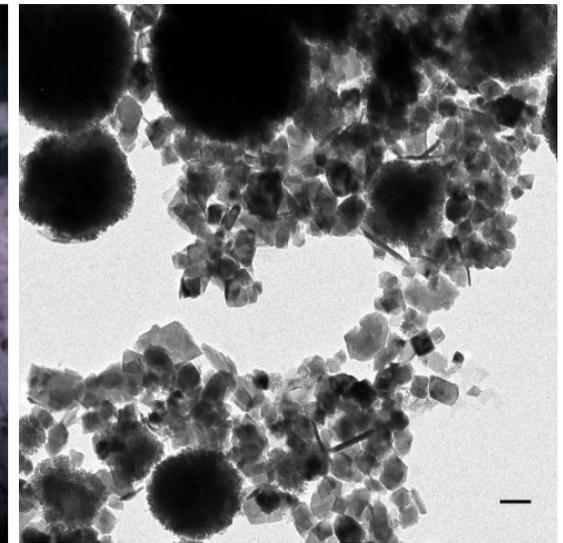
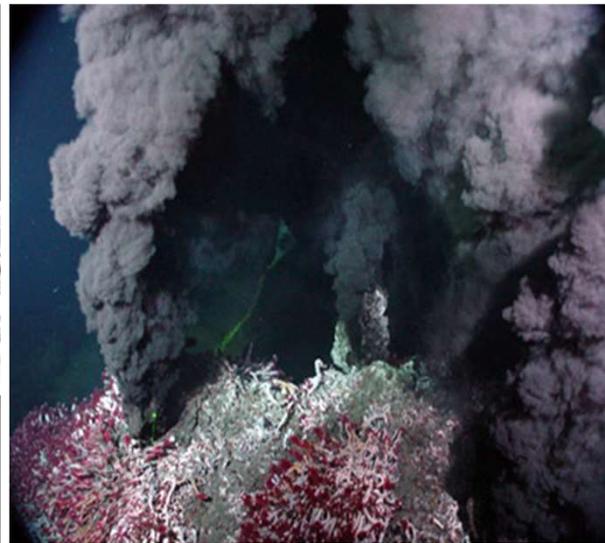
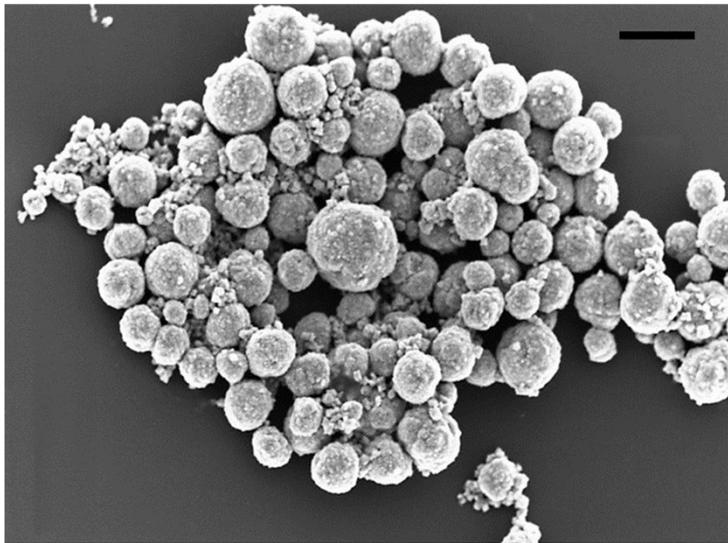
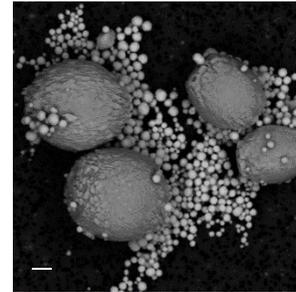


Biomining and iron sulfide production by hyperthermophilic archaea



Biomining, a key process by which organisms interact with their environment

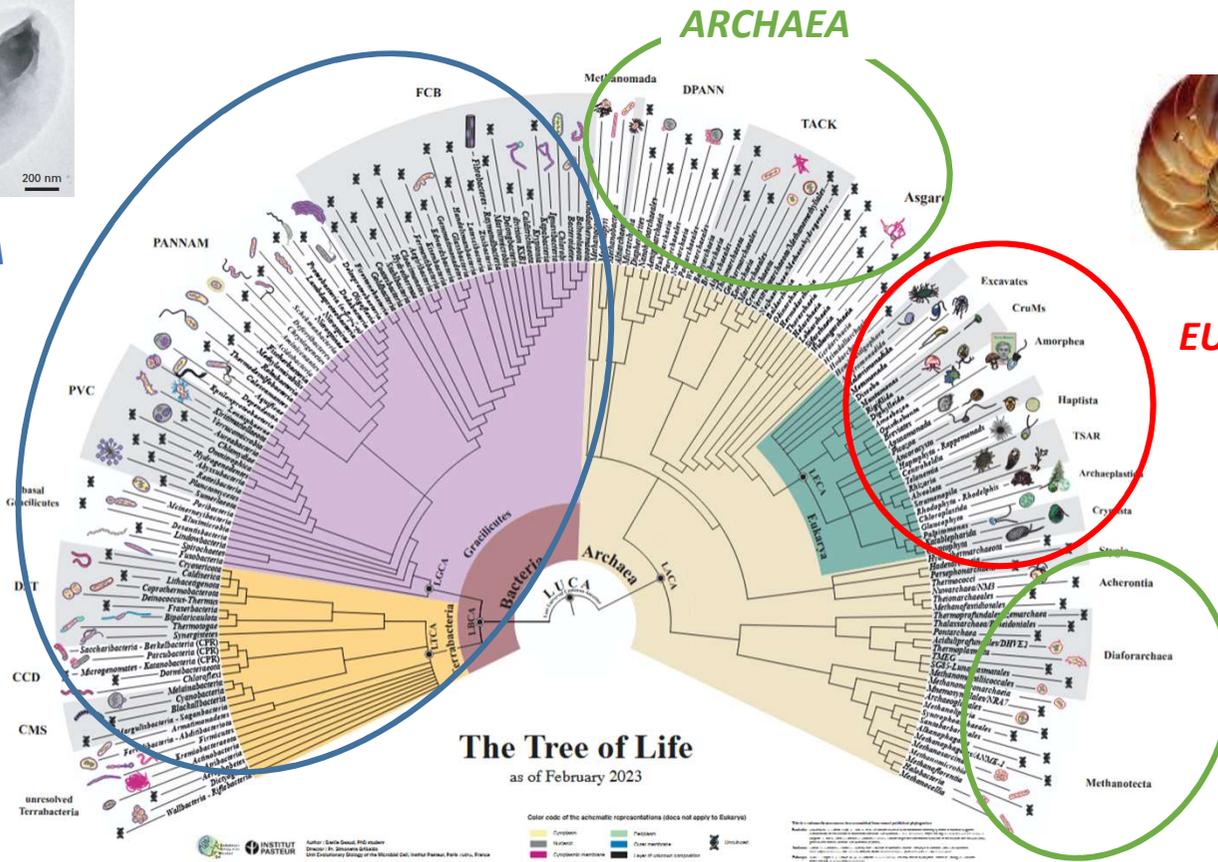
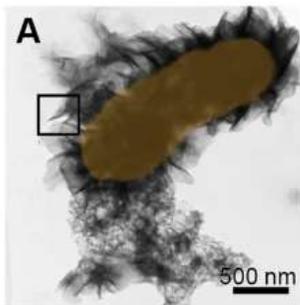
More recently
few examples



