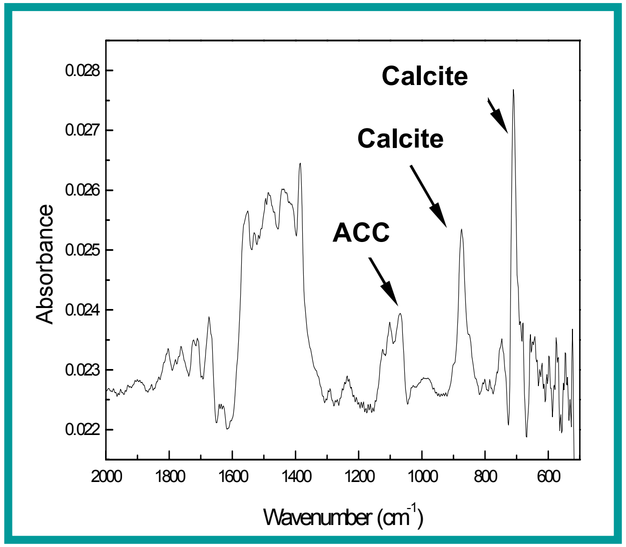


Formation and stabilisation of ACC may be due to:

- The low charge density, and high rigidity of the aggregates

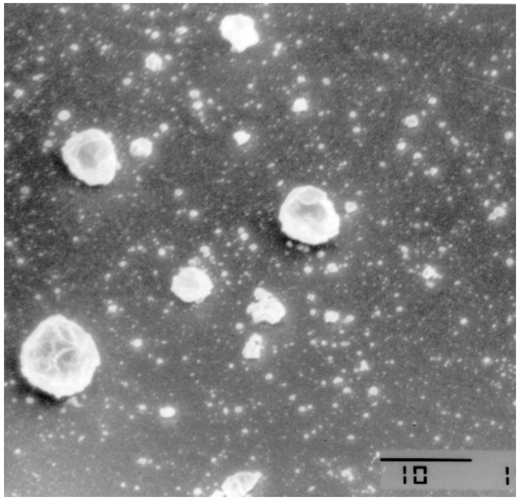
⇒ limits the nucleation and growth of the crystalline polymorphs.

- Binding of the aggregates to the ACC surface, and possible incorporation within the ACC particles

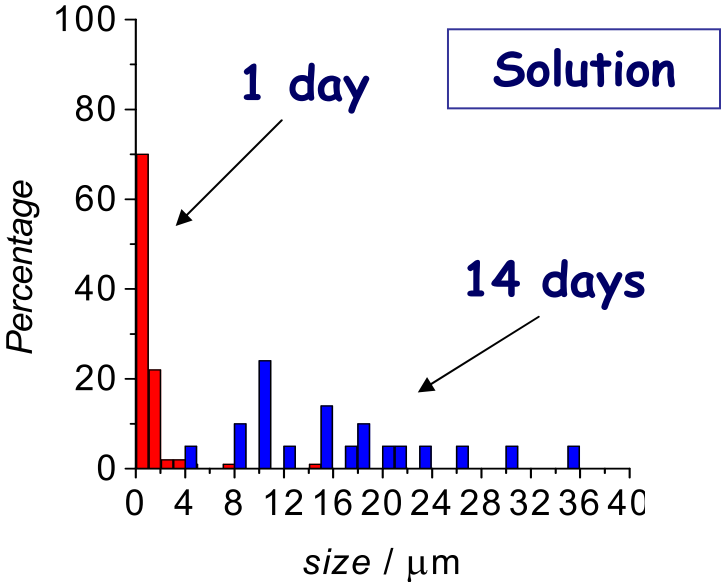


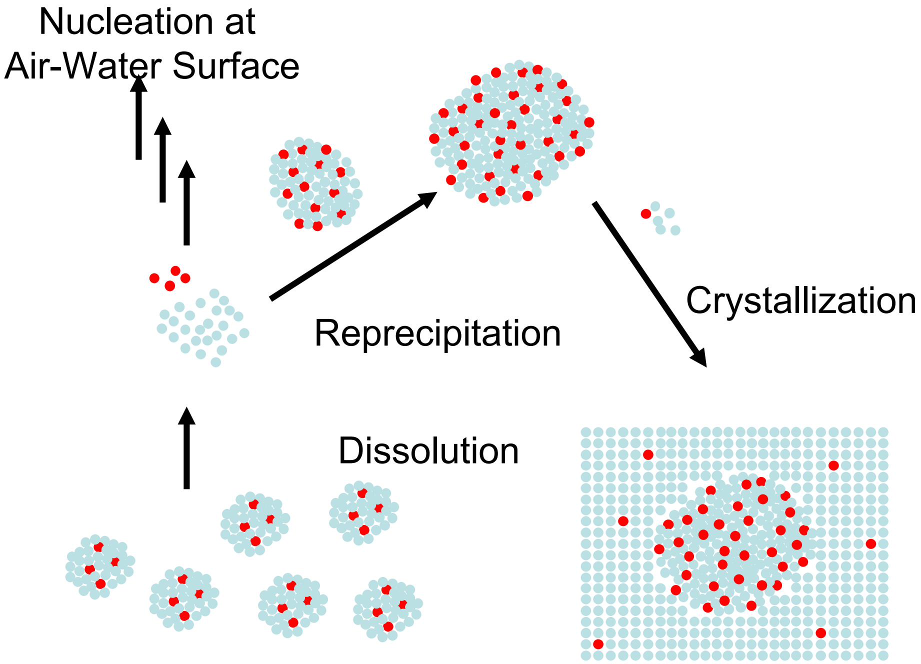
J.J.J.M. Donners et al. *Chem. Eur J.*, *8* 2561 (2002)

Mineralisation of the Aggregates



ACC continuously formed at air/solution interface ⇒ due to surface activity of dendrimer/surfactant aggregates





J.J.J.M. Donners et al. *Chem. Eur J.*, *8* 2561 (2002)

Using ACC in Crystal Synthesis

An amorphous material has no preferred shape ⇒ can be **easily moulded into any desired shape**

In contrast, crystals exhibit a preferred morphology with regular faces

Can an amorphous precursor material be used as a synthetic route to crystalline solids with controlled morphologies ?

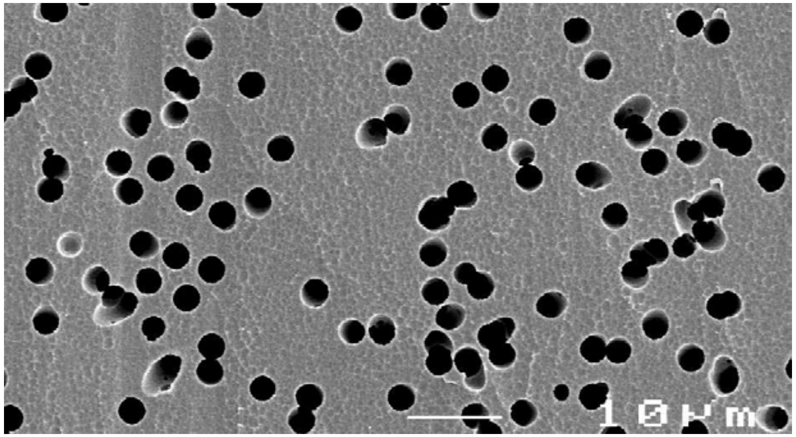
We will now provide a number of examples:

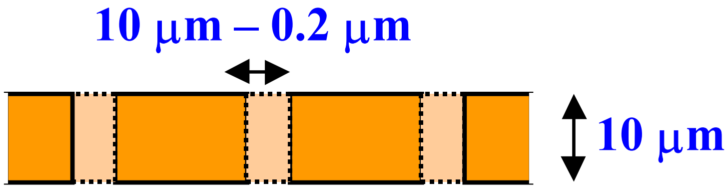
- Calcite single crystal rods
- Calcite thin films and fibres
- Porous calcite crystals

Calcium Carbonate Precipitation in Track Etch Membranes

Investigate transformation of ACC within a constrained environment ⇒ analagous to sea urchin embryo

Does this offer a mechanism of single crystal morphology control ?

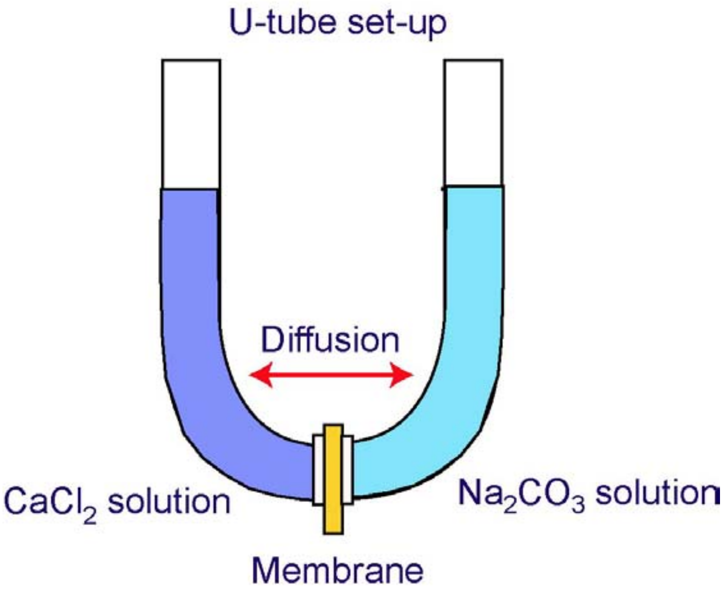


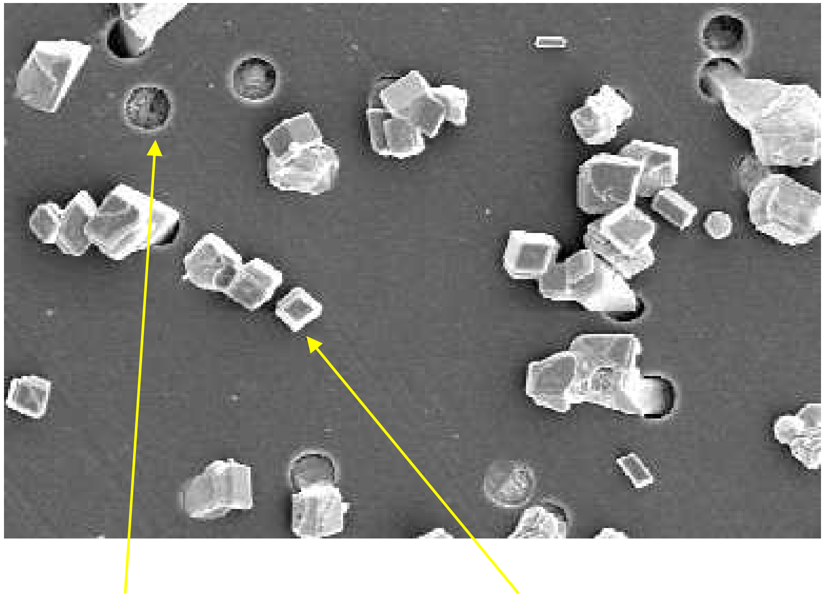


Track etch membranes offer perfect model system

Loste E., Meldrum F.C. (2001) *Chem. Comm.* 10, 901-902.

Methodology



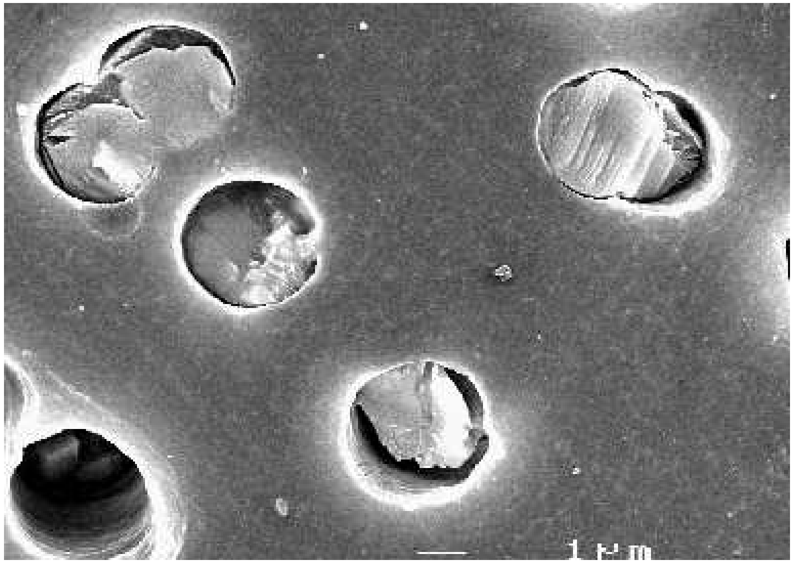


- Double diffusion technique used
- Low temperatures (0-4 °C) applied to stabilise ACC phase

