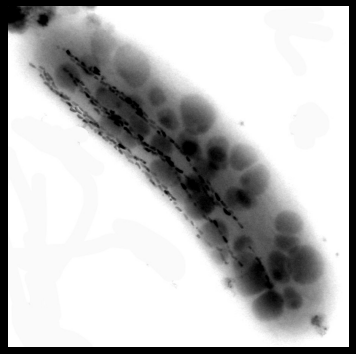
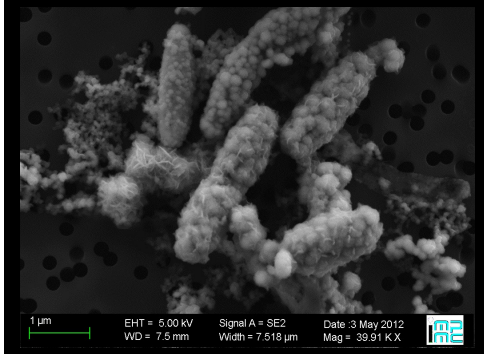
Cryo-ultramicrotomy (CEMOVIS), STXM, cryo-STXM and cryo-SEM study of mineral formation by microbes

Karim Benzerara, Marine Blondeau, Jean-Michel Guigner, Martin Sachse

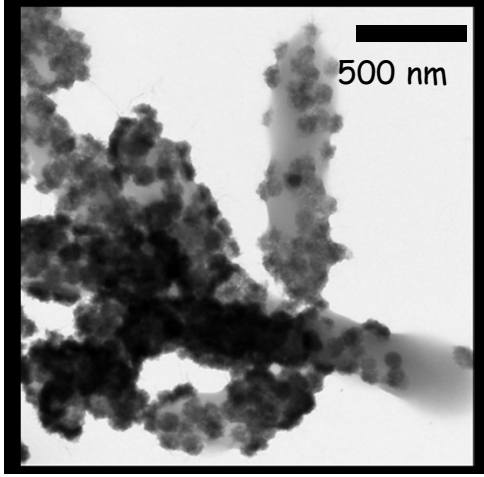
Diverse microorganisms form a wide diversity of mineral phases that are of interest to many research field



Nano-magnetites → ★ Hyperthermia, drug delivery, contrasting agents for medical imaging, magnetic sorting of molecules



Textured iron oxides → ★ New generation of Lithium-ion batteries



Ca-phosphates → ★ Infectious calcification (kidney stones);
★ Sequestration of pollutants

We have been particularly interested in cyanobacteria forming intracellular cabonates

★ Major producers of organic carbon for billion years

★ Induced atmosphere oxygenation 2.3 Gyr ago

★ Induce precipitation of carbonates and formation of stromatolites

★ Selectively and massively sequester Sr and Ra

Intracellular calcification is very surprising

Common assumptions for cell chemical composition:

pH < 7,9

$[Ca^{2+}]$ = 100 nM

$[HCO_3^-]$ = 30mM

→ Undersaturated with Ca-carbonates

Intracellular Ca-carbonate precipitation is thermodynamically impossible

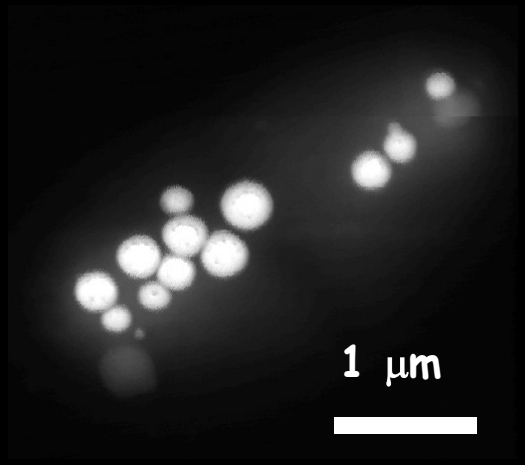
Cam et al. (2015) Geochimica Cosmochimica Acta

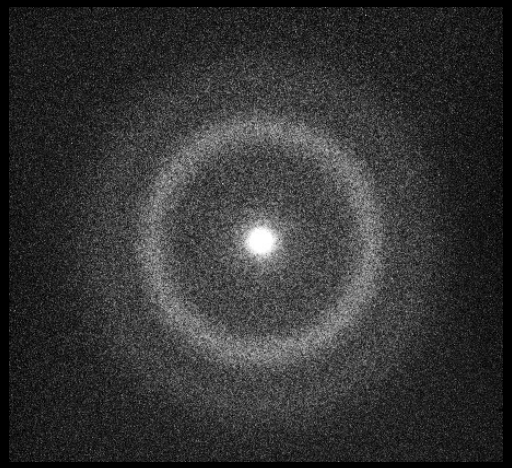
→ What is the speciation of Ca in the cells?

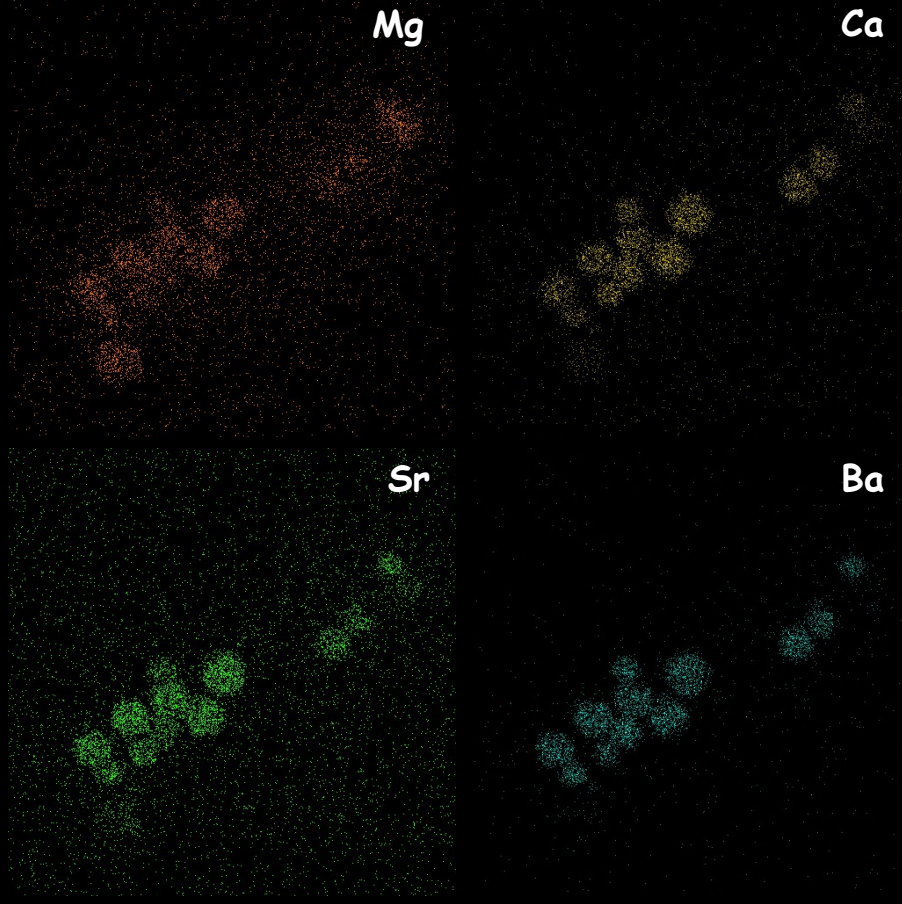
→ Are there different Ca pools?

→ Where are they located?

Room-T analyses by scanning transmission electron microscopy (STEM







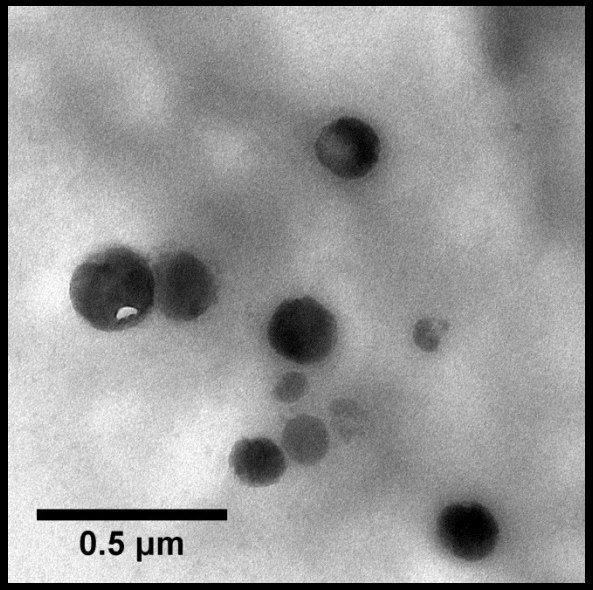
★ Up to 20 granules/cell: 0.13-0.19 μm^3 per cell ⇔ 2.4-3.5 fmol of *Ca*

★ *Amorphous* Ca-, Sr-, Ba-, Mg-carbonates

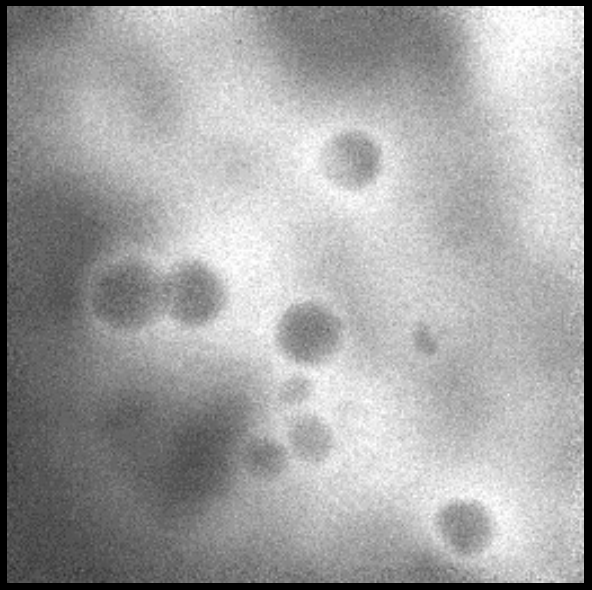
★ *Measure btw* 40 & 1000 nm in diameter

