Chemical Control of Biomineralization
(Part 2)

Outline

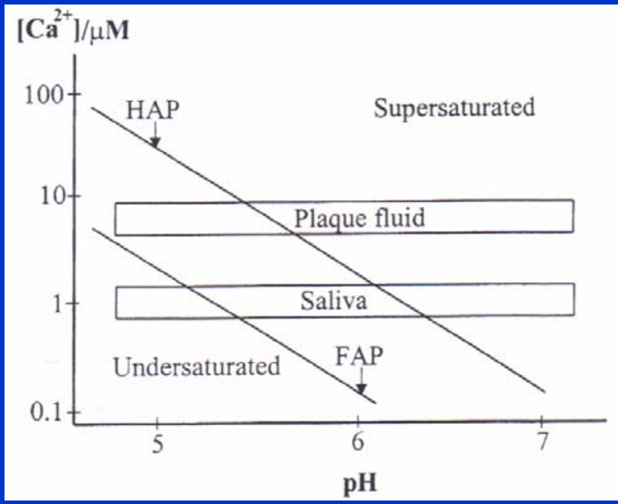
- *Crystal growth inhibition*
- *Crystal morphology*
- *Polymorphism*
- *Phase transformations*

Crystal growth inhibition

➢ *Many biological fluids are supersaturated with respect to certain inorganic minerals…*

BUT

Crystals do NOT form spontaneously



➢ *Example: saliva is supersaturated*

with respect to hydroxyapatite formation,

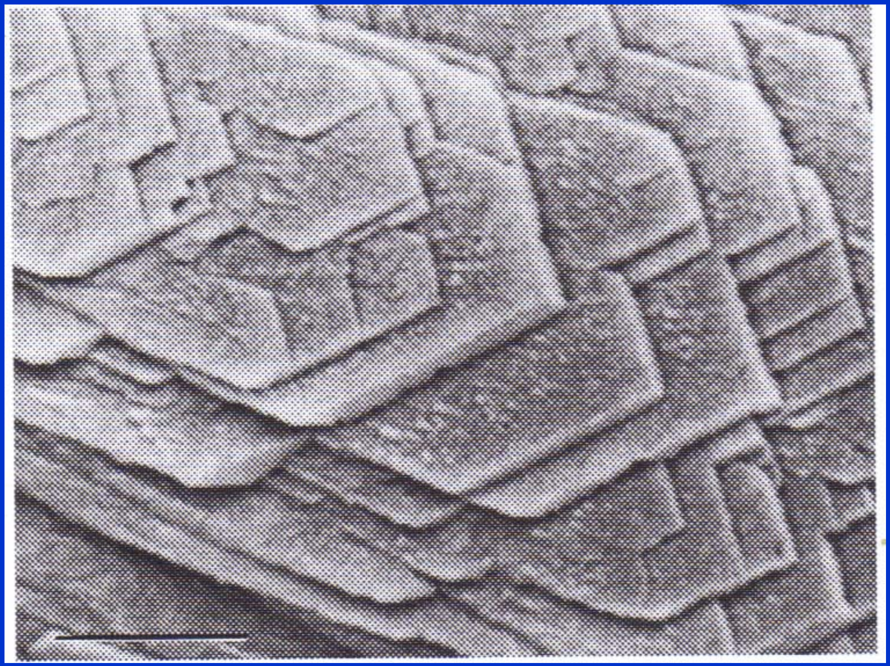
yet our teeth do not grow continuously.

➢ *The overgrowth is prevented by phosphoprotein macromolecules that bind to enamel crystals*

Crystal growth inhibition

- *In general, many different types of soluble additives, such as ions, organic molecules, macromolecules etc. can block incorporation of mineral ions into the crystal surface*
- *This is achieved by these species becoming anchored to the kink and step sites*
- *This interference gives rise to the inhibition of crystal growth and changes in the properties and morphology*
- *At very high additive levels the crystal steps “merge” and the crystal surface becomes irregular.*
- *Crystal growth eventually stops*

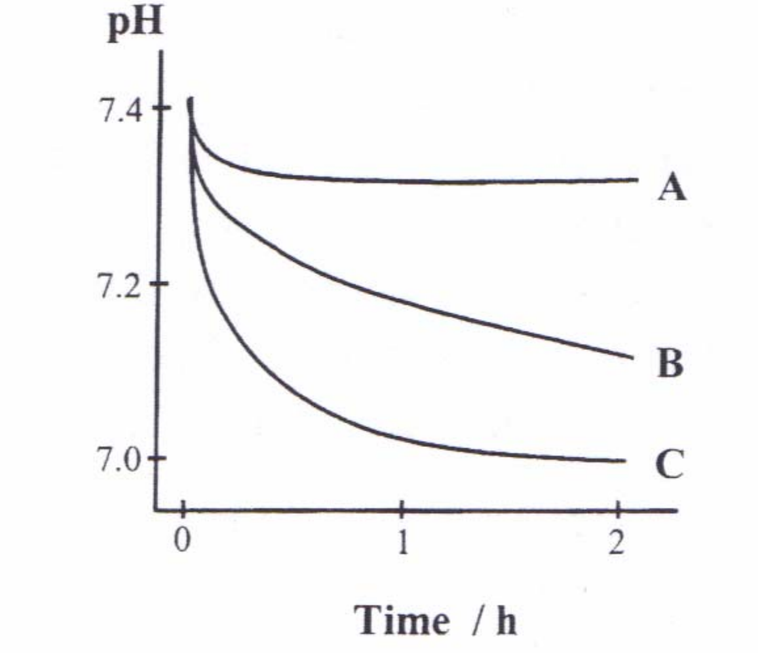
Calcite crystal growth



Calcite crystal grown in the presence of high levels of aspartic acid

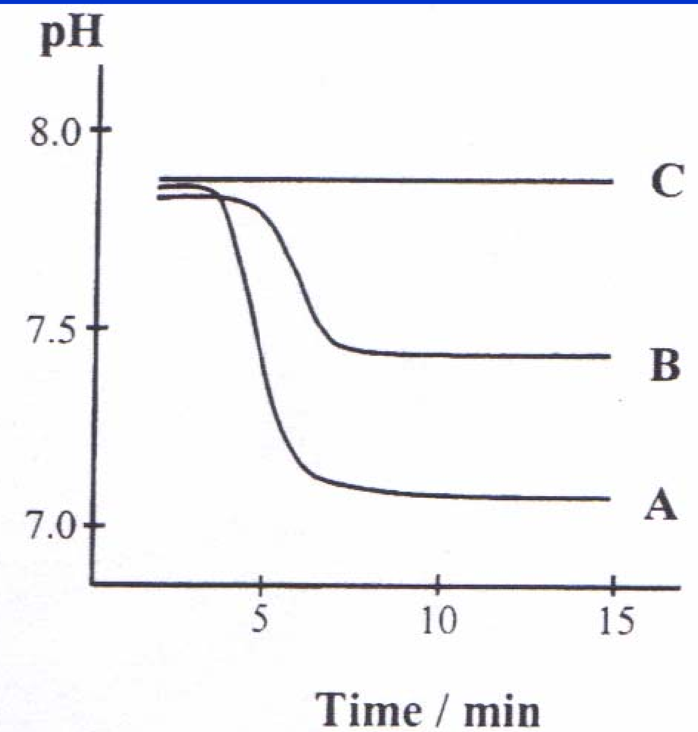
Incorporation of additives into the crystal lattice

- *There must be complimentarity in charge, size and polarization*
- *Example: fluoride in HAP*
- *Example: magnesium in HAP*



Effects of (A) Mg^{2+} and (C) F^- ions on HAP crystallization. Curve B, control (no additives).