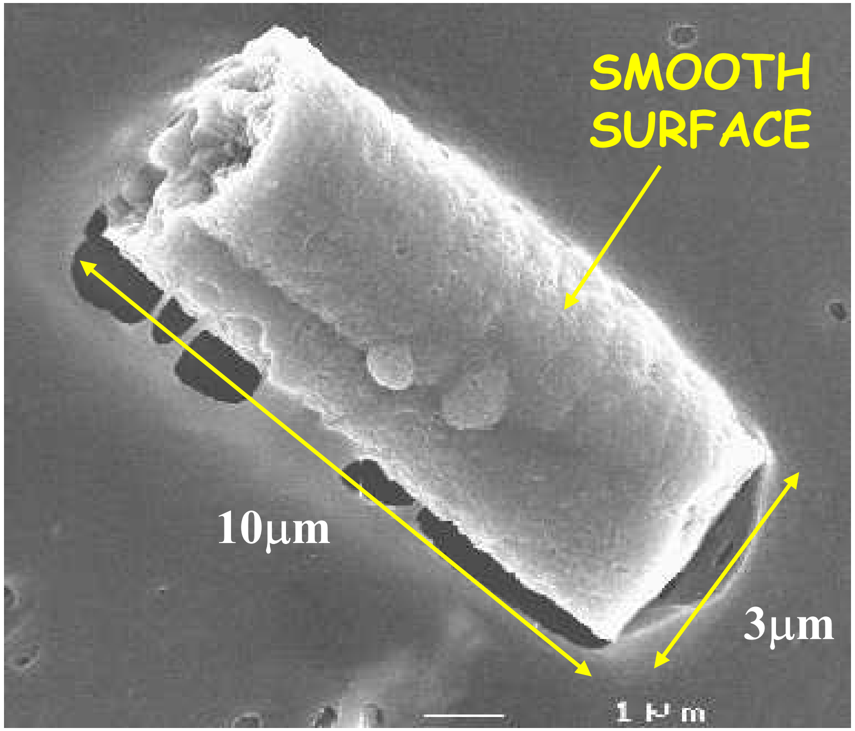
Control of $CaCO_3$ Morphology

$CaCO_3$ crystals grow within the membrane pores

⇒ exhibit rod-like morphologies and curved surfaces



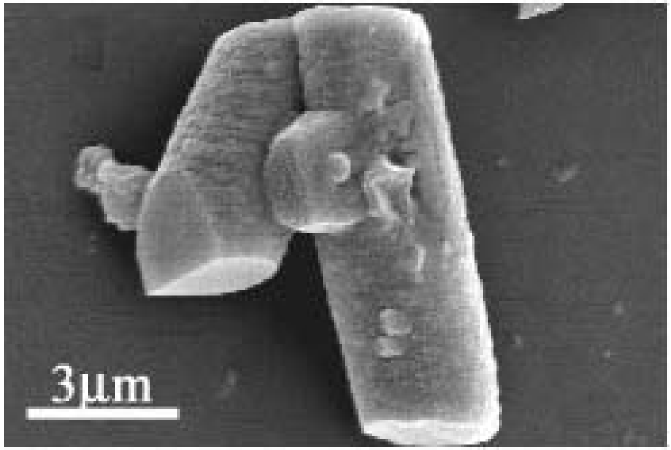
$CaCO_3$ in the membrane pores and isolated after dissolution of the membrane

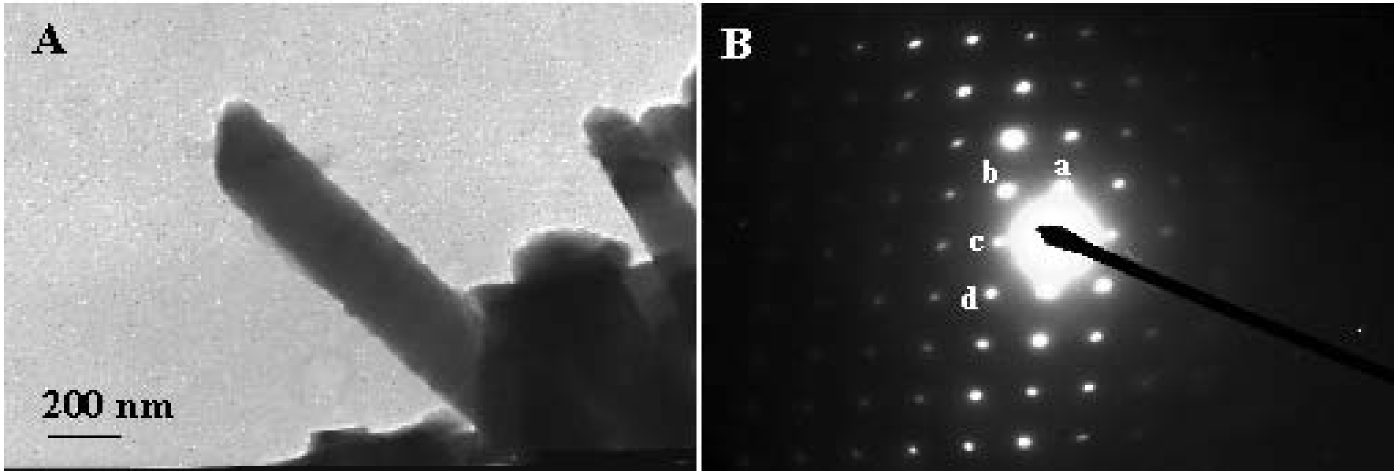


Crystal Structure

Planar faces are characteristic of calcite

Are particles SINGLE CRYSTALS ?

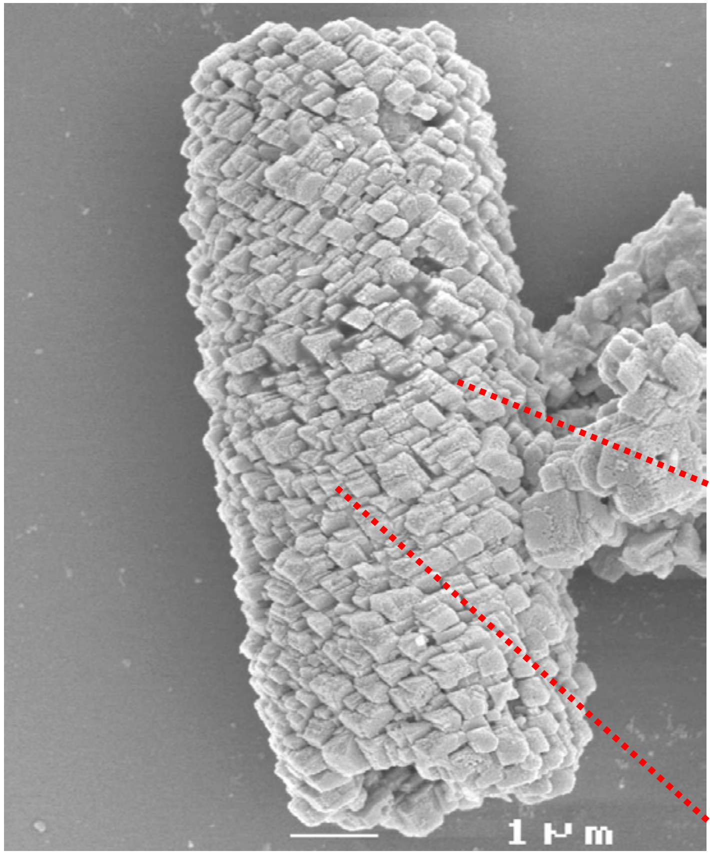




Selected area electron diffraction of 0.2 µm particles show SINGLE CRYSTAL CALCITE

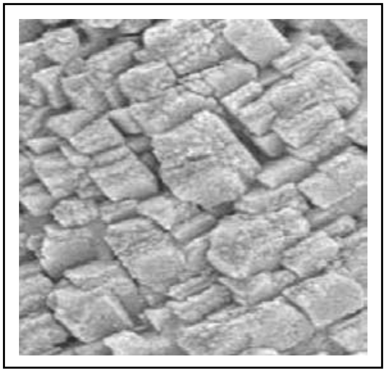
Crystal Overgrowth

Examine particle crystallinity and crystallography using crystal overgrowth ⇒ calcite crystals grown on particle surface

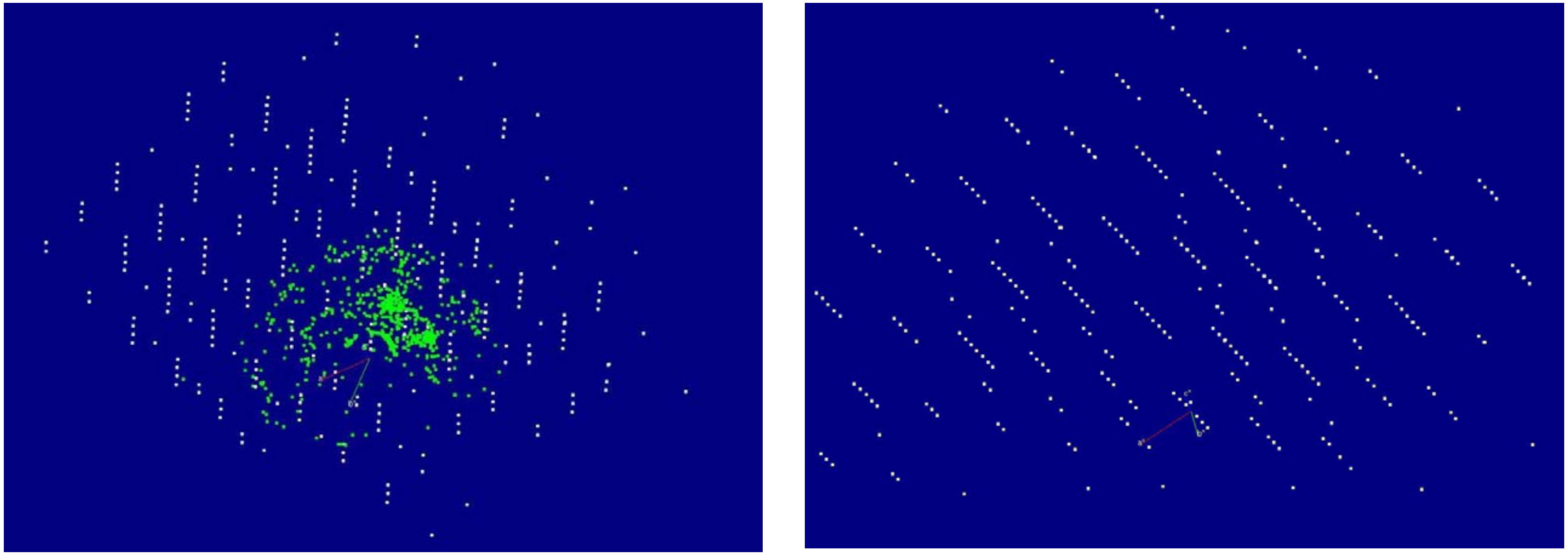


- Crystals aligned over particle surface ⇒ indicates single crystal

- No preferred orientation of crystals wrt rod axis apparent



3μm × 10μm Rod



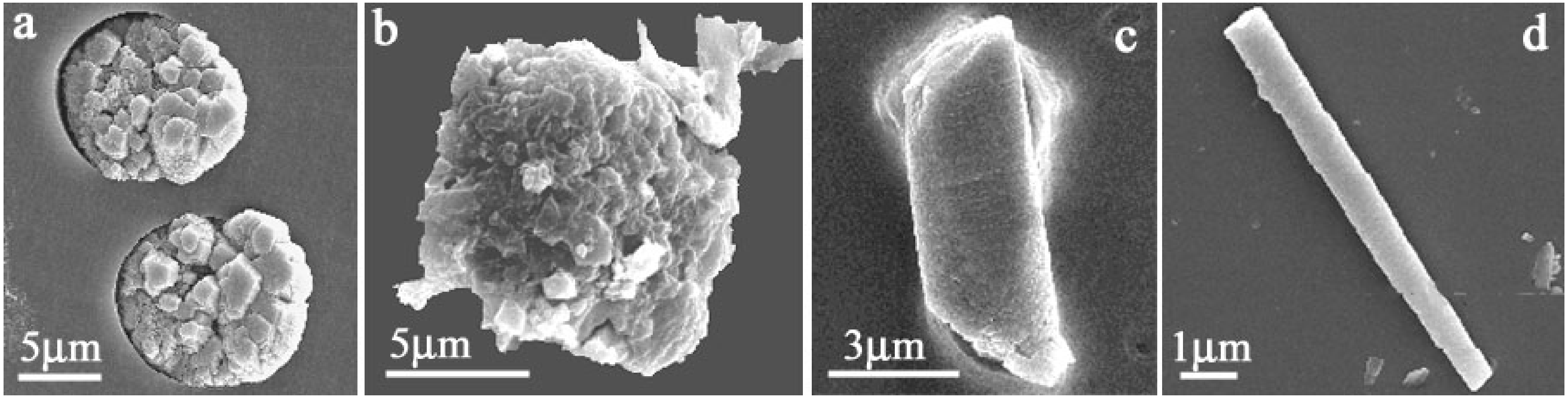
WHITE ⇒ SINGLE CRYSTAL CALCITE

$a = b = 4.9979$ Å, $c = 16.9898$ Å $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$

GREEN ⇒ No solution to unit cell

What is the Influence of the Pore Size ?