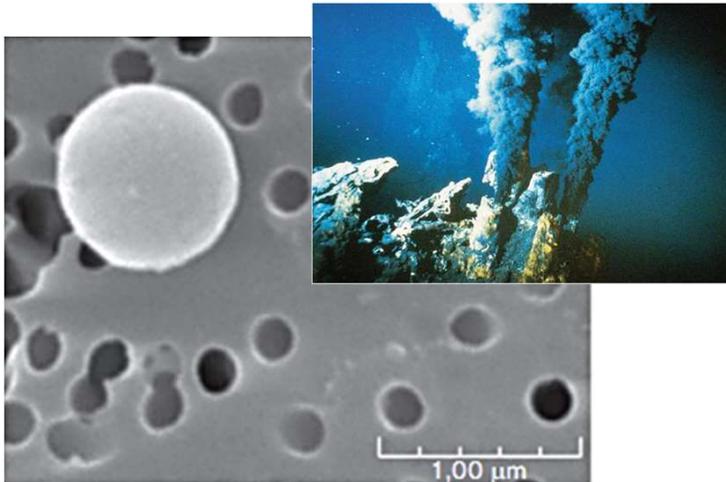
BACTERIA



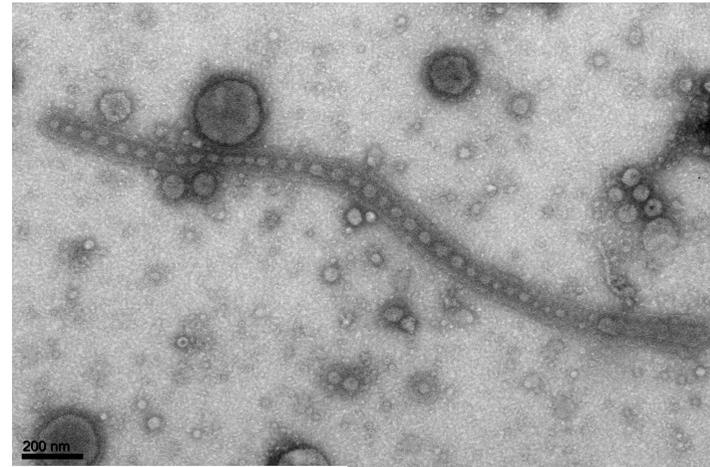
EUKARYA



Thermococcales, major inhabitants of the hot parts of hydrothermal sources

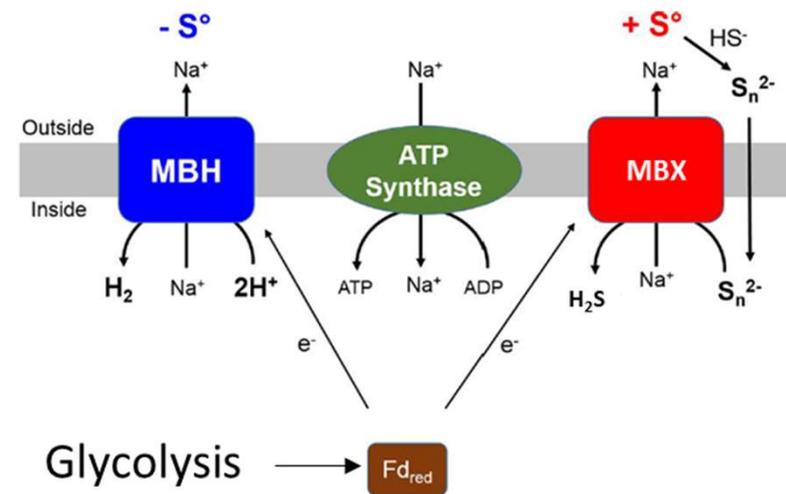


Gorlas et al., 2013



Gorlas et al., 2015

- *Archaea*
- Hyperthermophile (85°C)
- Strictly anaerobe
- Mainly heterotroph
- Vesicles and nanotubes producer
- Sulfur reducer



Two distinct pathways to produce ATP

Production of sulfur vesicles by Thermococcales



Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi



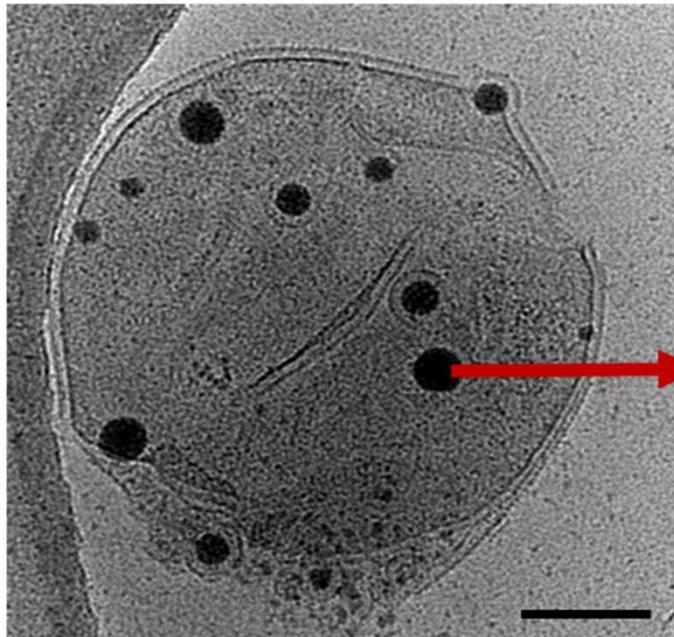
Research paper

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

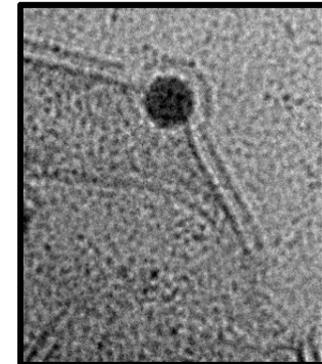
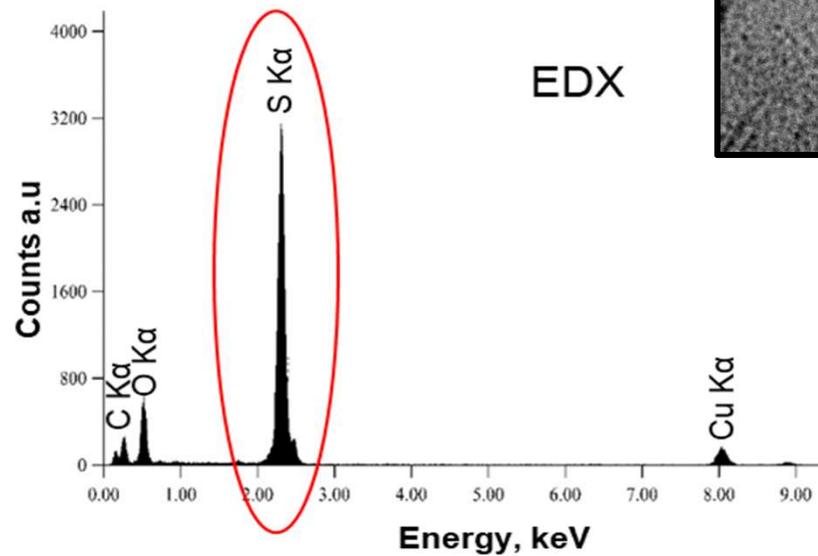