Inhibition by organic molecules



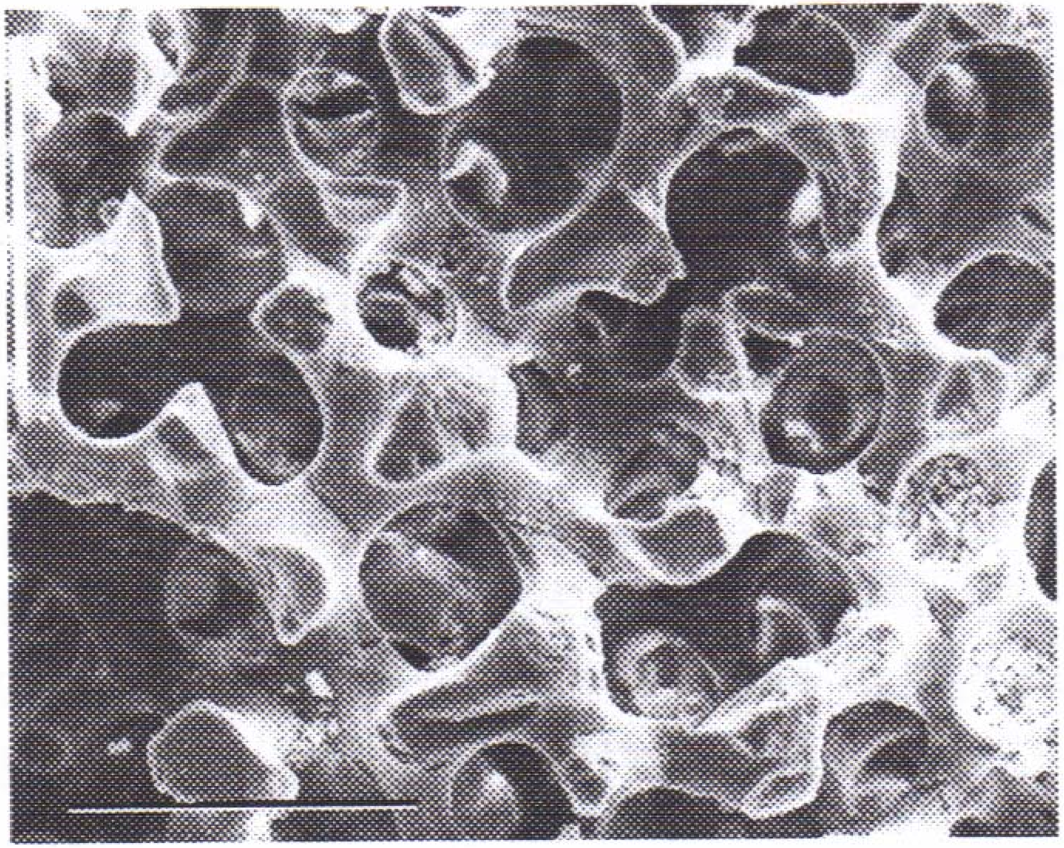
Calcite crystallization: (A) no additives; (B) coccolith polysaccharide added after 4 min; (C) polysaccharide added at beginning of experiment.

Effects of macromolecules on crystal morphology

Effect of amelogenin proteins on the growth inhibition of hydroxyapatite crystals

Molecular mass	Amount used (mg)	Amount adsorbed (mg m^{-2})	% Inhibition
25 000	0.33	0.26	22
	0.66	0.47	33
20 000	0.42	0.03	<5
	0.64	0.04	5

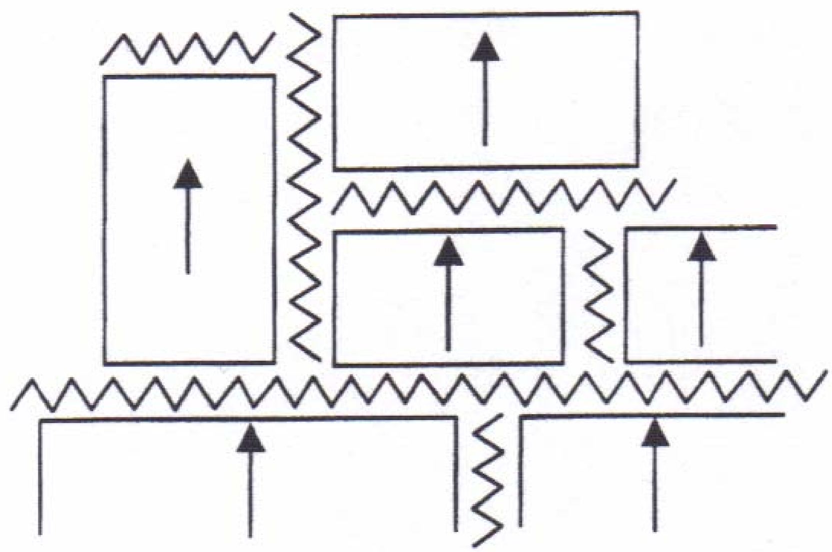
Effects of macromolecules on crystal morphology



Fractured shell of a mature sea urchin. Scale bar, 50 μm.

Mg-calcite

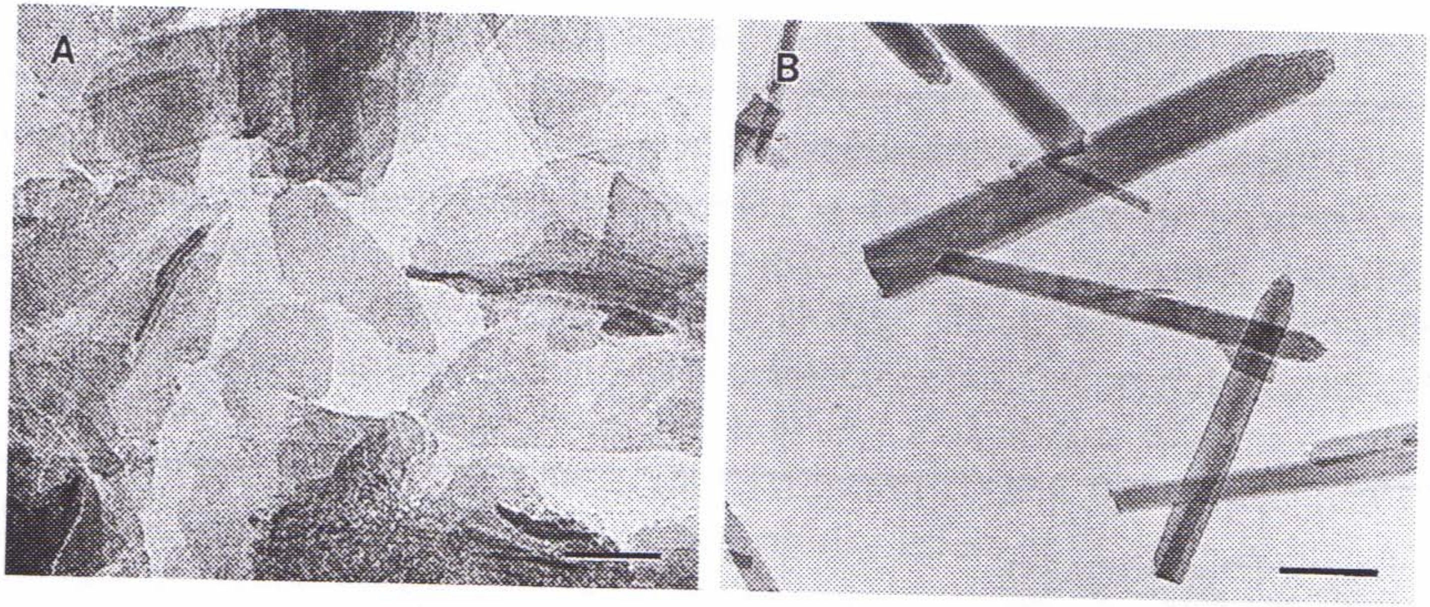
Where are the macromolecules ???



Domain structure in a single crystal of an inorganic mineral with intercalated organic macromolecules at the coherent interfaces.

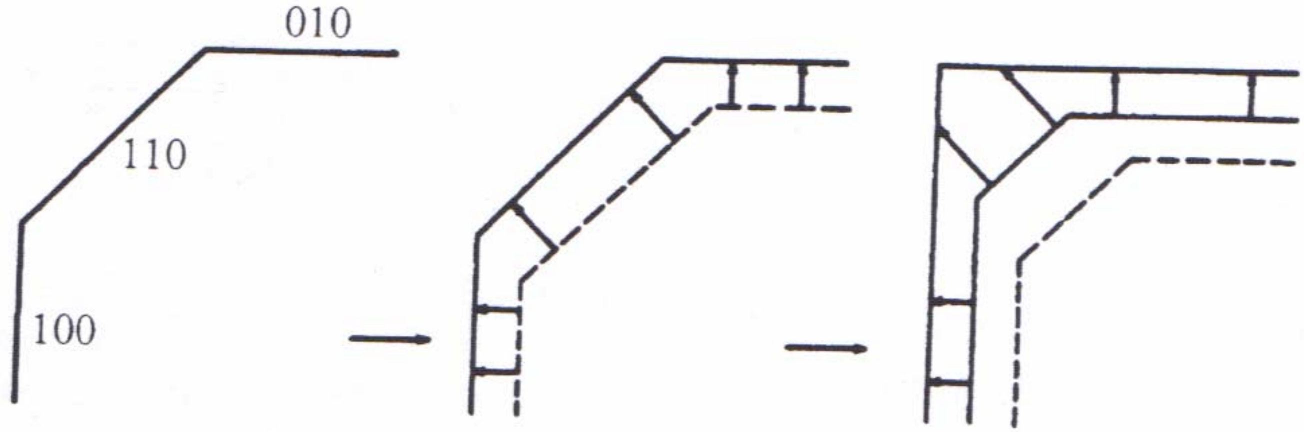
Crystal morphology

- *Crystal inhibition modifies crystal morphology (habit)*



Hydroxyapatite crystals: (A) plate-shaped; (B) needle-like morphology. Scale bars, 100 nm.