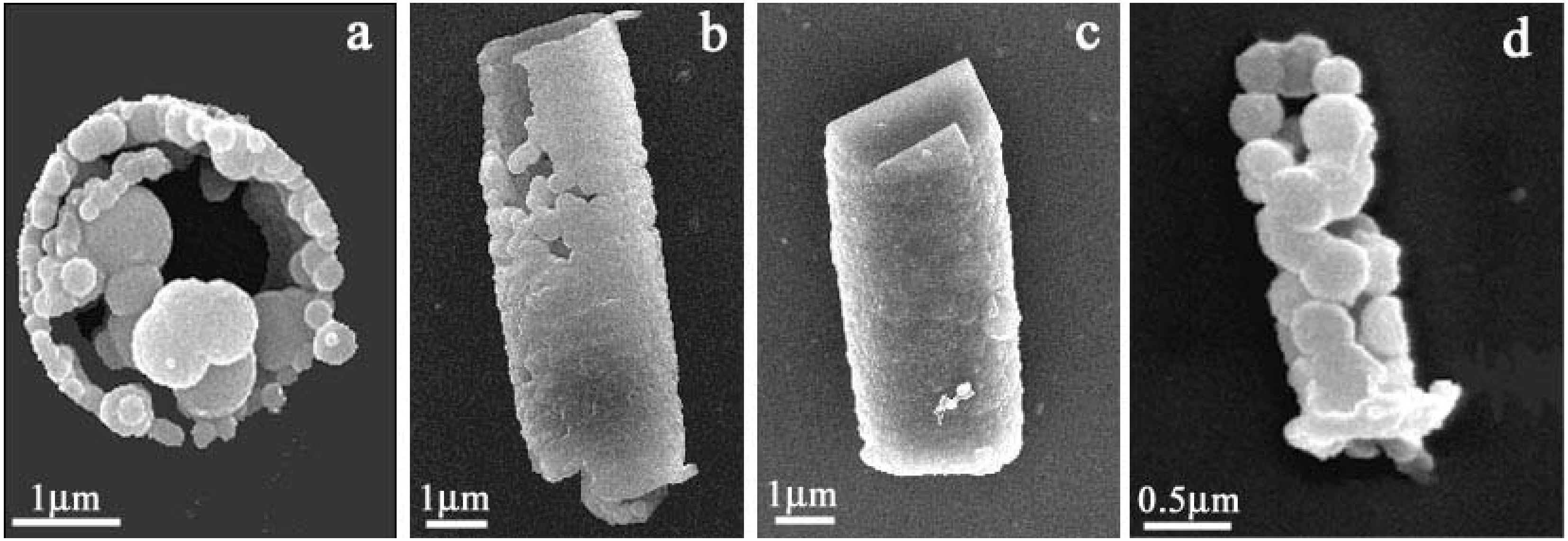
Grow crystals in 10μm, 3 μm, 0.8 μm and 0.2 μm pores



- Perfect replication in 3 μm, 0.8 μm and 0.2 μm pores
- Polycrystalline aggregates in 10 μm pores
- Why ??

Loste , Park, Warren, Meldrum (2004) *Adv Func. Mater.* 14(12), 1211-1220.

Mechanism of Particle Formation

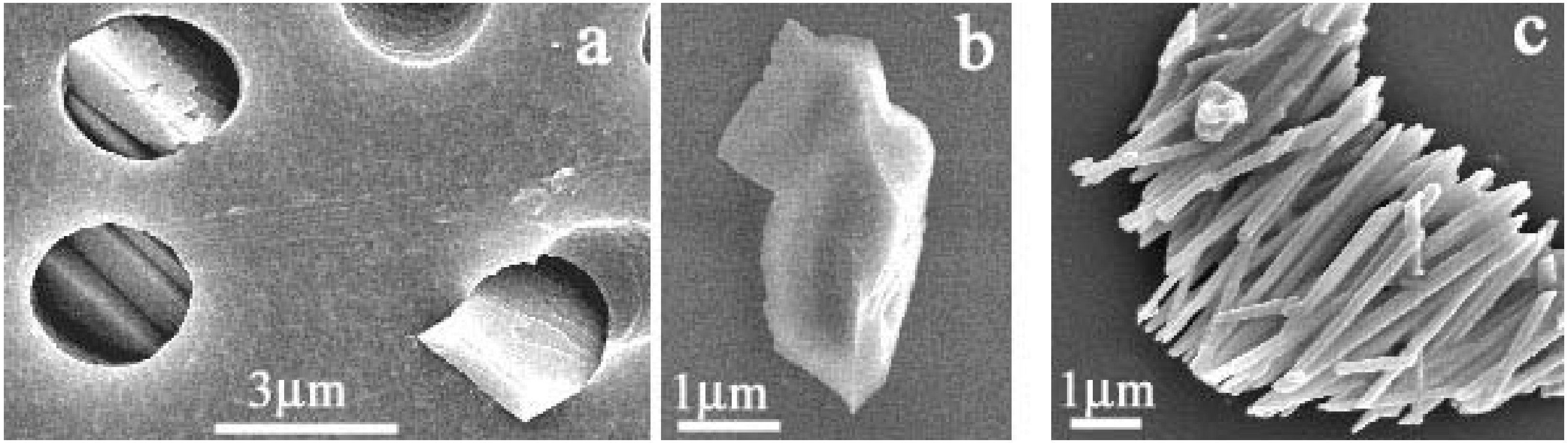


Particles in (a-c) 3 µm pores and (d) 0.8 µm pores after (a, b) 15 mins (c) 1hr and (d) 15 mins

- In 3 µm, 0.8 µm and 0.2 µm pores, ACC completely fills pore before crystallisation occurs ⇒ SINGLE CRYSTAL

- 10 µm pores are too large and crystallisation proceeds before pore is filled ⇒ POLYCRYSTALLINE

0.1 M $CaCl_2$ / Na_2CO_3 at Room Temperature

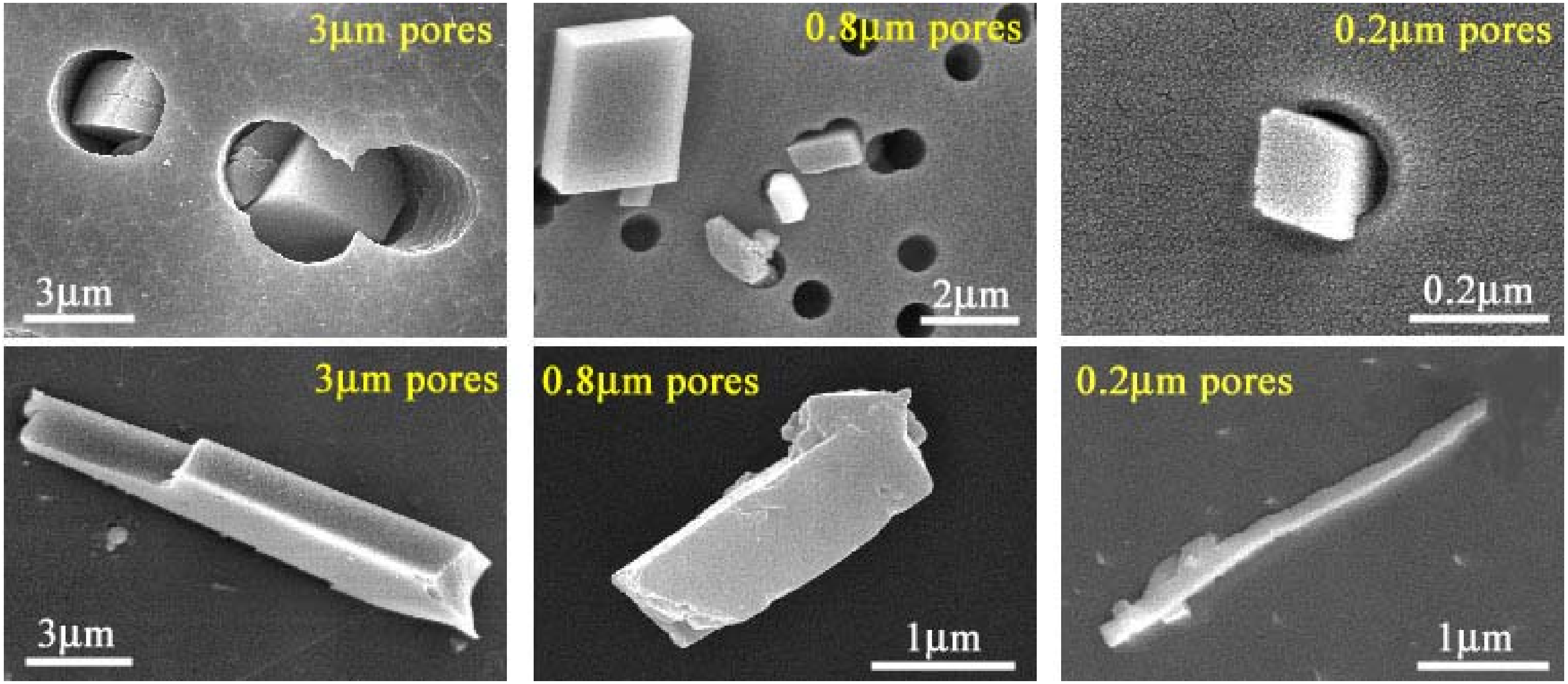


- Intra-membrane particles increasingly differed from rods with increasing pore diameter

- Particles from 3μm membranes show irregular shapes and crystalline faces

- Only the 0.2μm membranes gave perfect rod-like morphologies

0.008 M $CaCl_2$ / Na_2CO_3 at Room Temperature



- No *ACC* was observed
- Particles have regular morphologies and planar surfaces characteristic of crystalline materials

Summary

- An amorphous material has no preferred morphology $\Rightarrow$ can shape into any form

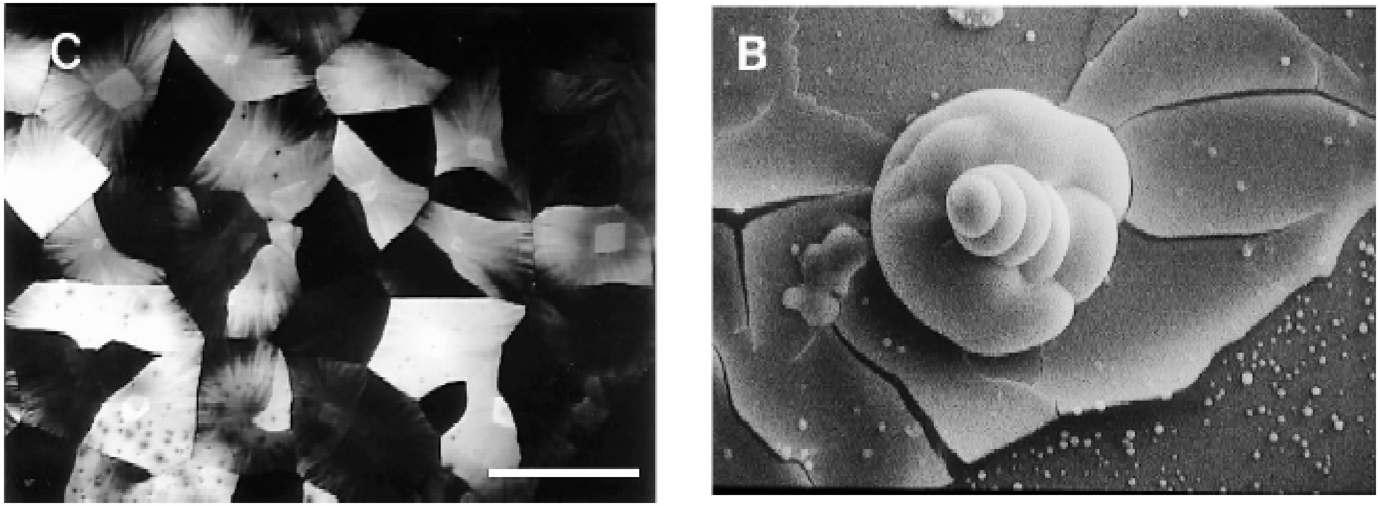
- Subsequent crystallisation generates a **single crystal with identical morphology**

Polymer Stabilised ACC

Previous Experiments ⇒ used temperature to stabilise ACC

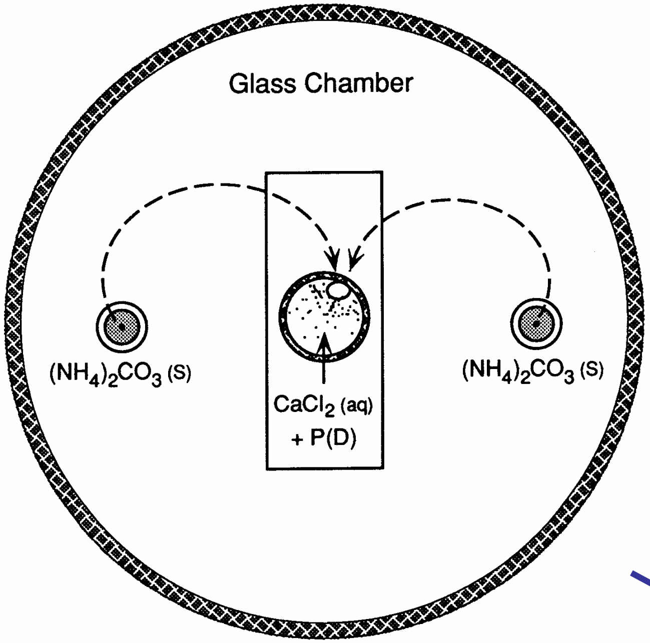
Can also used polymer additives ⇒ Laurie "PILP" Gower
Poly(aspartate)
Poly(acrylic acid)

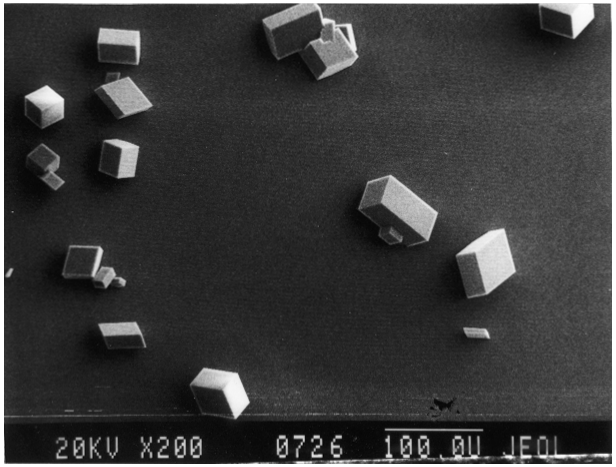
Stabilises ACC particles and gives them "liquid-like" properties



Gower, Tirrell, *J. Cryst. Growth*, (1998), 191(1-2), 153.