★ Found in >30 species of cyanobacteria, everywhere in the world and diverse environments

Energy filtered-TEM analyses at Ambient-T



Zero loss image

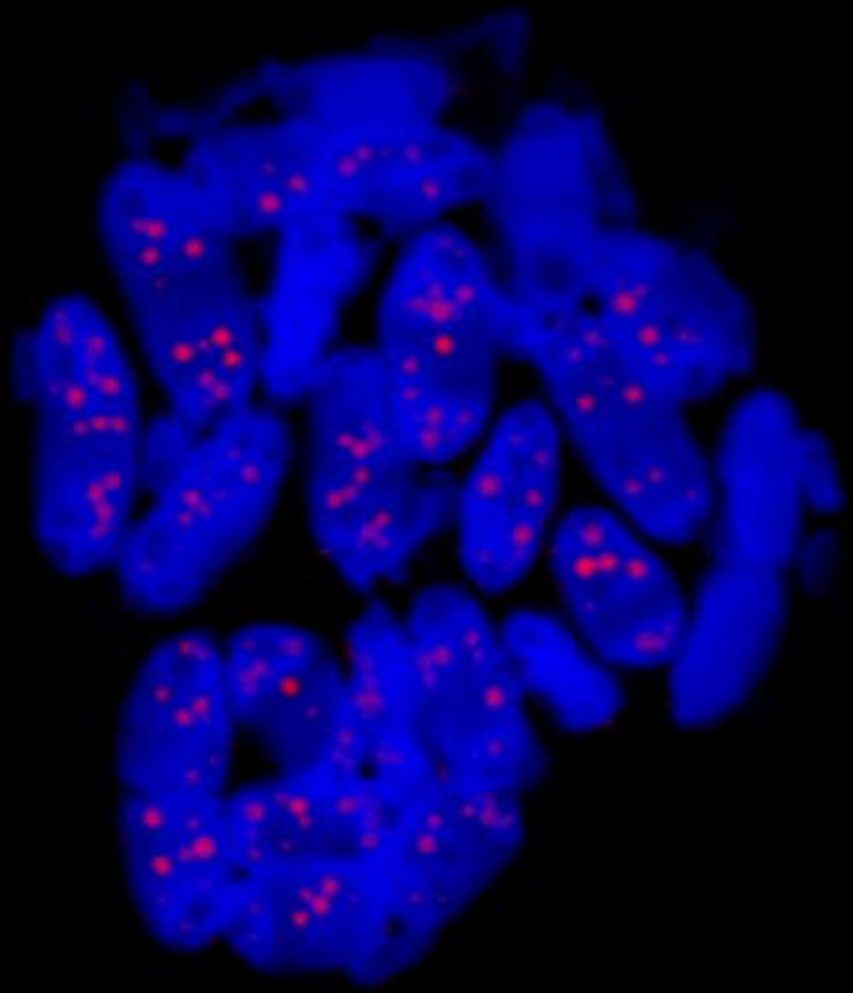


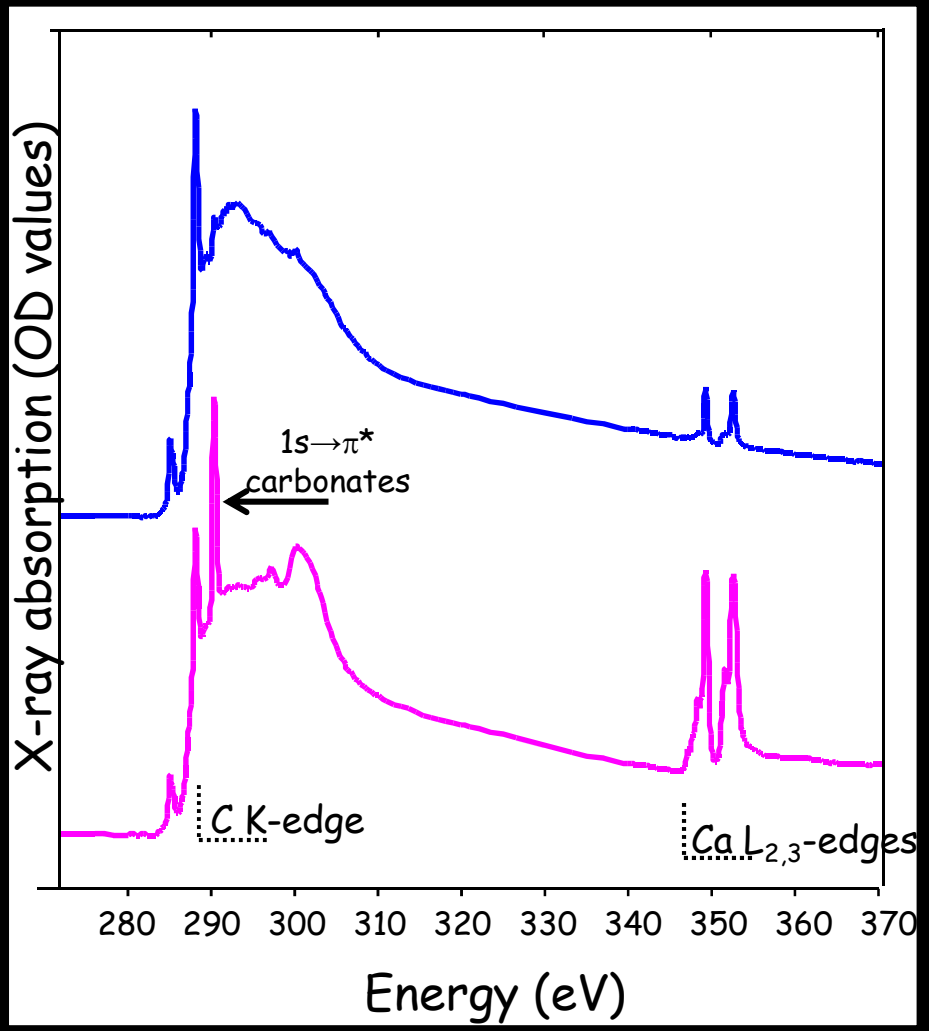
C image

Ca image

★ Some C-rich material around the Ca-inclusions?

Room-T analyses by scanning transmission x-ray microscopy (STXM)



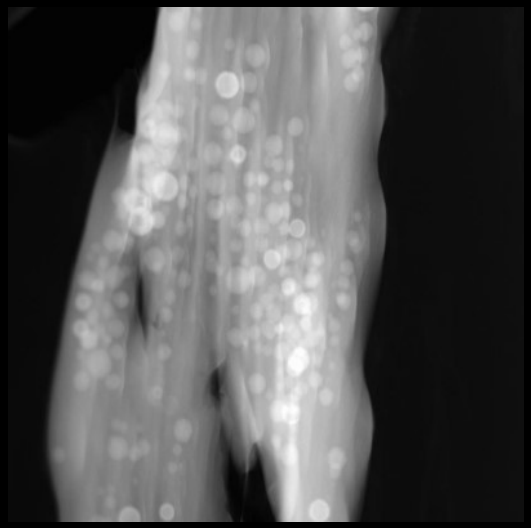


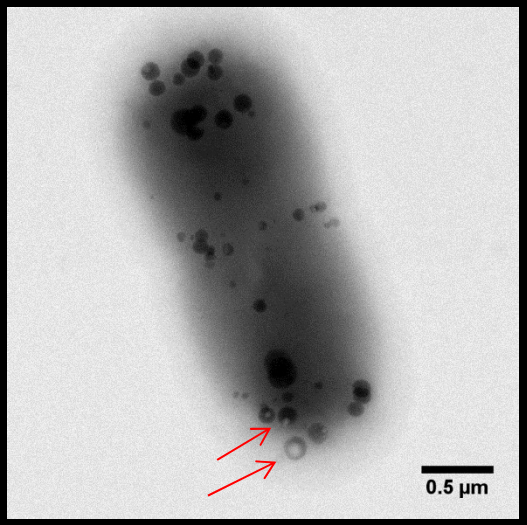
★ Granules are carbonates

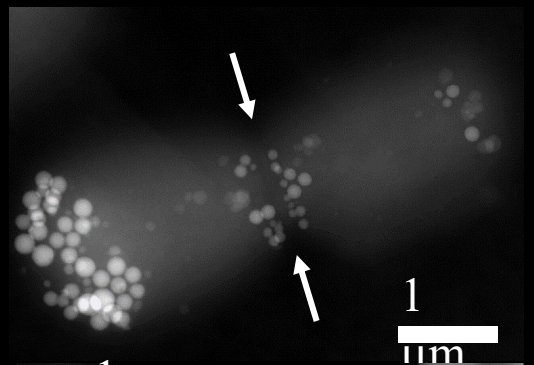
★ Some Ca in polyphosphates as well

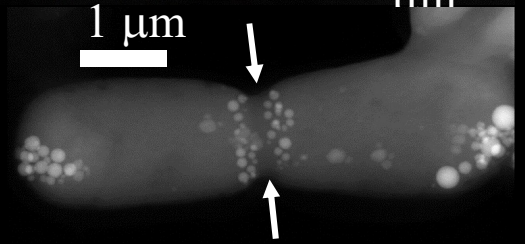
★ Ca $L_{2,3}$-edges consistent with amorphous Ca-carbonates

