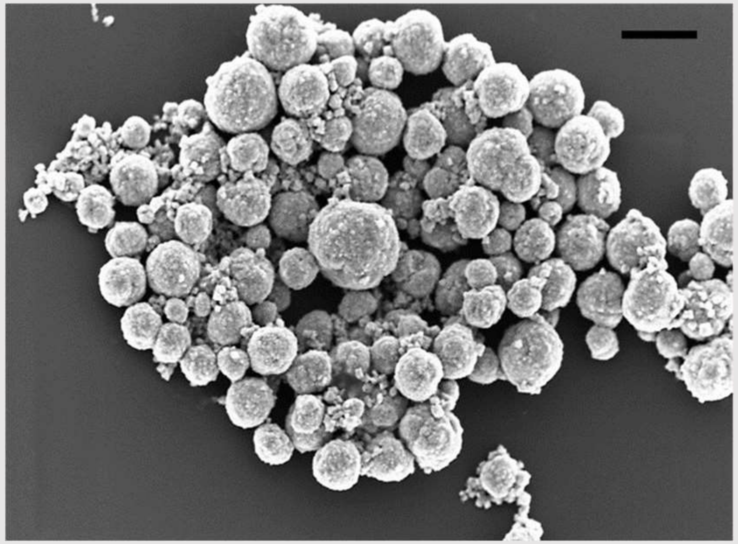
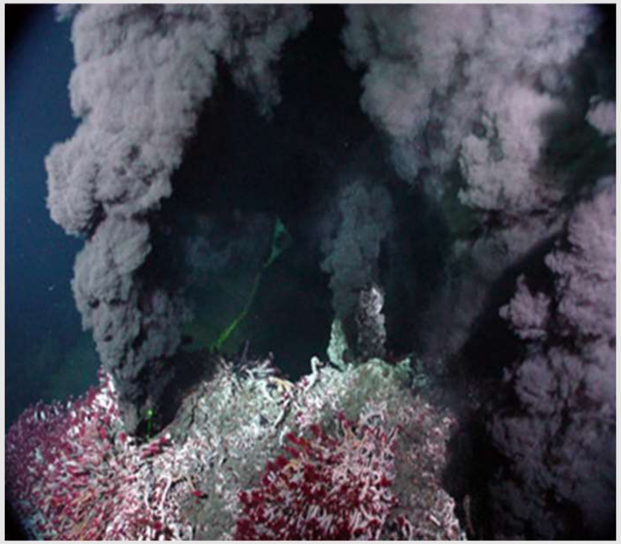
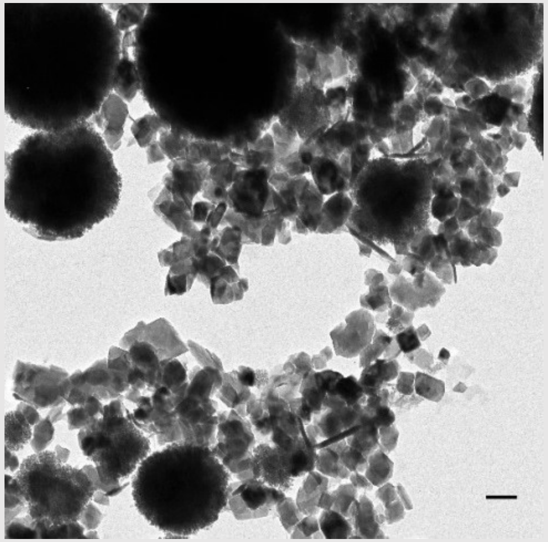
Laboratoire de
Biologie Cellulaire
des Archées

Mineralization of iron-sulfides induced by Thermococcales



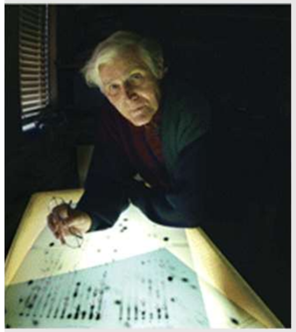


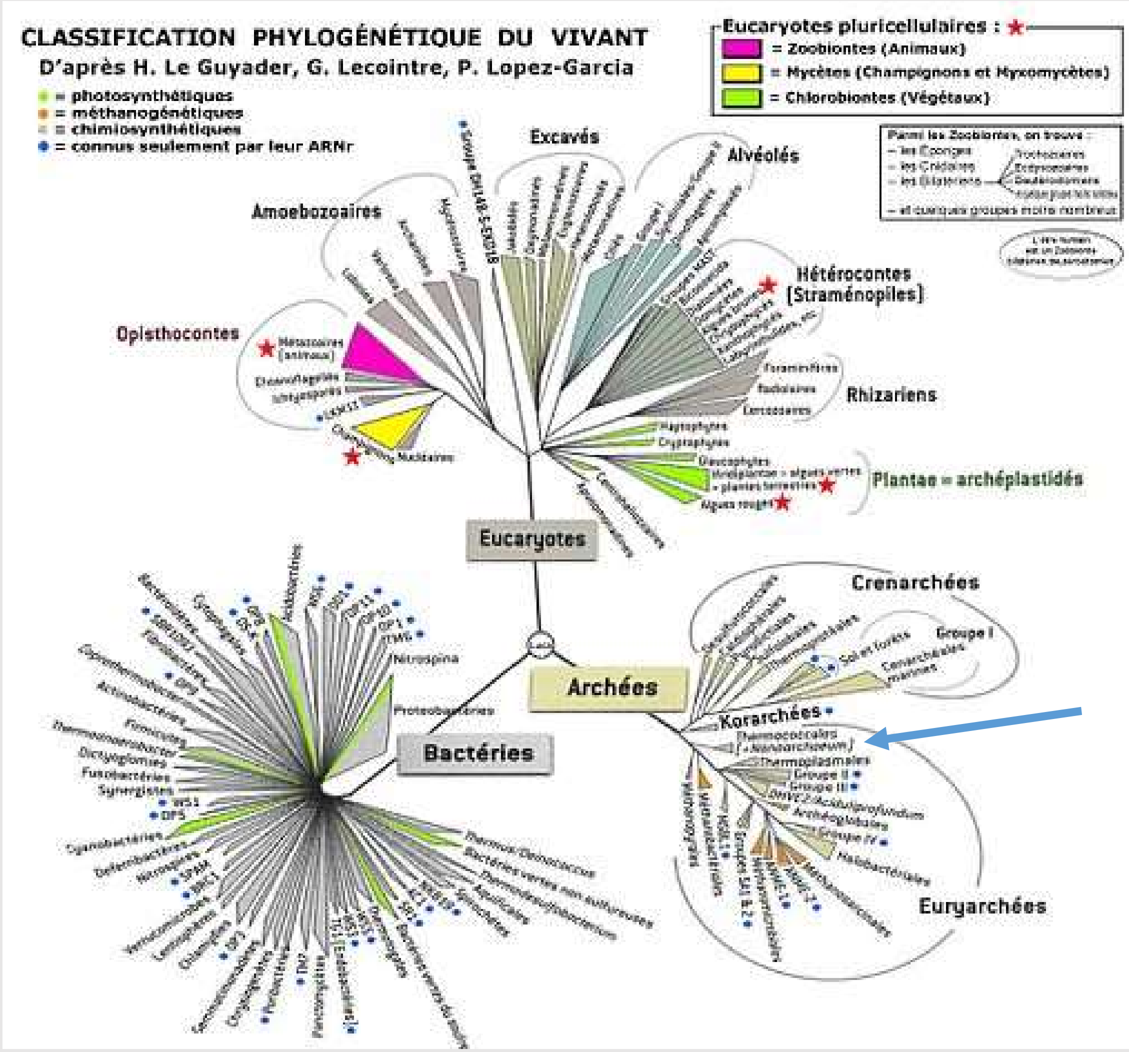


18/01/22

Aurore Gorlas

Archaea, the third domain of life





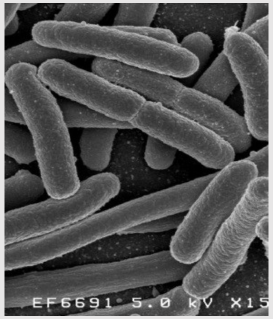
3 main phyla

Crenarchaeota
Euryarchaeota
Thaumarchaeota

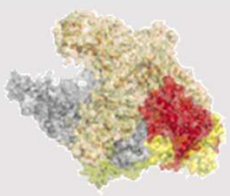
(Woese *et al.*, 1977; Woese *et al.*,1990)

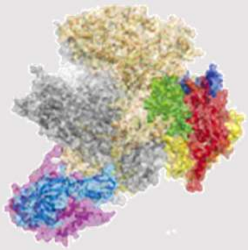
Archaea, the third domain of life

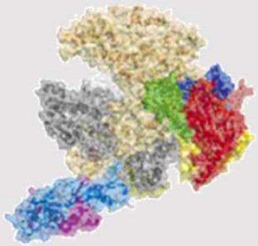
Bacteria *Eucarya* *Archaea*



Morphological characteristics
Compartmentalization
Genes organization



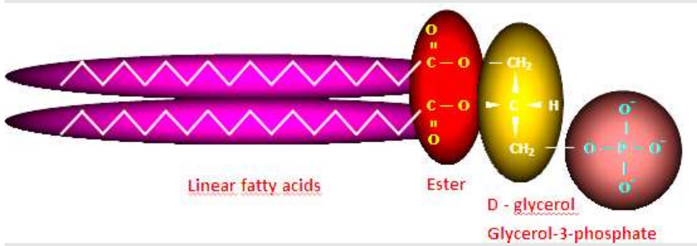


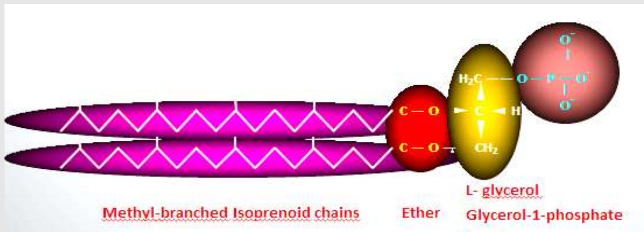


Replication, transcription, translation mechanisms

3D Structures of RNA pol

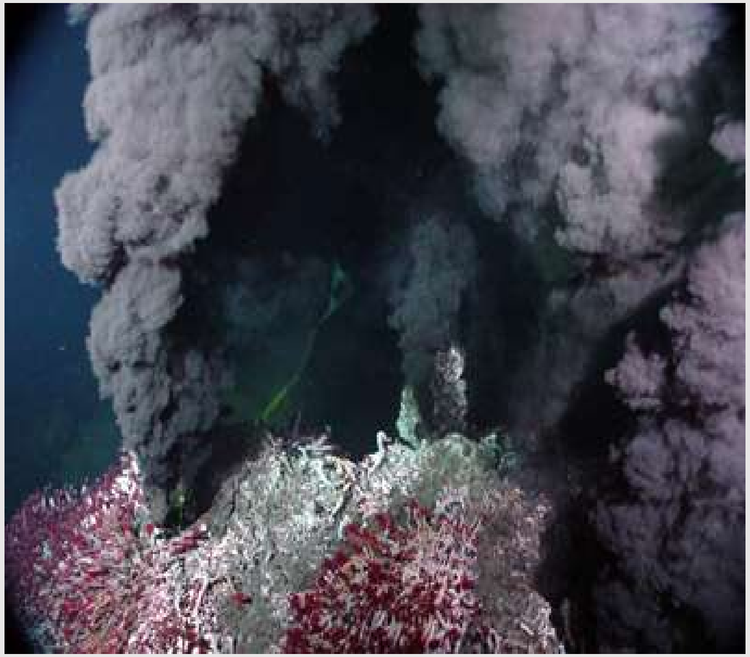
(Werner et al., 2008)