Experimental Setup for $CaCO_3$ Crystallization



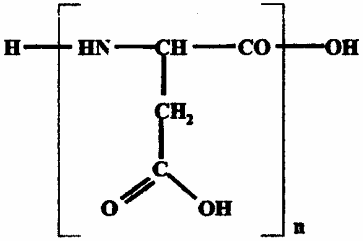


Calcite of rhombohedral habit

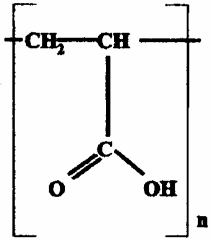
w/ P(D)

Calcite thin film (0.5mm thick)

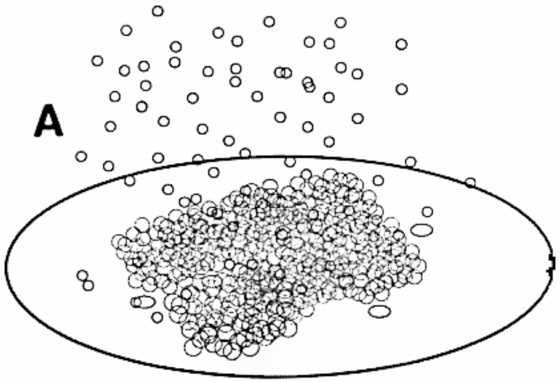
Poly-L-Aspartic Acid
(PLAsp)



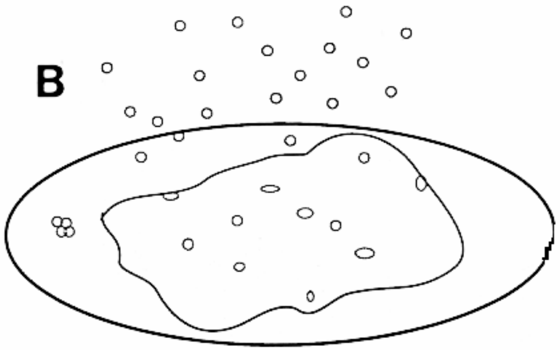
Poly-Acrylic Acid
(PAA)



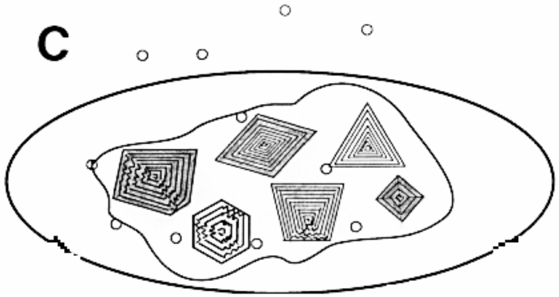
Polymer-Induced Liquid-Precursor Process



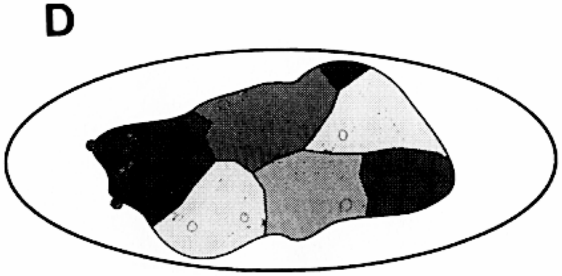
Liquid-Liquid Phase Separation



Deposition of Precursor Film



Solidification and Crystallization



Birefringent $CaCO_3$ Film

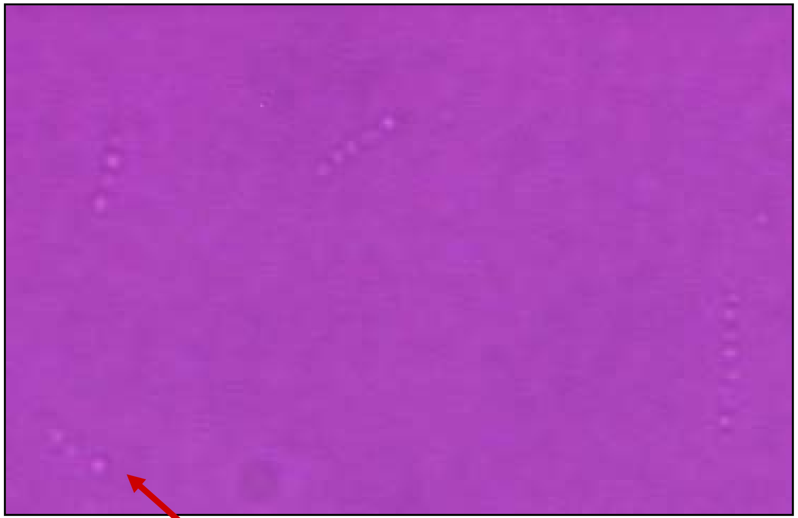
Gower, L.B. & Odom, D.J., *J. Crystal Growth*, 210/4, 719-734 (2000).

Stages of PILP Process

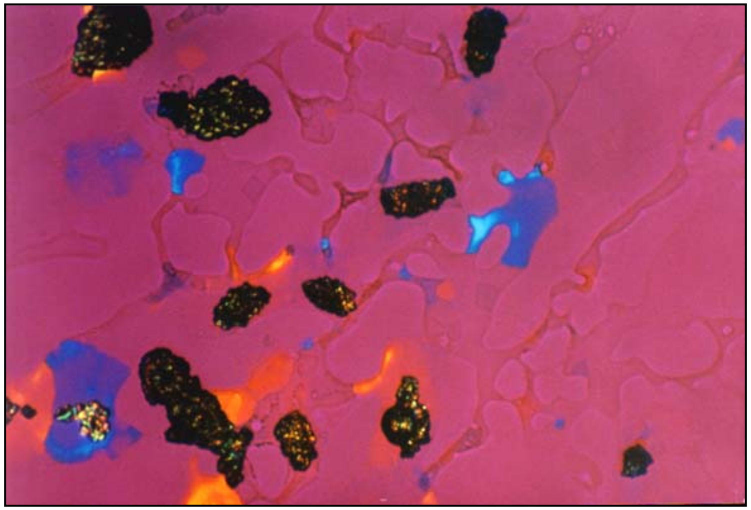
$$CaCl_2(aq) + P(aq) \xrightarrow{(NH_4)_2CO_3(v)} CaCO_3\text{-}P\text{-}H_2O(PILP) \longrightarrow CaCO_3\ (s)$$

Stage I
Precursor deposition

Stage II
Precursor transformation

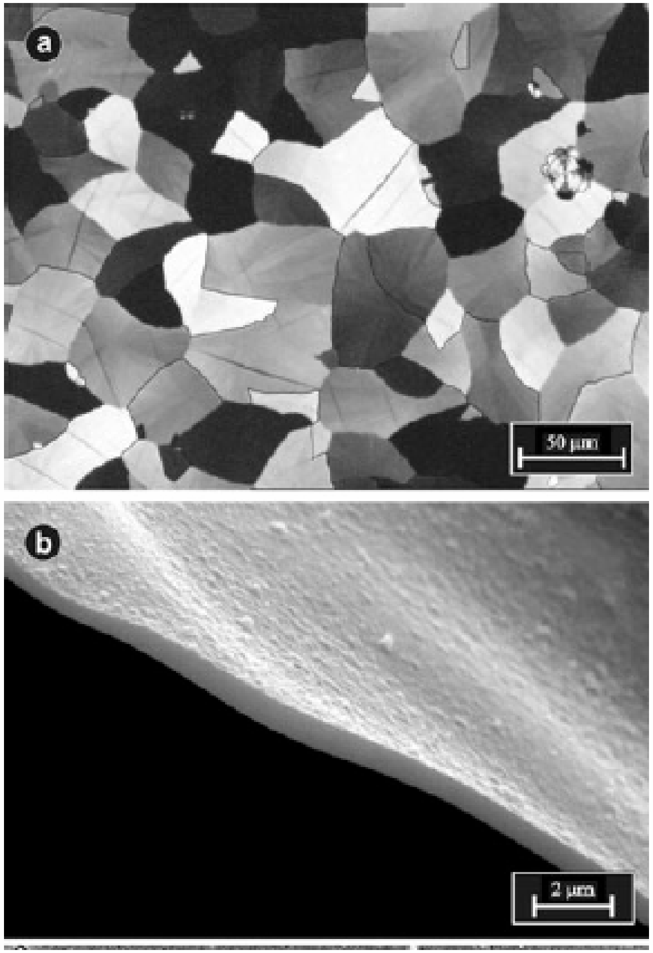


- droplets of precursor phase (2 –4 mm) observed in situ



- amorphous precursor film
- birefringent crystalline patches
- aggregates of small crystals

Film Structure



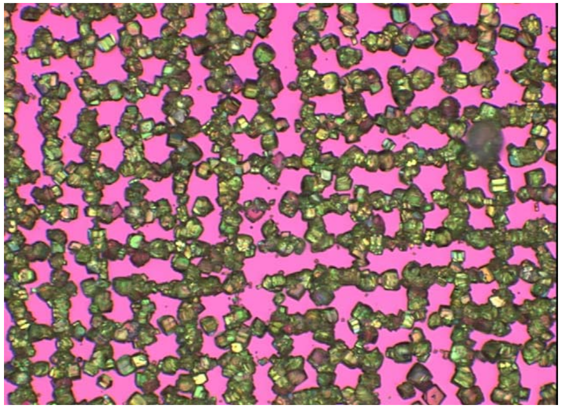
- Crystalline thin film formed after heating at 400 °C for 2 h.
- Typical domain sizes range from 50–200 mm in diameter
- Average film thickness is 600 nm.

Volkmer, Harms, Gower and Ziegler *Angew. Chem. Int. Ed.* 2005, 44, 639 –644

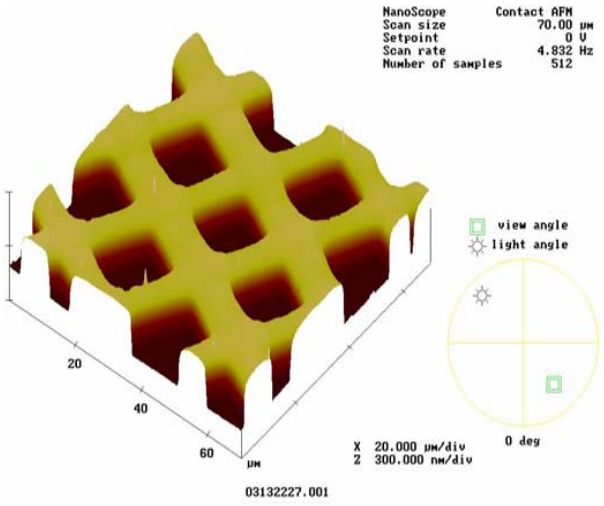
Templating $CaCO_3$ Films via PILP

Microcontact printed SAM tempate: COOH terminated thiol / Au

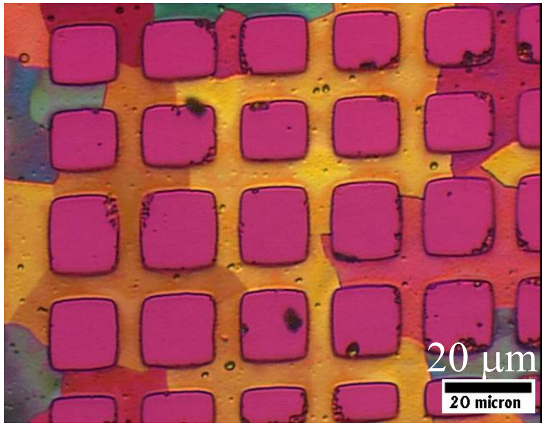
Reaction conditions: 12 mM $CaCl_2$ + 42 mM $MgCl_2$, 2 mg/ml PAA, 4°C