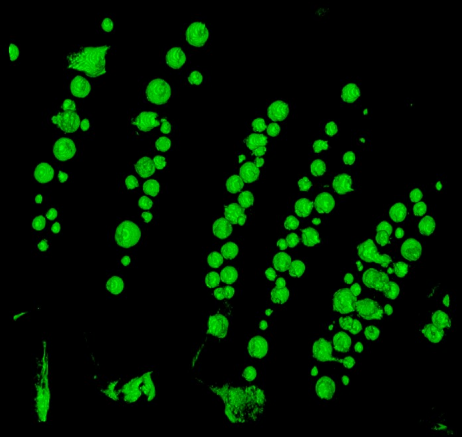
Limitations with ambient-T analyses

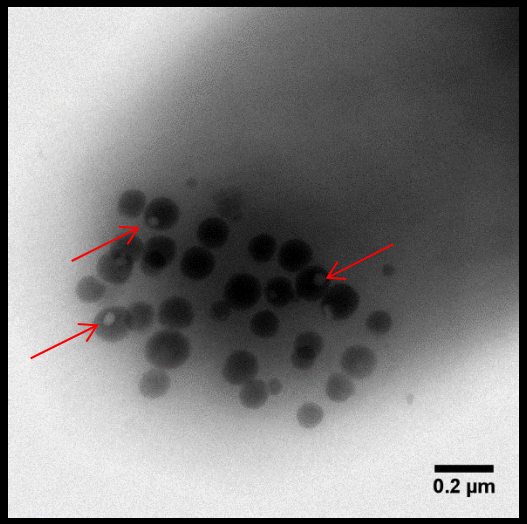












★Cells and Ca-inclusions get flattened upon prep

★Beam damages

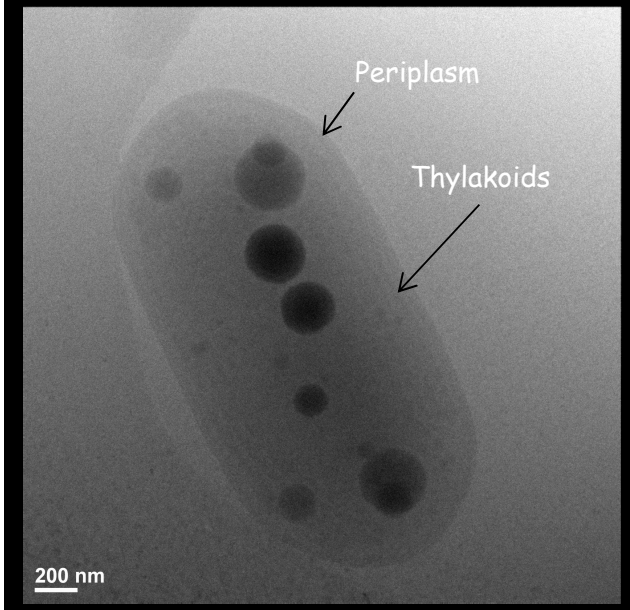
★Loss of diffusible elements upon washing (?)

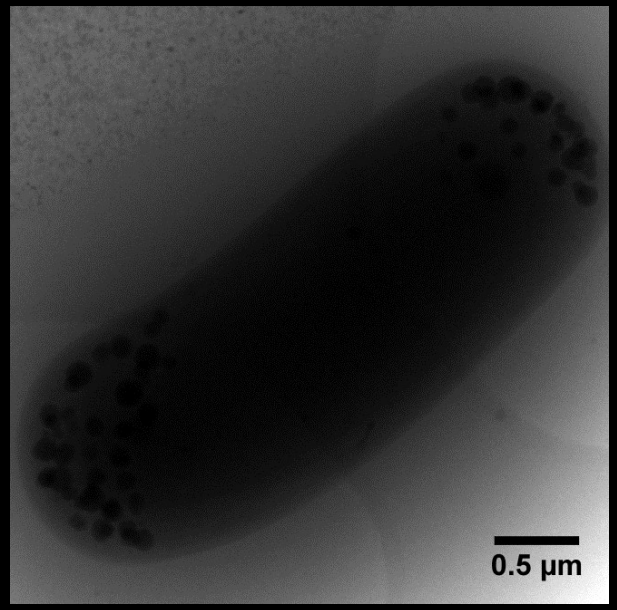
★Poor ultrastructural info

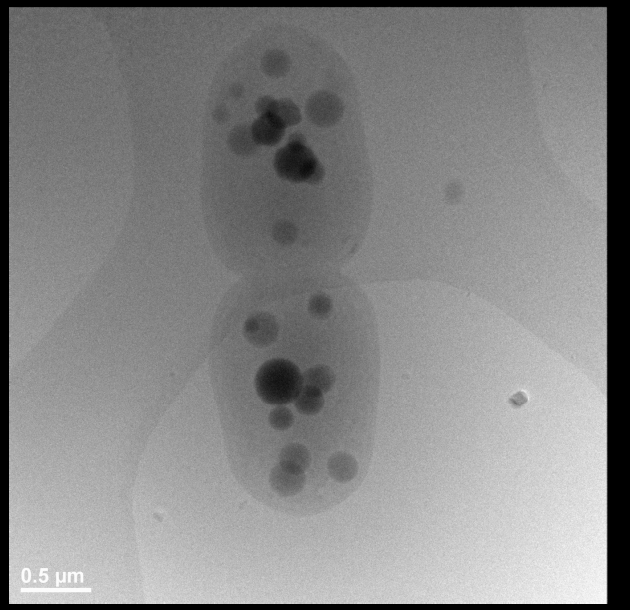
★For some species we lose $CaCO_3$ upon drying

We need cryo

Manual plunge and freeze, cryo-TEM JEOL 2100 (IMPMC – Jean-Michel Guigner)



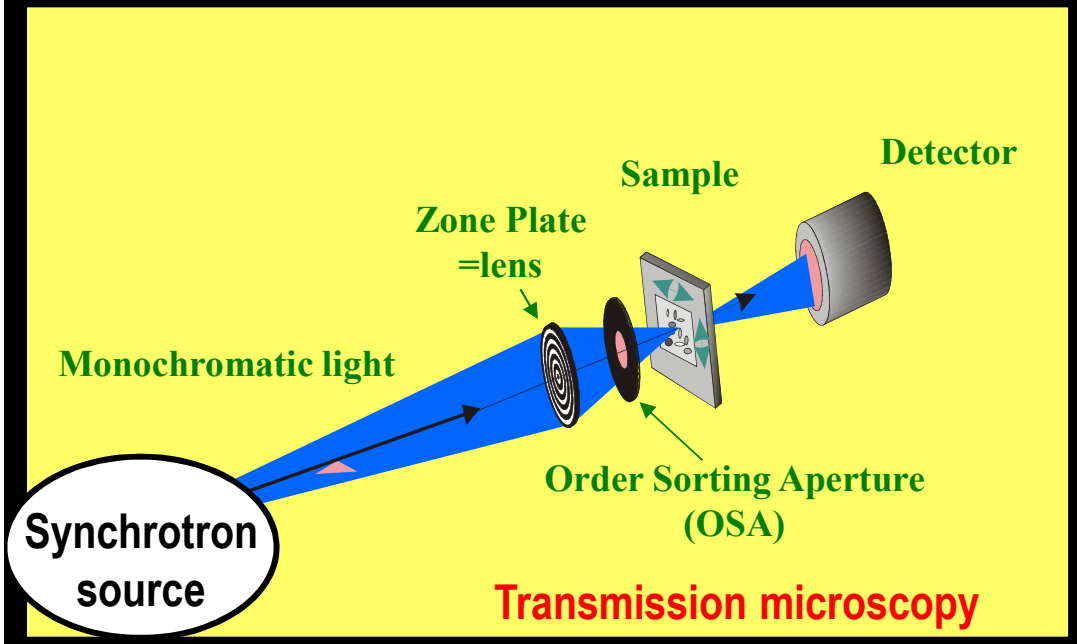


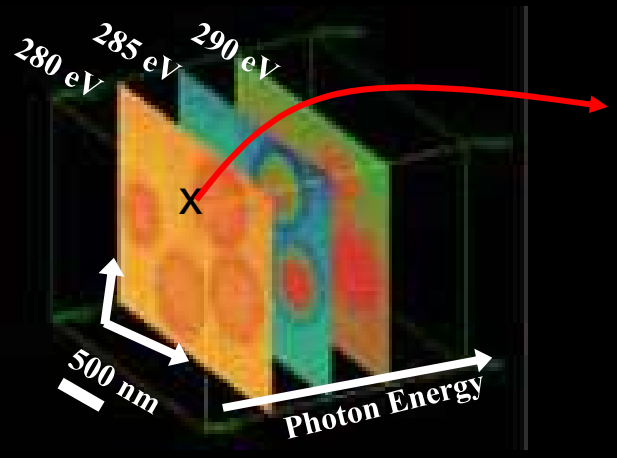


A little too thick for TEM

Cryo-Scanning Transmission X-ray Microscopy (STXM)