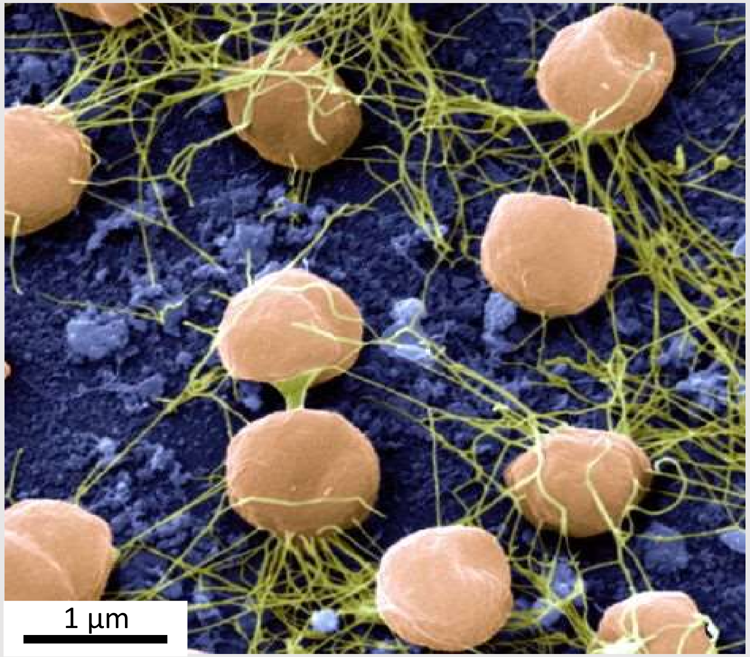




Lipids

Thermococcales





Pyrococcus, Thermococcus **and** ***Paleococcus***

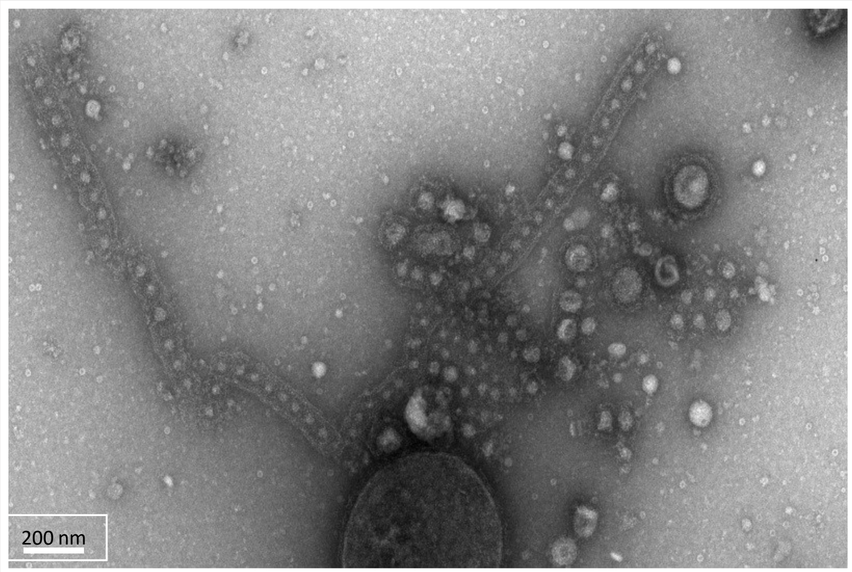
- Hyperthermophile
- Strictly anaerobe
- Mainly heterotroph
- Sulfur reducer
- Two types of metabolisms :
  S(0) respiration, fermentation

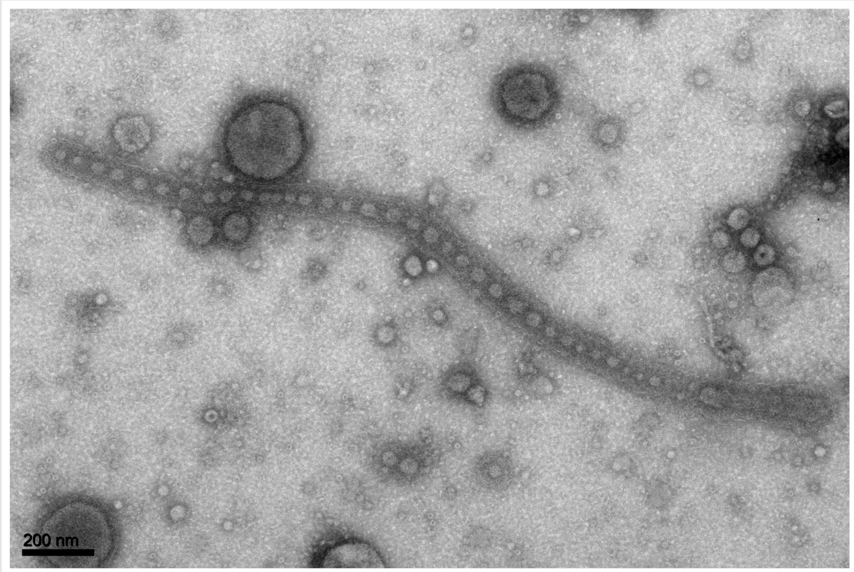
About 50 species described:

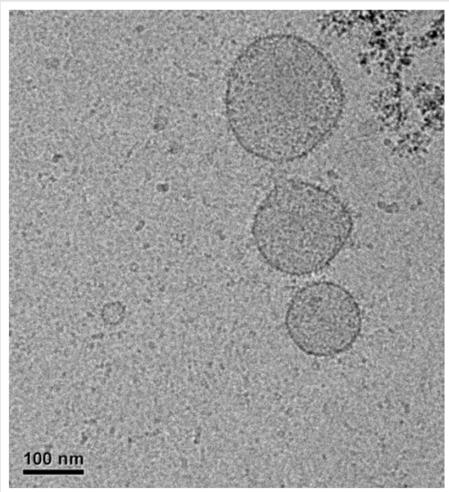
T. prieurii: Gorlas et al., 2013a

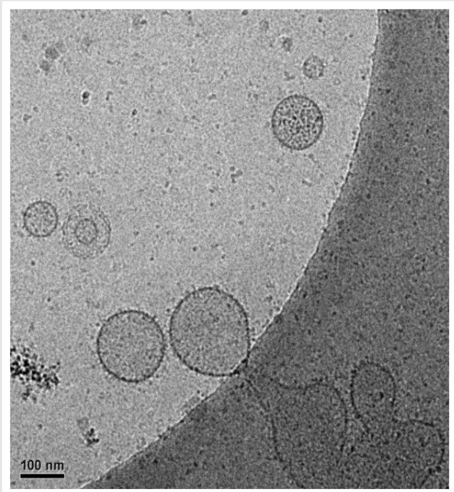
T. nautili: Gorlas et al., 2014

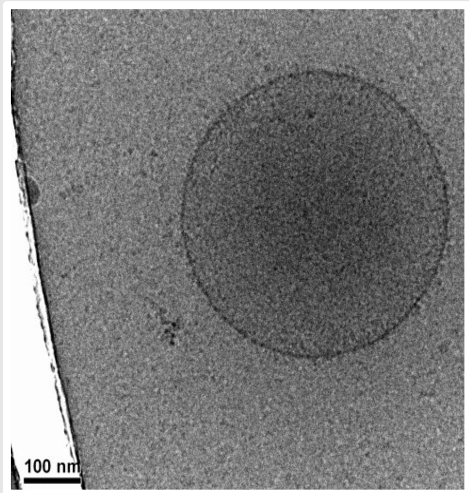
Observation of purified membrane vesicles and nanopods from Thermococcales (TEM and Cryo-EM)











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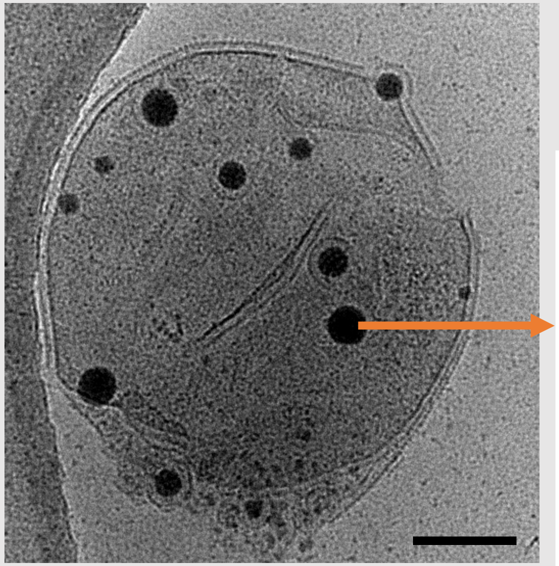
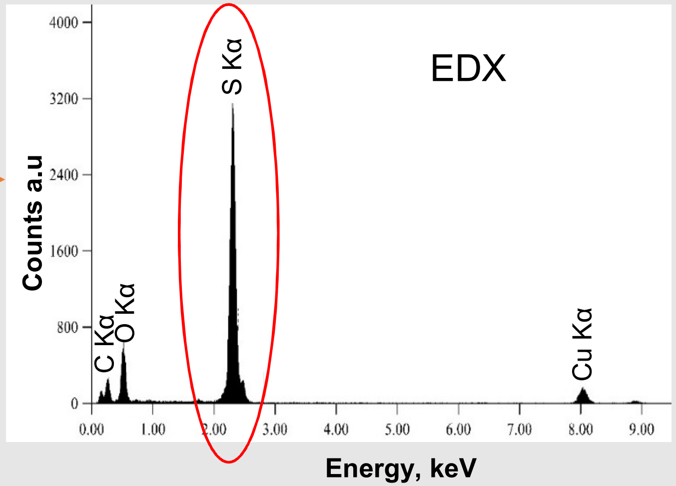
Research paper

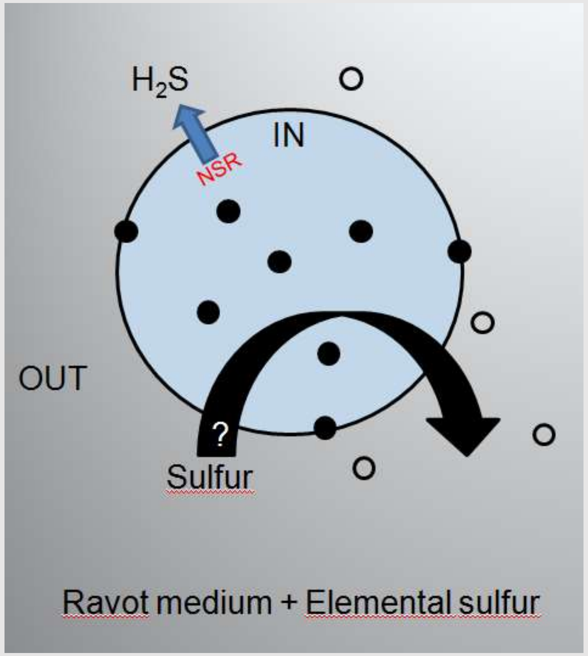
Sulfur vesicles from *Thermococcales*: A possible role in sulfur detoxifying mechanisms

A. Gorlas [a,*], E. Marguet [a], S. Gill [a], C. Geslin [b], J.-M. Guigner [c], F. Guyot [c], P. Forterre [a,d]



Thermococcales cell





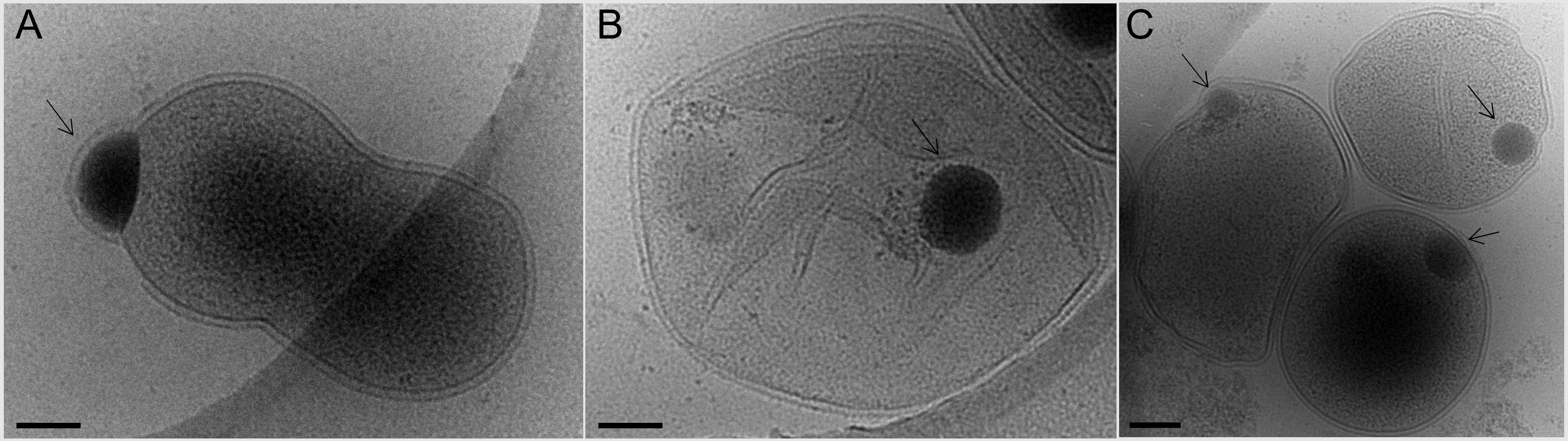
NSR: Sulfur oxydoreductase

S(0) electron acceptor

Detoxification of polysulfides?