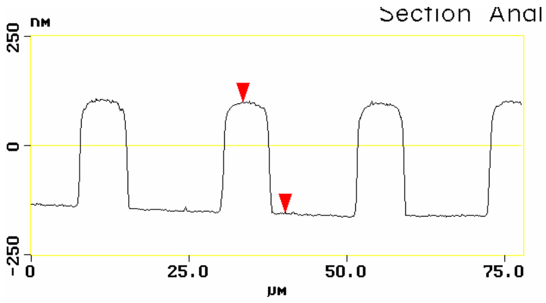
Patterning without polymer



AFM image

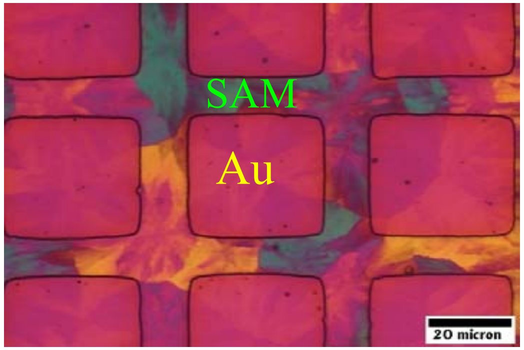


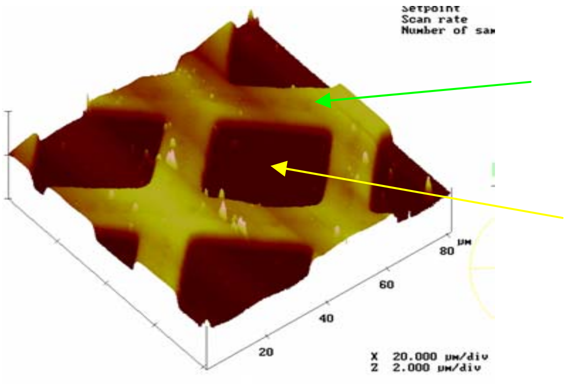
Patterning via PILP process



AFM height image and line scan

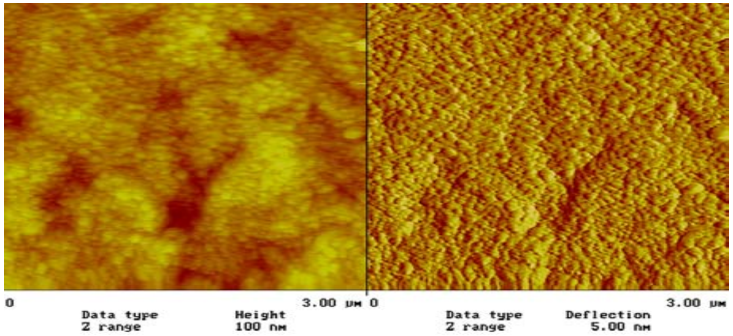
Calcite Film Topology On Different Substrates

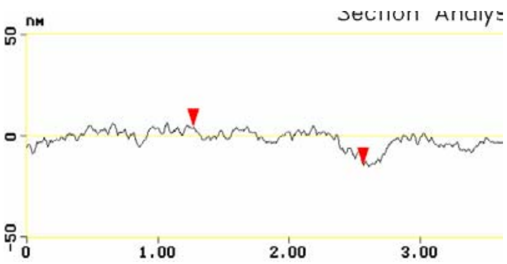




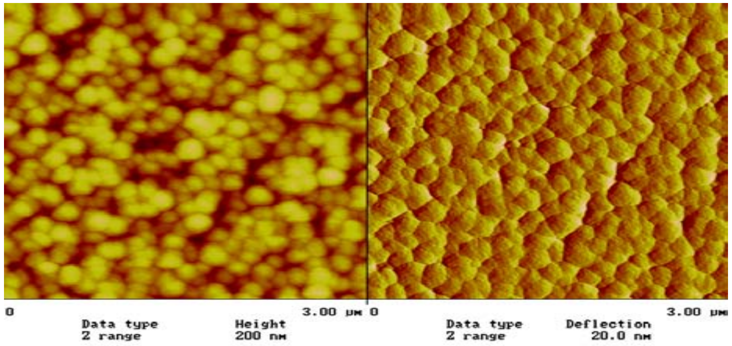
Thick $CaCO_3$ film on COOH-terminated SAM

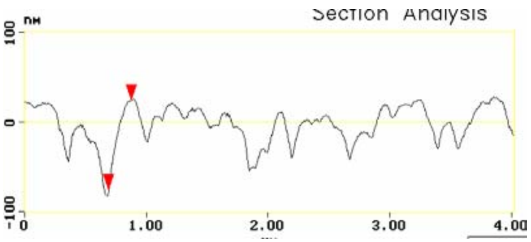
Thin $CaCO_3$ film on bare gold surface





Thick $CaCO_3$ film on COOH-terminated SAM

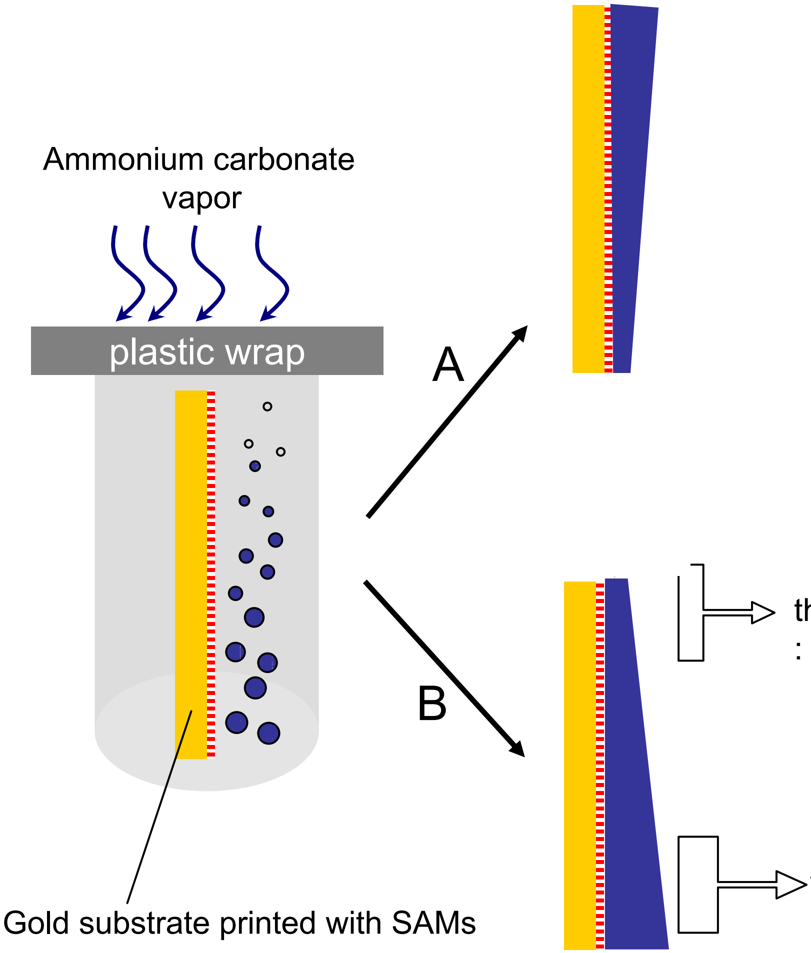


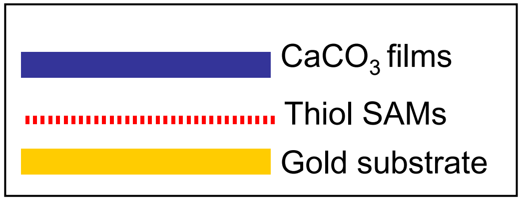


Thin $CaCO_3$ film on bare Au surface

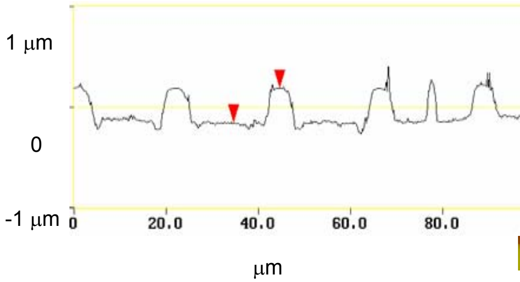
"height" image "deflection" image Section analysis across the film

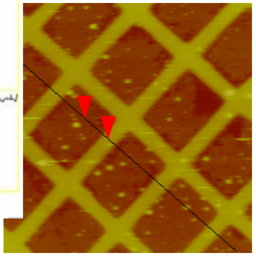
Mechanism of Amorphous Film Formation: Heterogeneous Surface Nucleation or Colloid Deposition?



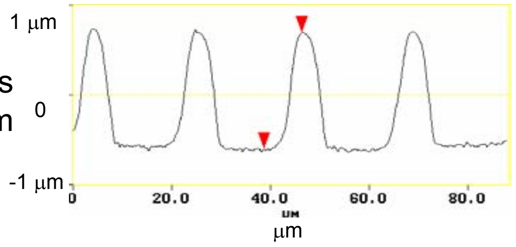


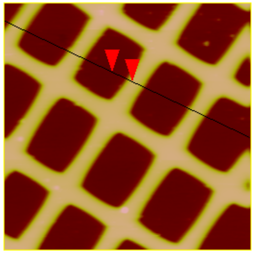
thickness : 350nm





thickness :1300 nm



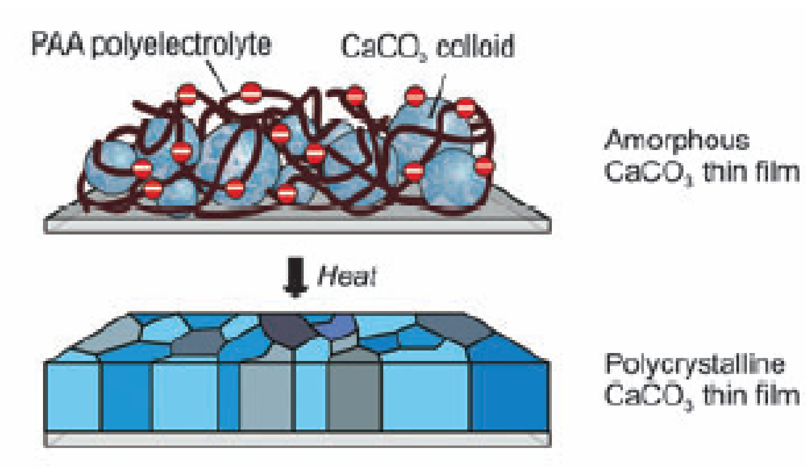


Laminated $CaCO_3$ Thin Films via PILP Method

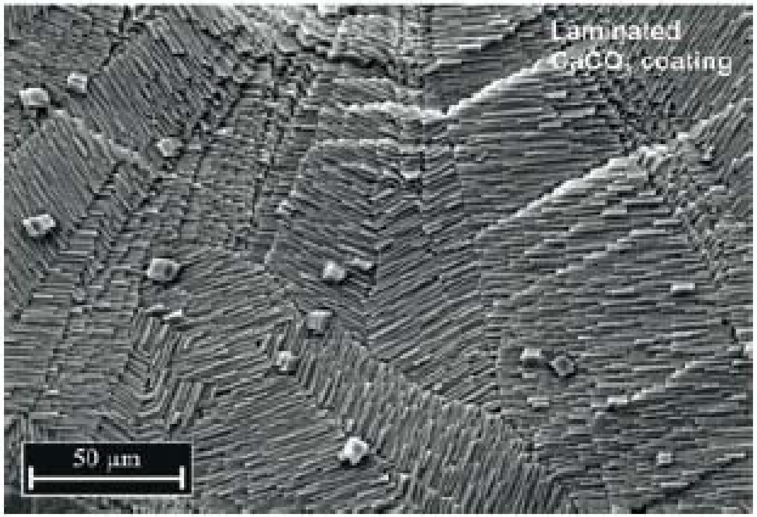
Polycrystalline calcite films formed via “PILP” process