A. Gorlas ^{a,*}, E. Marguet ^a, S. Gill ^a, C. Geslin ^b, J.-M. Guigner ^c, F. Guyot ^c, P. Forterre ^{a,d}

With presence of S(0) in growth medium



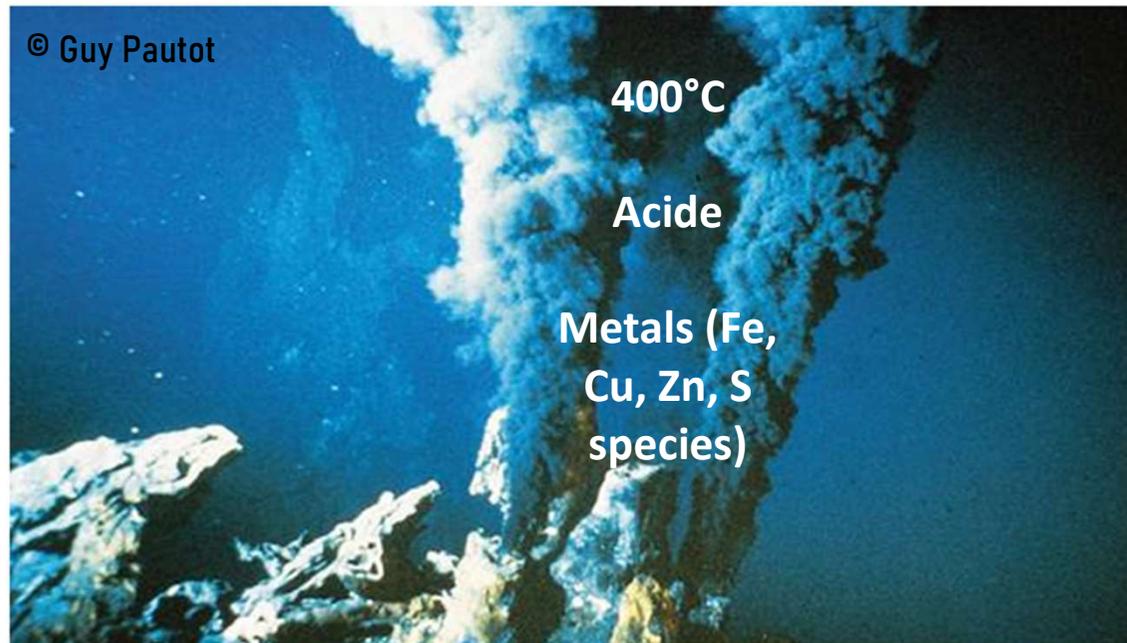
Thermococcales cell



Sulfur transport

Hydrothermal vents, a site for microbe-mineral interactions

Hydrothermal deep-sea vent : an extreme environment for life



Strong physico-chemical gradients

Iron- and sulfur-rich systems

FeS₂ pyrite formation (> 200°C) (Rickard, 1997; Rickard & Luther, 2007)

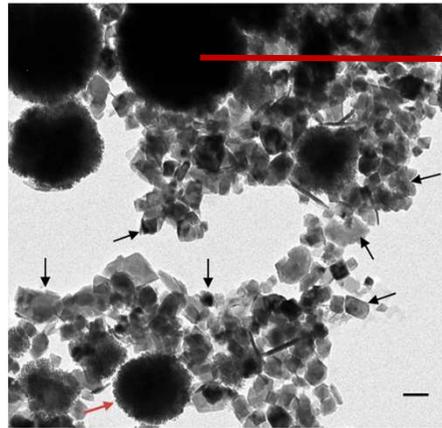
However at lower temperatures (< 150°C) FeS₂ are also produced by a still unknown mechanism which might involve the living compartments. 4

(Juniper & Fouquet, 1995; McCollom, 2007)

Biological induced mineralization by Thermococcales

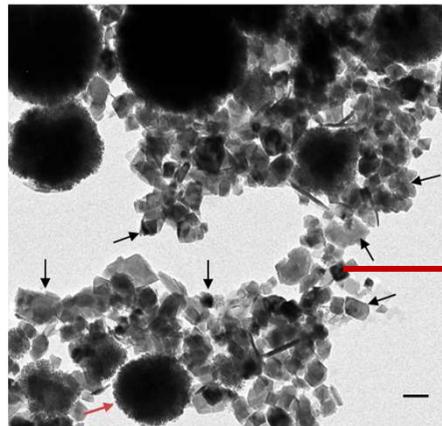
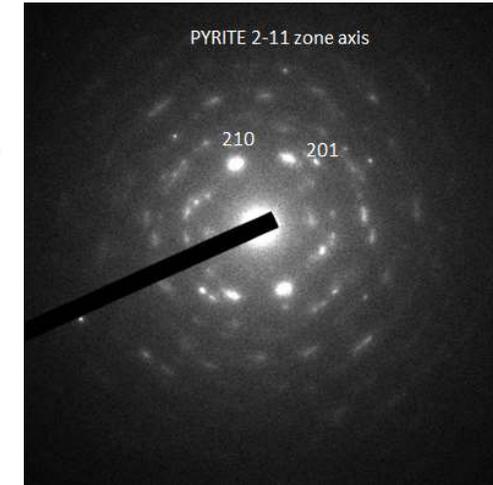
Rapid (<60 hours) formation of iron-sulfide minerals

- Fe(II) mineralization medium
- Strict anoxia
- 85°C
- With **presence of S(0)** in growth medium



Pyrites (FeS_2) replacing former cells and vesicles of *Thermococcales*

Pyrite spherules



Extracellular $\text{Fe(II)Fe(III)}_2\text{S}_4$ greigite mineralization on EPS (Extracellular polymeric substance)



Does this hyperthermophilic (i.e. $>80^{\circ}\text{C}$) biosphere contribute significantly to the formation of minerals that build up the chimney and to the biogeochemistry of the hydrothermal system?

Physiological level:

Determine the **physiological and physico-chemical** conditions which **influence** and **control the rates of biominerals** produced

Genomic level:

Explore the **molecular mechanism of biomineralization**

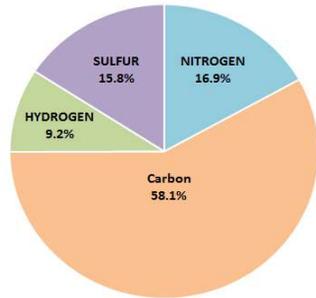
Ecological level:

Search for **biosignatures of hyperthermophiles** in black smokers

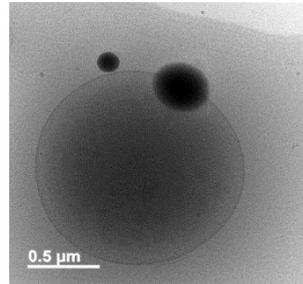
Thermococcales metabolism influences the production of iron-sulfide biominerals

In S(0) conditions (with Fe²⁺ solution at 5mM)

% intracellular of Carbon, Nitrogen, Hydrogen and Sulfur

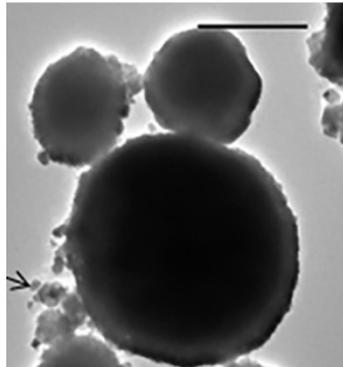


CHNS analyses



CryoEM

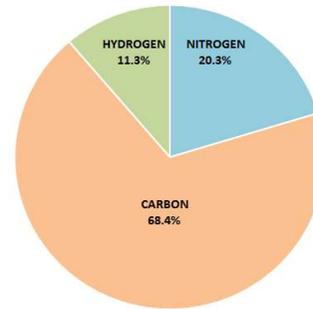
✓ Sulfur vesicles



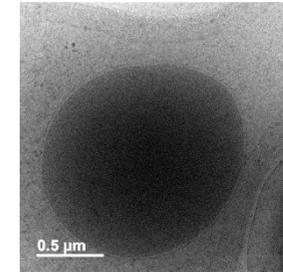
- ✓ Amorphous FeS
- ✓ Pyrite
- ✓ Greigite

In L-cystine condition (with Fe²⁺ solution at 5mM)

% intracellular of Carbon, Nitrogen, Hydrogen and Sulfur

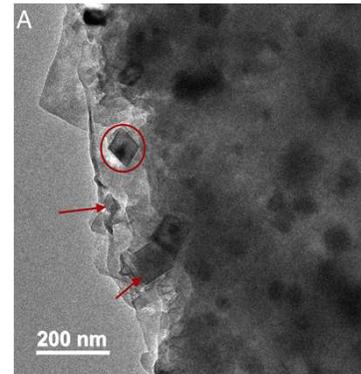


CHNS analyses



CryoEM

X No sulfur vesicles



- ✓ Amorphous FeS
- X No pyrite
- ✓ Greigite

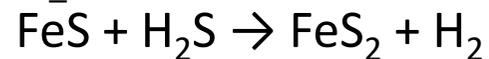
Essential role for intracellular sulfur and/or sulfur present in sulfur vesicles for pyrite formation

Thermococcales metabolism influences the production of iron-sulfide biominerals

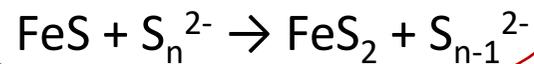
Pyrite formation, 2 major pathways

(Rickard, 1997; Rickard & Luther, 2007)

H₂S pathway :



Polysulfide pathway :



In both S(0) and L-cystine conditions :