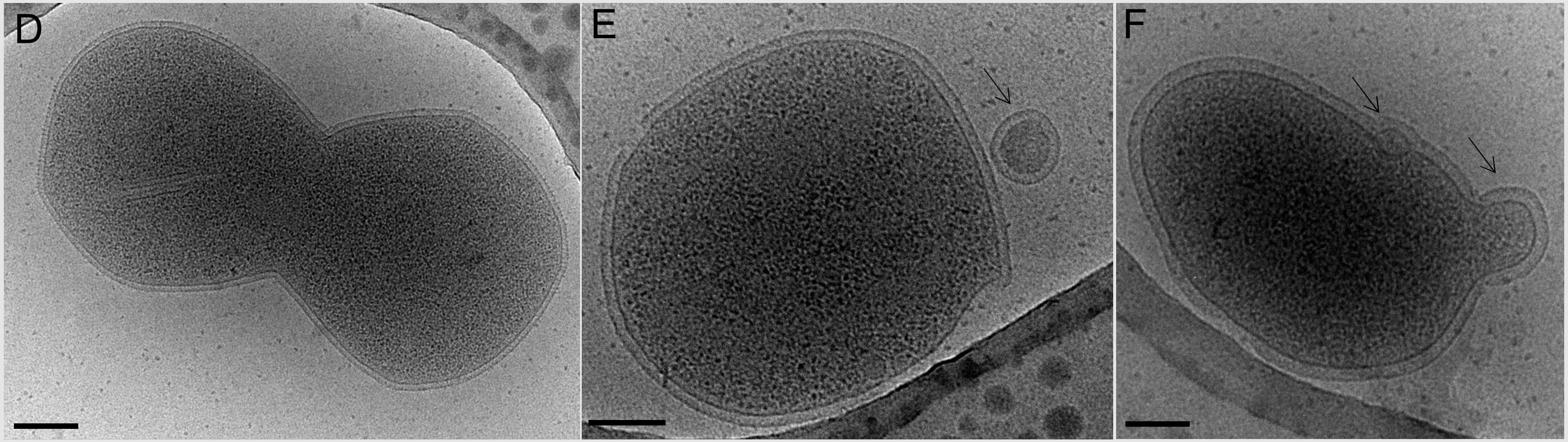
S transported as polysulfides

Cultures in Ravot medium without S(0) supplemented with L-cystine



→ Only one sulfur vesicle per cell

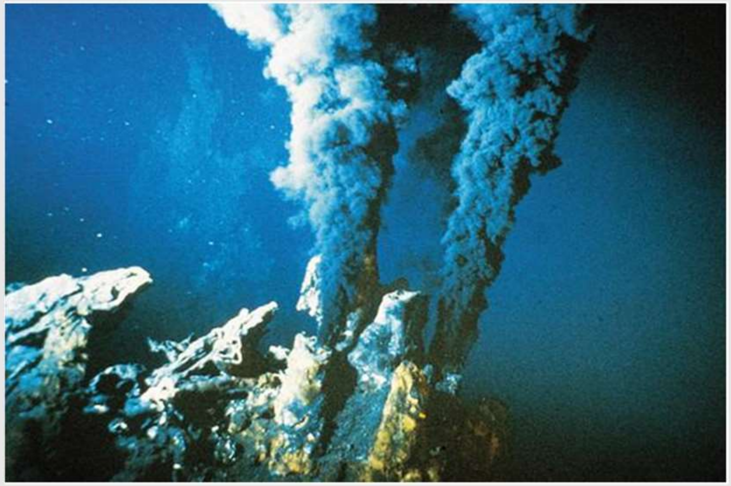
Second subculture in Ravot medium supplemented with L-cystine



→ No sulfur vesicles

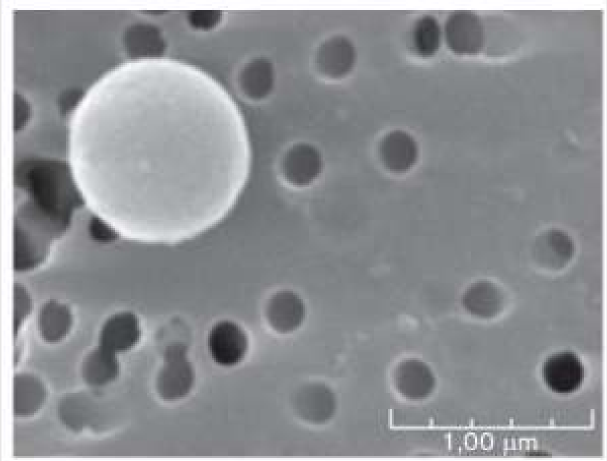
(Gorlas et al., 2015)

Hydrothermal deep-sea vent :
an extreme environment for life



→ Strong physico-chemical gradients
Iron- and sulfur-rich systems
FeS_2 pyrite formation (> 200°C)

***Thermococcales* :** major inhabitants of the hydrothermal sources



→ Hyperthermophile
Strictly anaerobe

However at lower temperatures (< 150°C) FeS_2 are also produced by a still unknown mechanism which might involve the living compartments.

Does this hyperthermophilic (i.e. >80°C) biosphere contribute to the formation of iron-sulfide minerals ?

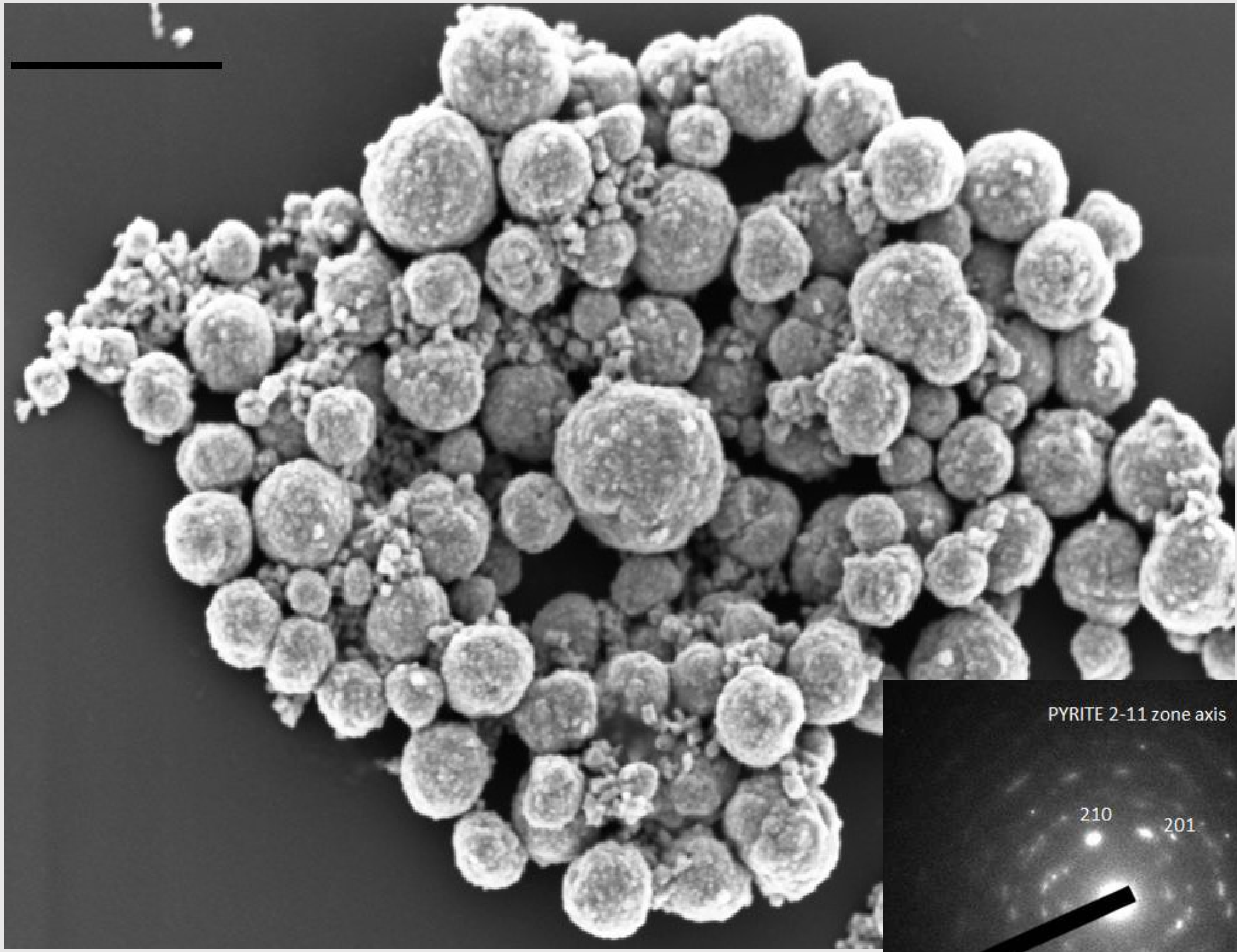
Rapid (<100 hours) formation of pyrites (FeS_2) replacing former cells and vesicles of Thermococcales

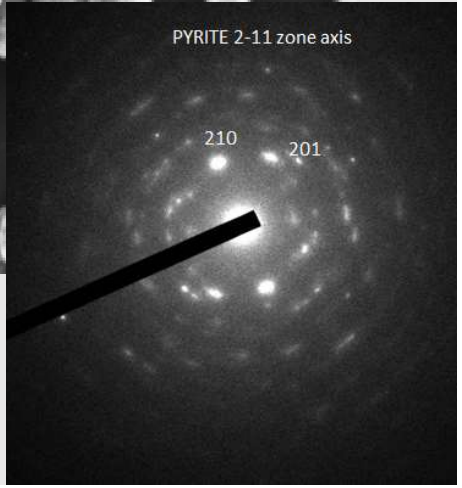
Thermococcus prieurii

85°C, Strict anoxia
144 hrs in Fe^{2+}
mineralization
medium

With presence of S(0)

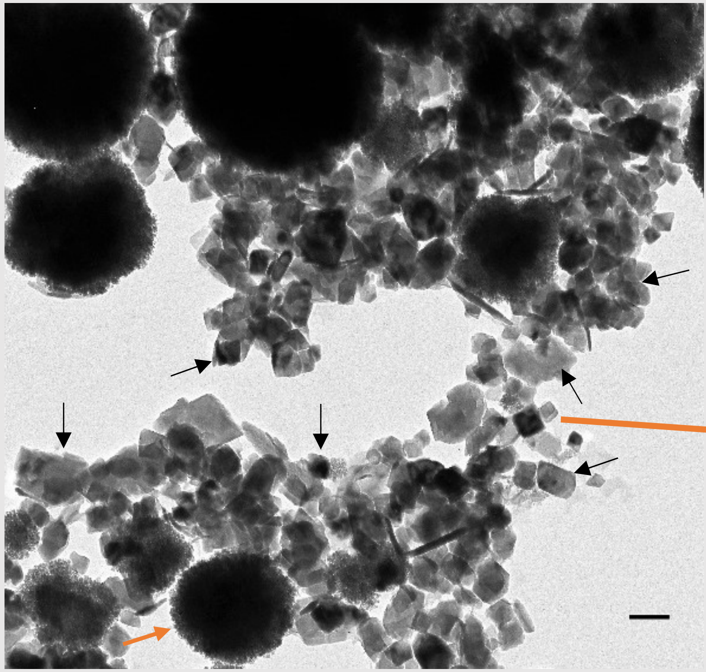
SEM Image, scale bar : 1 micron

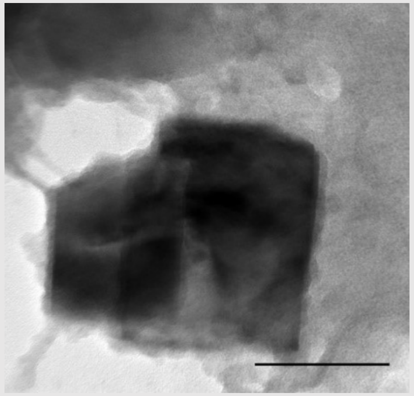




Gorlas et al., 2018 PlosOne

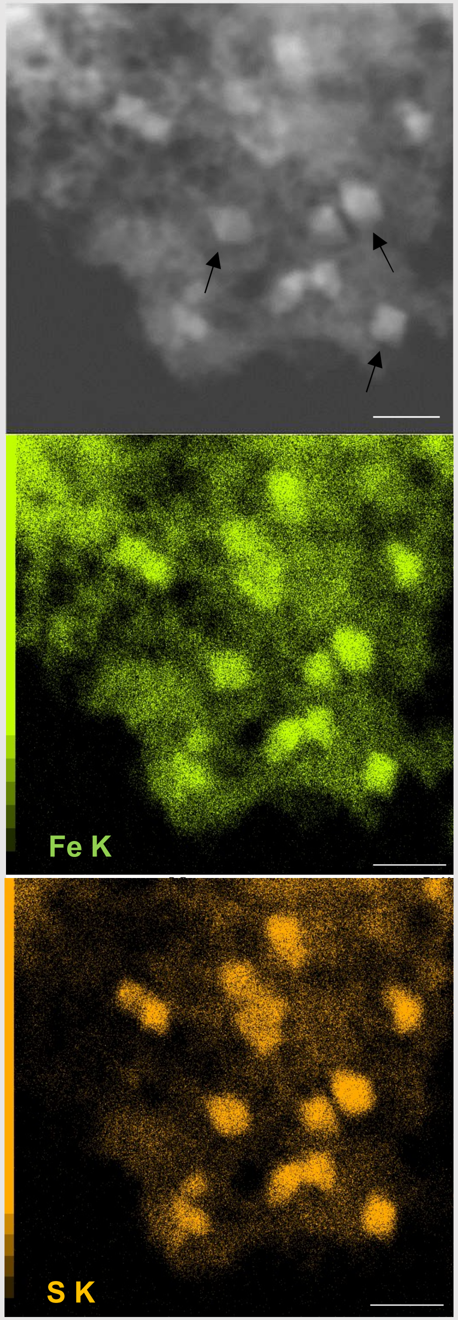
Fe_3S_4 ($Fe(II)Fe(III)_2S_4$) greigite mineralization