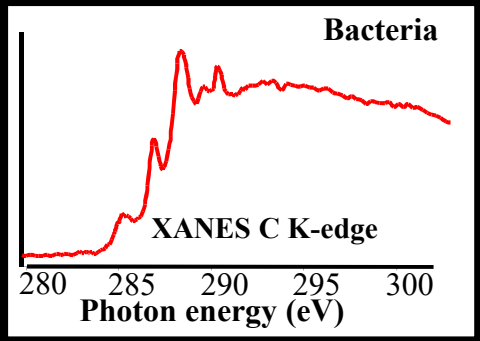
Berkeley, Saskatoon





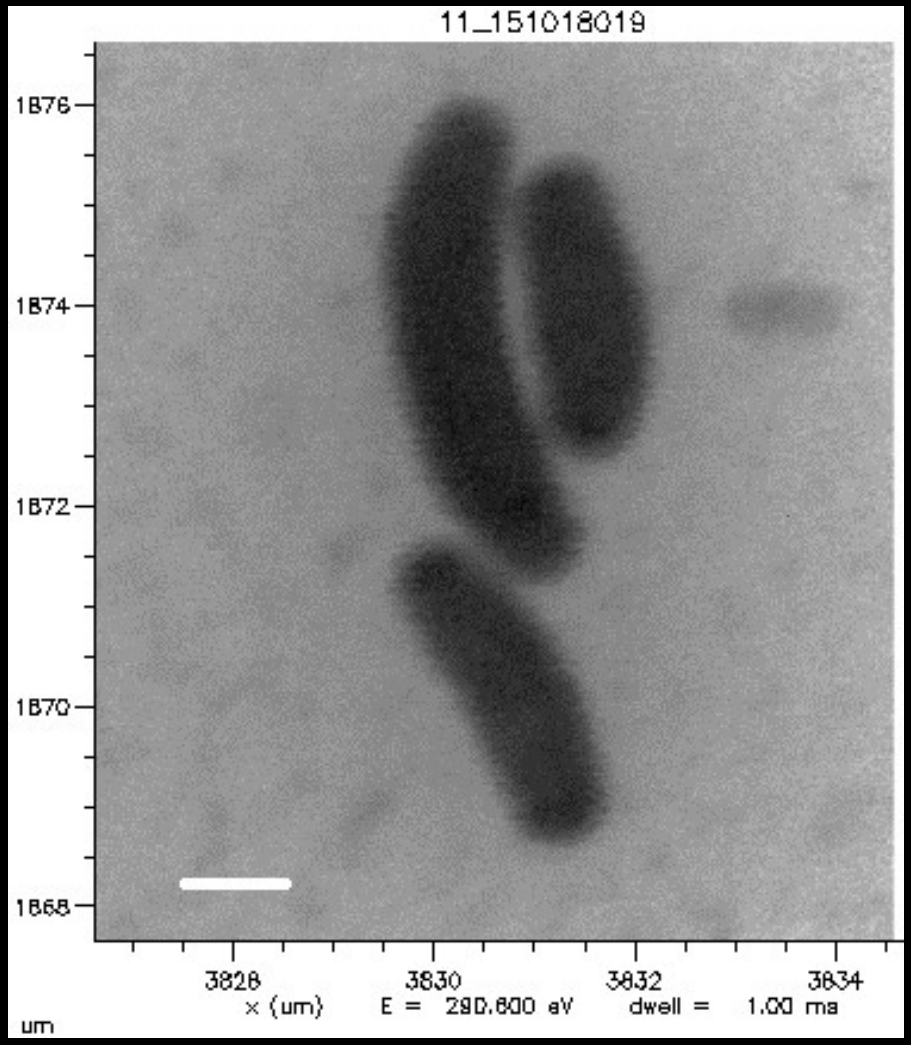
Imaging w. 25 nm resolution

X-ray Absorption Near Edge Spectroscopy (XANES)



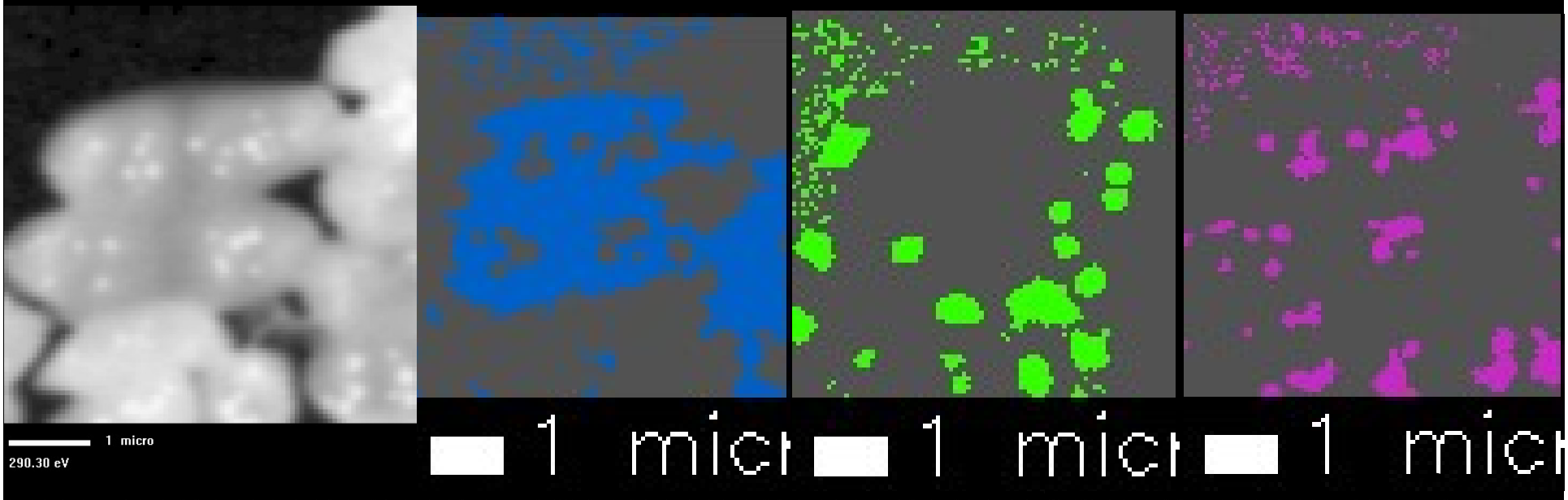
Ca & C speciation at 25 nm resolution

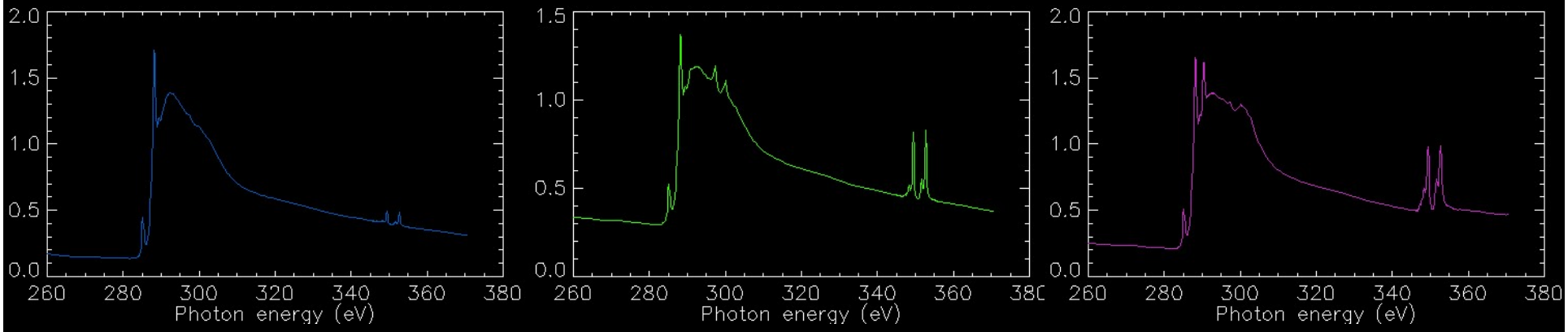
Synchrotron microscopy study of intracellular phases at the C K-edge and Ca $L_{2,3}$-edges



XANES spectra allow localizing and identifiying different species of C and Ca.

There are 3 reservoirs of Ca in the cells



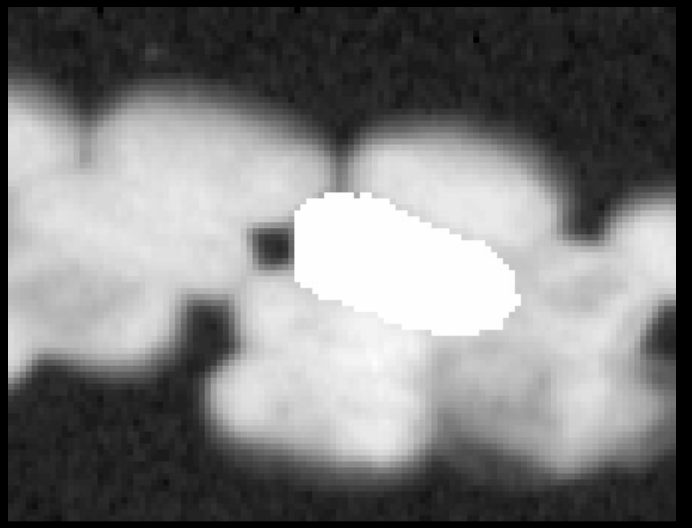


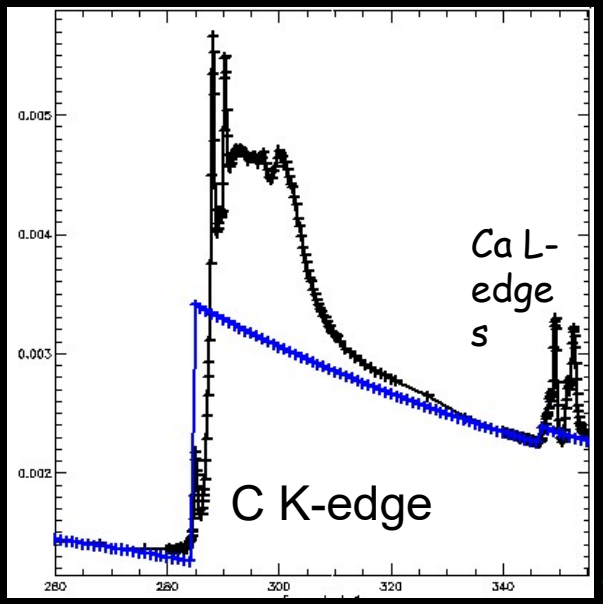
Ca complexed by proteins

Ca in Polyphosphates

Ca in carbonates

Quantification of the reservoirs





Benzerara et al. in prep

★ C in carbonates = 1 – 2 % of total cell C

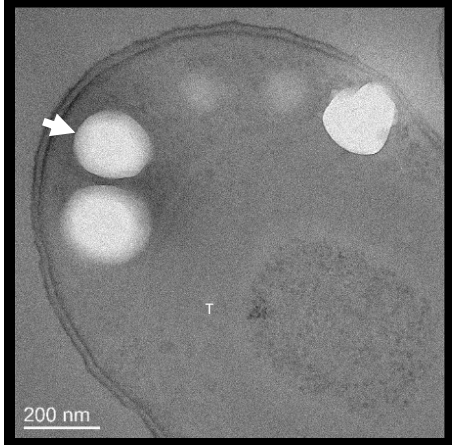
★ In one cell, there is ~ 760 fg of C and 60 fg of Ca (⇔ 3M!).