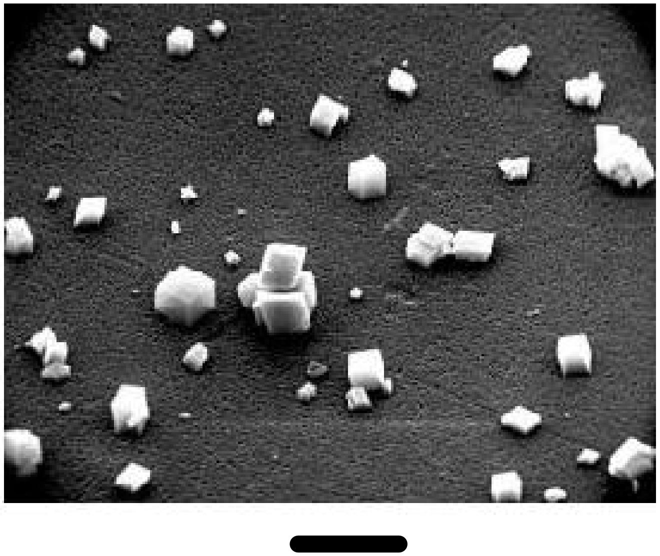
Growth along certain crystallographic planes



Changes in crystal morphology due to face-specific growth rates cubic system.

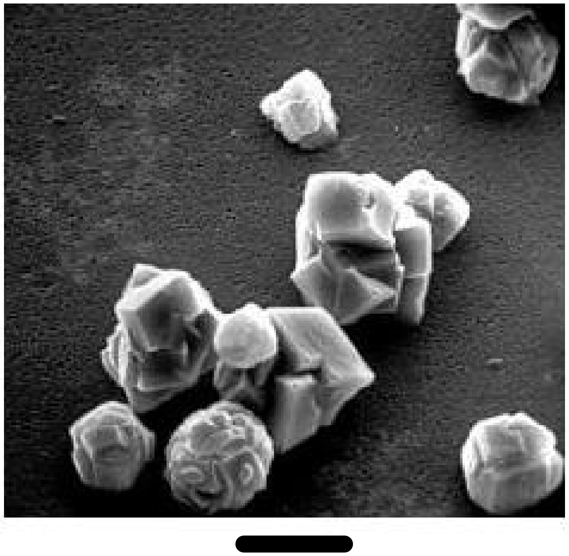
Another example of crystal inhibition

CaCO3 crystals (Untreated)

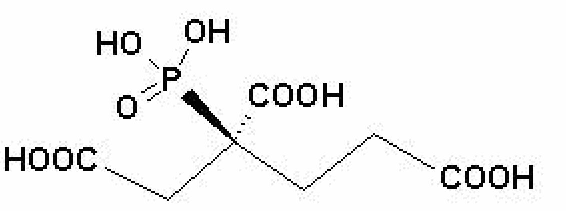


Bar = 20 μ

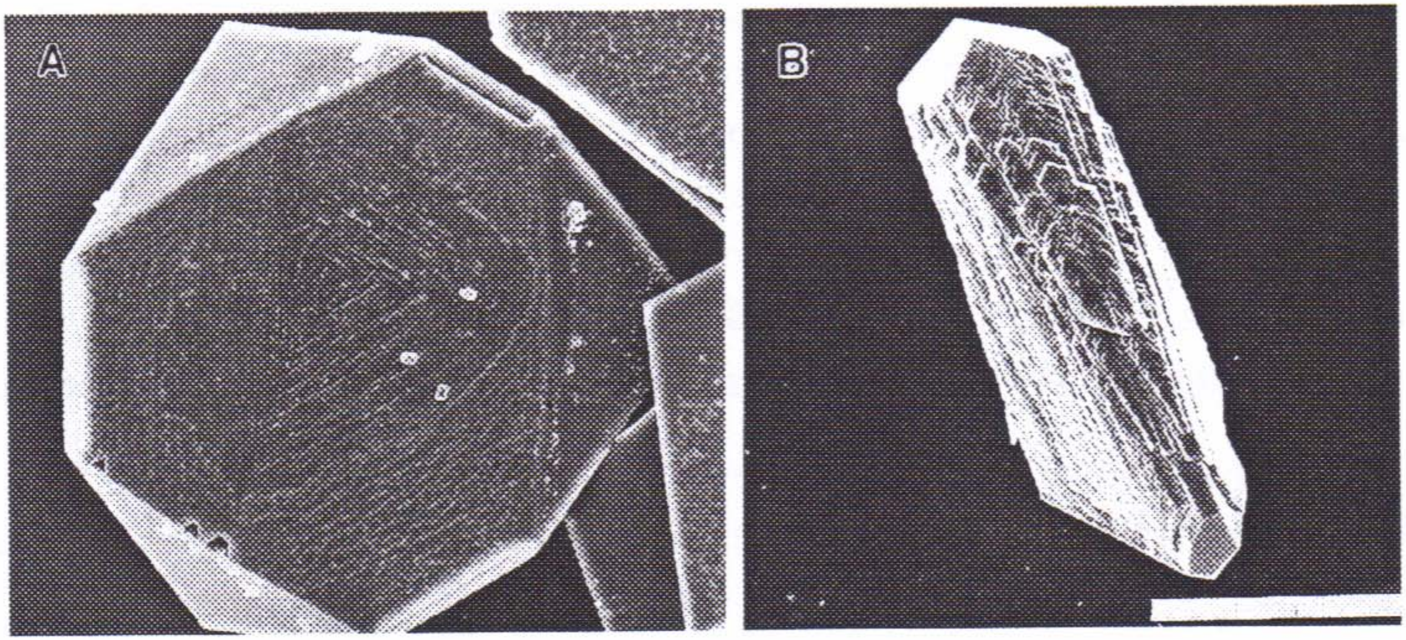
CaCO3 crystals (treated with PBTC)