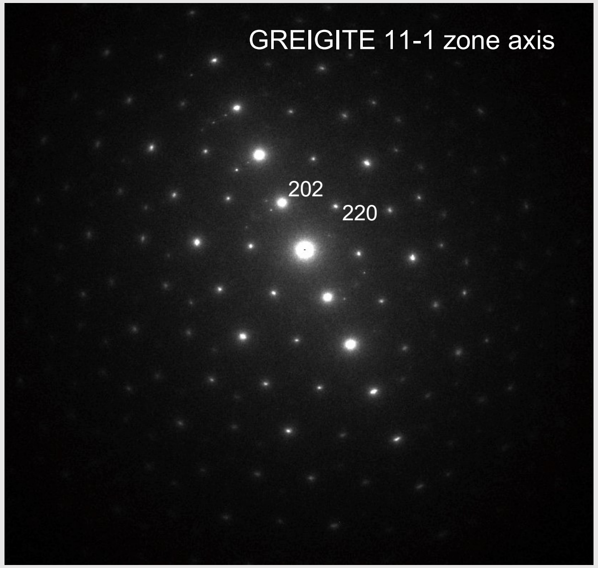


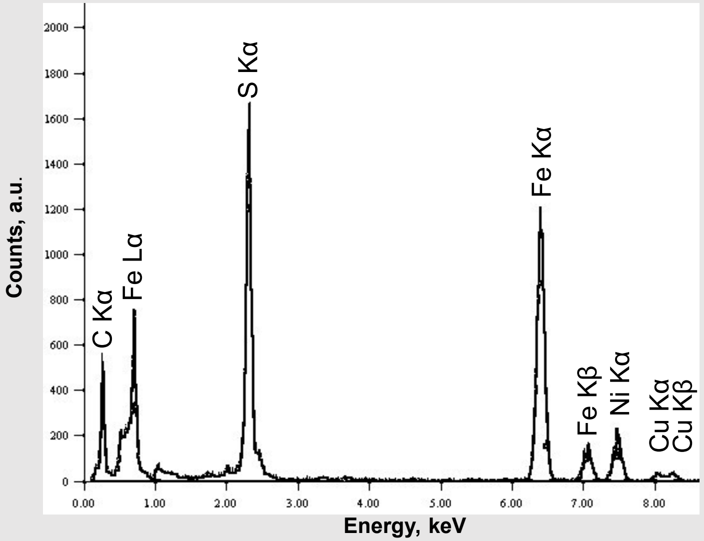


Nanocrystals of greigite

Where does Fe(III) come from?

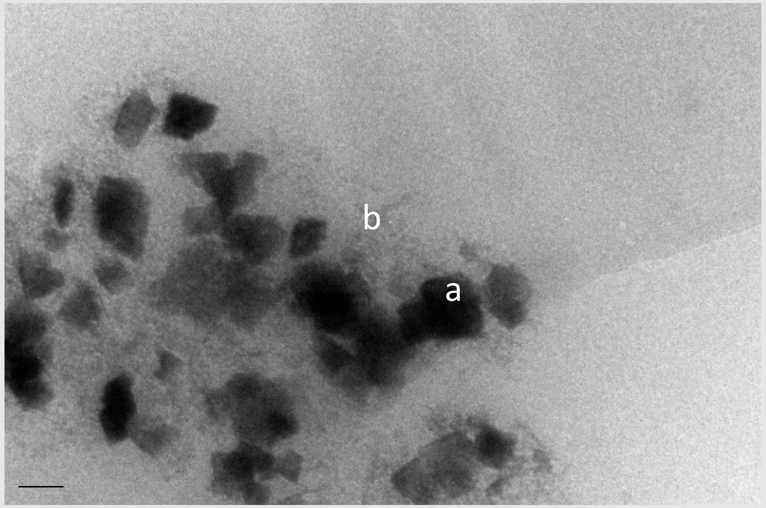






Gorlas et al., 2018 PlosOne

Iron phosphate discovered on cells debris



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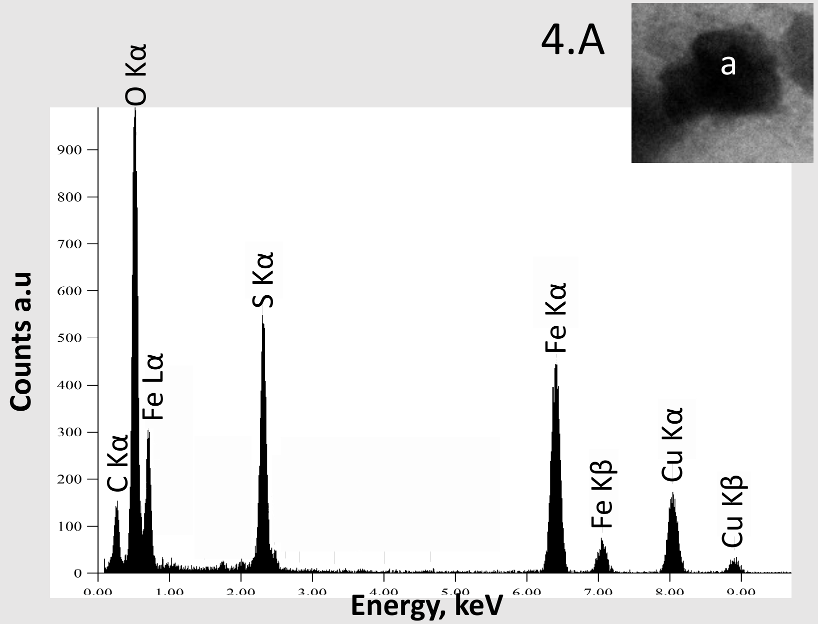
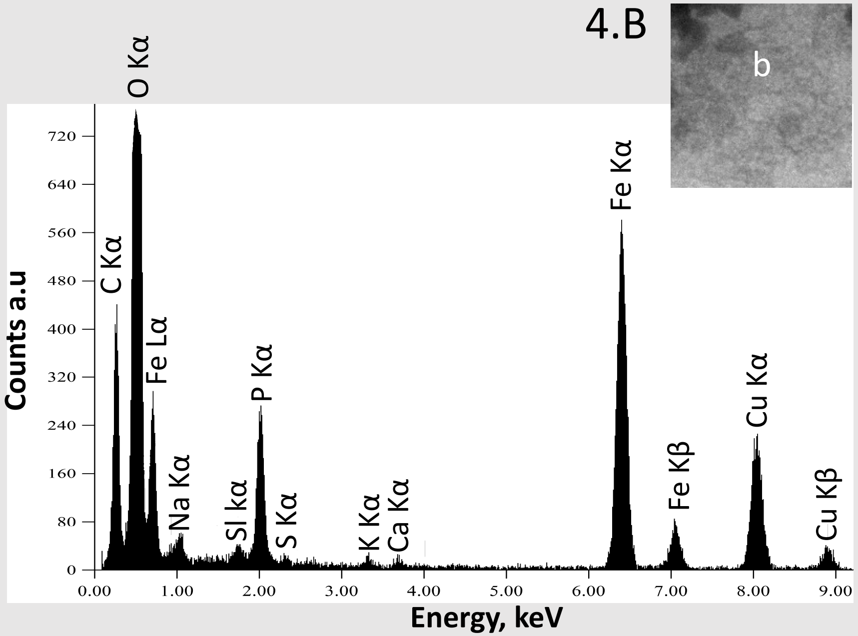
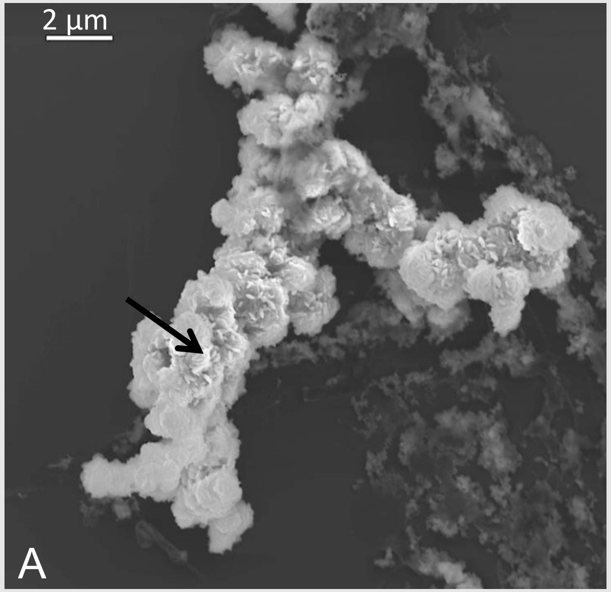




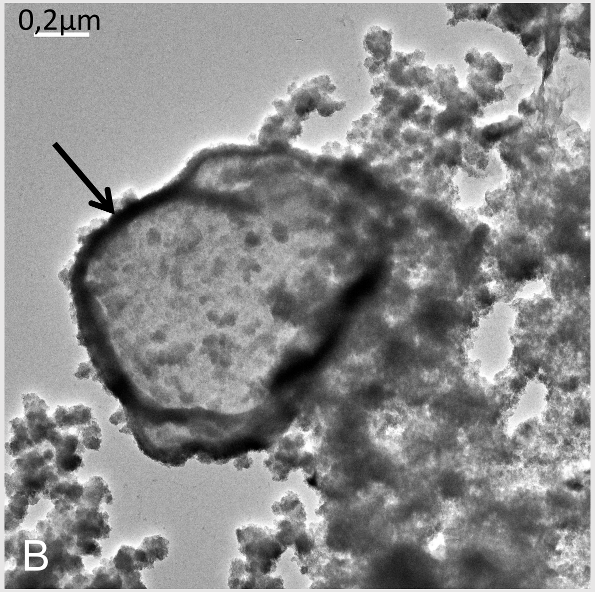
Gorlas et al., 2018 PlosOne

Iron (III) phosphate are the first solid phases formed at the contact of cells. Which mechanism for Fe(II) oxidation?

