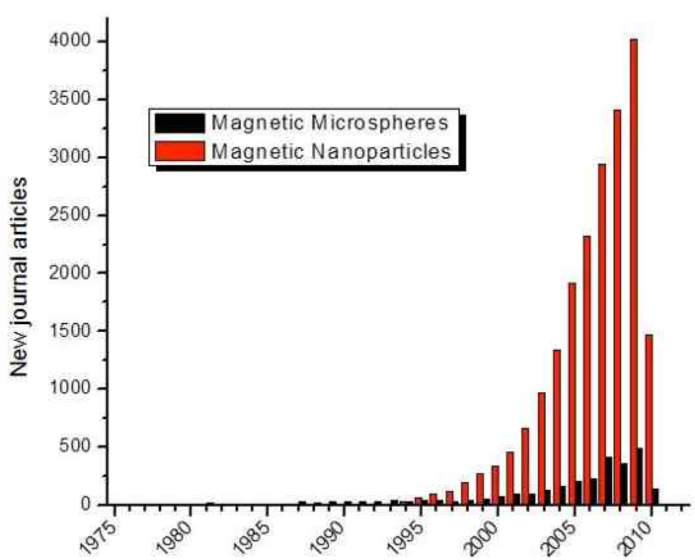
Magnetic nanoparticles from bacteria

M.L. Fdez-Gubieda, A. Muela, J. Alonso, A. García-Prieto, L. Olivi, R. Fernández-Pacheco, J.M. Barandiarán

Club de Magnetismo, Noviembre 2013

Magnetic nanoparticles



Interest of magnetic nanoparticles

Are magnetic particles useful?

Patent update

Urs Hafeli, Conference Magnetic Carriers, Minneapolis, Mayo 2012.

Magnetic nanoparticles

» Applications

- Catalysis
- Data storage
- Energy storage
- Biomedical applications:
  - Diagnostic
  - Therapy
  - Analysis

» Preparation methods

- Chemical routes
- Mechanical routes: ball milling
- Photolitography
- ………

- Narrow Size distribution
- Well define shape
- Coatings

Biosynthesis: Magnetic Nanoparticles

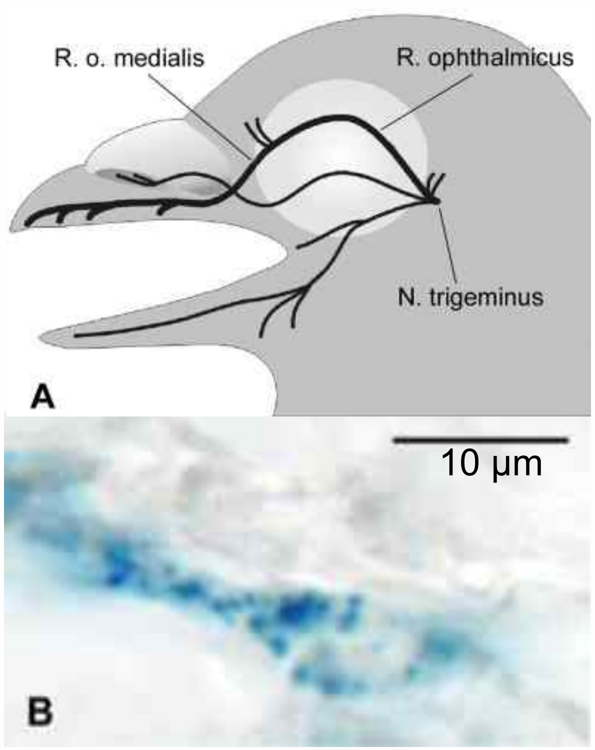
living organisms

Name	Composition	Magnetic order	
Hydroxides			
Ferrihydrite	**$FeOOH.nH_2O$**	**AFM(?)**	**Fe storage (Ferritine core)** **Plants, animals**
Goethite	α–FeOOH	AFM, weak FM	Limpets
Lepidocrocite	γ–FeOOH	AFM(?)	Chiton
Oxides			
Greigite	Fe_3S_4	ferrimagnet	Bacteria. Magnetotaxia
Magnetite	**Fe_3O_4**	**ferrimagnet**	**Microrganisms to humans** **•Magneto-reception** **•Magnetotaxia**