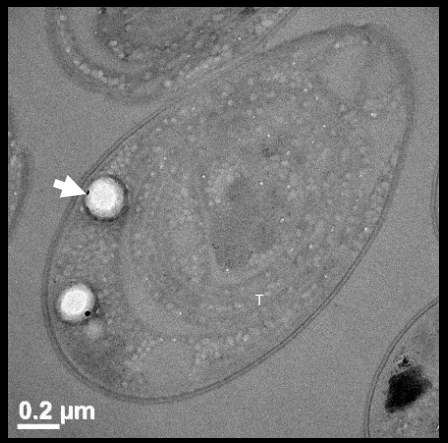
★ About 2/3 of Ca is in the carbonates, 1/3 complexed by proteins or in Polyphosphates

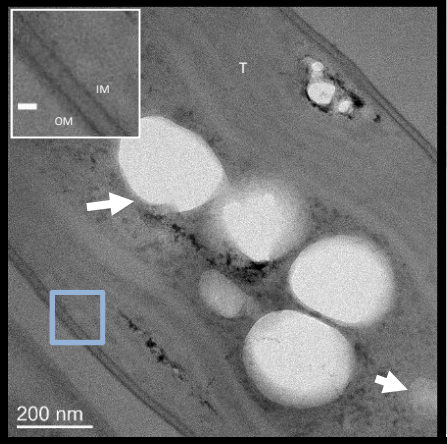
→ Need to section for ultrastructural details

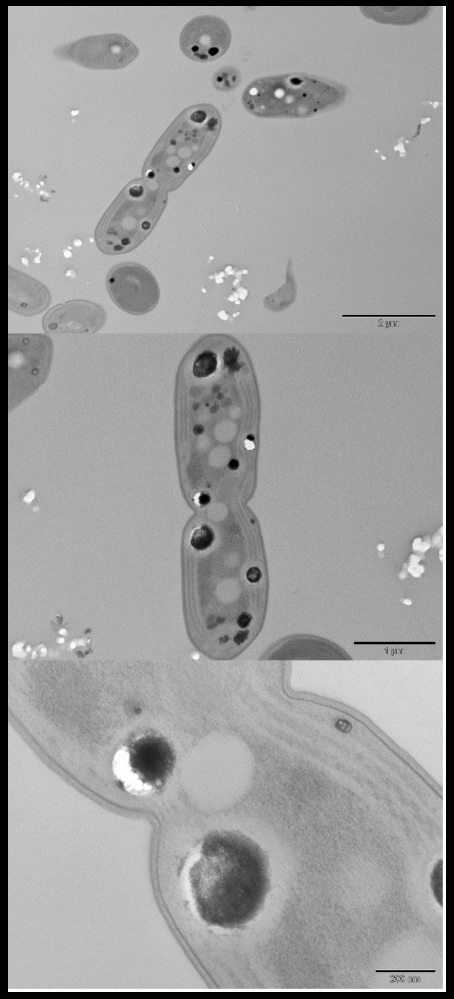
With standard room-T protocols, amorphous $CaCO_3$ inclusions disappear

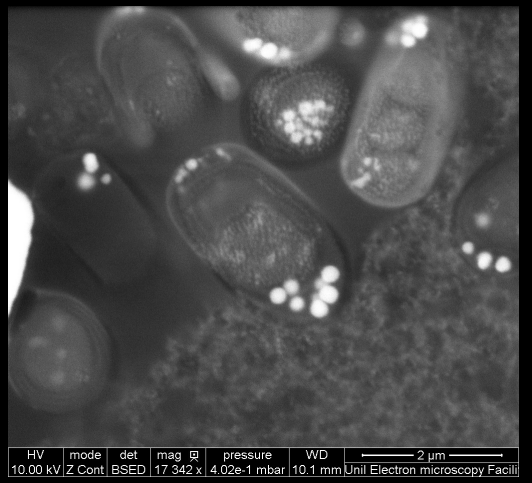
Holes or Ca-phosphates replacing $CaCO_3$ in sections

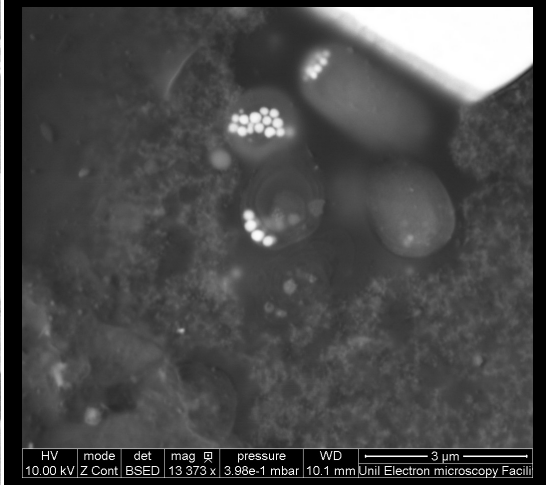












Li et al. (2016) Minerals

Preservation by Cryo Electron Microscopy of Vitreous Sections (CEMOVIS) @ Institut Pasteur w. Martin Sachse

High pressure Freezing (2kbars, -196°C)
(Vitrification)

↓

Amorphous ice

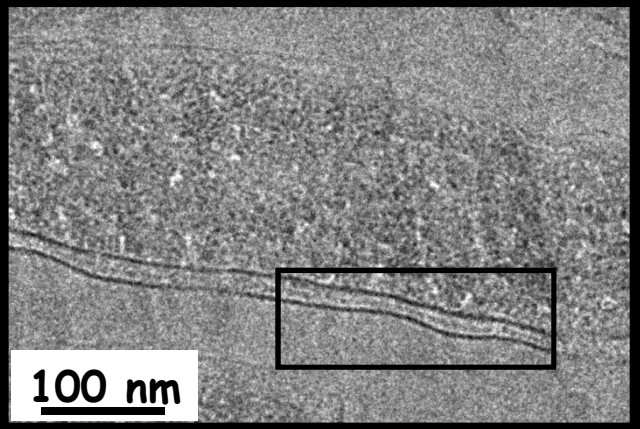
↓

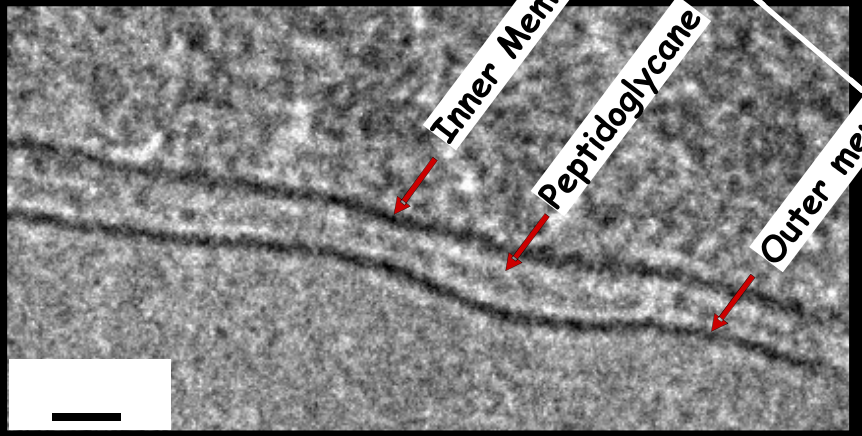
Section (cryo-ultramicrotomy)

↓

TEM low dose mode

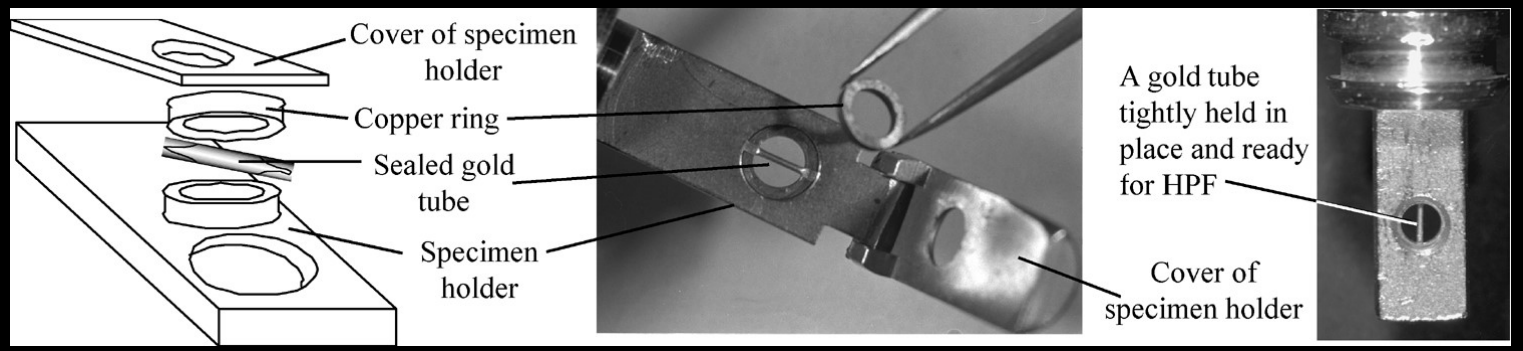
↻ Native « hydrated » conditions





e.g., Miot et al. (2011) Geobiology, in press; Murat et al. (2010) PNAS 23, 5593-5598

Freezing of cell suspension in gold capsules [HPM 010]



(Shimoni and Müller, 1998)