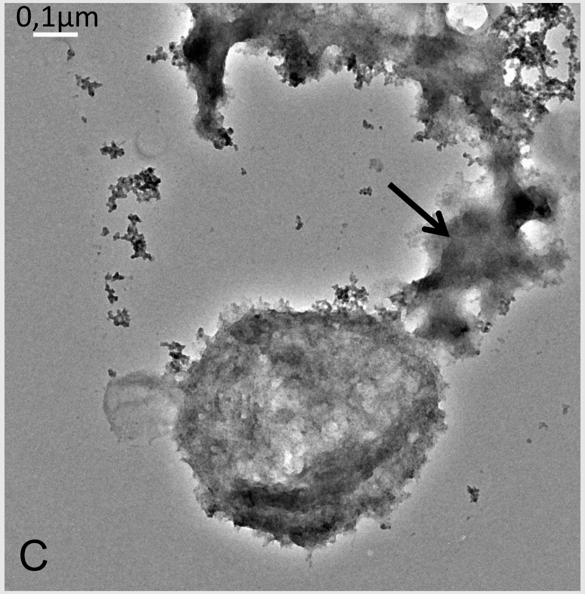
5 hours mineralization

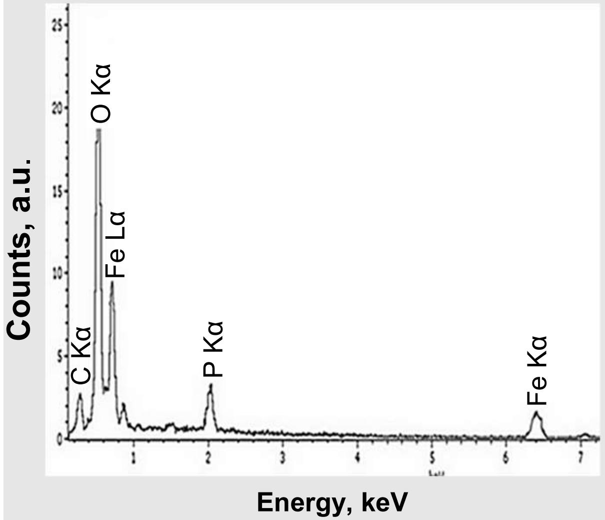


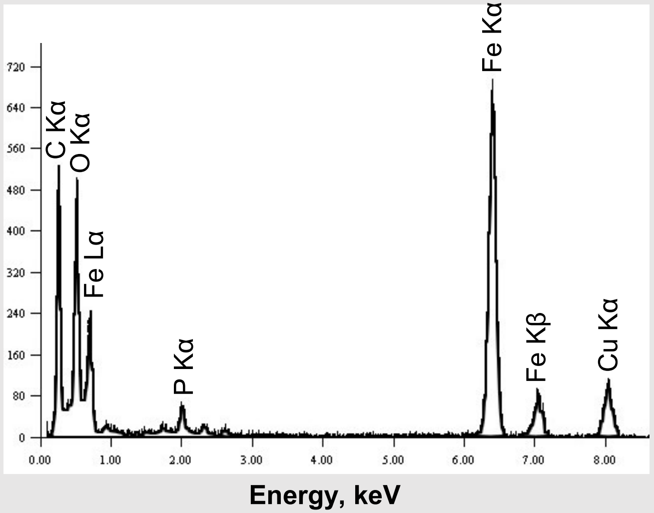
24 hours mineralization

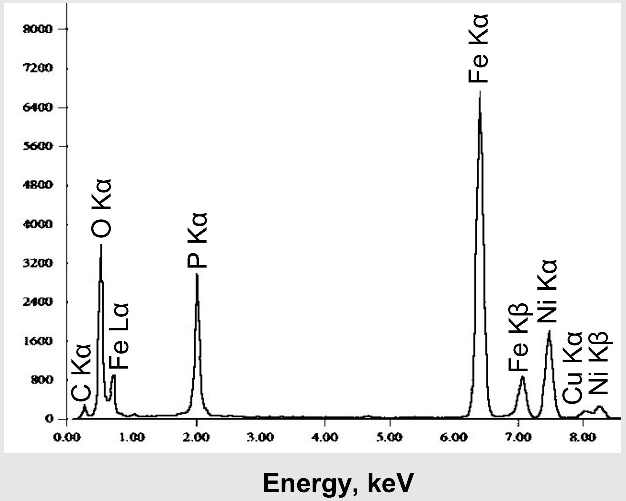


48 hours mineralization









$FeS + 2H^+ \rightarrow Fe^{3+} + 1/2H_2 + HS^-$. $Fe^{3+} + H_2PO_4^- \rightarrow FePO_4 + 2H^+$

Gorlas et al. 2018 PlosOne

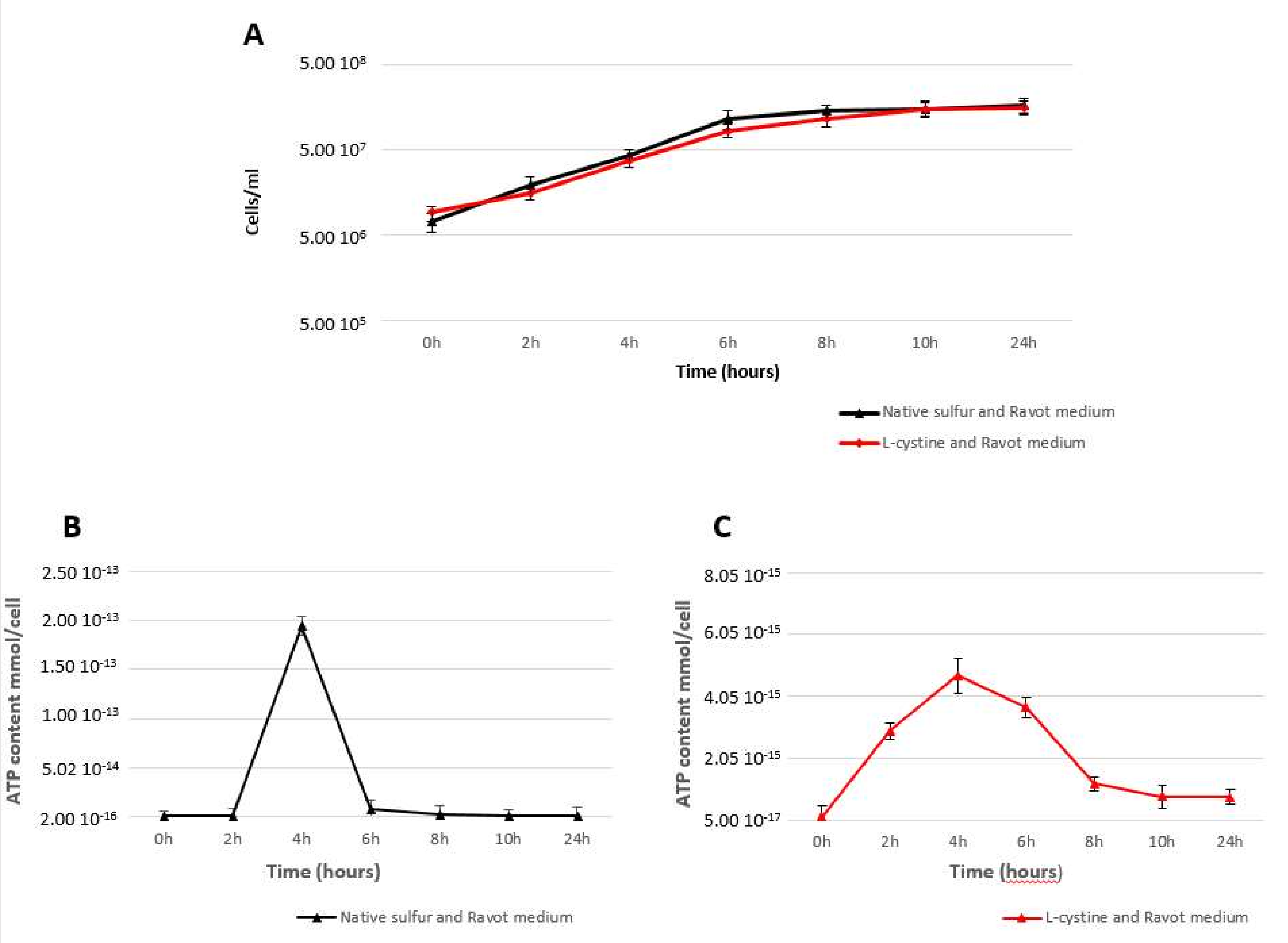
Are iron-sulfides formation linked to metabolism of Thermococcales ?

Model strain:
T. kodakarensis

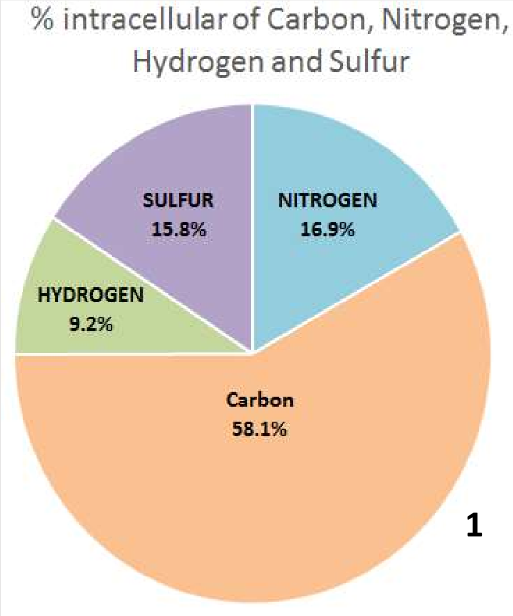
Two culture conditions:

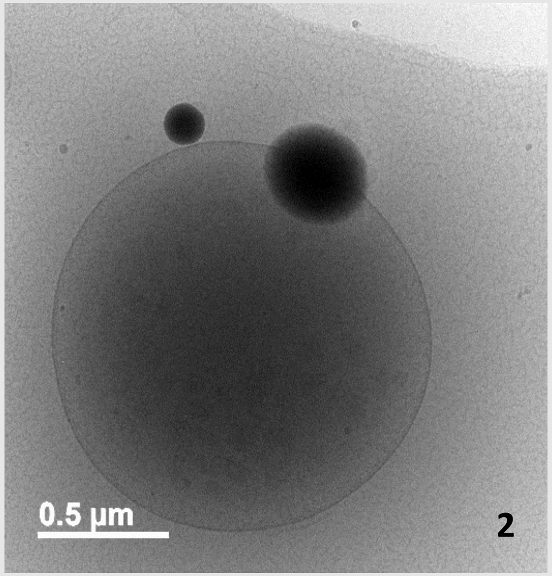
With presence of S(0)

With L-cystine compouds

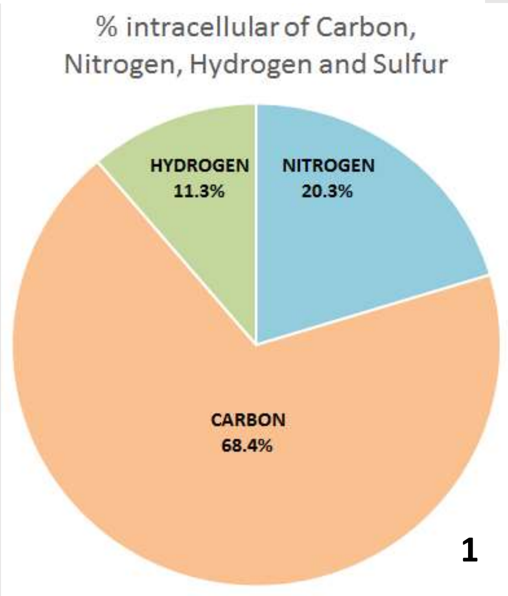


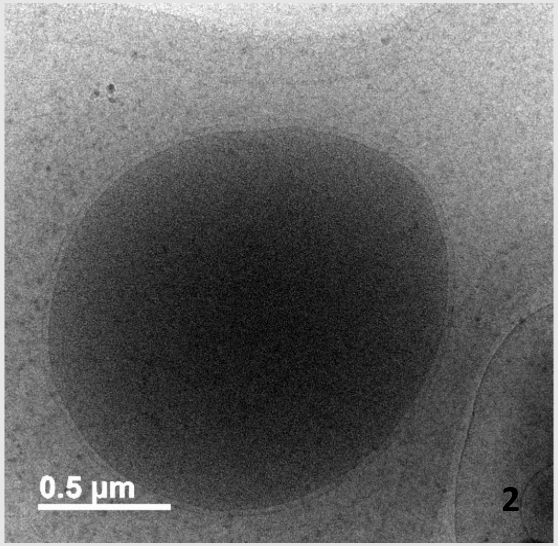
Gorlas et al. 2022 Env Microbiol





With presence of S(0)
- Intracellular sulfur
- Sulfur vesicles production





With presence L-cystine
- No traces of intracellular sulfur
- No production of SVs

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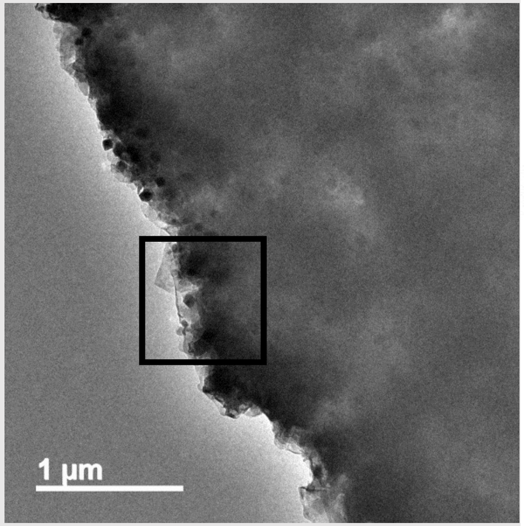
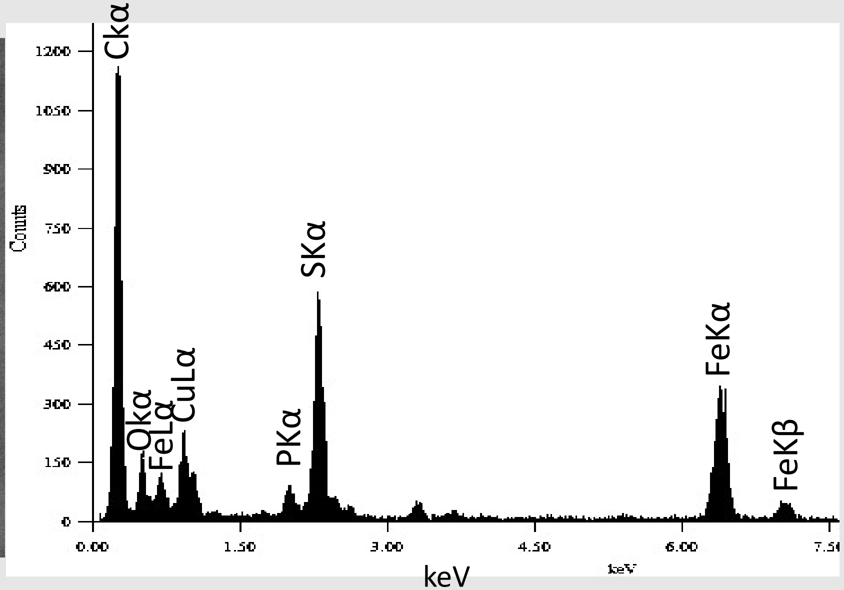
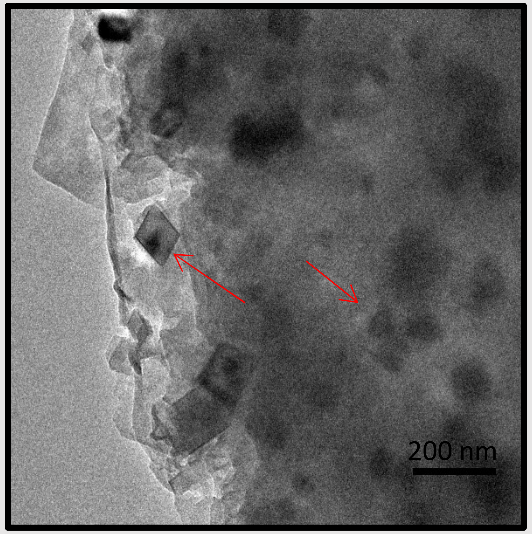
With cystine instead of S(0) in the medium only Fe_3S_4 greigite. No pyrite