Biosynthesis: Magnetite Nanoparticles

Magnetoreception:

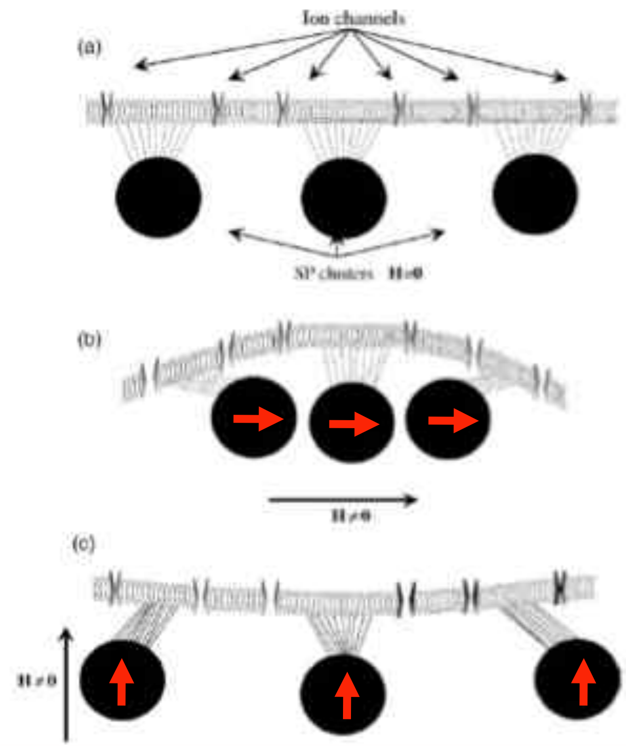
animal senses field direction, uses information to navigate (e.g., bird, salmon, bee, turtle)



PIGEONS

beak NPs of magnetite

G. Fleissner, et al., J. Comparative Neurol. (2003).
CV Mora et al.; Nature (2004)

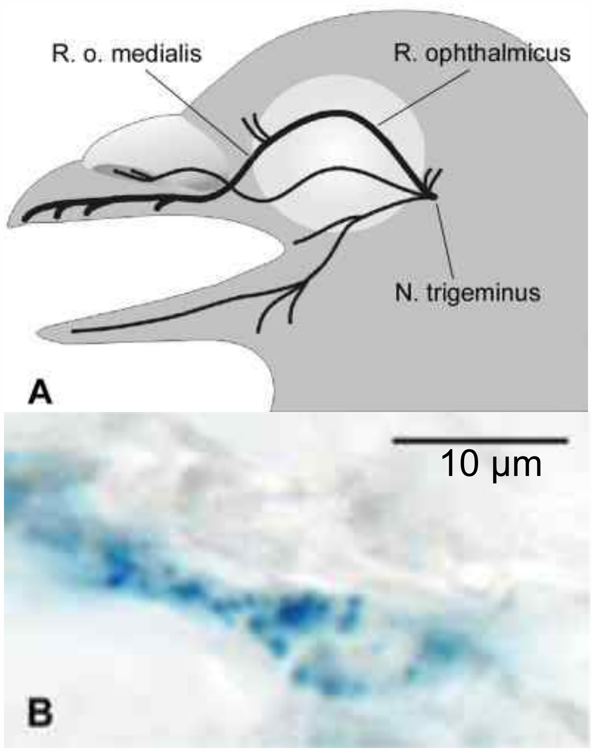


Magnetic information is transduced into neuronal impulses by using a magnetite-based magnetoreceptor