Bar = 10 μ

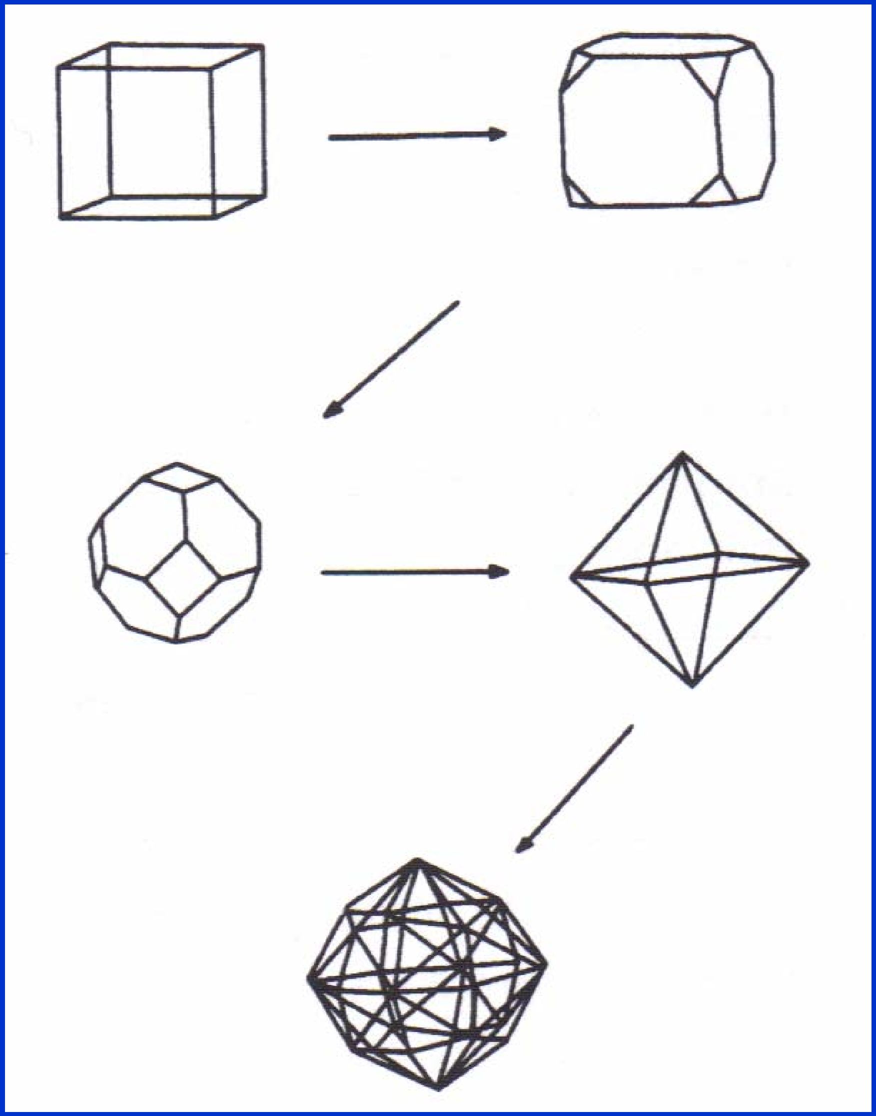


Habit modification

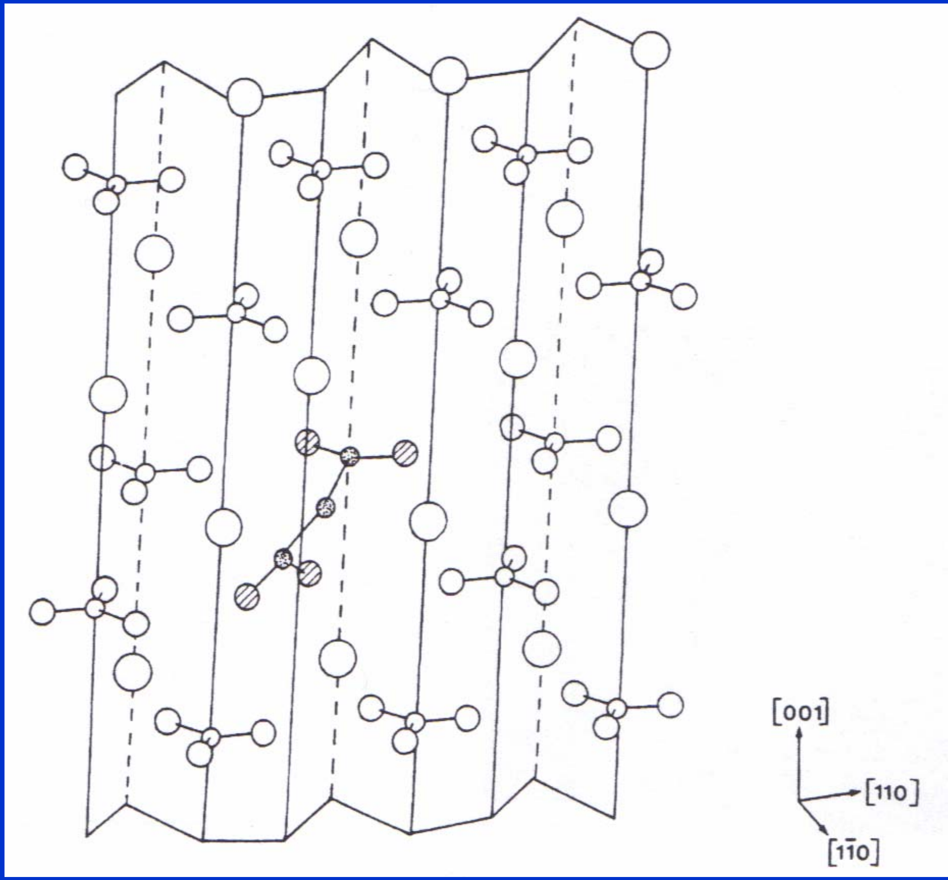


Calcite crystals grown in the presence of: (A) Li^+, plate with hexagonal {001} faces and rhombohedral {104} side faces; (B) HPO_4^{2-}, prism with rhombohedral {104} top faces and {1$\bar{1}$0} side faces. Scale bars, 10 μm and 20 μm, respectively.

NaCl crystals grown in the presence of urea

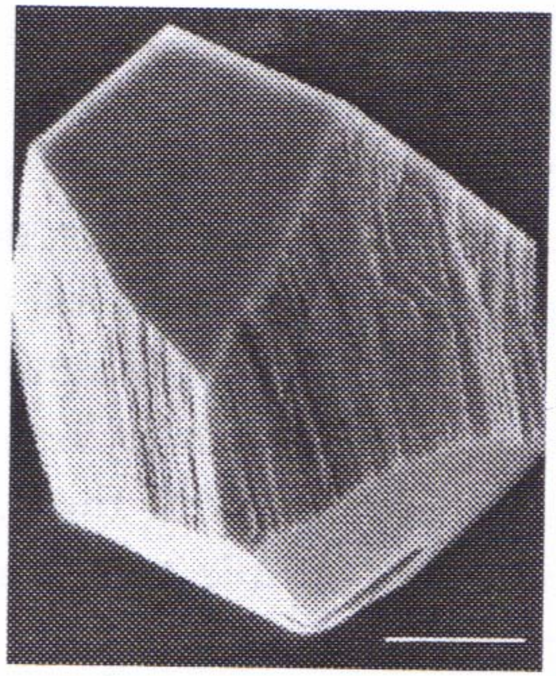


Surface interaction of additives with mineral surfaces



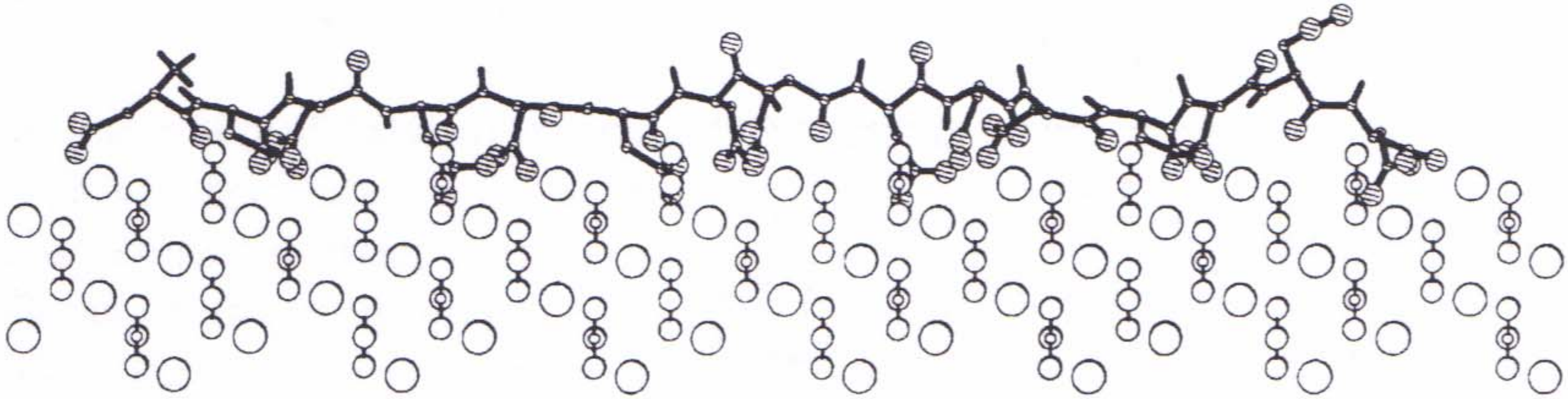
Drawing of calcite {1$\bar{1}$0} crystal face with surface-adsorbed malonate anion.

Morphology effects of carboxylates on calcite

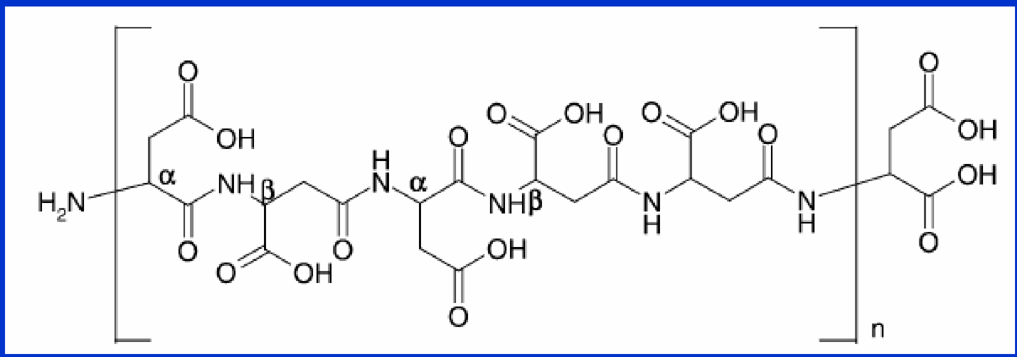


Prismatic calcite crystal formed in the presence of γ-carboxyglutamate. Scale bar, 10 μm.

Interaction of macromolecules with mineral surfaces



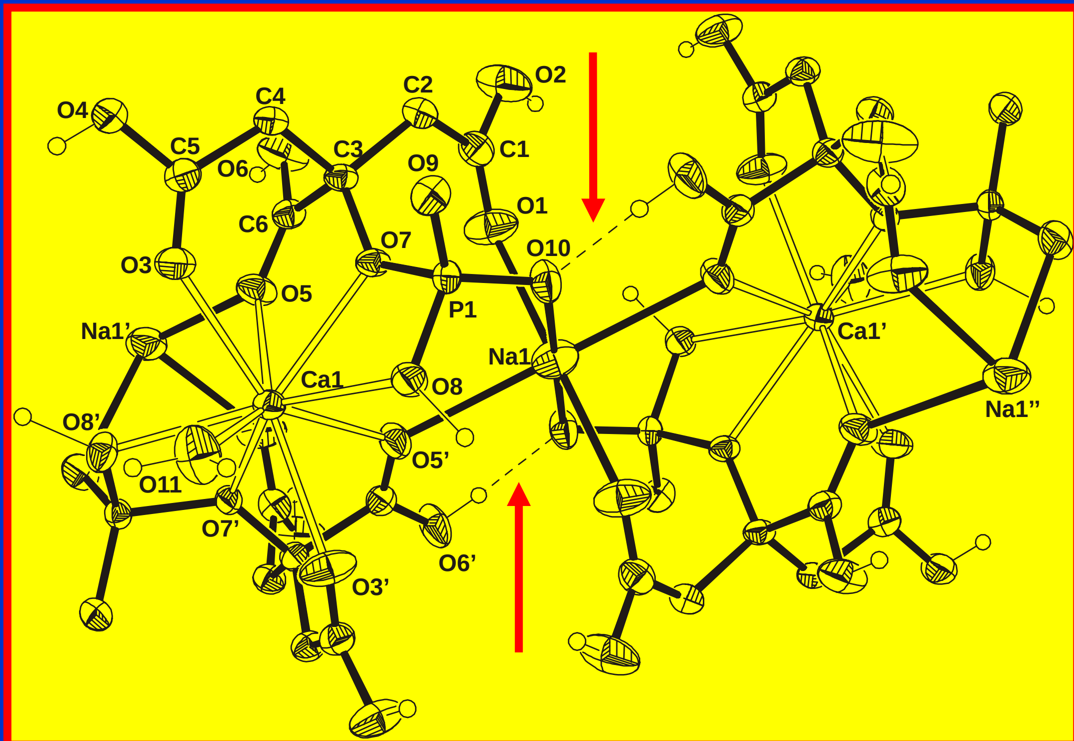
Computer model showing side view of the calcite {1$\bar{1}$0} face with surface-bound polyaspartate ([Asp]$_{11}$).