⇒ Used as substrate to further deposit calcite

⇒ Complex, oriented thin film structures produced

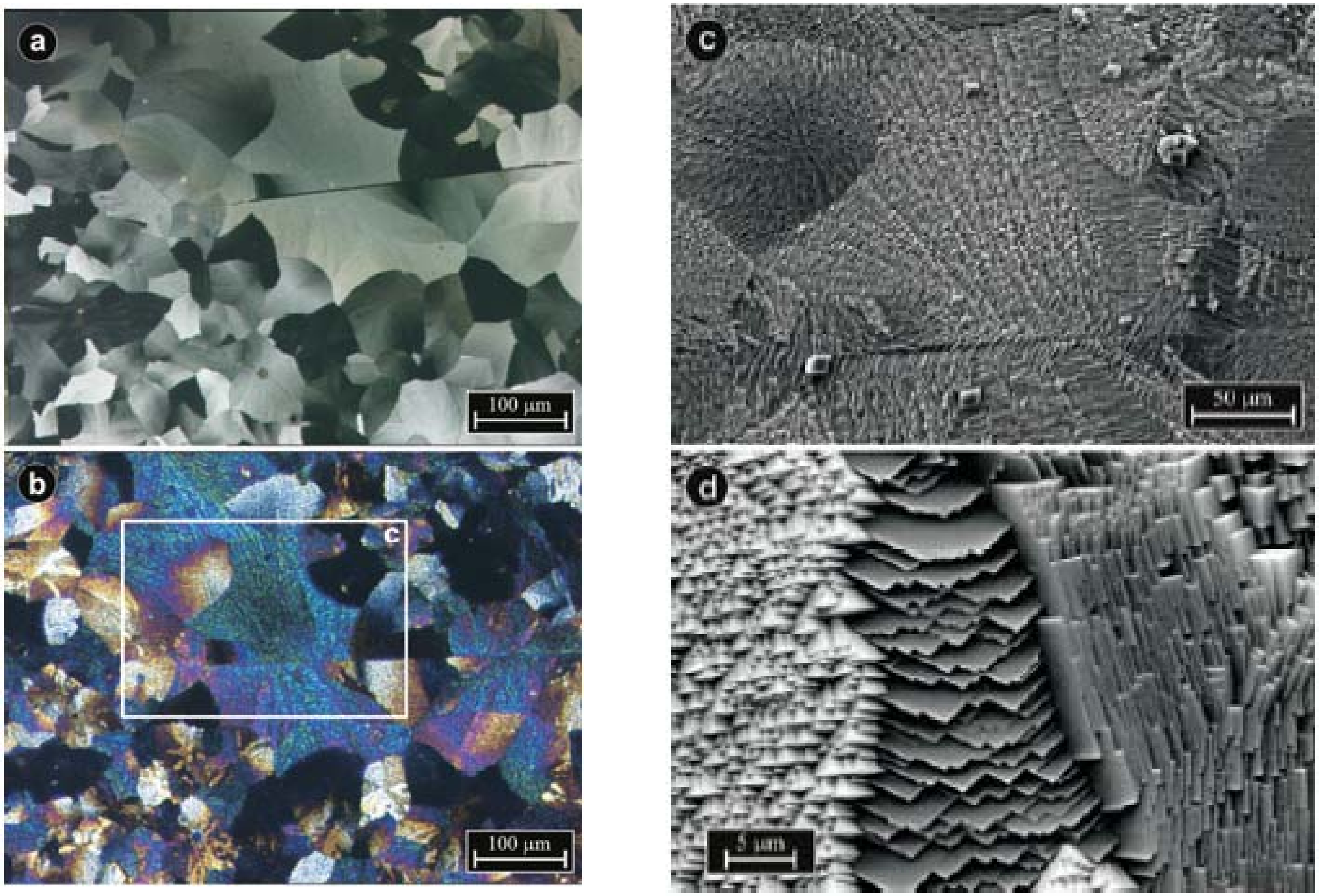


Films grown using flow system



Continued growth WITHOUT polymer

Volkmer, Harms, Gower and Ziegler *Angew. Chem. Int. Ed.* 2005, 44, 639 –644

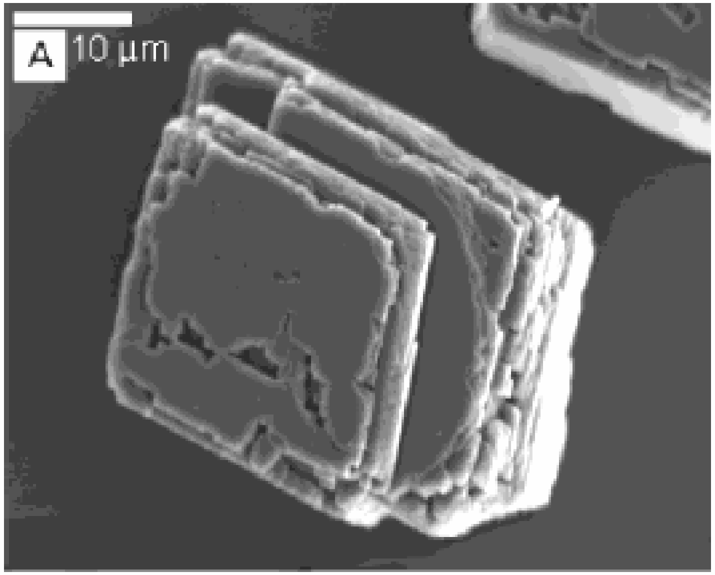


Polycrystalline calcite thin film (a) before and (b) after calcite overgrowth (b).

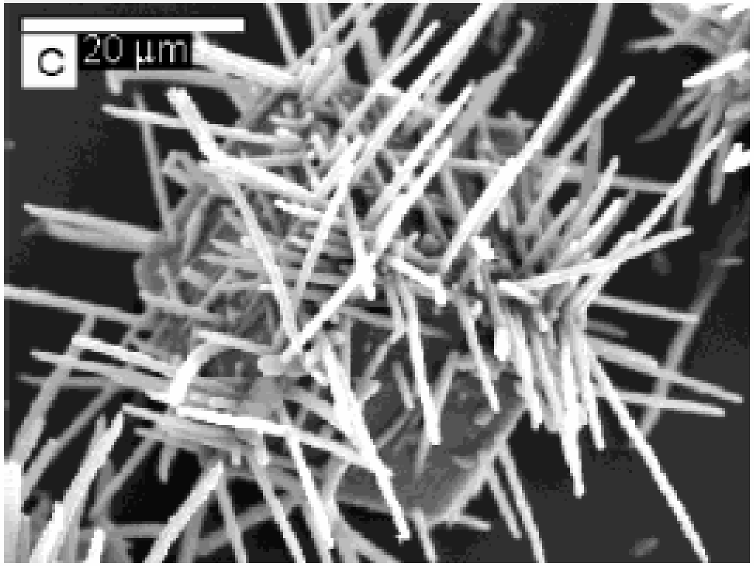
(c,d) Images showing abrupt changes of crystal orientations at the domain boundaries.

Calcite Fibres via an ACC Precursor Phase

ACC deposited on pre-formed rhombohedral calcite seed crystals



No stabilising polymer present

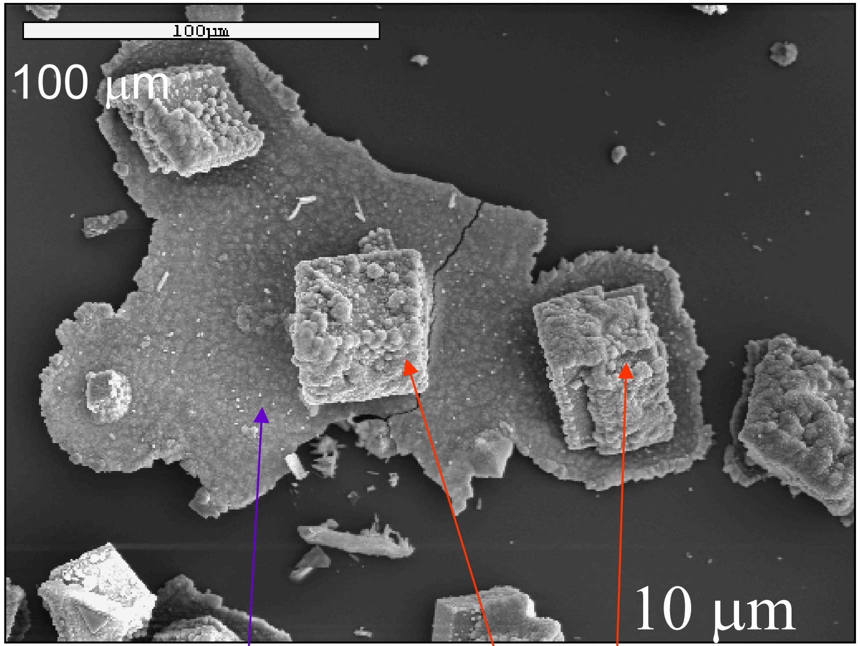


ACC stabilised using poly(acrylic acid)

ACC stabilised ⇒ SINGLE CRYSTAL CALCITE FIBRES formed

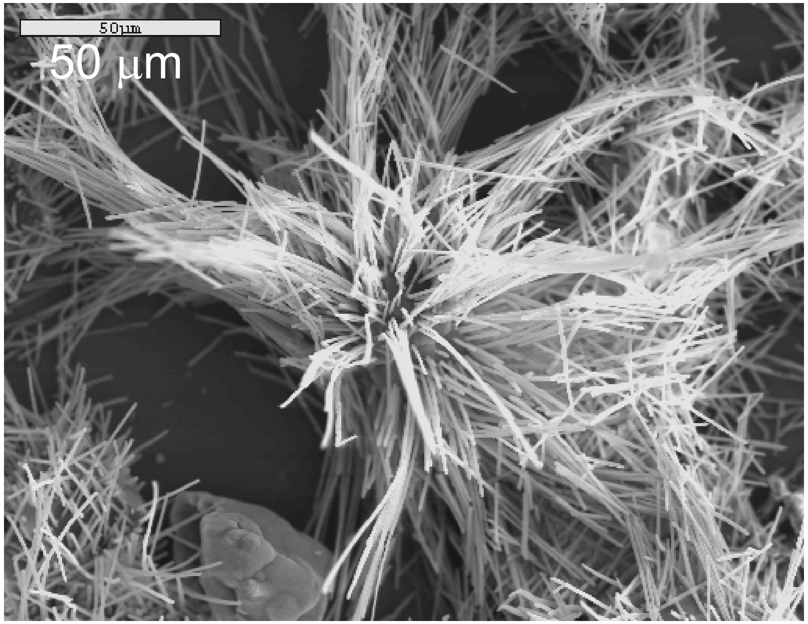
Olszta, Gajjeraman, Kaufman, and Gower *Chem Mater*. 2004, 16, 2355-2362.

Deposition of PILP Phase on Calcite Seeds



PILP film

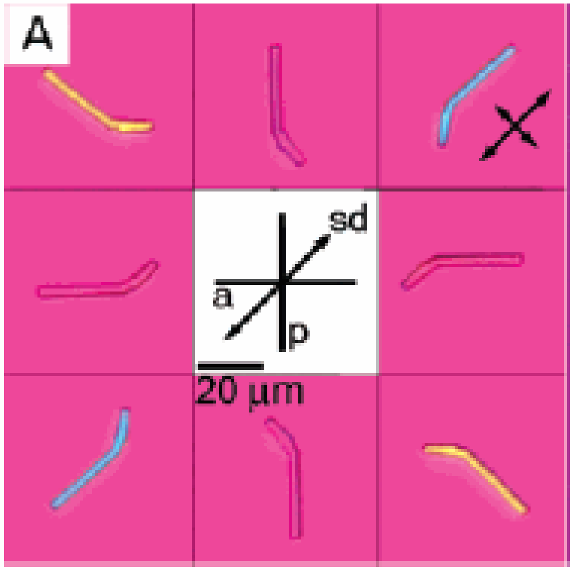
calcite “seeds”

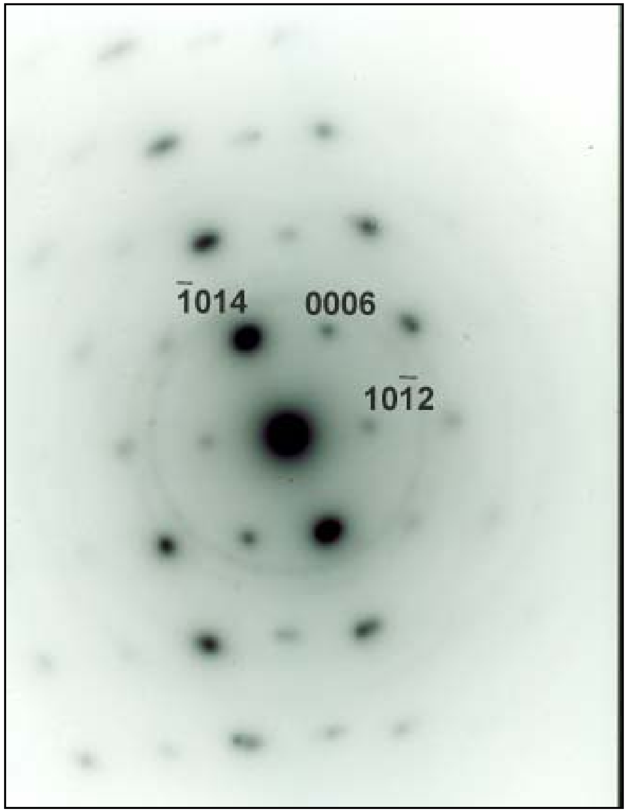


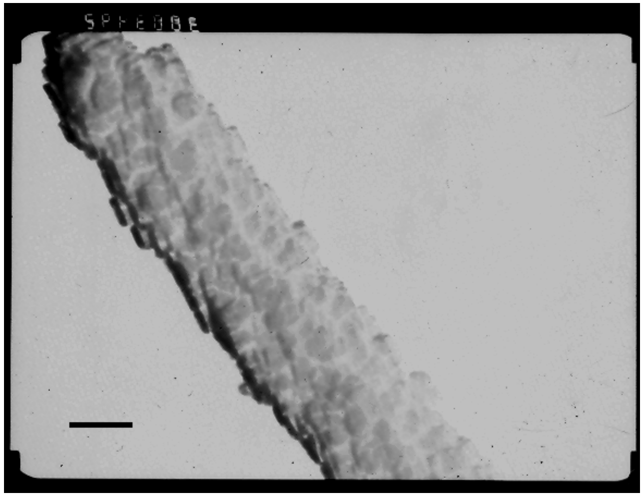
$CaCO_3$ fiber overgrowths, 540-800 nm in diameter

Seed crystals placed at base of dish ⇒ ACC particles fall on to crystals

Characterisation of Fibers



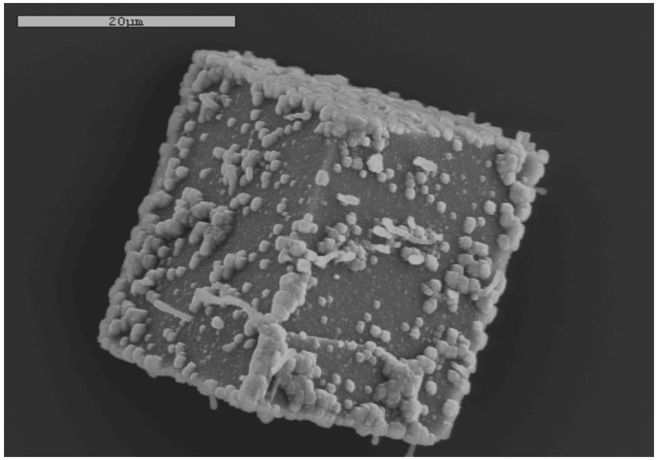




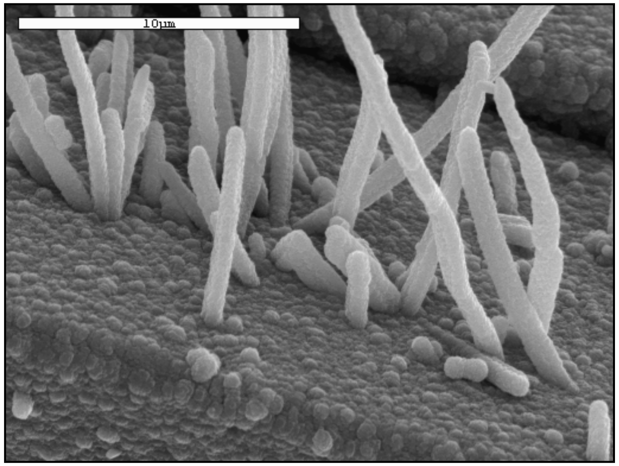
Polarised light microscopy and electron microscopy