Production of H₂S

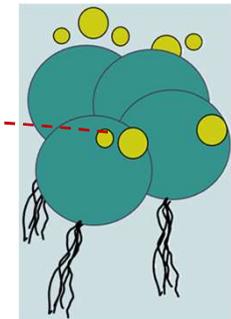
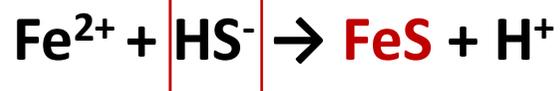
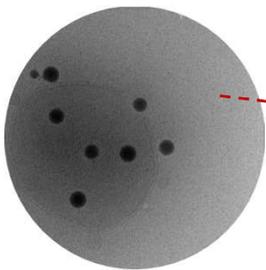
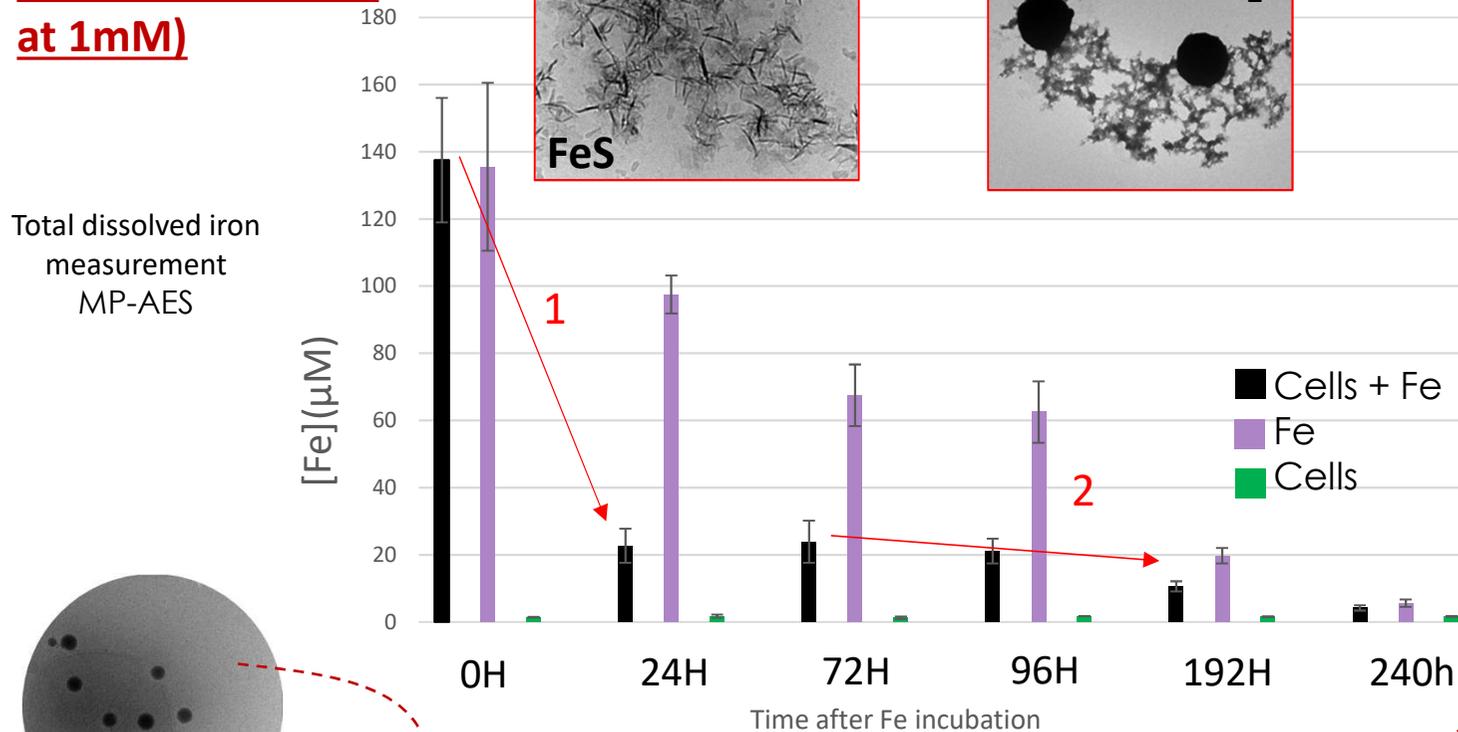
Sulfur intracellular and/or sulfur vesicles (source of polysulfides ?) act as precursor for pyrite formation

Pyrite production : successive precipitation phases

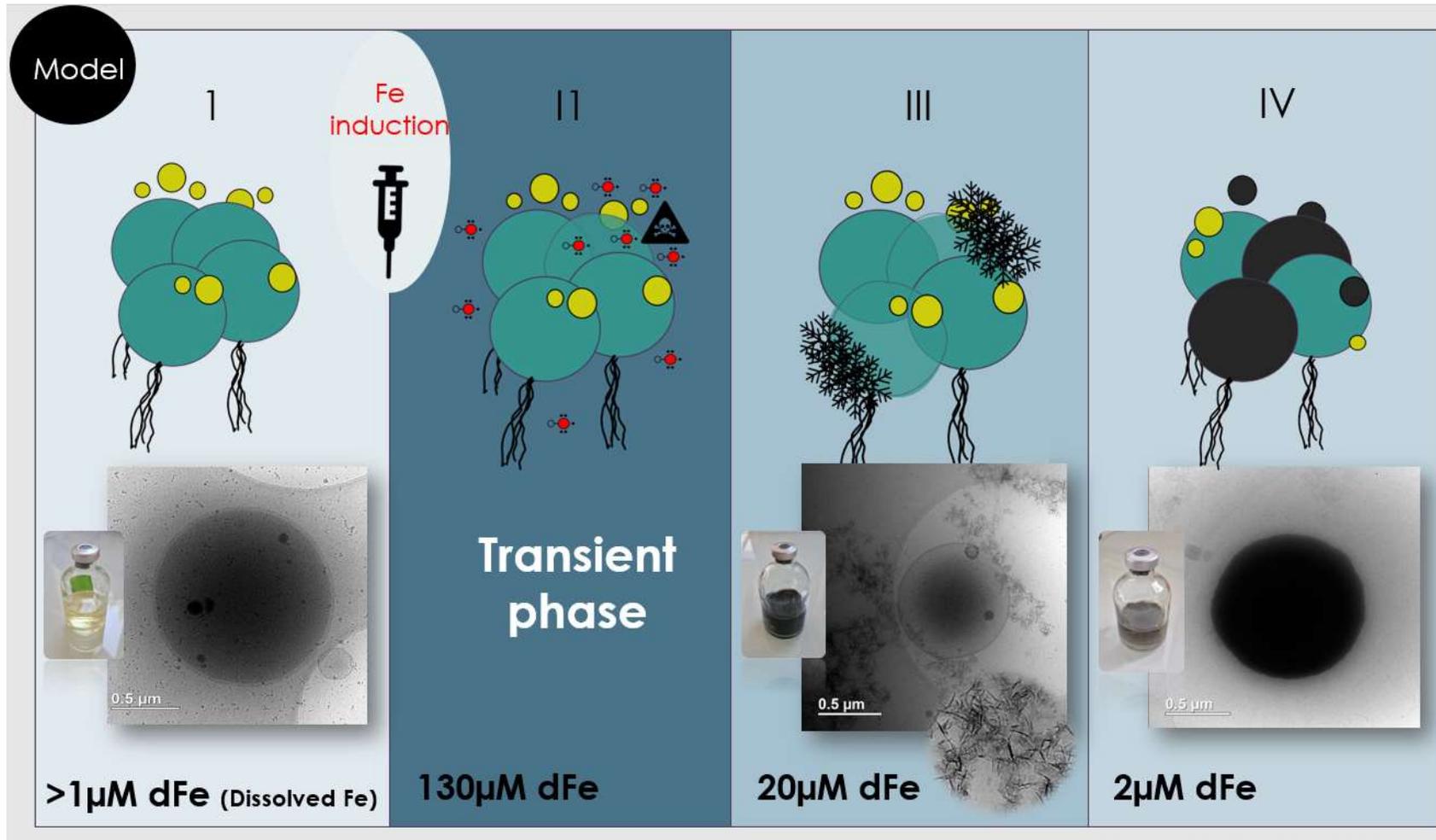
In S(0) conditions
(with Fe²⁺ solution
at 1mM)