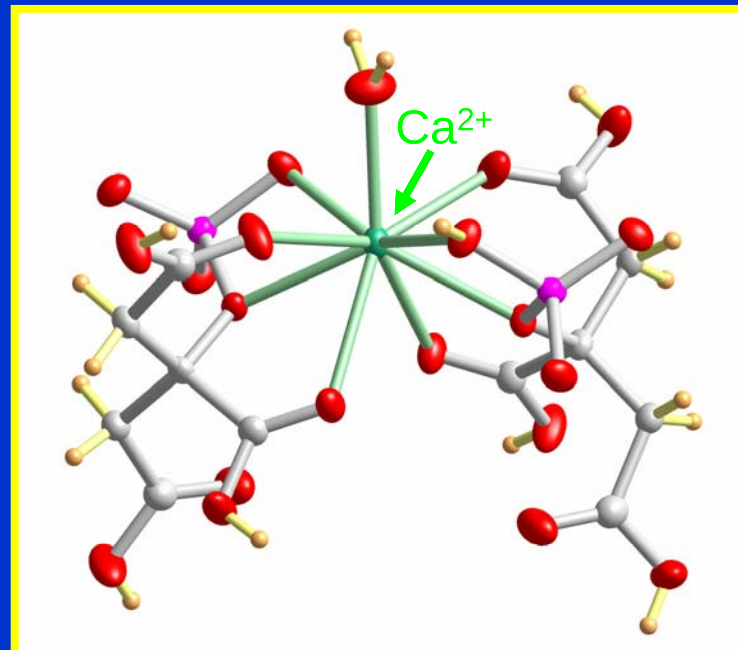


Biological inhibitors of hydroxyapatite crystallization from aqueous solution

Mg^{2+}
CO_3^{2-}
Pyrophosphate ($P_2O_7^{4-}$)
Polyphosphates
Nucleotide polyphosphates
adenosine triphosphate
guanosine diphosphate
glucose 1,6-diphosphate
Cartilage proteoglycans
Dentine phosphoproteins
Polycarboxylates
Phospholipids
Phosphocitrate

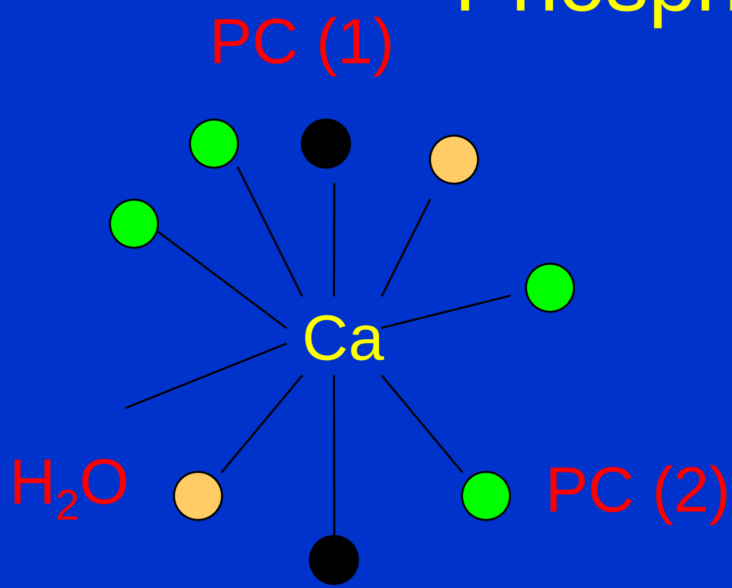
Molecular Structure of Ca/Na/Phosphocitrate Hybrid

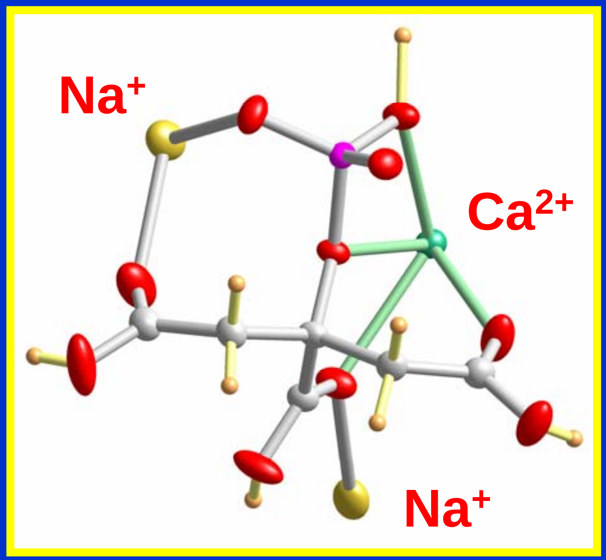




K. Demadis, *Inorg. Chem. Commun.* **2003**, *6*, 527.

Coordination Environment of Calcium and the Phosphocitrate Ligand





- Ca-O=C(carboxylate) bonds

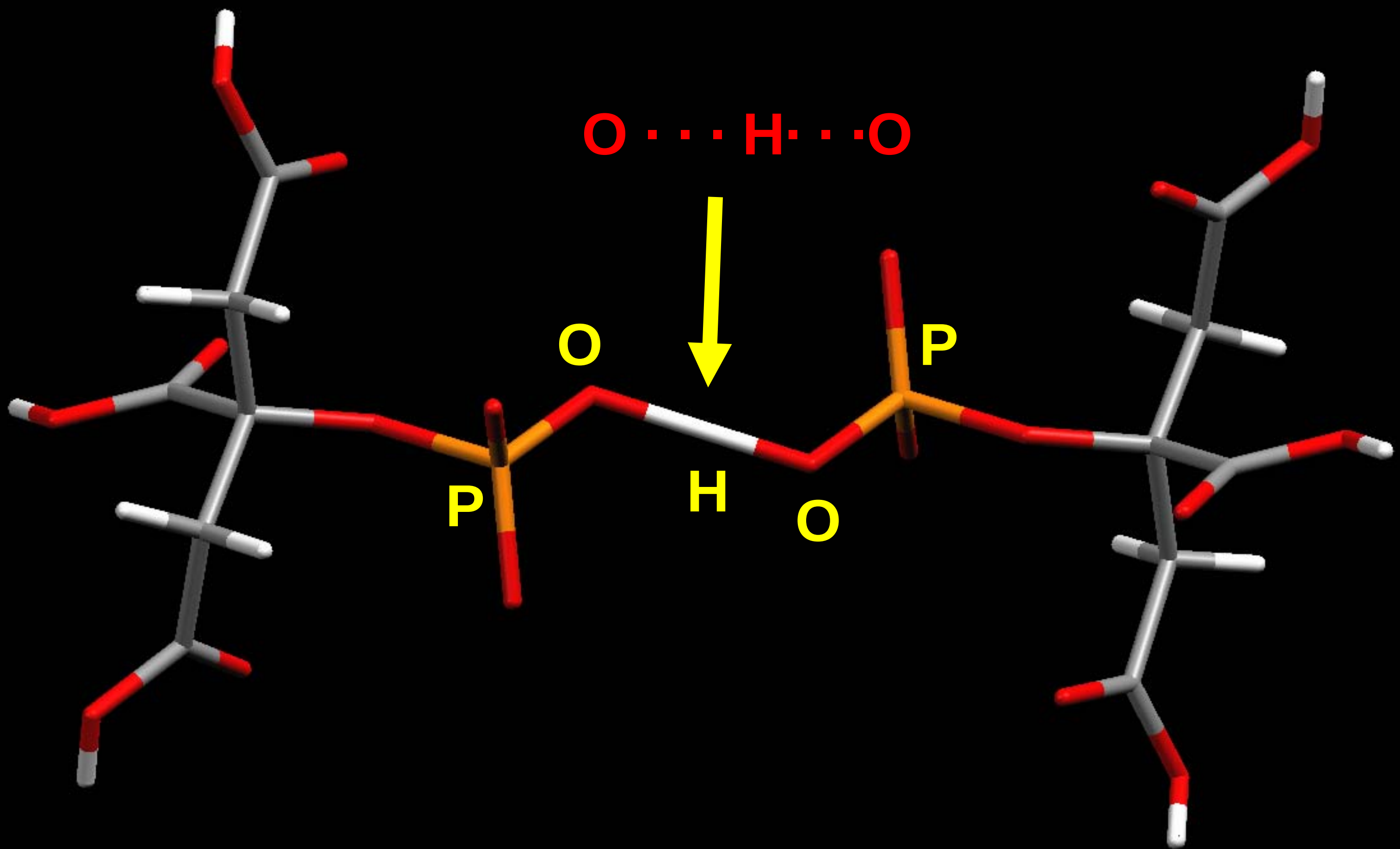
  2.446(2)-2.586(2) Å
- Ca-O-P(phosphate) bond

  2.527(2) Å
- Ca-O(phosphate ester) bond

  2.477(1) Å
- Ca-O(H_2O) bond

  2.388(2) Å

The P-O···H···O-P Hydrogen Bond in the Structure of CaNaPC



Demadis *et al. J. Am. Chem. Soc.* **2001**, *123*, 10129.