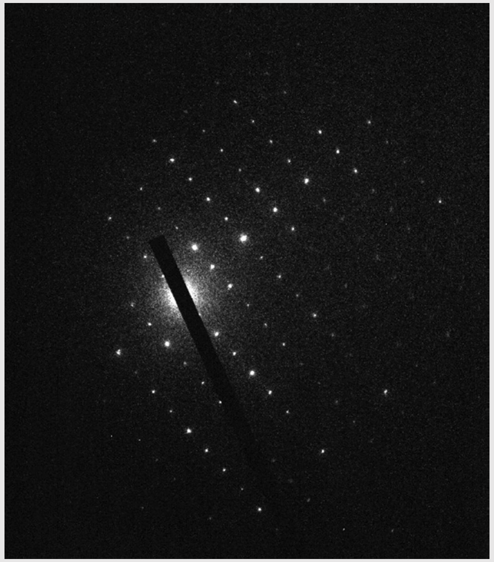


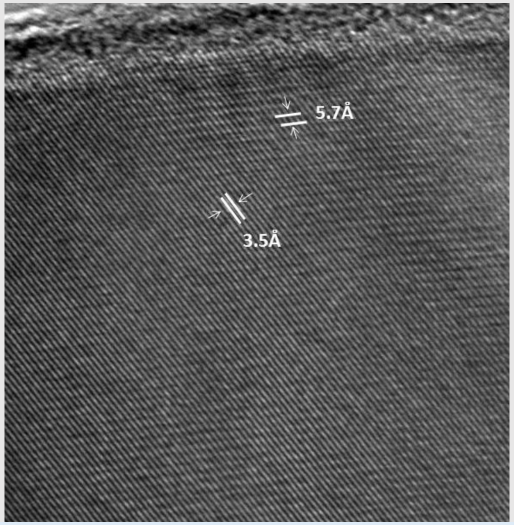


$FeS \rightarrow Fe_3S_4$

Still requires oxidation

Metastable oxidation of Fe^{2+} by cystine, by other organic matter or by H^+ ?





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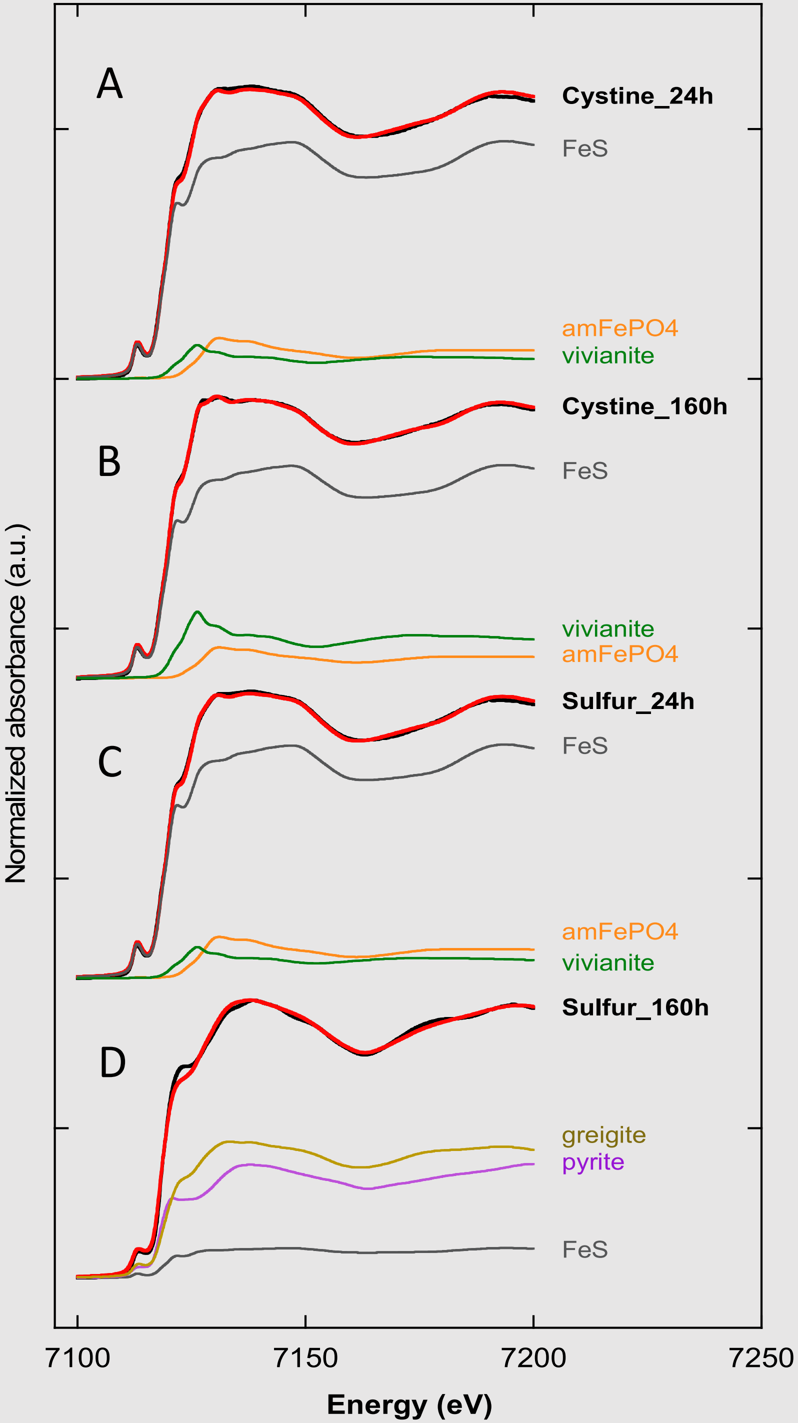

Mineralization in presence of S(0) or in presence of L-cystine

Sample	FeS %	$amFePO_4$ %	vivianite %	greigite* %	pyrite %	*Sum*	χ^2_R 10^{-4}	R_{factor} 10^{-5}
Cystine_24h	86 (2)	11 (1)	8 (2)	-	-	105	*11.3*	*9.5*
Cystine_160h	78 (2)	8 (1)	16 (1)	-	-	102	*3.7*	*3.3*
Sulfur_24h	85 (2)	11 (2)	8 (2)	-	-	104	*7.7*	*6.7*
Sulfur_160h	11 (14)	-	-	41 (9)	49 (13)	101	*21.2*	*19.9*

S(0) acts as a precursor for pyrite formation on Thermococcales cells and vesicles

Thermococcales **metabolism** influences the production of iron-sulfide biominerals

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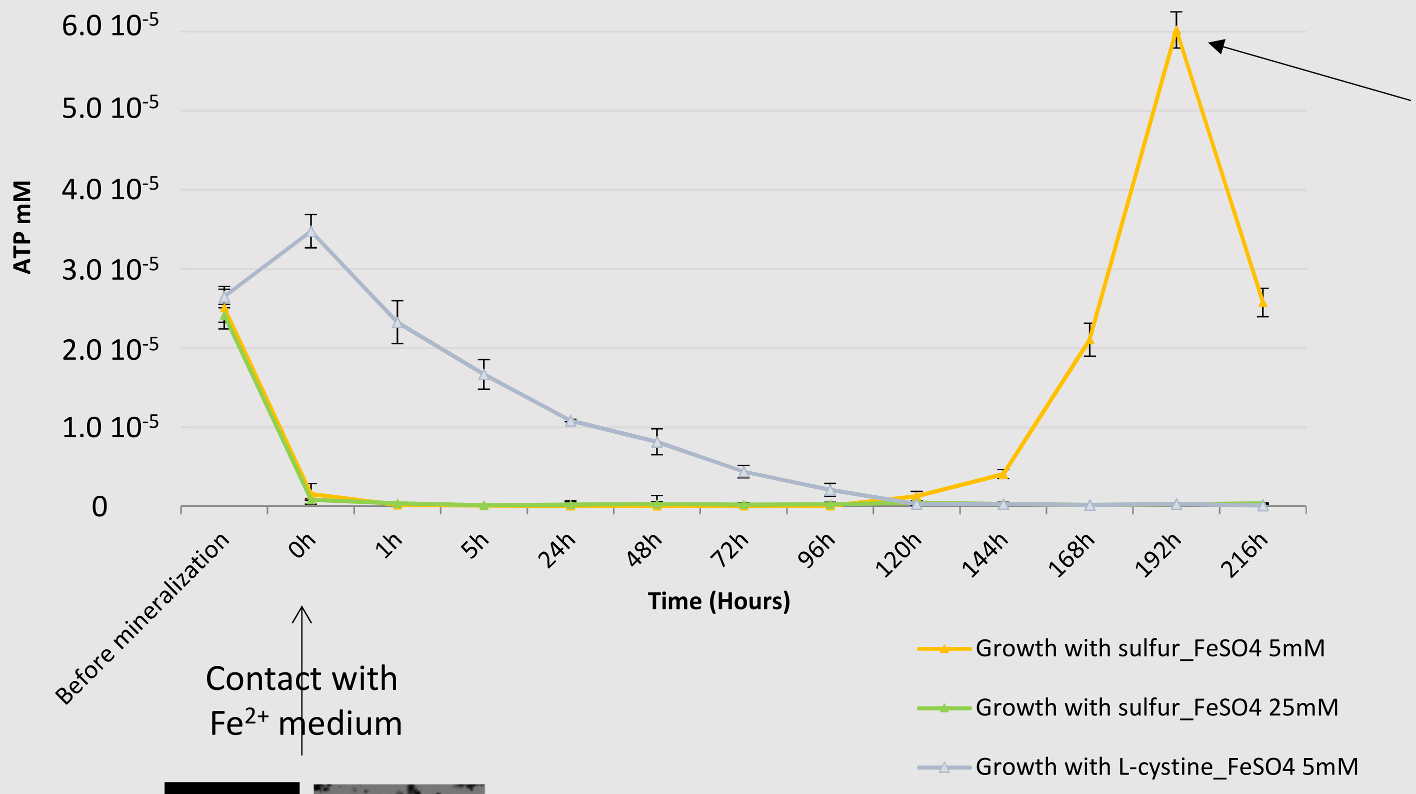
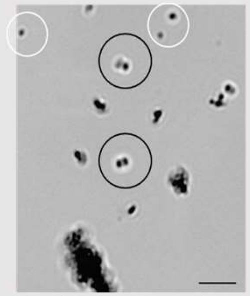
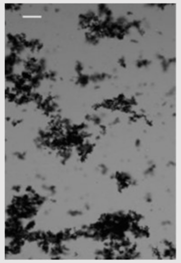
ATPmetry analyses during biomineralization process

- Massive mortality during initial encrustation in phosphate
- Phosphate and nutrients release and transformation to greigite and pyrite
- Some cells survive and multiply using lysed cell materials