Tom
MARIOTTE



Pyrite production : successive precipitation phases



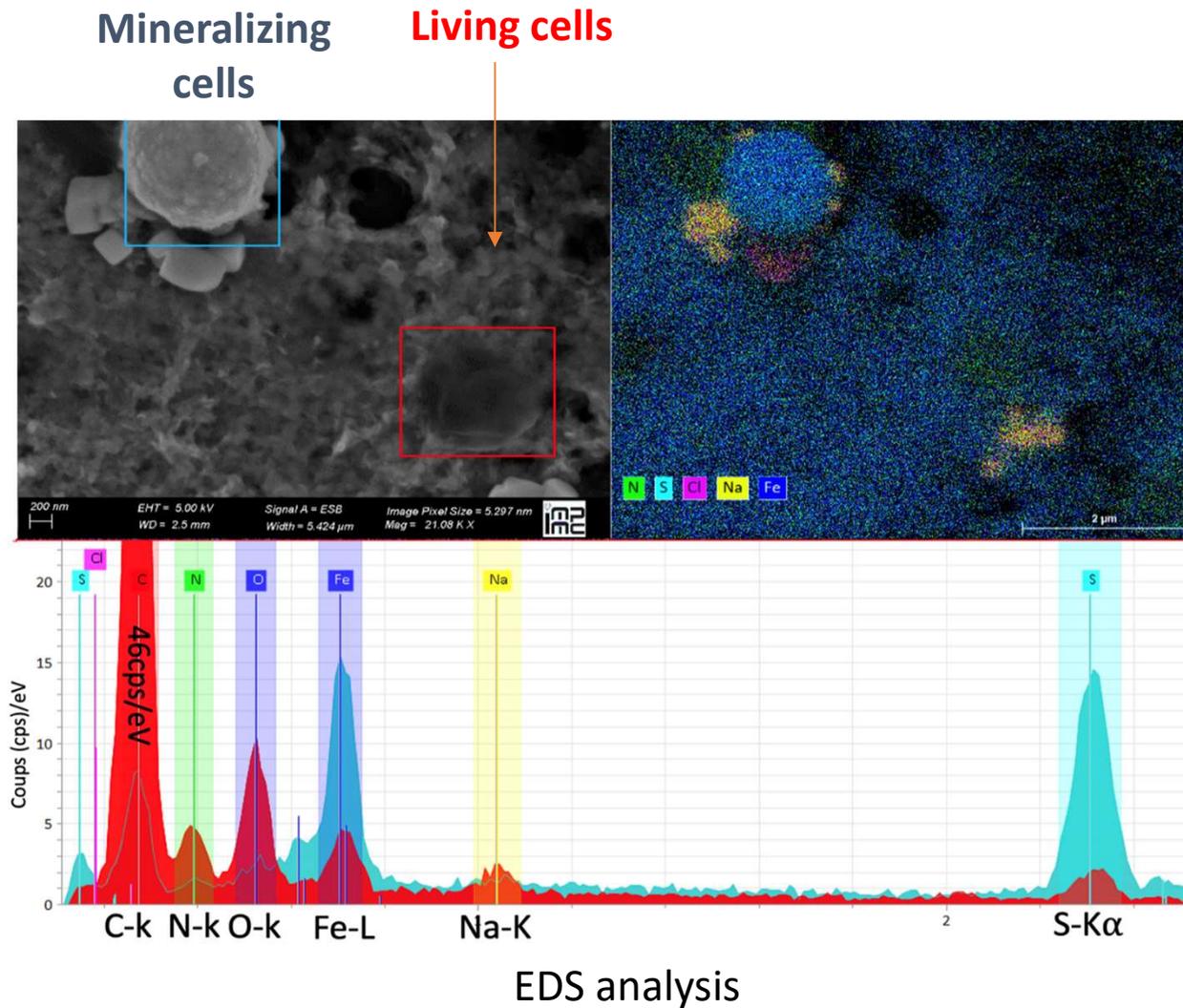
↘ chemical toxicity

↘ physical toxicity

Population heterogeneity

In S(0) conditions
(with Fe²⁺ solution
at 1mM)

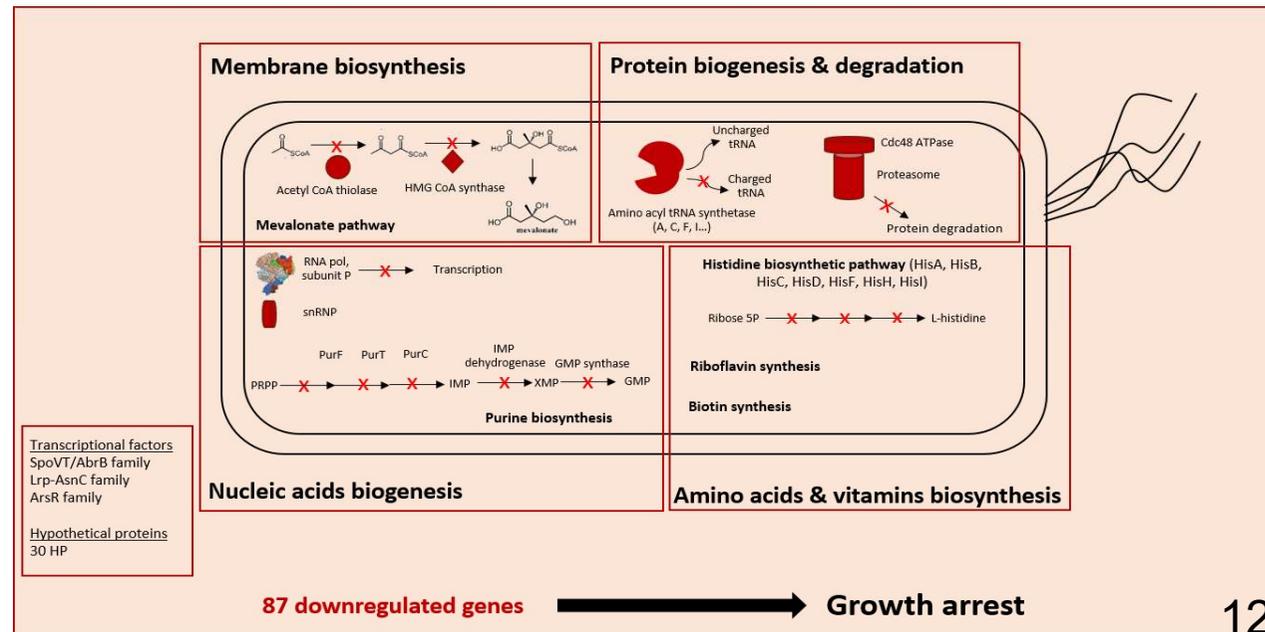
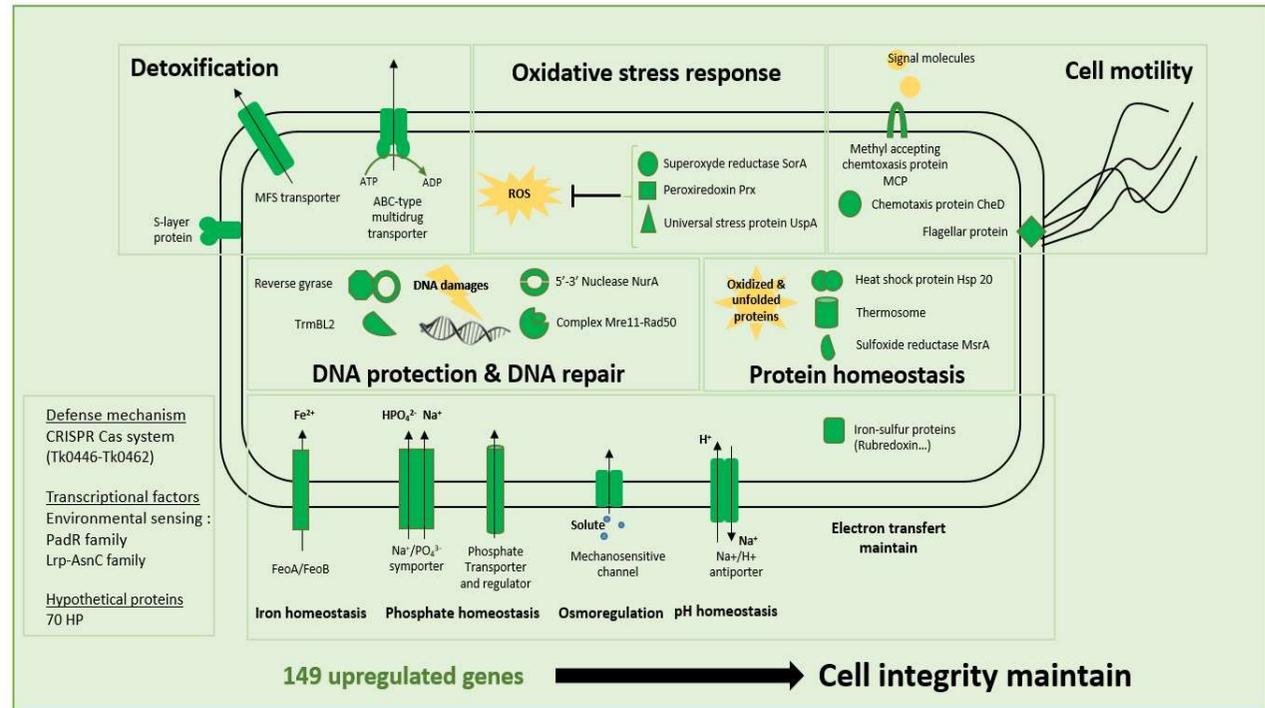
First pyrite after
72H of Fe
induction