Induced Calcification Protocol

- Three Groups of Male Hooded Wistar rats (200 g each)
- Solutions of NaPC and CaNaPC in 0.1 M *Tris*-HCl buffer (pH 7.2)
- Three treatments: **A** (control)
  B (NaPC)
  C (CaNaPC)
- Subcutaneous injection of 200 μL of 0.1 % $KMnO_4$
- ~9.6 mg doses (as H_5PC) were given to Groups B and C
- Therapy was given on alternate days
- Calcification of plaques proceeded for 10 days

Inhibitory Activity of NaPC and CaNaPC on Plaque Growth

treatment groups	***treatment dosage***	***plaque weight (mg)***	***plaque weight reduction (%)***
Group A	0	211 ± 9	0
Group B	9.7	147 ± 9	30
Group C	9.6	***11 ± 4***	***95***

Demadis *et al. J. Am. Chem. Soc.* **2001**, *123*, 10129.

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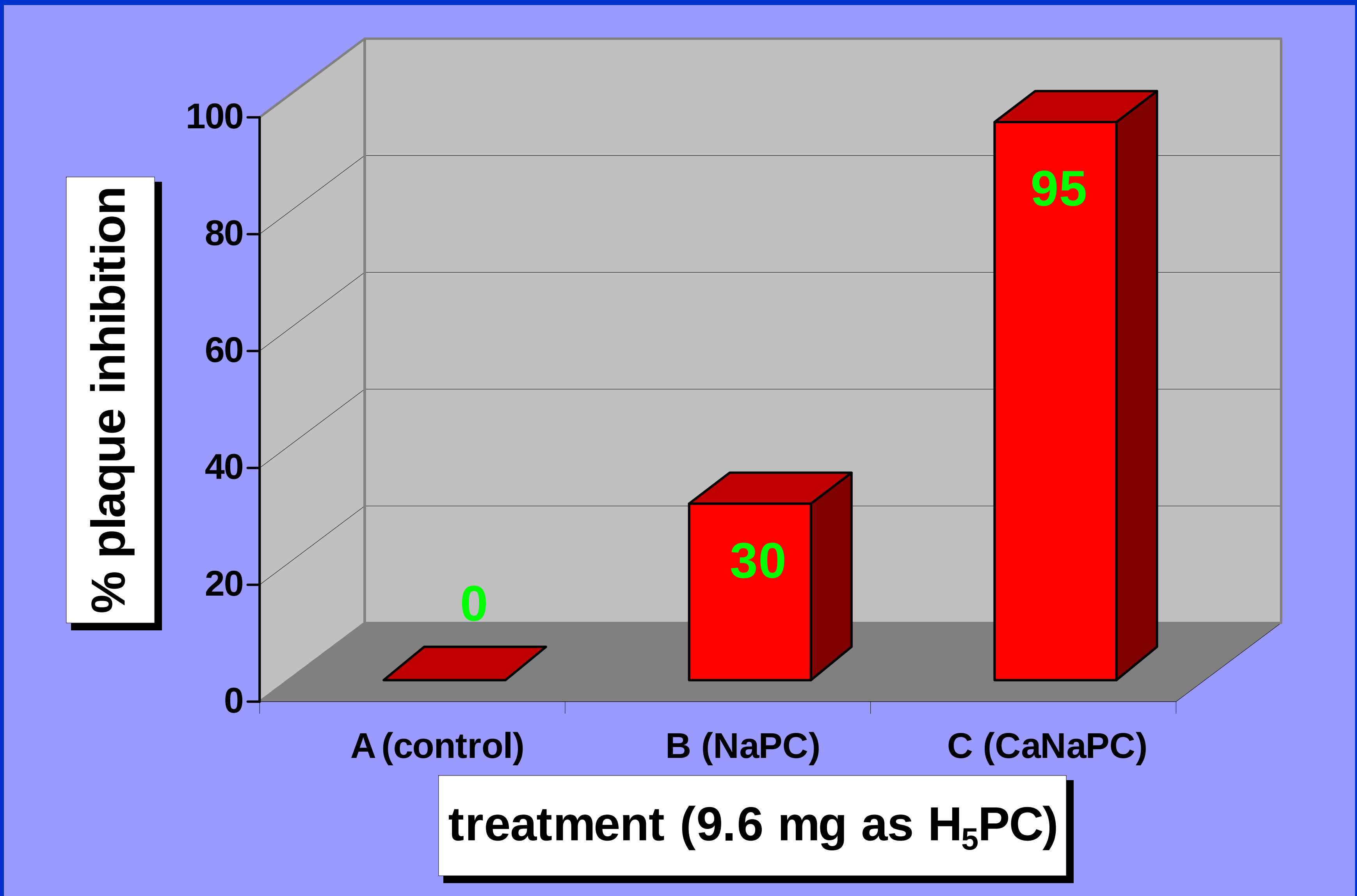
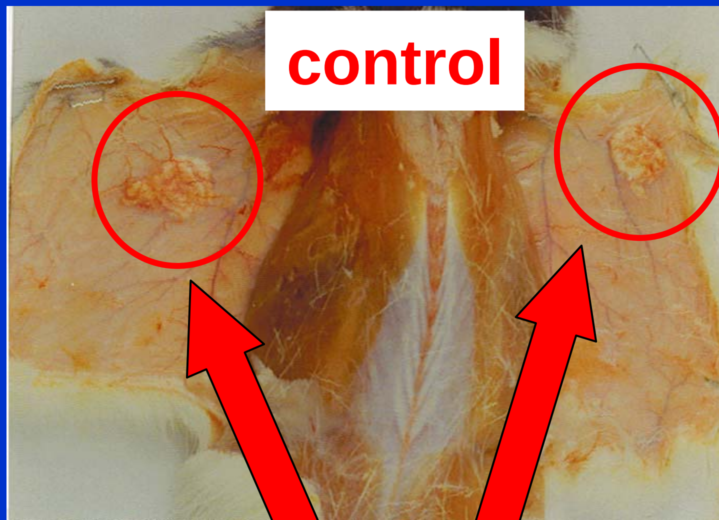
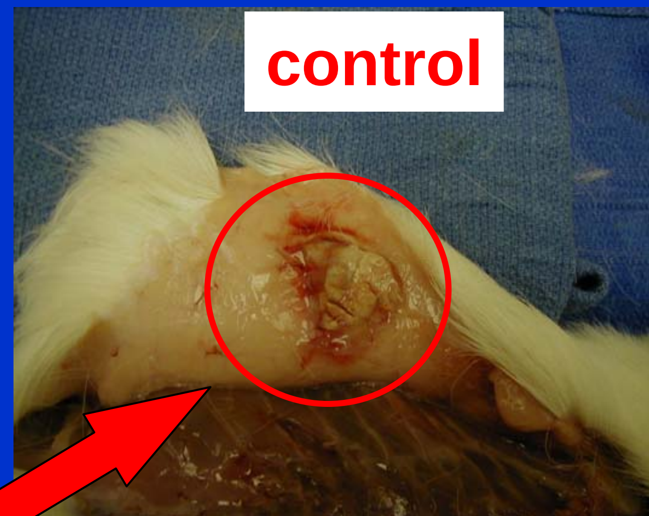
Plaque Calcification Inhibition (%)