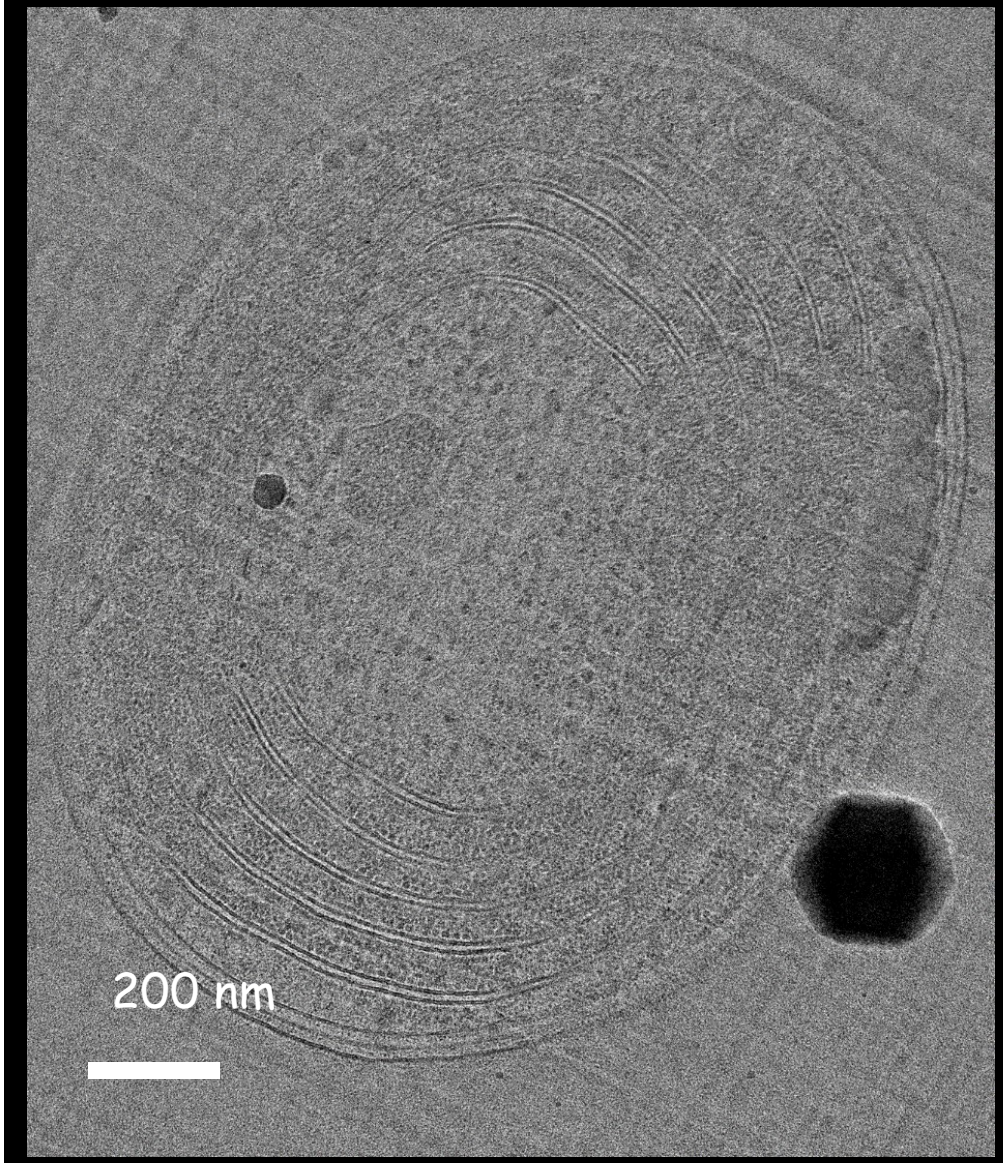
Mounting of capsules for sectioning

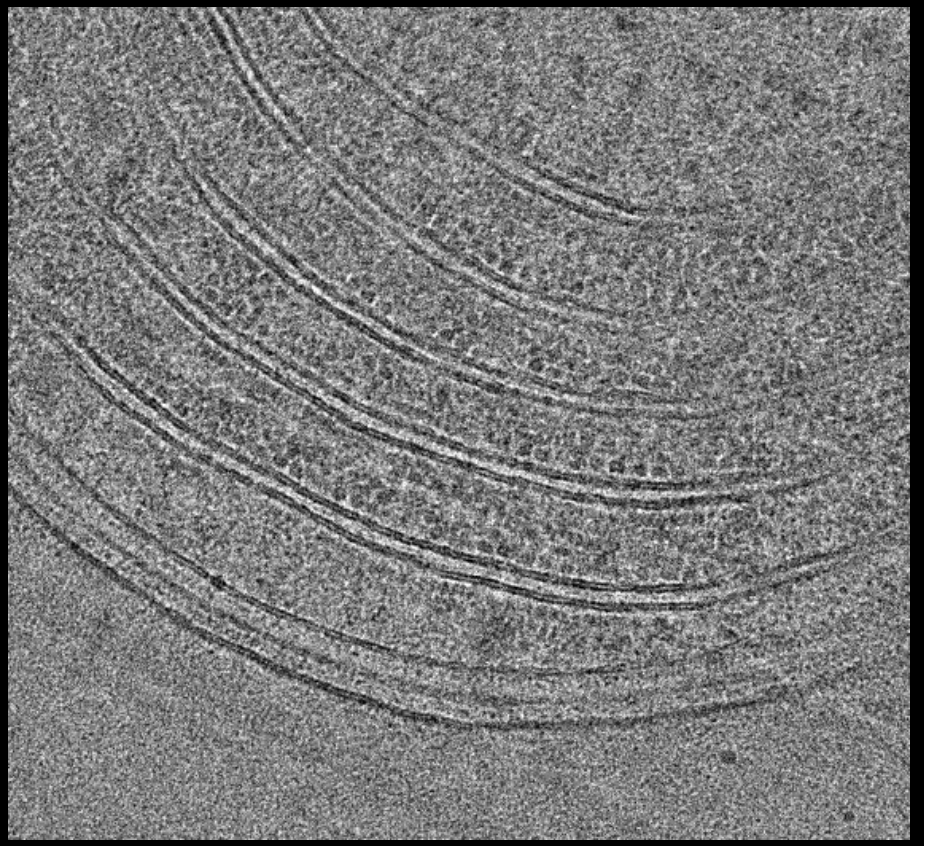
Cryoglue
(Richter, 1994).
@ -140°C – viscous
@-160°C – hard for cutting

Lately problems with cryoglue: not hard enough at -160°C. Chatter……

Solution:
Change ratio
ethanol : 2butanol from 3:2 to 3:1

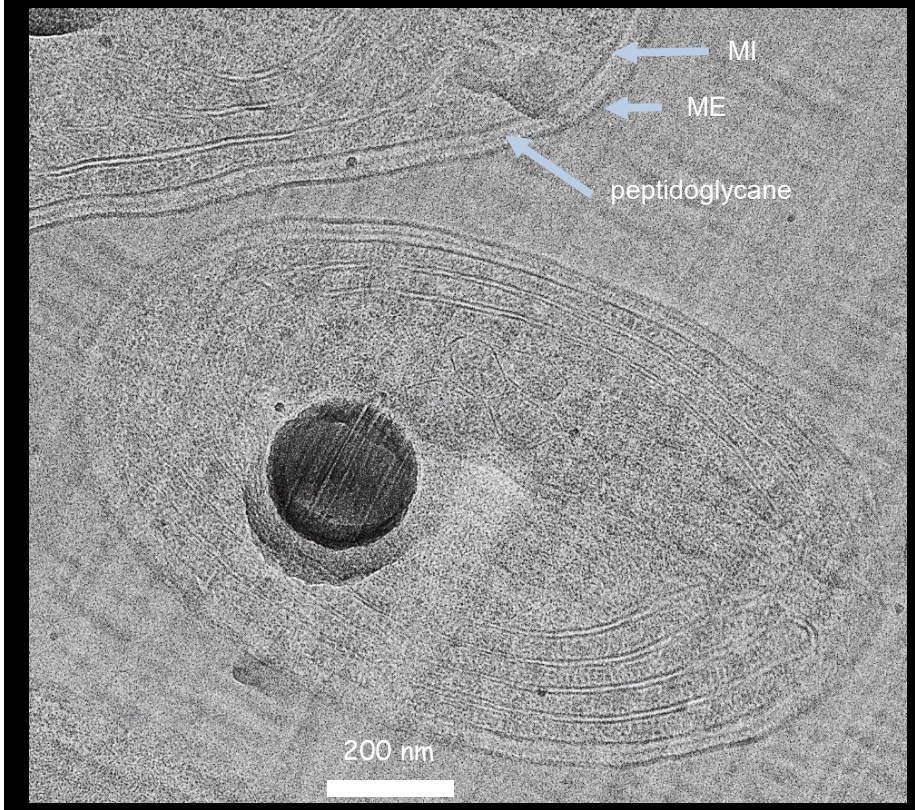
Low dose Cryo-TEM

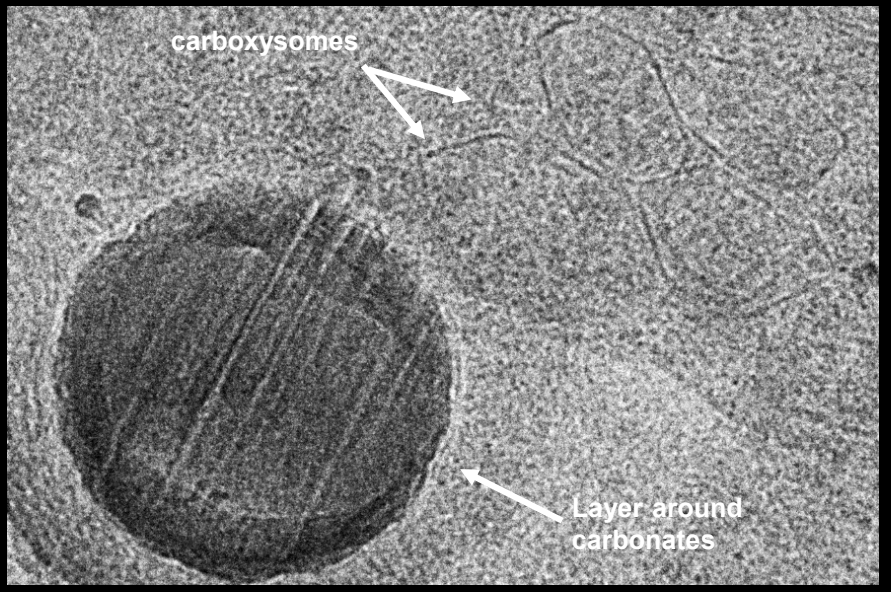


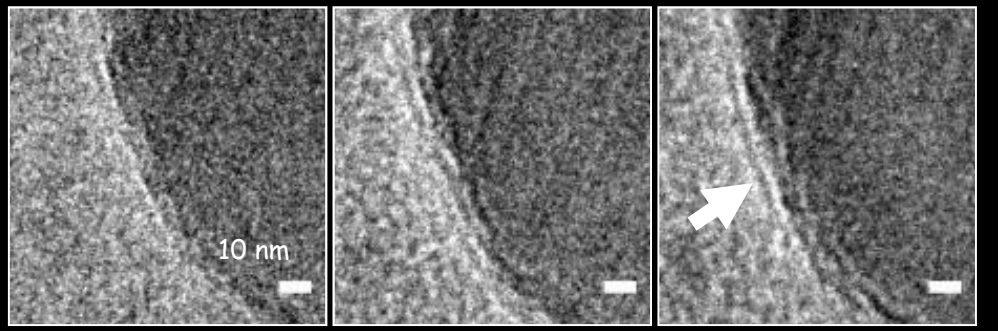


Cyanothece sp. PCC 7425

A layer (lipid monolayer or proteins) has been systematically observed around carbonates