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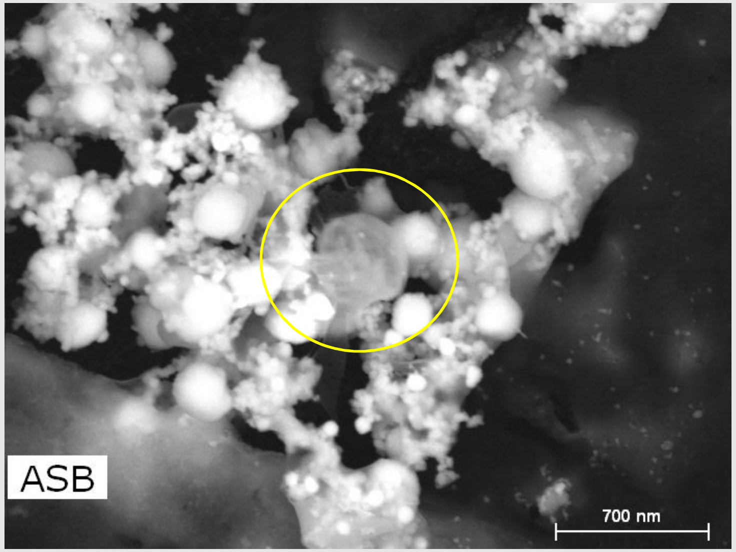
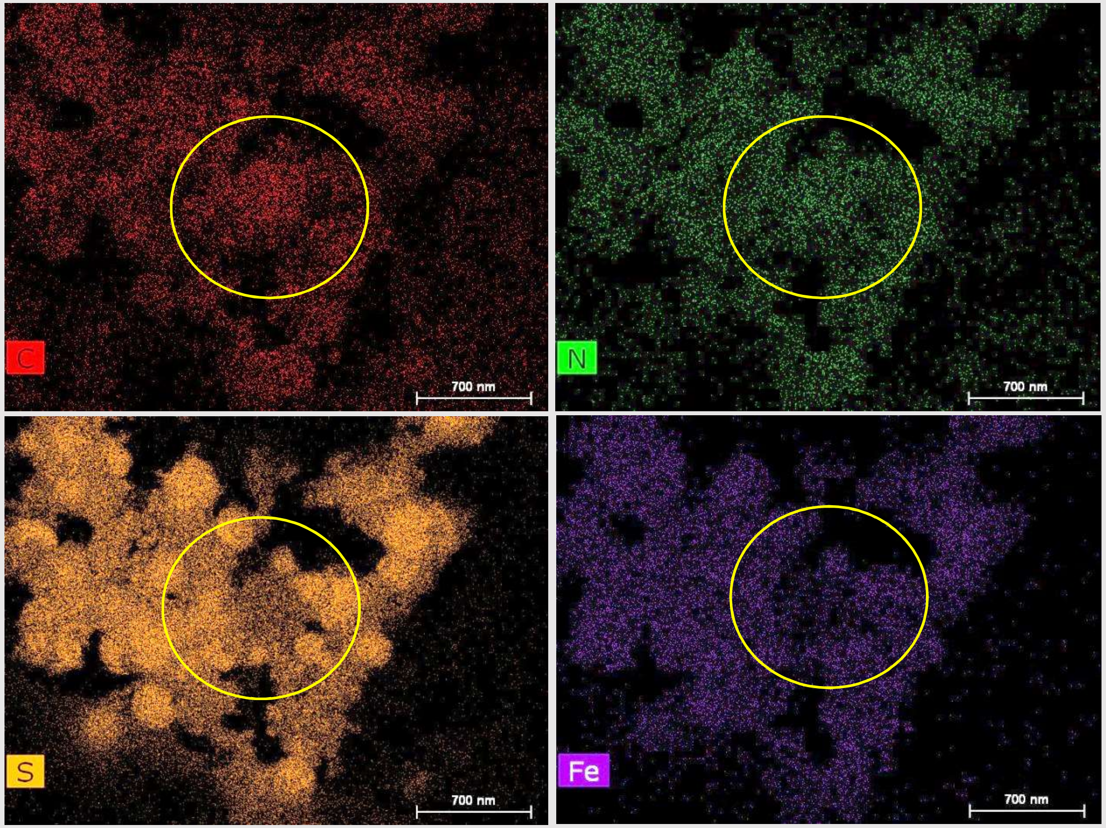
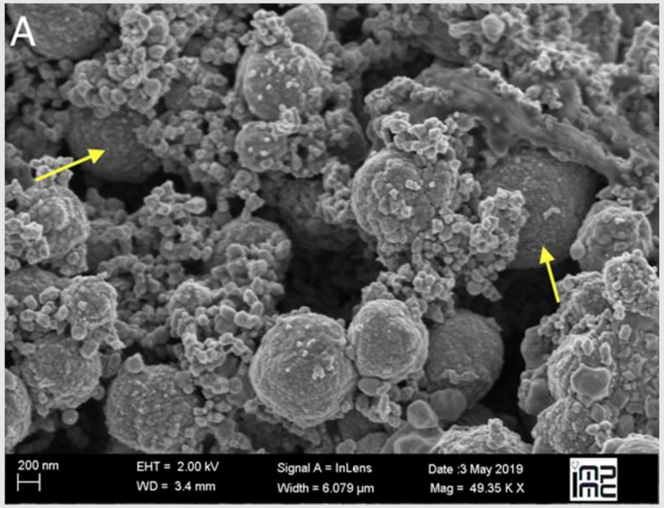
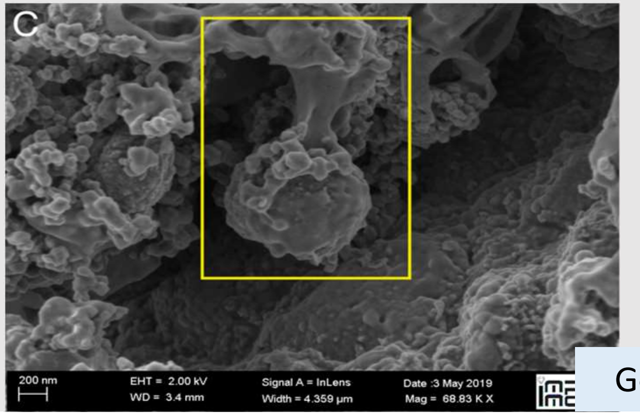
Presence of living cells after long time experiments.









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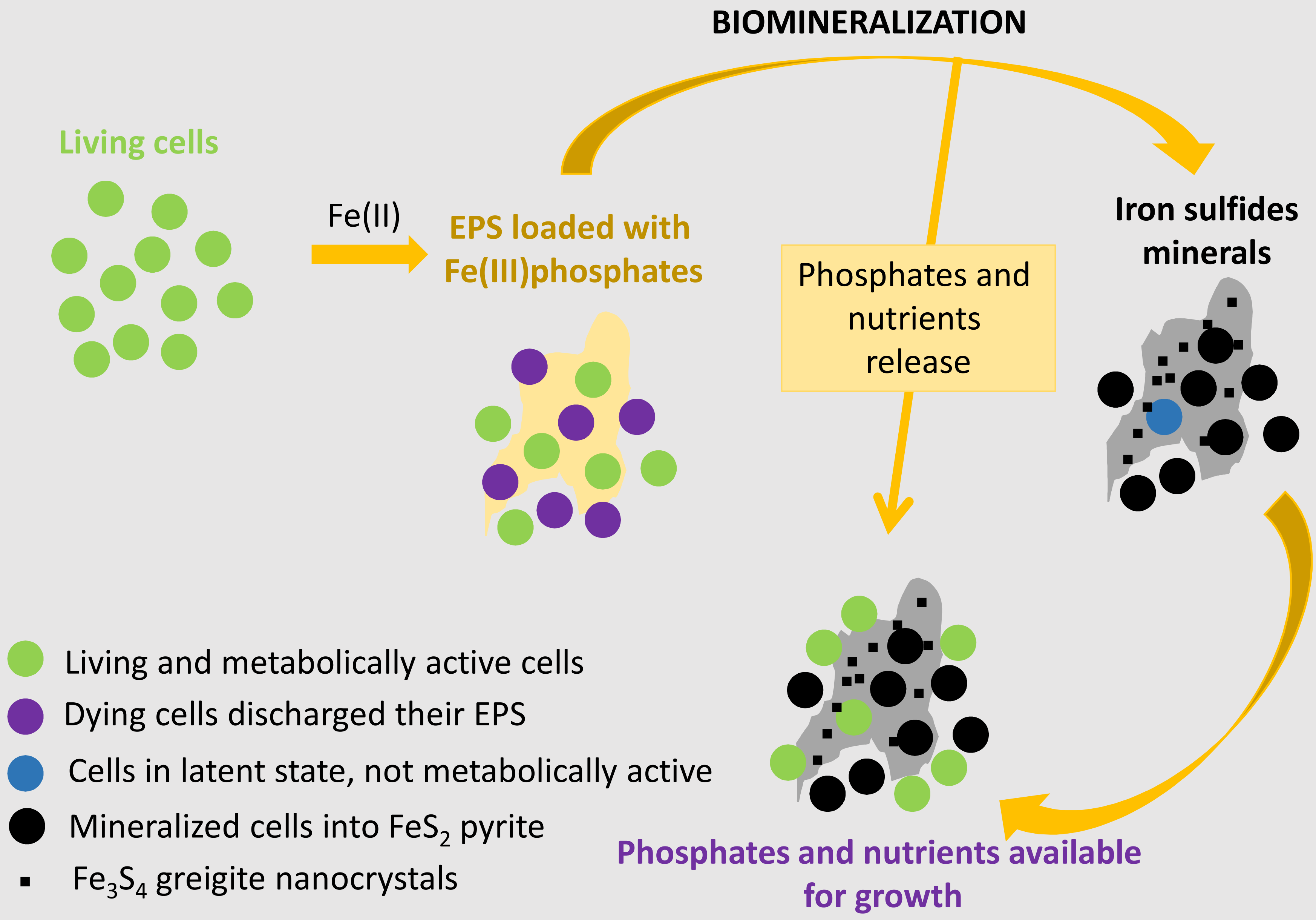
Model of the biomineralization mechanism



Project: Biosignatures of hyperthermophilic archaea


Aurore GORLAS

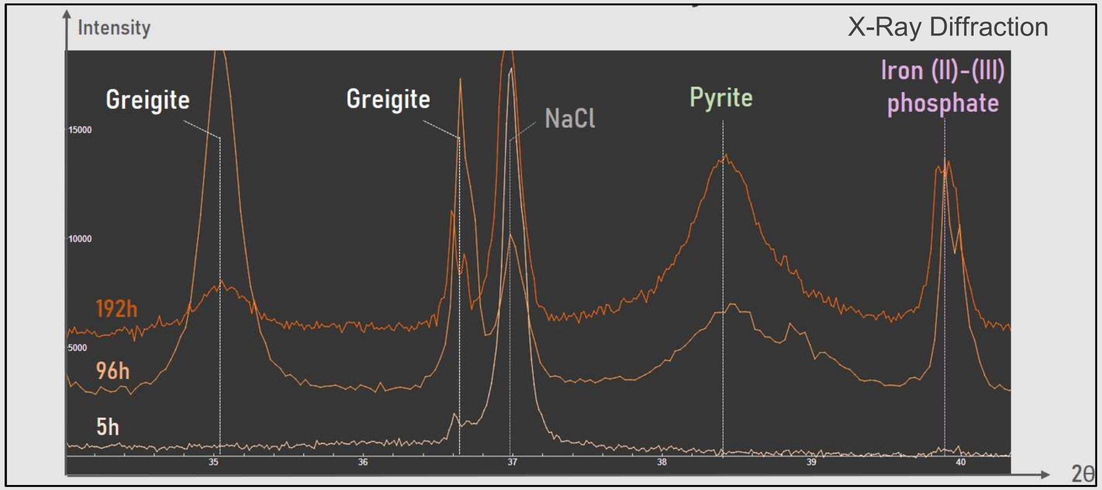

Chloé TRUONG


François GUYOT


Sylvain BERNARD

- Identify **biosignatures** of hyperthermophilic archaeon

- Mineralogical characterization



- Experimental fossilization

- Rock samples observation

Project: Molecular mechanism of biomineralization

- Characterization of the **molecular mechanism** involved in biomineralization process

Aurore GORLAS

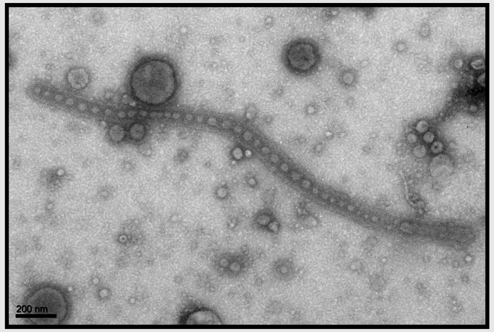
Tom MARIOTTE

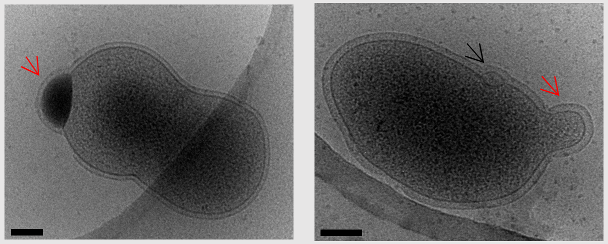
- Iron related genes

T. kodakarensis genes	Function
TK0707	Iron(III)-siderophore homolog
TK0714	Iron(II) transport protein B
TK0715	Iron(II) transport protein A
TK0957	Iron(II) transport protein B
TK0958	Iron(II) transport protein A
TK1055	Bacterioferritin homolog
TK1999	Ferritin-like protein

Funding:
ANR JCJC HYPERBIOMIN (2020 – 2024)
Hyperthermophiles and their biomineralization mechanism