Plaque Calcification and Inhibition

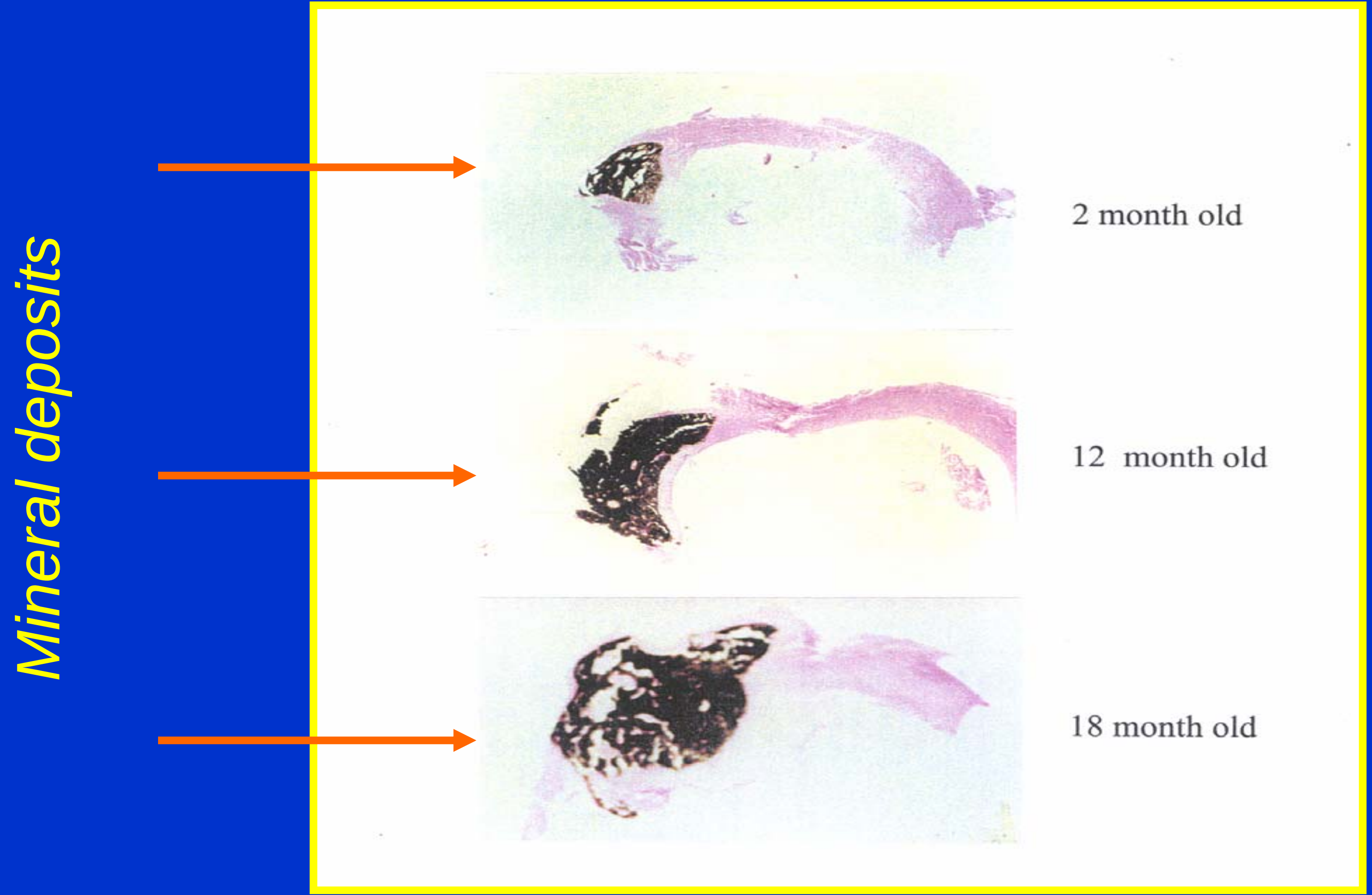




severe plaque calcification

complete absence of plaque

Spontaneous Osteoarthritis of Meniscus of 2, 12 and 18 months old Guinea Pigs

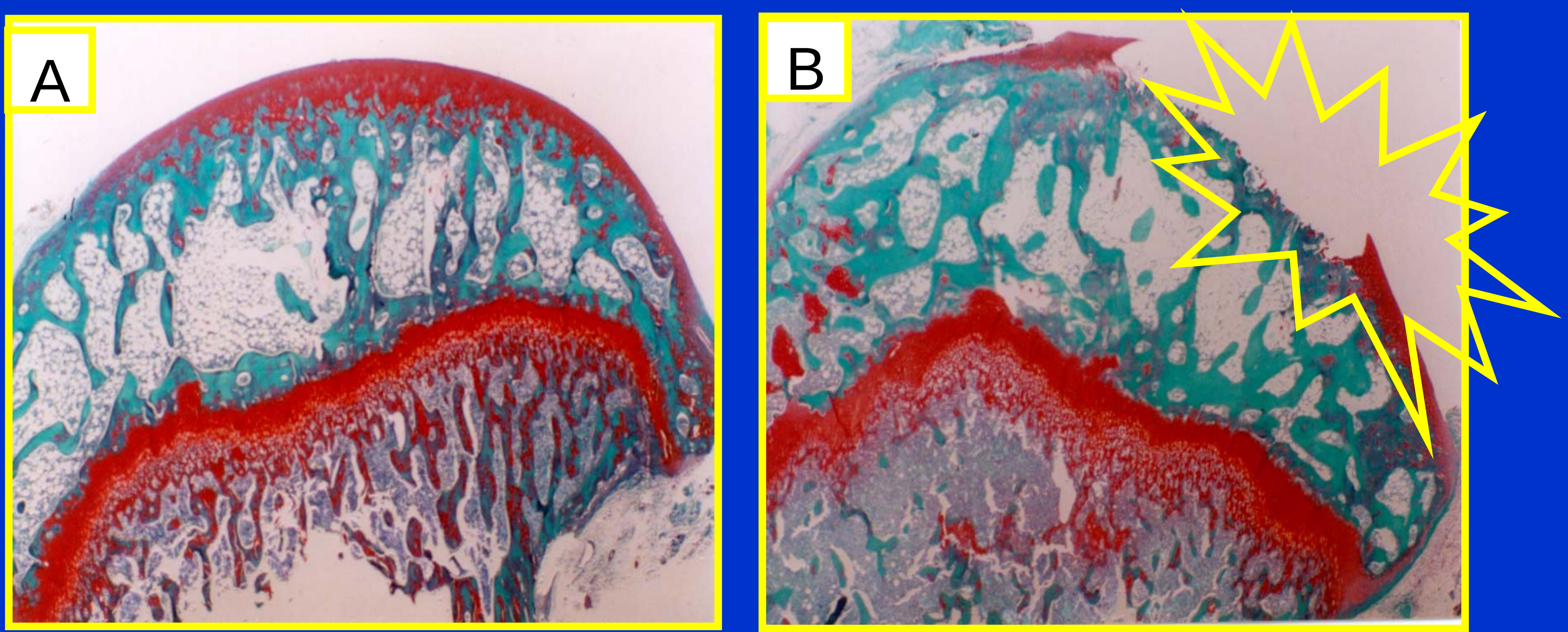


Cheung, H.S.; Sallis, J.D.; Demadis, K.D. *Arthritis & Rheumatism* ***2006****, 54*, 2452.

Histology of 6-month old guinea pig tibia plateau

treated with CaNaPC (40mg/wk) for 2 months

untreated control

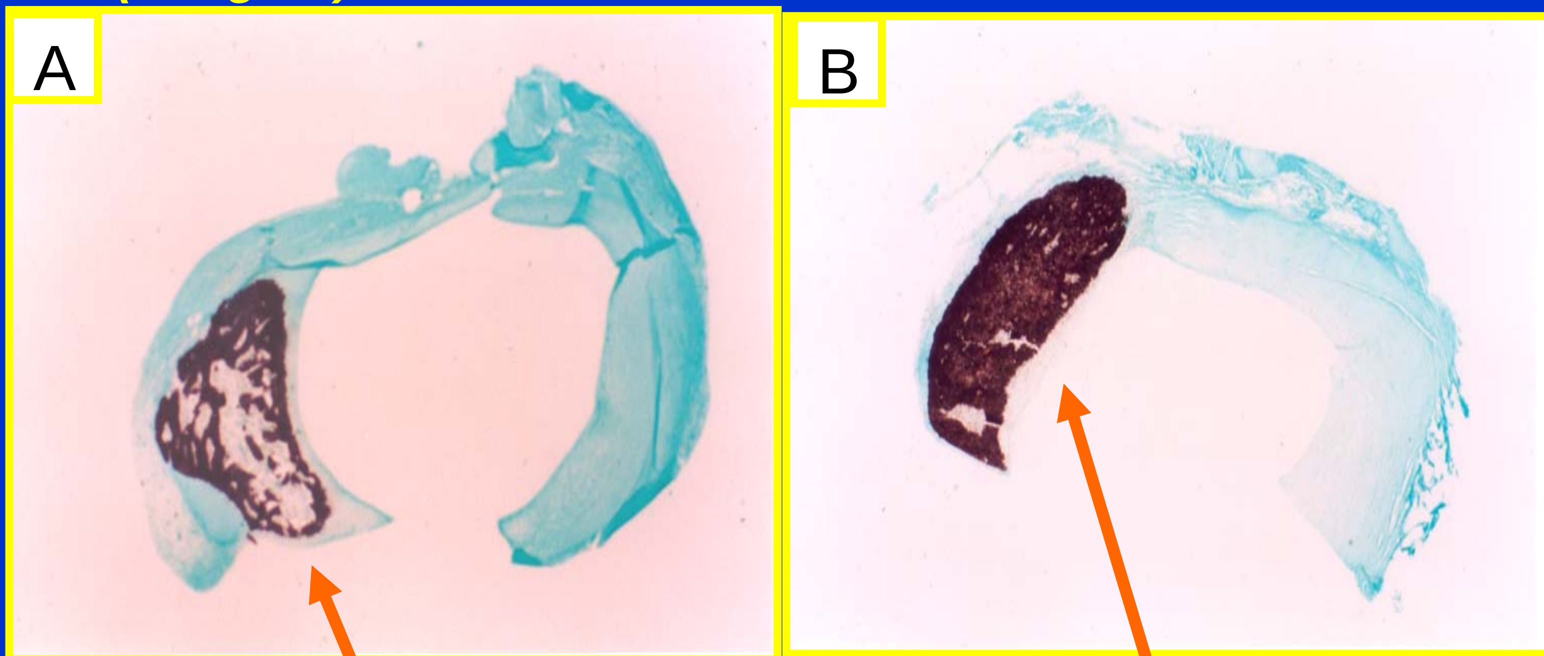


Cheung, H.S.; Sallis, J.D.; Demadis, K.D. *Arthritis & Rheumatism* ***2006***, *54*, 2452.