⇒ Fibres single crystals of calcite

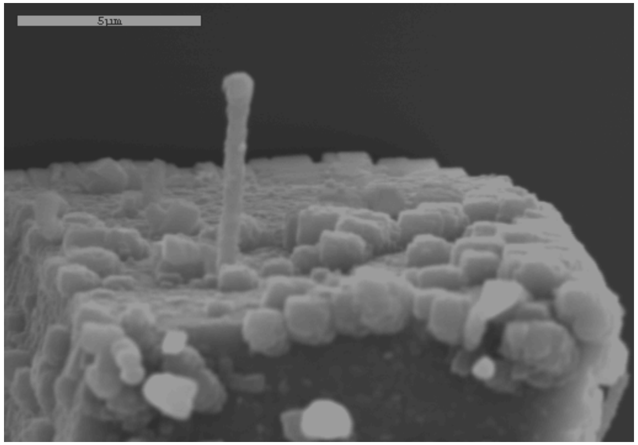
Development of Fibres



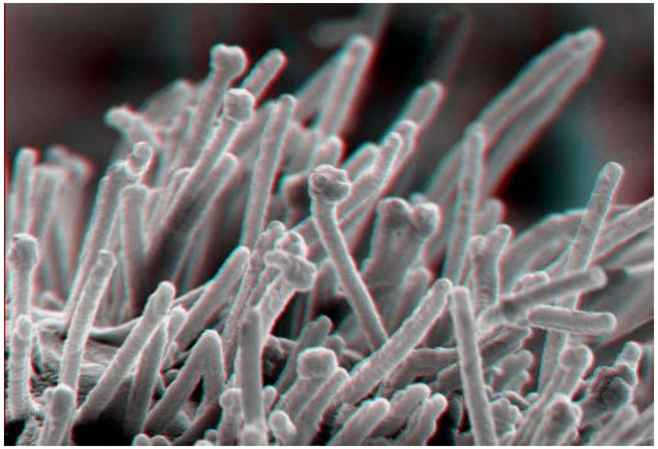
PILP Deposition on Seed



Formation of Flux Droplet

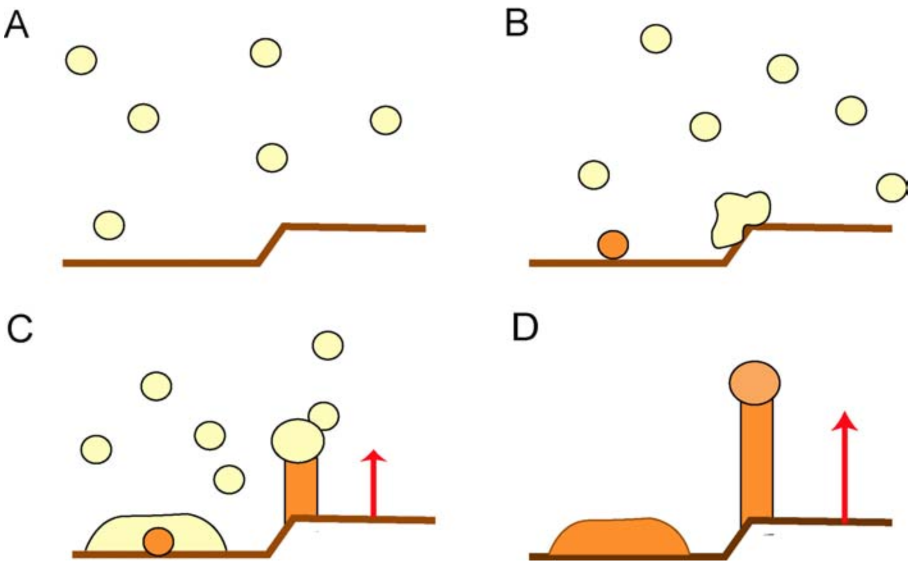


One-dimensional Growth



Solidification of Bobble

Suggested that the ACC Phase has Liquid-like Properties …



- Liquid-like ACC particles condense at selected sites on substrate crystal
- Begin to crystallise and act as a site for further condensation

⇒ FIBRES OF CALCITE FORM

Summary

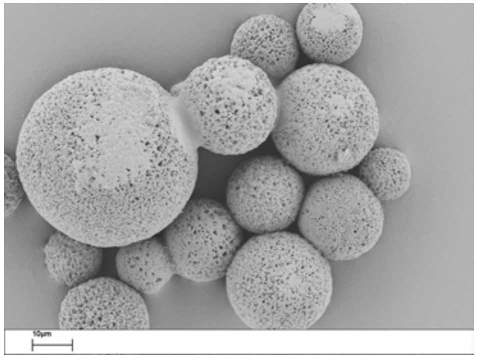
Addition of polymers is an effective method for stabilising ACC

⇒ Production of crystalline thin films

⇒ Infiltration to form 3D solids

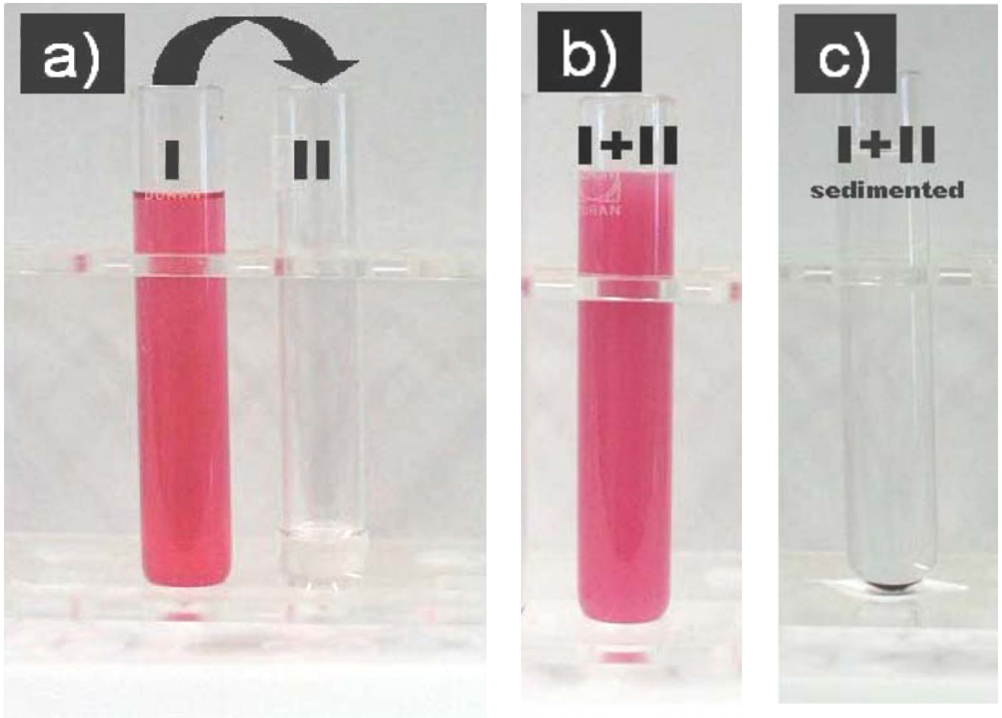
Not restricted to $CaCO_3$

- Calcium Phosphates (Laurie Gower)
- Organic crystals - DL-glutamic acid (Helmut Coelfen)

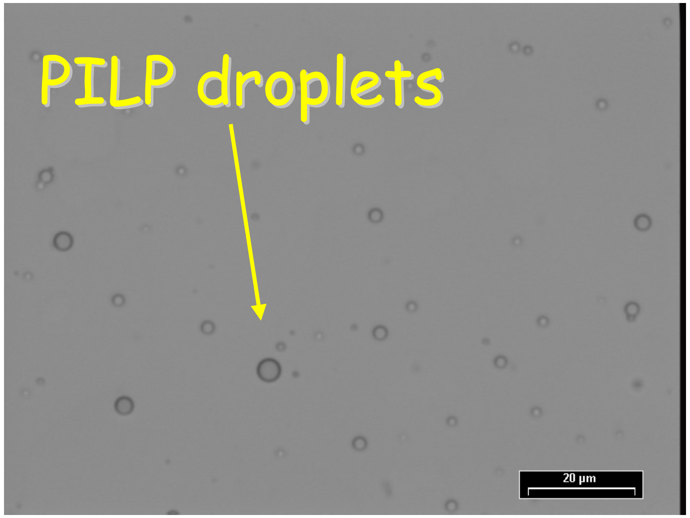


Wohlrab, Colfen, Antonietti, *Angew. Chem. Int. Ed* (2005), 44, 4087-4092

Crystallisation of Glutamic Acid via PILP Method

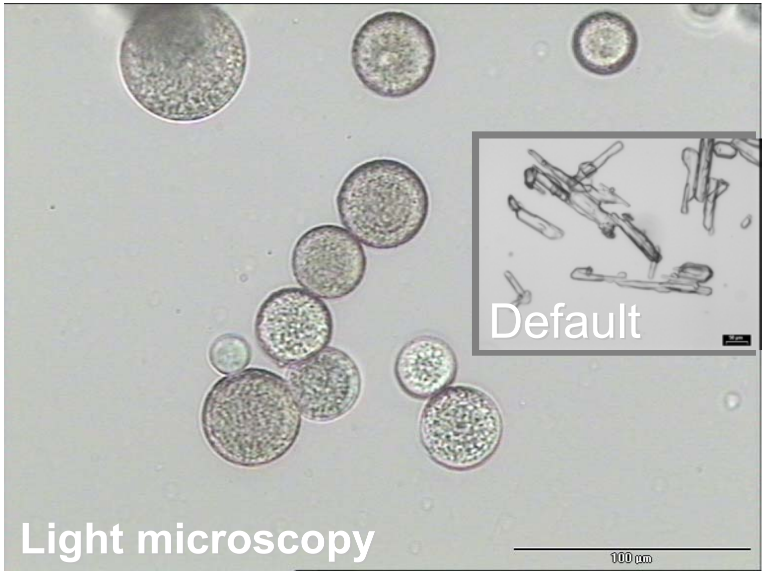


I = ethanol + 0.01 % amaranth, II = 10 g/l DL glutamic acid + 1 wt.-% PEI_{600}

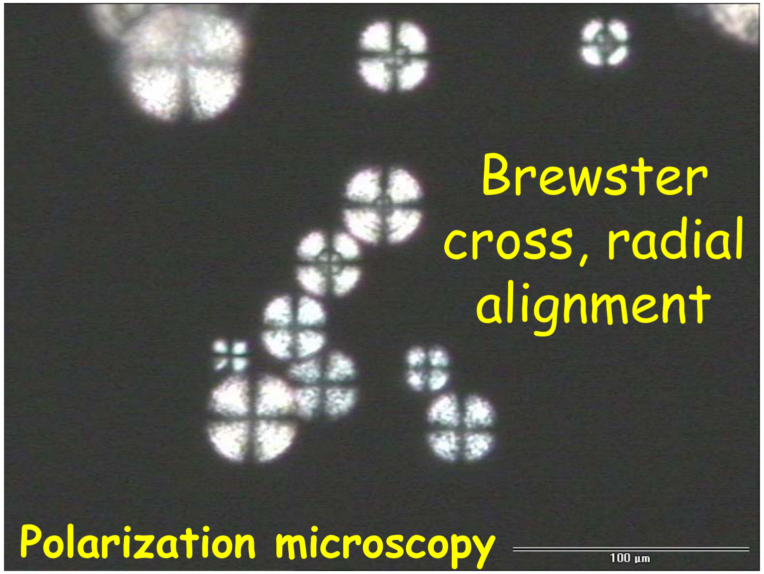


DL-glutamic acid after addition of ethanol precipitant to saturated aqueous solution (1 wt.% PEI (M_w = 600)