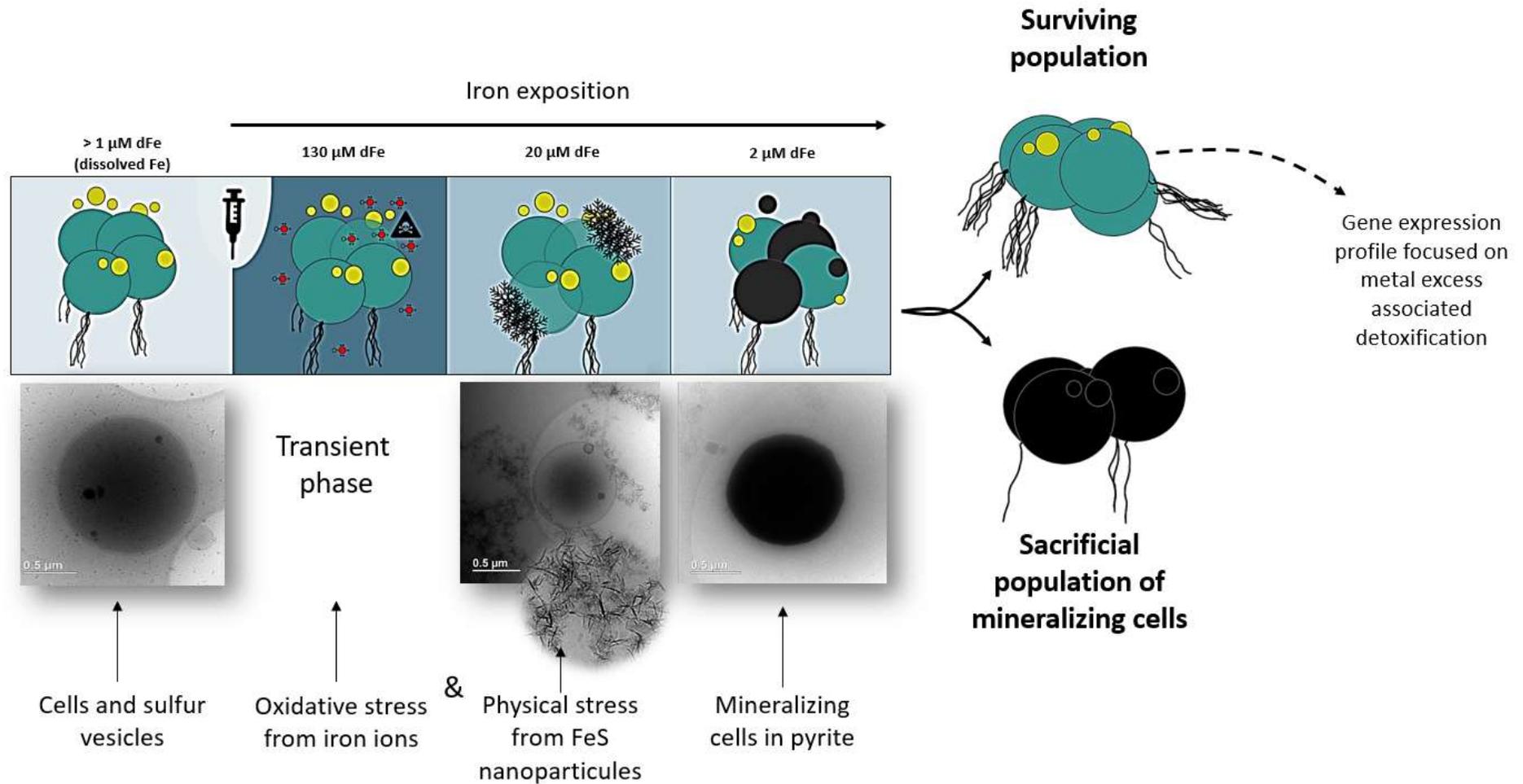
**In S(0) conditions
(with Fe²⁺ solution
at 1mM)**

Transcriptomic
analyses

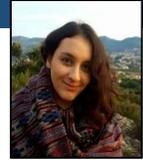
First pyrite after
72H of Fe
induction



Iron sulfides produced by Thermococcales: an iron detoxification mechanism

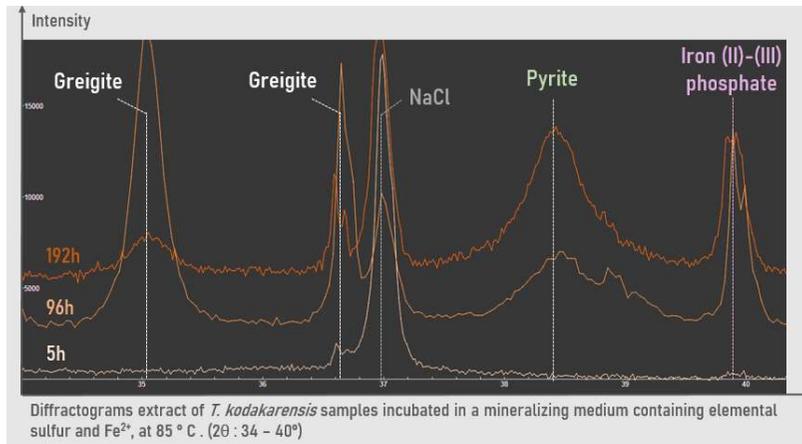


Mineralogical characterization during mineralization process



Chloé TRUONG

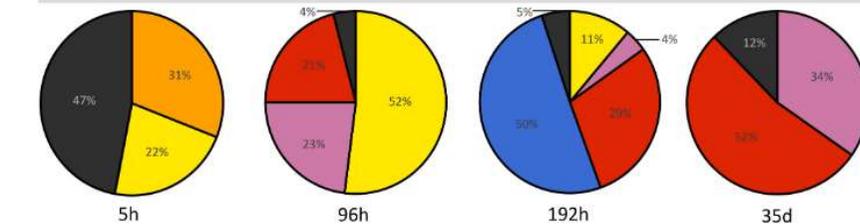
In S(0) conditions (with Fe²⁺ solution at 5mM)