M Blondeau, M Sachse et al. 2018 (Frontiers in Microbiol)

→ Existence of a compartment can explain low cytosolic Ca / high Ca where $CaCO_3$ precipitates

Conclusions

★Cryo-analyses provide unique info (e.g., ultrastructure)

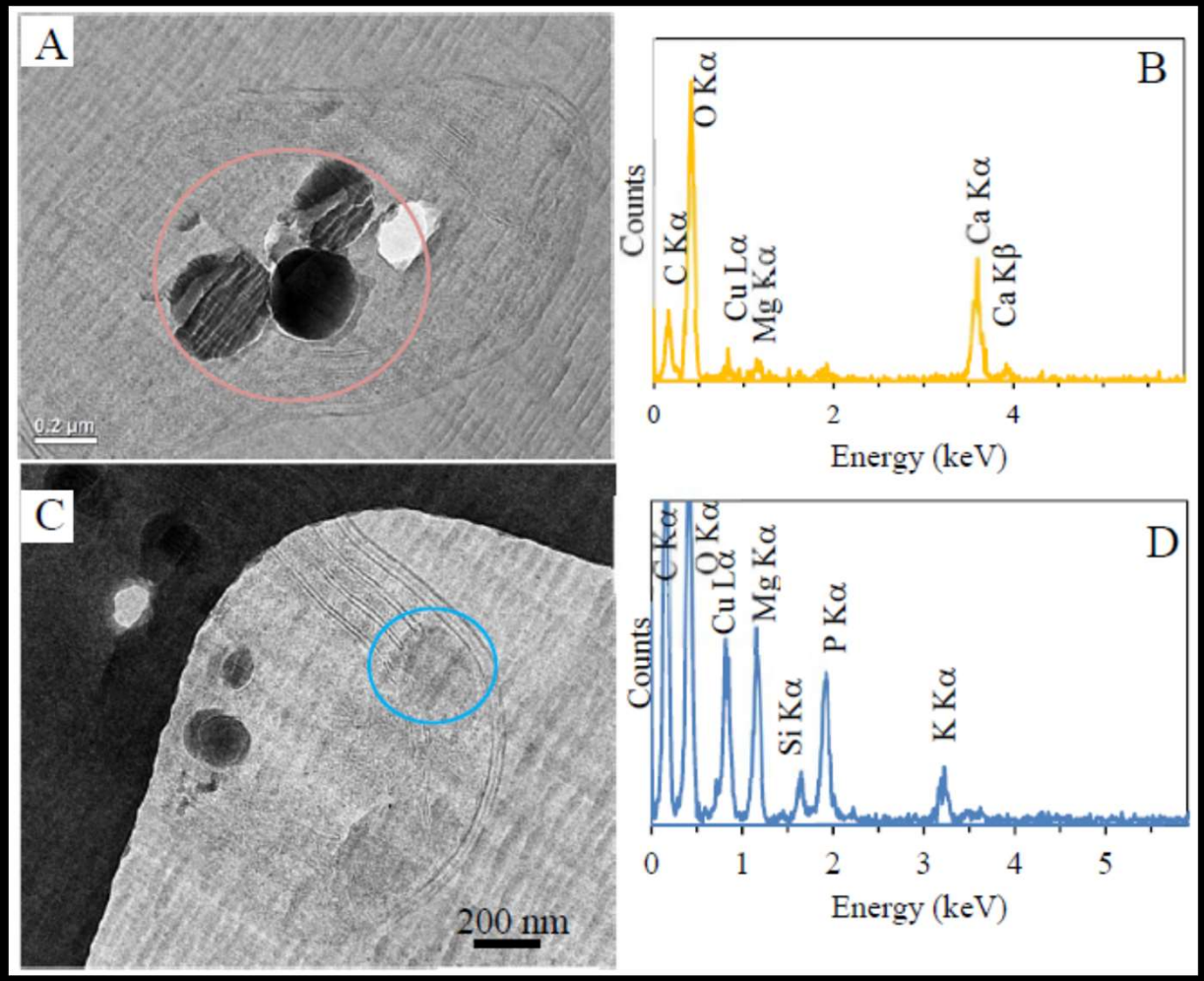
★Cryo allows to circumvent some artefacts associated with ambient T sample prep

Yet,

★ need high cell density and therefore centrifugation

★Need several developments…

Perspectives: important to improve analytical efforts in cryo

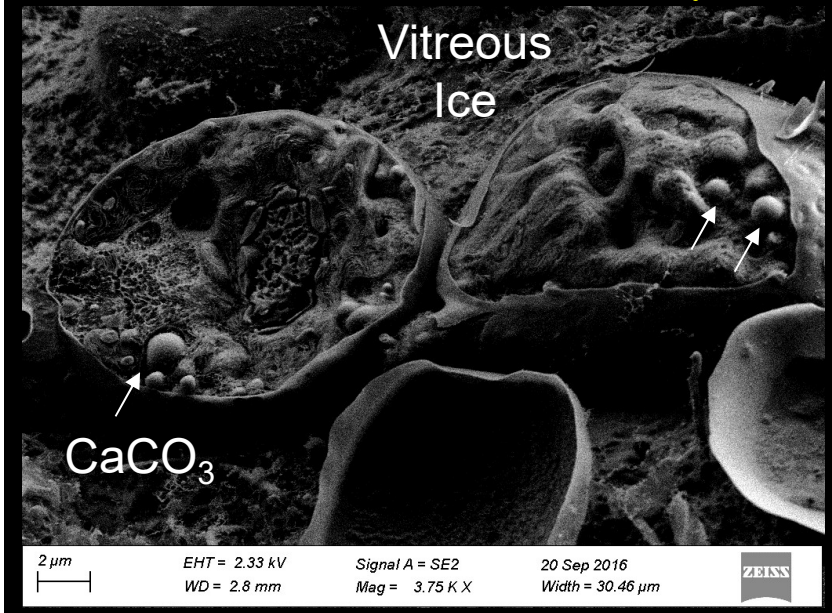


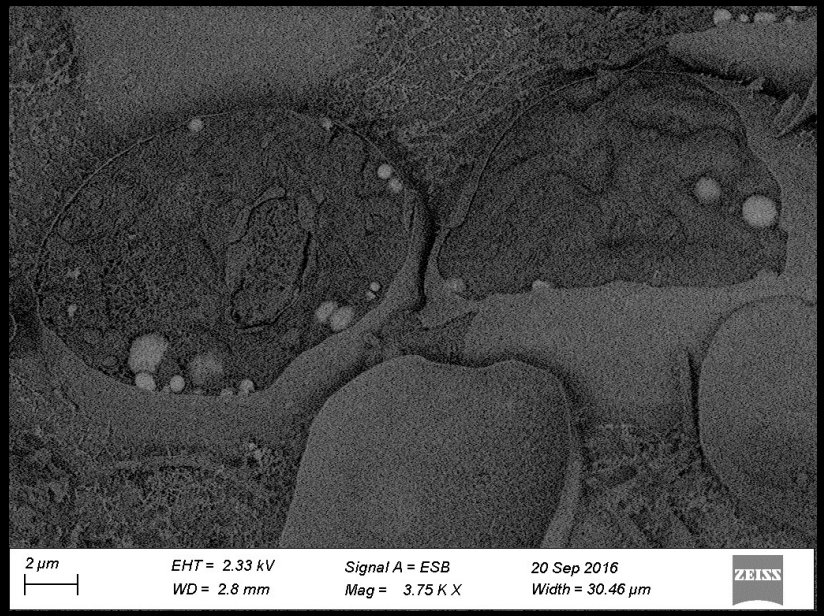
EDXS: poor quality spectra and fast destruction of the sample

Alternative = sublimation in the TEM and analyses at room T

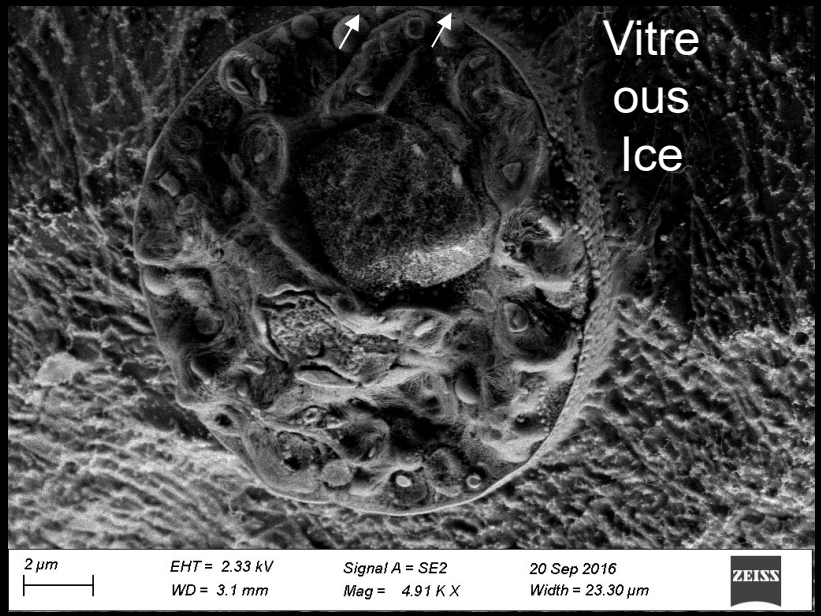
→ Correlative would be awesome (CLSM/EM/synchrotron (XRF, tomography…))