- Role of membrane vesicles in biomineralization





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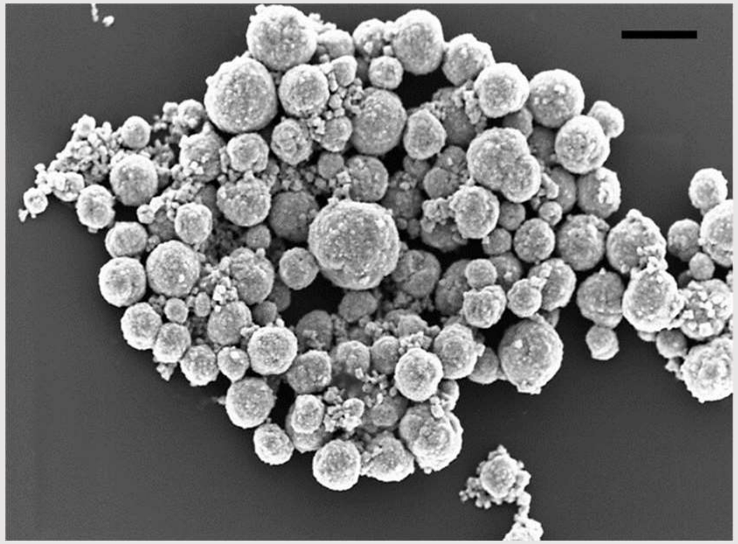
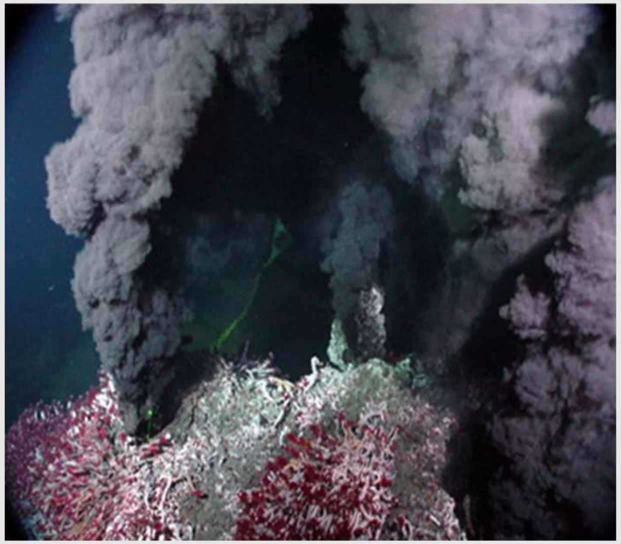
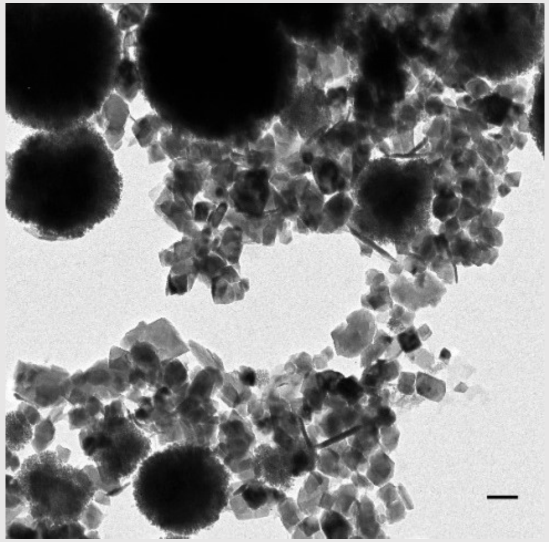






Some Conclusions

- **In presence of *Thermococcales* strict anoxic oxidation of S^{2-} and Fe^{2+} occurs**

- **H^+ is a possible candidate electron acceptor**

- **Fe(III) and polysulfides are formed**

- **Fe(III) phosphates and oxides precipitate**

- **Bioavailable phosphate is then released thanks to (poly)sulfide precipitation**

Objectifs

Does this hyperthermophilic (i.e. >80°C) biosphere contribute significantly to the formation of minerals that build up the chimney and to the biogeochemistry of the hydrothermal system?

- Determine the **physiological and physico-chemical** conditions mimicking the fluctuating environment which **influence** and **control the rates of biominerals** produced by *Thermococcus kodakarensis*

- Explore the **molecular mechanism of biomineralization**, using the genetically tractable strain ***Thermococcus kodakarensis*** **as model**

- Identify the **adaptive strategies employed by hyperthermophiles** to cope with their harsh metal-rich high temperature environment.

Objectifs

First task _ Determine and analyze quantitatively the iron biominerals produced under different physico-chemical conditions mimicking the fluctuating environment of hydrothermal chimneys

- Determine the parameters which could influence the formation of iron sulfide minerals (Fe_3S_4, FeS_2, FeS), and iron phosphates in relation with the cells

    T°C effects (from 80°C to 150°C)
    pH effects (from 4.5 to 8)
    Continuous cultures

HT bioreactor

- Characterize and compare the biominerals formed when *T. kodakarensis* grows in other growth conditions

    Sulfur respiration (H_2S production) VS Fermentation (H_2 production)
    Minimal medium can be use to mimic more closely the oligotrophic hydrothermal environment

Second task _ Understand the biomineralization mechanisms of *T. kodakarensis* at the genomic level

To date, no information is available about the relationships between production of biominerals and putative biomineralization related-genes in *Thermococcales*

- Identify all molecular partners involved in *Thermococcales* biomineralization mechanism (Transcriptomic experiments, Mutant strains..)

T. kodakarensis genes	Function	Comments
TK0707	Iron(III)-siderophore homolog	
TK0714	Iron(II) transport protein B	Probably, two genes organized in operon
TK0715	Iron(II) transport protein A	
TK0957	Iron(II) transport protein B	Probably, two genes organized in operon
TK0958	Iron(II) transport protein A	
TK1055	Bacterioferritin homolog	
TK1999	Ferritin-like protein	

- Determine the involvement of membrane vesicles in mineralization process
  - Mutant strains deficient in MV production
  - Mutant strains overproducing MVs

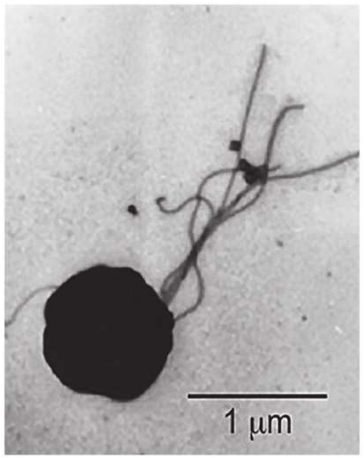
Objectifs

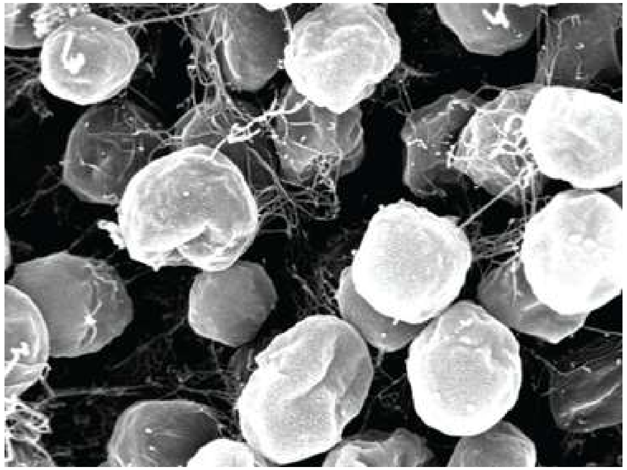
Third task _ Understand the role of biominerals at the ecological level

Abundant precipitation of minerals is deleterious to most cells but growth of non-mineralized and metabolically active cells in long time experiments of mineralization has been observed

- Identify the role of iron sulfide minerals on the nutriments in the hydrothermal medium
  - Metabolomic experiments of the culture
  - Metabolomic experiments of the biomineralizing media

- Investigate the relationships between two euryarchaeon in the reconstituted ecosystem
  - Co-cultures of *T. kodakarensis* and *M. jannaschii* in bioreactor





Partenariats

Name	First name	Current position	Role & responsibilities in the project (4 lines max)	Involvement (person/month) throughout the project's total duration
GORLAS	Aurore	Assistant professor (MC CIN)	Coordinator and manager of the project, supervisor engineer and graduate student. Task 1, Task 2, Task 3	48
LORIEUX	Florence	Assistant engineer	Genetic constructs. Task 2	6
To be recruited by ANR		PhD student	Biomineralization, genetic constructs, molecular biology. Task 1, subtask 2.1	36
To be recruited by ANR		Technician	Bioreactor set up, Batch and Fed-Batch Thermococcales cultivation and co-cultures. Task 1, Task 3	12
To be recruited by ANR		Master student	Biomineralization, electron microscopy analyses and X-ray diffraction analyses. Task 1	6

Collaborations:

Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie (IMPMC, Paris) with teams « Minéralogie environnementale » and « Biominéralogie »

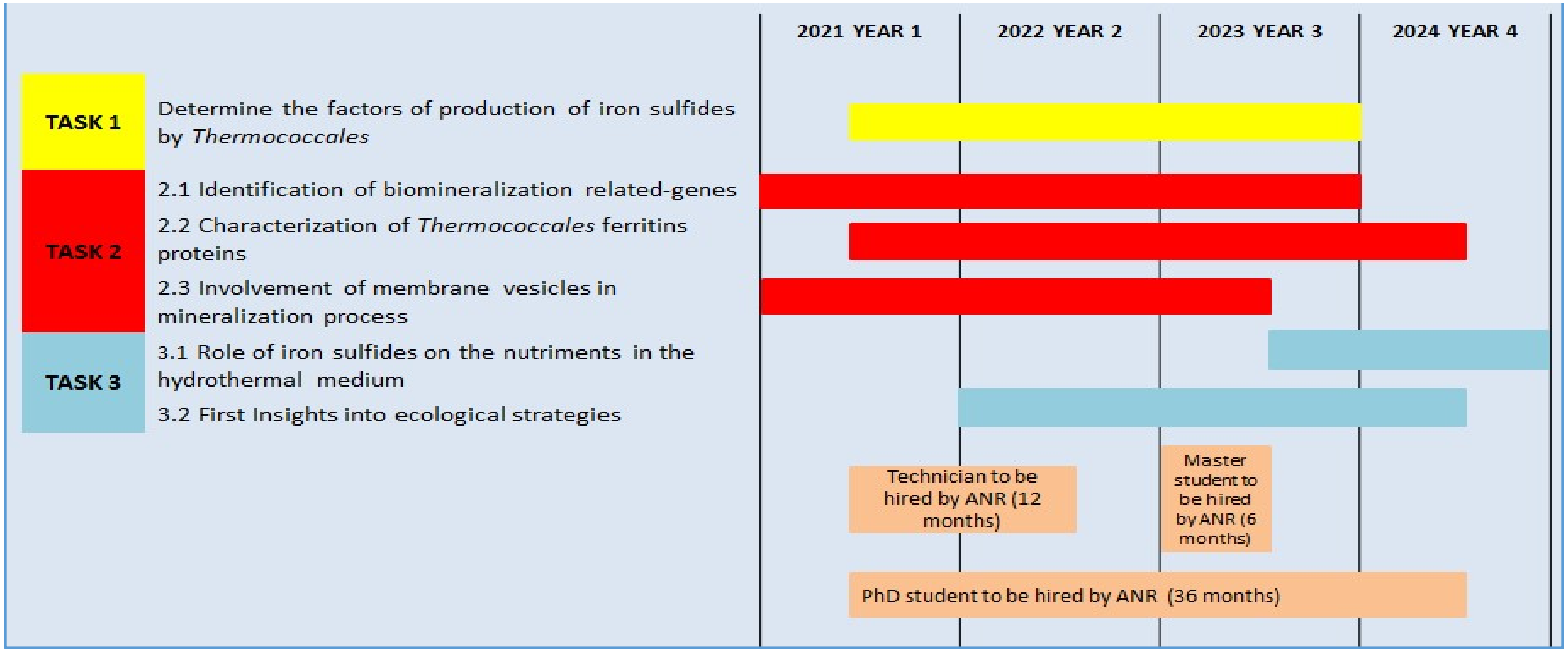
TEM, SEM, Cryo-EM, XRD, EDXS, STEM-EDX, STEM-EELS facilities

Synchrotron facilities through my collaboration with IMPMC (Dr Pierre Le Pape)

Institut Universitaire Européen de la Mer (IUEM, Plouzané) with team “Laboratoire de Microbiologie des Environnements Extrêmes”

Impacts espérés

By the multidisciplinary and experimental scientific aspects of the project, HYPERBIOMIN will generate interest in a large audience in microbiology, biogeochemistry, geology, astrobiology, bioremediation to cite the most obvious fields



- Submission of scientific manuscripts to publication in high-impact life sciences journals
- Presentation at international conferences such as Extremophiles 2022, and Goldschmidt Conference 2021 and 2022 and national meetings such as GdR Archaea 2022
- New courses and pedagogical projects about biomineralization and the use of biominerals in biotechnology