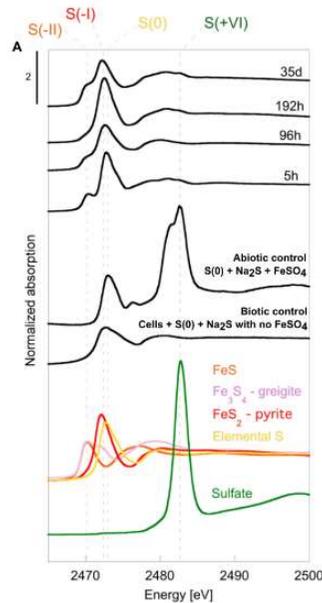
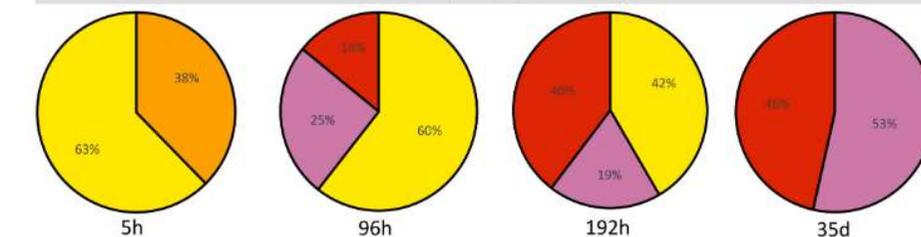
DRX analyses

■ S(0)
 ■ FeS
 ■ FeS₂
 ■ Fe₃S₄
 ■ FePO₄OH
 ■ NaCl

A Relative proportions of mineral phases



B Relative proportions of sulfur species



XANES data

Phosphorus-iron-sulfur dynamics :

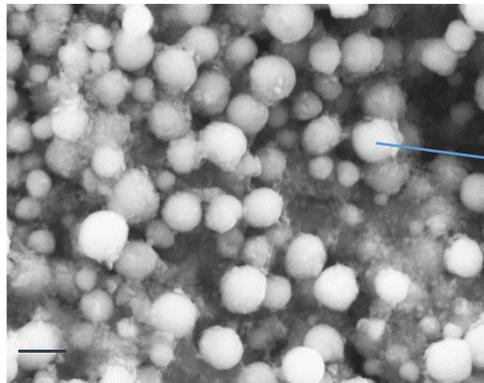
FeS (5h) < greigite-pyrite (96h) < pyrite - Iron II-III phosphate (192h) < greigite-pyrite (35d)

Sulfur redox swing from S(0) to S⁻² (H₂S/FeS) and then to S⁻¹ (FeS₂)

Iron redox evolution : Fe²⁺ in FeS and pyrite, Fe³⁺ in greigite and Iron II-III phosphate

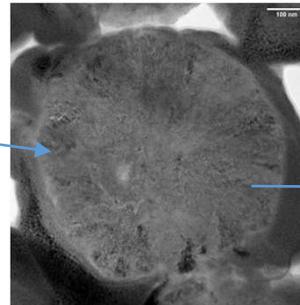
Characterization of biogenic pyrite spherules

In S(0) conditions (with Fe²⁺ solution at 5mM) – 96h

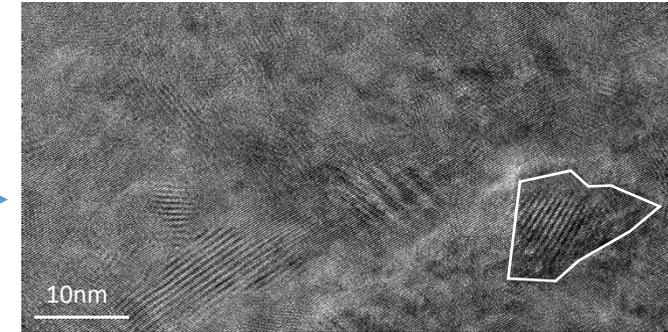


**Pyrite spherules (200nm – 1μm)
Ultra-smooth surfaces**

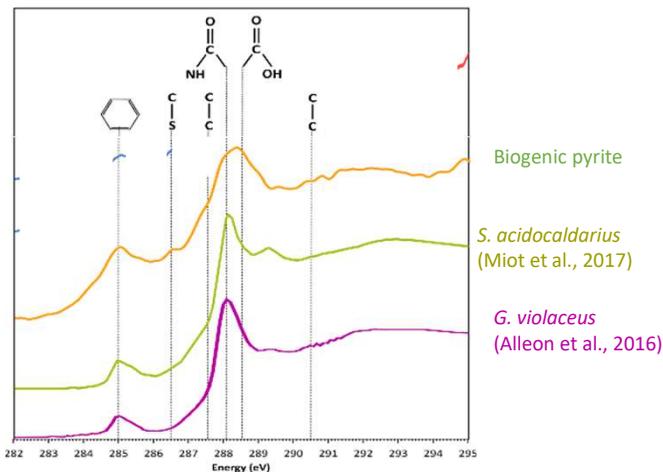
FIB section



Microtexture of pyrites



**Polycrystalline pyrite
Small domains (15 nm)**



XANES data

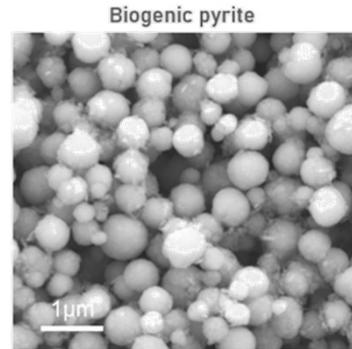
**Presence of organic compounds trapped
within the spherules**

aromatic groups, amide groups and carboxylic groups...

Abiotic syntheses of pyrite VS biogenic pyrite spherules

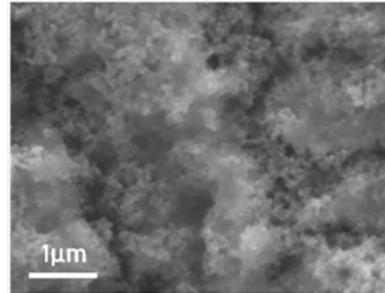


Chloé
TRUONG

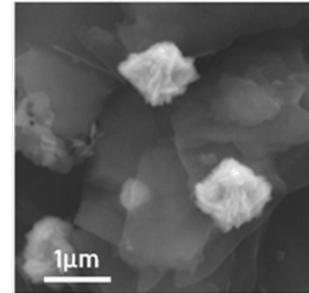


Pyrite spherules (200nm – 1μm)
Ultra-smooth surfaces
Polycrystalline pyrite
Crystallographic domains (15 nm)
Presence of organic compounds

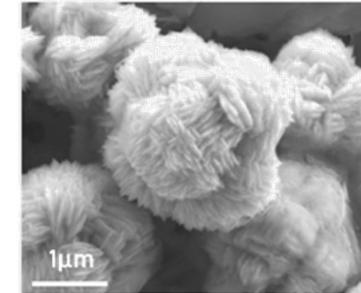
Negative control (no MO)