Cryo-SEM observations

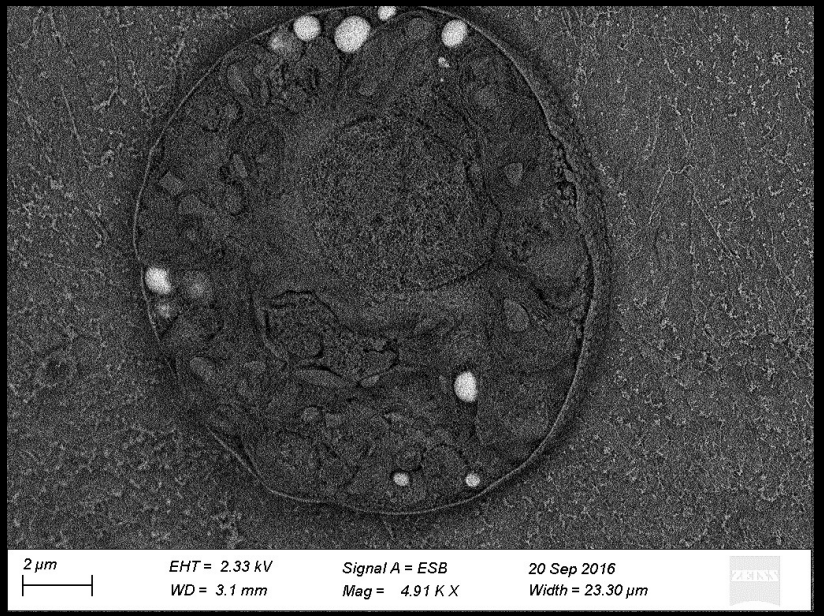




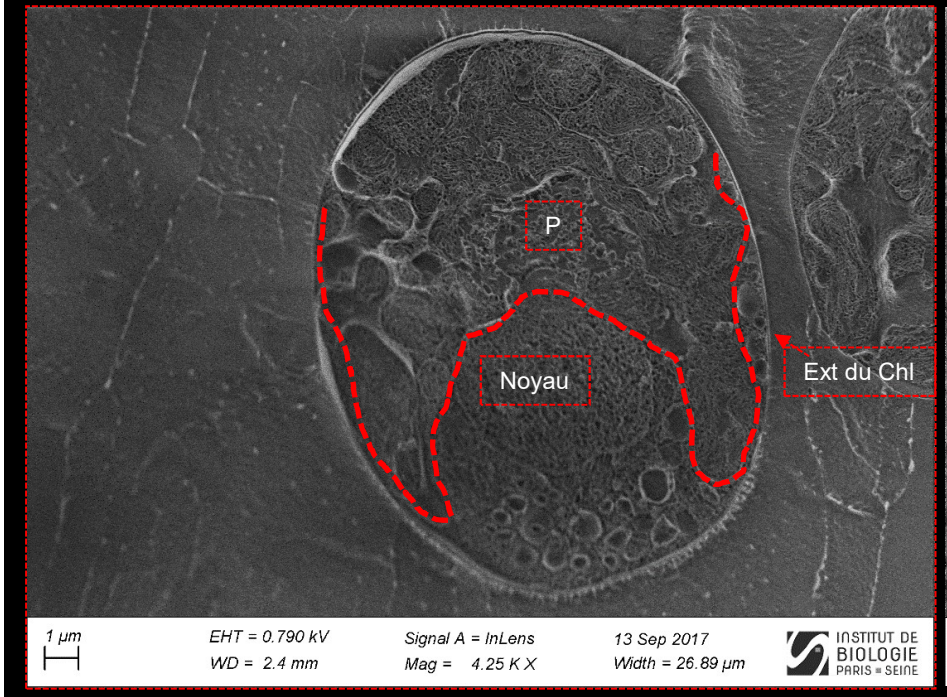
SE2, 2.33 keV

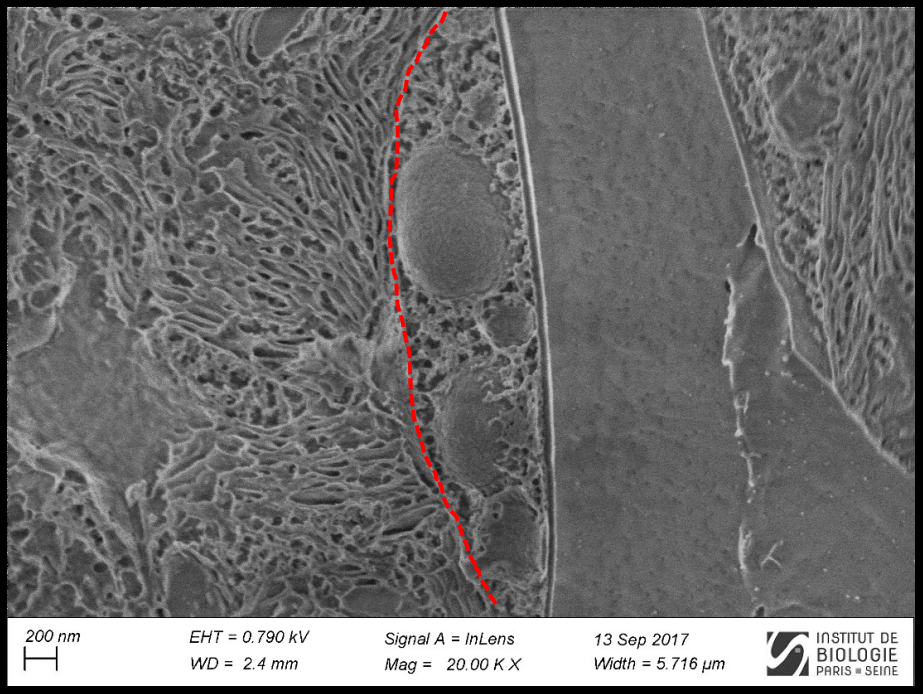


ESB, 2.33 keV



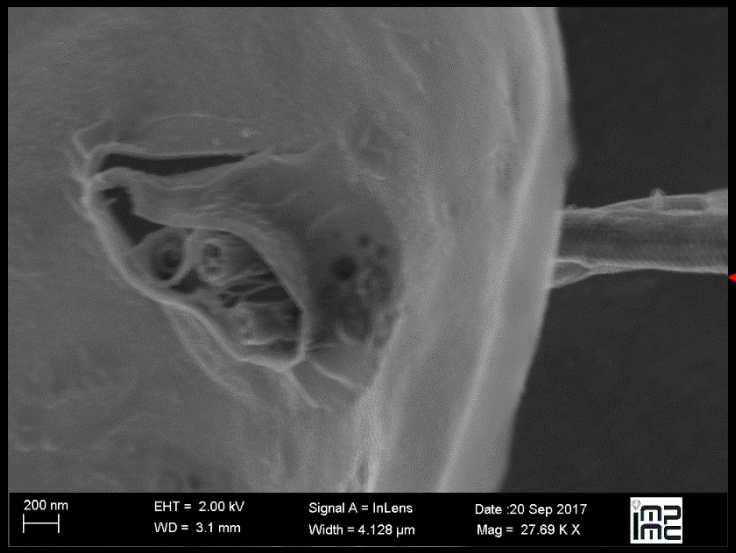
Cryo-SEM observations

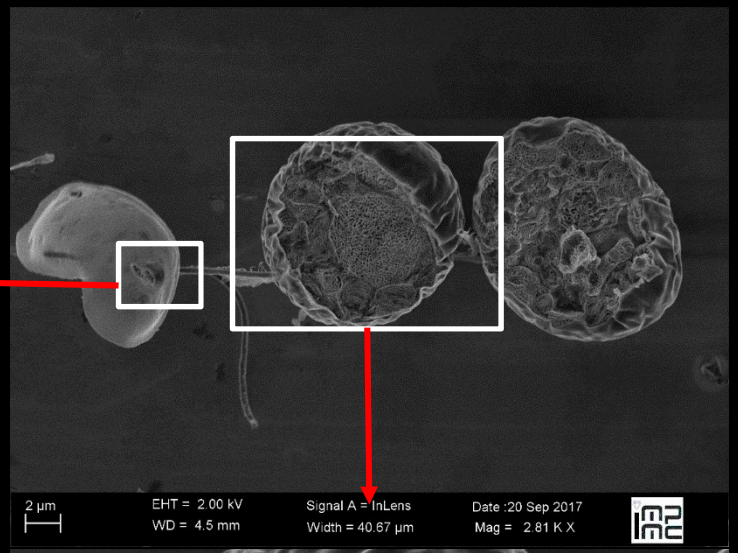


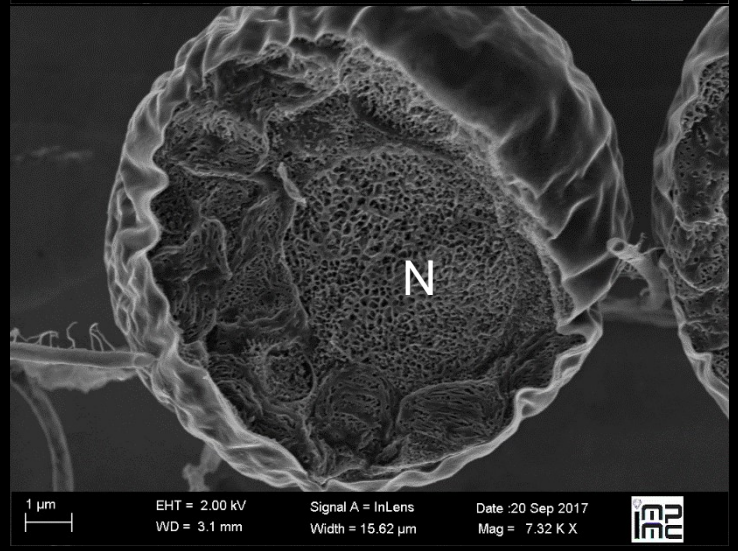


★ $CaCO_3$ inclusions stuck to the wall of the cells outside the chloroplast
★ Difficult to say if they are located within a compartment

Room-T SEM observations of sublimated cryofractured cells







- ★ EDXS analyses can be performed
- ★ Better field depth

Acknowledgments

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F Skouri-Panet : IMPMC

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