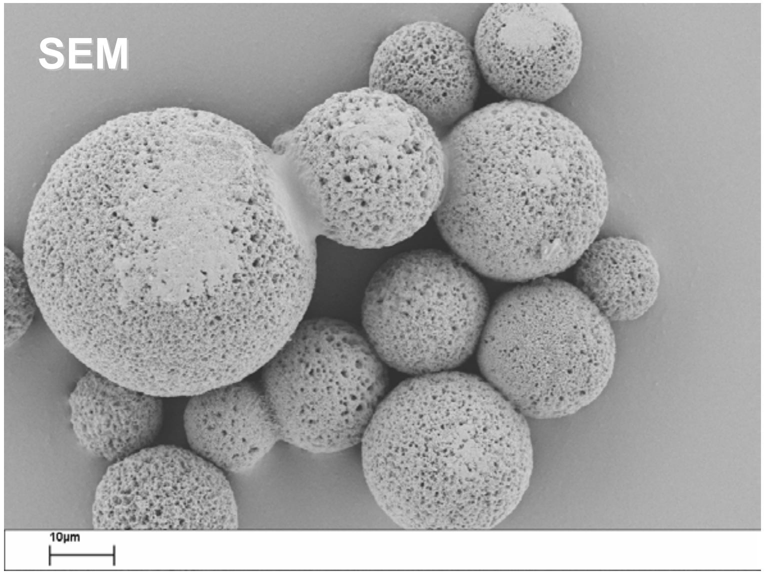
Porous D,L Glu Spheres via PILP Process

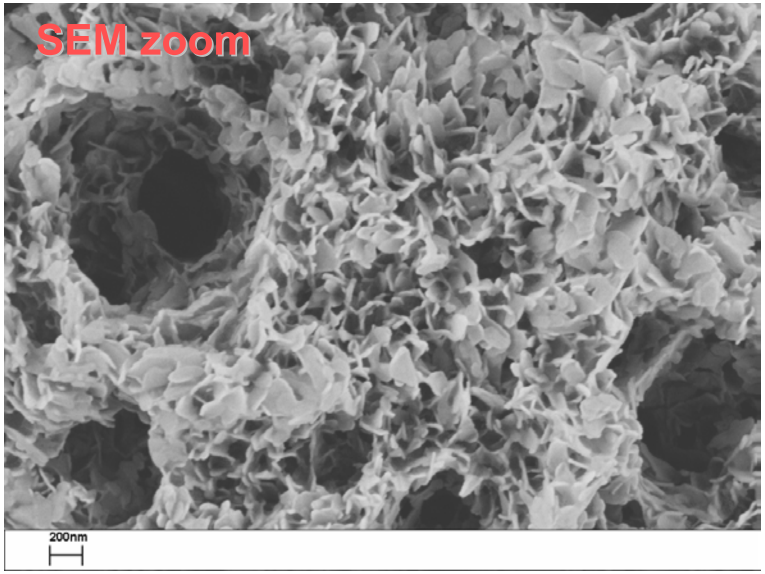

Light microscopy

Default


Polarization microscopy

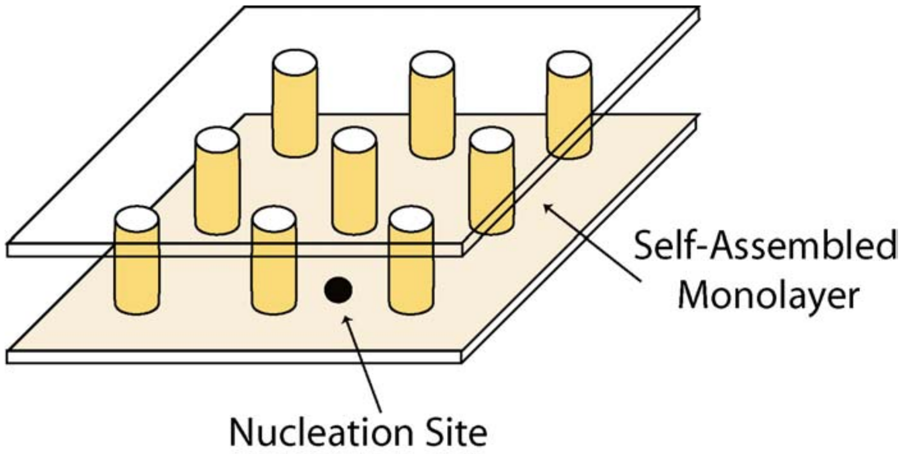
Brewster cross, radial alignment

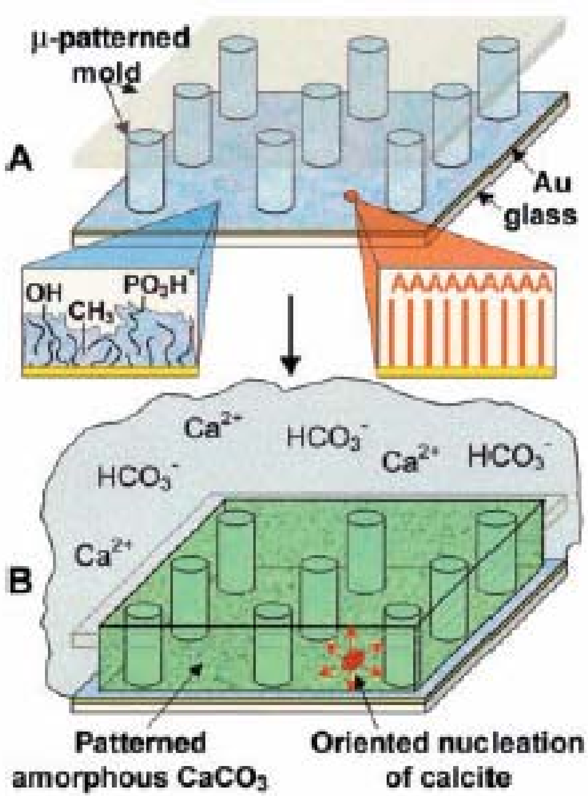

SEM


SEM zoom

Micro-Patterning Single Crystals of Calcite

ACC was formed within a mould perforated with a regular array of pillars

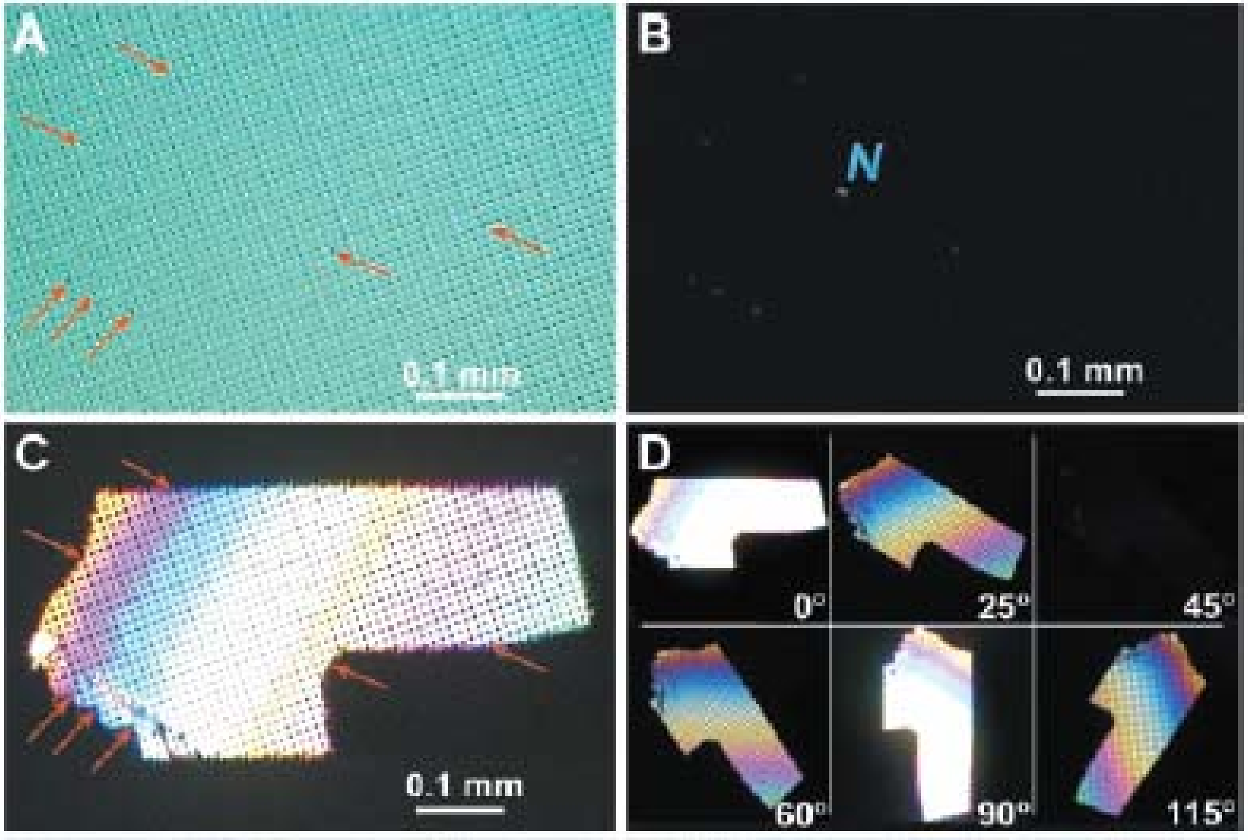




- ACC formation promoted by SAM terminated with OH, CH$_3$ and PO$_3$H groups
- Nucleation was initiated at ONE POINT

J. Aizenberg, Muller, Grazul, Hamann *Science* 2003, 299, 1205-1208

Large Single Crystals of Calcite Formed

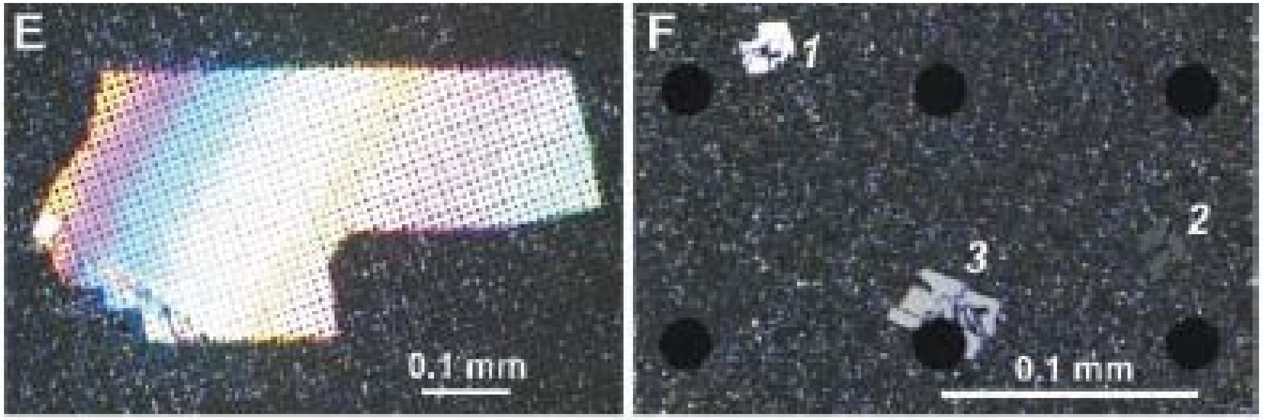


A) ACC film :   B) ACC film in polarised light:

C) Single calcite crystal after 2.5 hrs

D) Polarised light micrographs of single crystal

Separation of Pillars Varied ...



(E) Remaining ACC layer finally ruptures into a polycrystalline calcitic film. (F) Polycrystalline film formed in a framework of 20 μm posts with 100 μm separations. 3 nucleation sites studied, at different separations from pillars.

- Large single crystals of calcite, perforated with holes formed when the pillars were separated by less than 15 μm

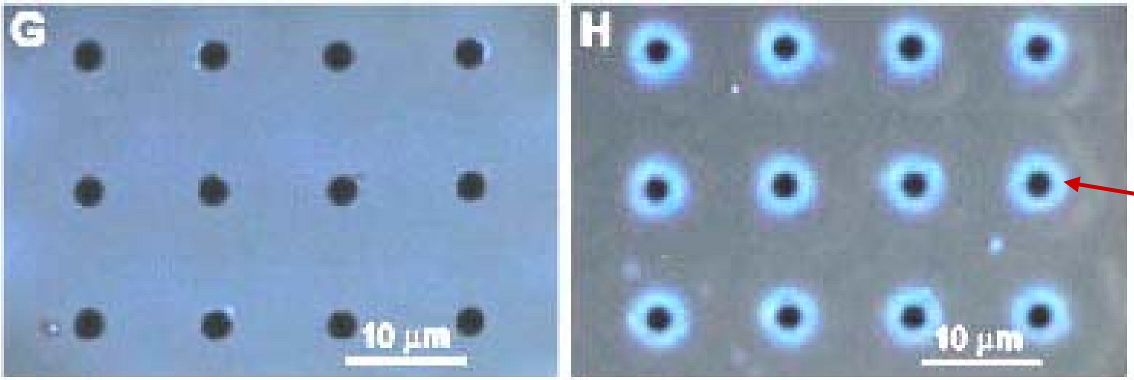
- Polycrystalline calcite films formed at pillar separations > 15 μm

- Sites < 15 μm from pillars nucleated single crystals

Pillars Behave as "Micro-sumps"

- Water is released from ACC on transformation into calcite via a dissolution/ reprecipitation mechanism ⇒ needs to be removed

- Pillars act as site to remove water ⇒ if too far apart crystallisation terminates



Pores larger in product crystal than template

Fluorescence micrographs of:
(G) ACC film containing a fluorescent additive
(H) Product calcite crystal showing exclusion of dye from crystal and its location around the pillars

Posts act as micro-sumps to remove water, impurities and stress from growing crystal

Summary

- ACC films stabilised at disordered SAMs with OH, CH_3 and PO_3H groups ⇒ functional groups on macromolecules extracted from biogenic ACC

- Transformation from ACC to crystalline calcite occurs via a dissolution/ reprecipitation mechanism

- Formation of a single crystal from ACC is dependent on the elimination of water from the structure ⇒ a maximum pattern length of 15 μm was suggested

Suggests that solid crystals of sizes > 15 μm cannot be produced via an ACC precursor

⇒ Compare with echinoderm skeletal elements

Conclusions

Biogenic ACC

⇒ Stable (hydrated), Transient (anhydrous)

⇒ Stabilised by specific macromolecules

⇒ Represents a "family" of phases / possess short range order

⇒ May provide a blueprint of transformation product

ACC shown to prove a versatile synthetic precursor phase to crystalline calcite

⇒ Thin films

⇒ 3D morphologies

⇒ Crystallisation proceeds by dissolution/ reprecipitation route