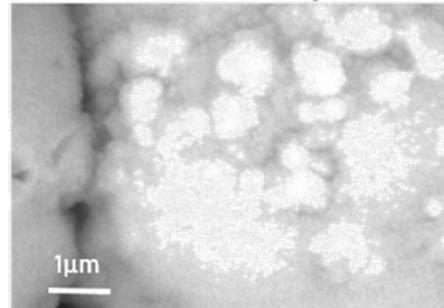
Graphitic carbon



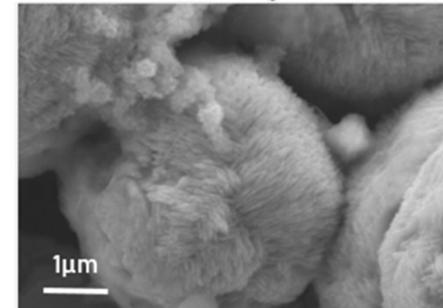
YE + tryptone



Cellular envelopes



Cellular lysates



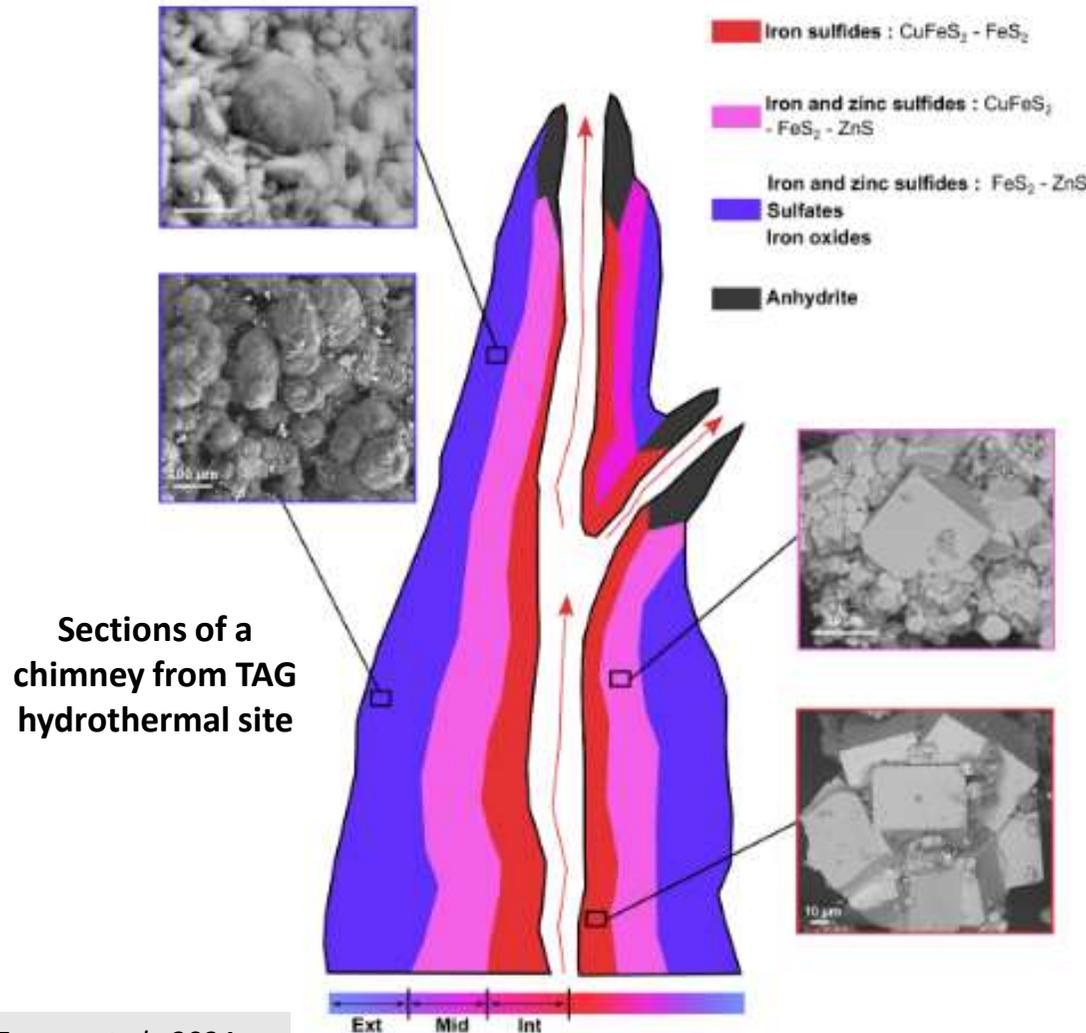
Negative control (no MO) – No pyrite (need MO)
Intracellular material – No pyrite (no reactive surface)

Graphitic carbon - sand-rose-like crystals, domains > 200 nm, no organic compounds
YE + tryptone - sand-rose-like crystals (5μm), domains > 100 nm, no organic compounds
Cellular envelopes – pyrite spherules (10μm), domains > 50 nm, few organic compounds
Cellular lysates – pyrite spherules (2-10μm), domains < 10 nm, organic compounds

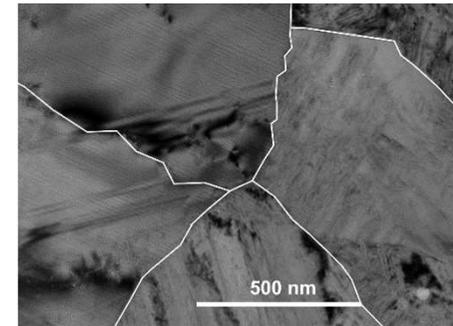
Truong *et al.*, 2024a
Truong *et al.*, 2024b

Biogenic pyrite spherules : biosignatures ?

Mineral biosignatures in black smokers ?



Sections of a chimney from TAG hydrothermal site



External portion contain pyrite spherules

- 2 – 5 μm in size
- Mosaic disoriented domains of pyrite spherules
- Domains > 100 nm
- Presence of organic compounds

Greater similarity between pyrite spherules from cellular lysates and those from the TAG site

Truong *et al.*, 2024a
Truong *et al.*, 2024b

Biosignatures



Metabolism
Thermodynamic model



François
GUYOT



Sylvain
BERNARD



Guillaume Morin



Pierre Le Pape

Metal stress response



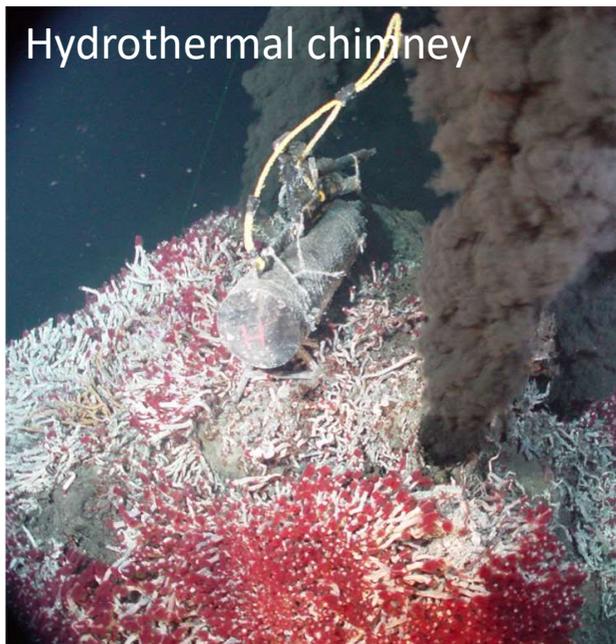
Alexandre
GELABERT



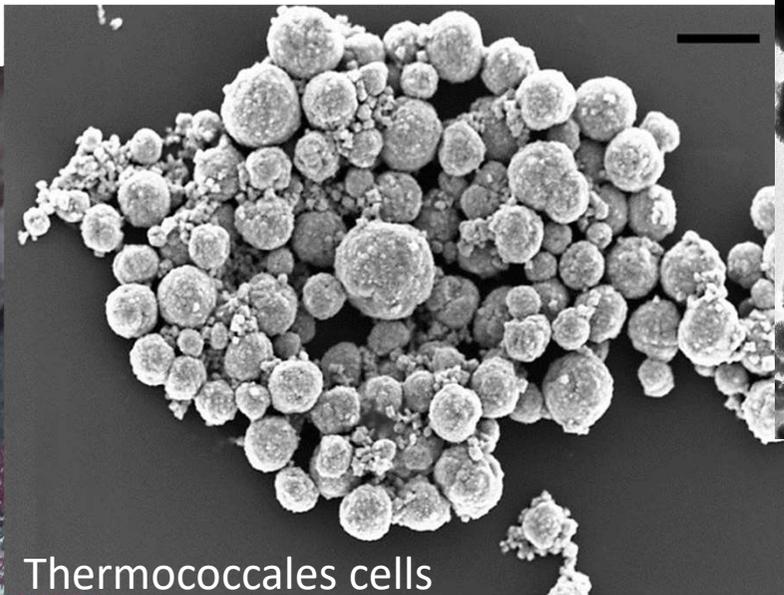
Biom mineralization



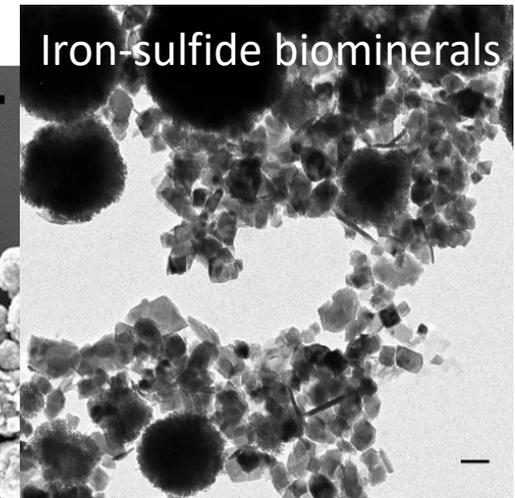
Thank you for your attention



Hydrothermal chimney



Thermococcales cells



Iron-sulfide biominerals