Xcross-sections of meniscus of 6-month old guinea pigs

treated with CaNaPC (40mg/wk) for 2 months

untreated control



resorption of calcified deposits

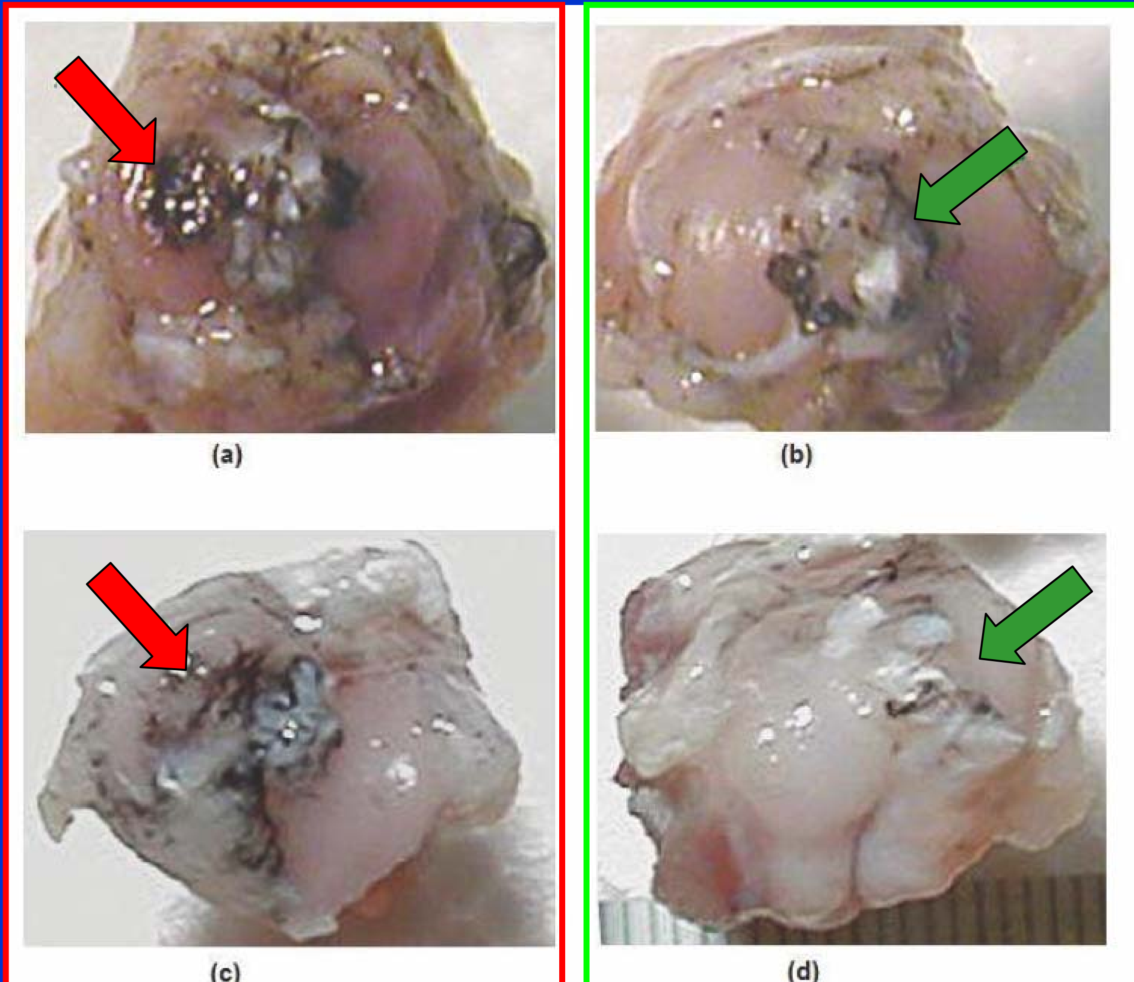
massive calcification

Cheung, H.S.; Sallis, J.D.; Demadis, K.D. *Arthritis & Rheumatism* ***2006***, *54*, 2452.

Cartilage surface after coating with carbon black

control femoral condyle

control tibial plateau



CaNaPC treated femoral condyle

CaNaPC treated tibial plateau

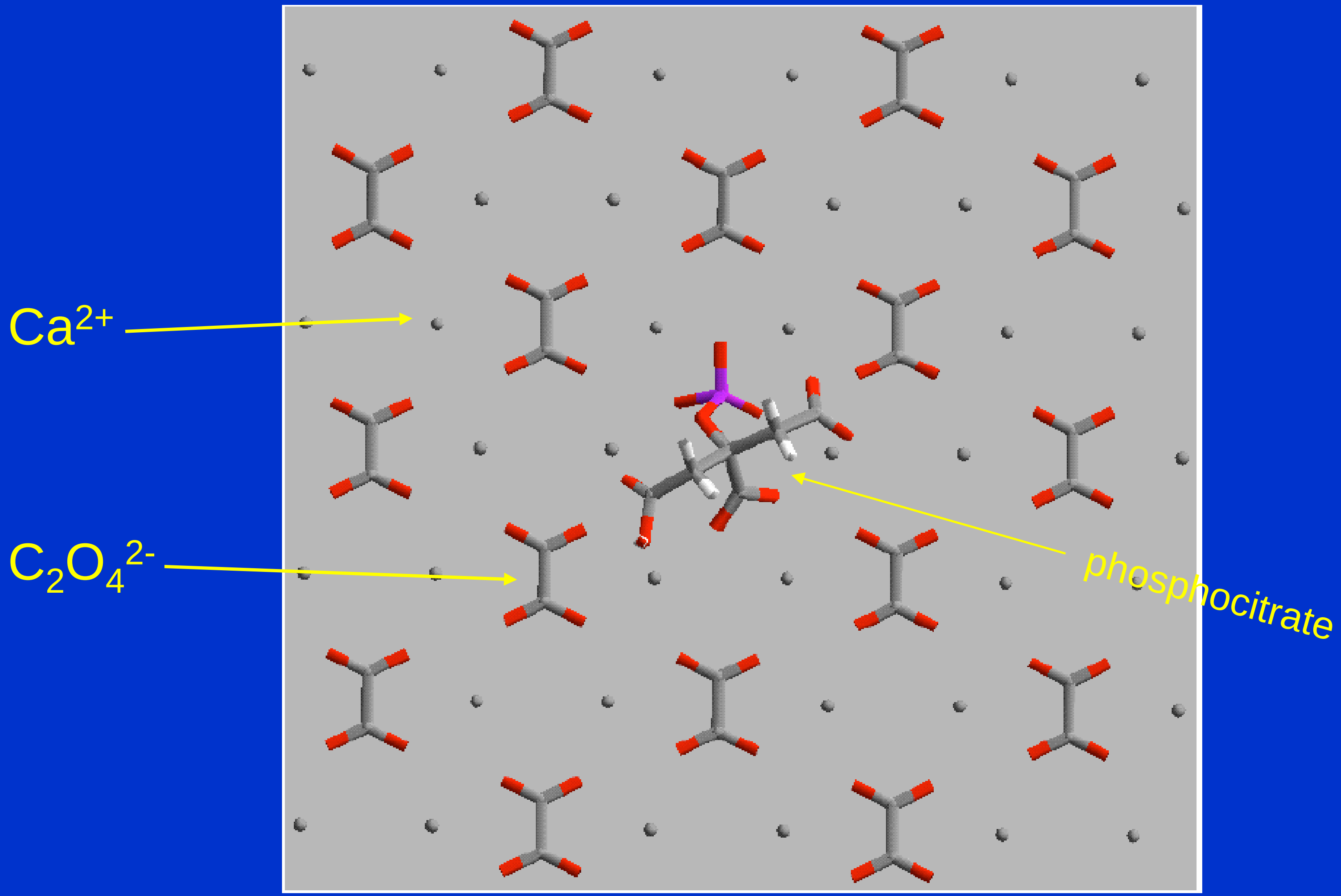
discolored surface,
surface ulcerations,
pitting lesions

white, glistening
cartilage surface,
few erosions,
little synovial thickening

Conclusions

- CaNaPC has a unique structure:
  - ✯ polymeric inorganic-organic hybrid structure
  - ✯ 9-coordinate Ca
  - ✯ Ca-O=C(R) bonds
  - ✯ ”Acidic” polymer
- CaNaPC a powerful inhibitor of biological calcification *in vivo*
- Possible explanations for *anti*-calcification activity:
  - ✯ Enhanced bioavailability of “PC”
  - ✯ More effective stereospecific interaction between CaNaPC and hydroxyapatite

Ca-Oxalate-Phosphocitrate Interactions



Wierzbicki, Cheung, *J. Mol. Struct. (Theochem.)* **1998**, *454*, 287.