Biosynthesis: Magnetite Nanoparticles

Magnetoreception:

animal senses field direction, uses information to navigate (e.g., bird, salmon, bee, turtle)



beak NPs of magnetite

PIGEONS

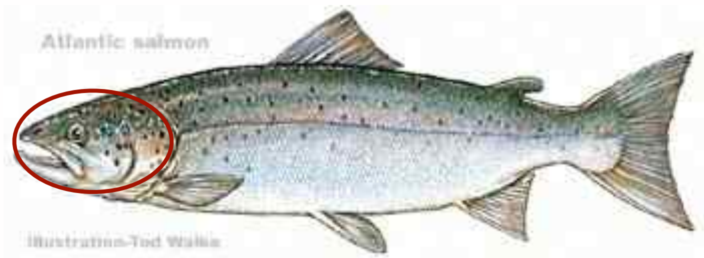
Christoph Daniel Treiber et al; NATURE, VOL 484, 19 APRIL 2012, 367

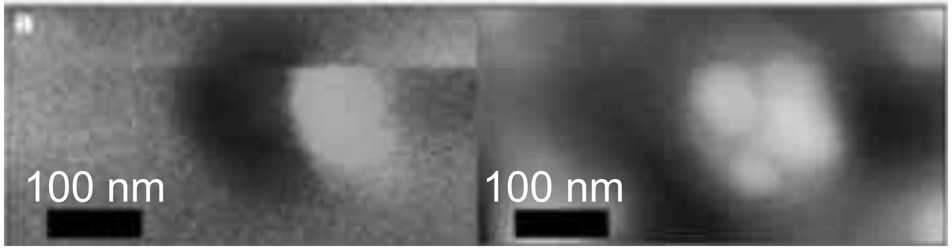
Clusters of iron-rich cells in the upper beak of pigeons are macrophages not magnetosensitive neurons

Biosynthesis: Magnetite Nanoparticles

Magnetoreception:

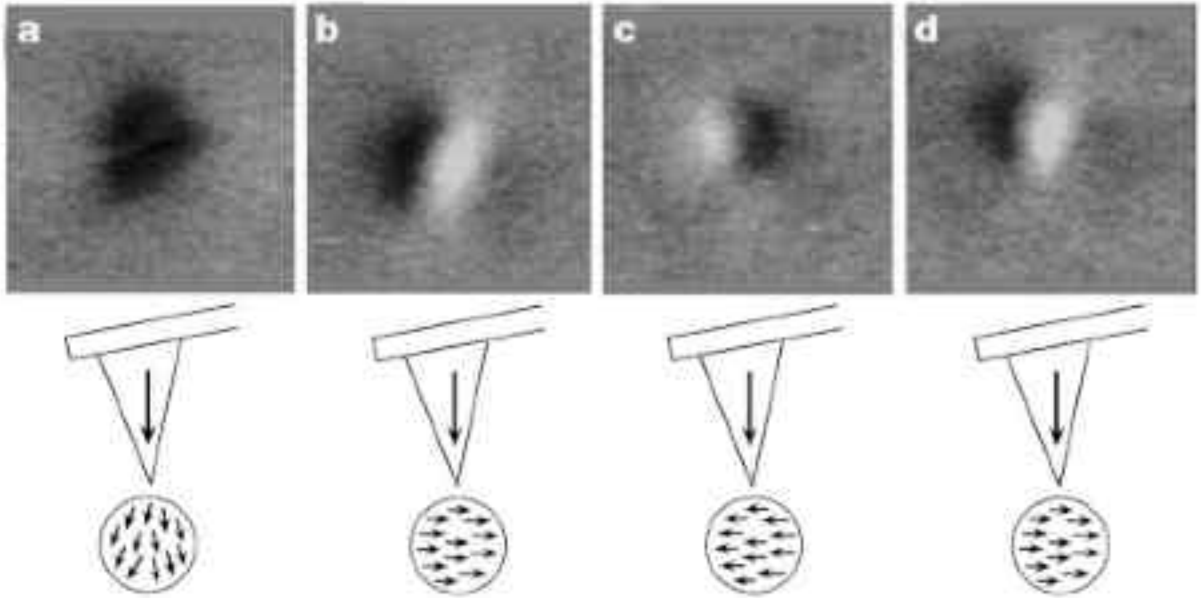
animal senses field direction, uses information to navigate (e.g., bird, salmon, bee, turtle)





rainbow trout
salmon

area in the olfactory lamellae

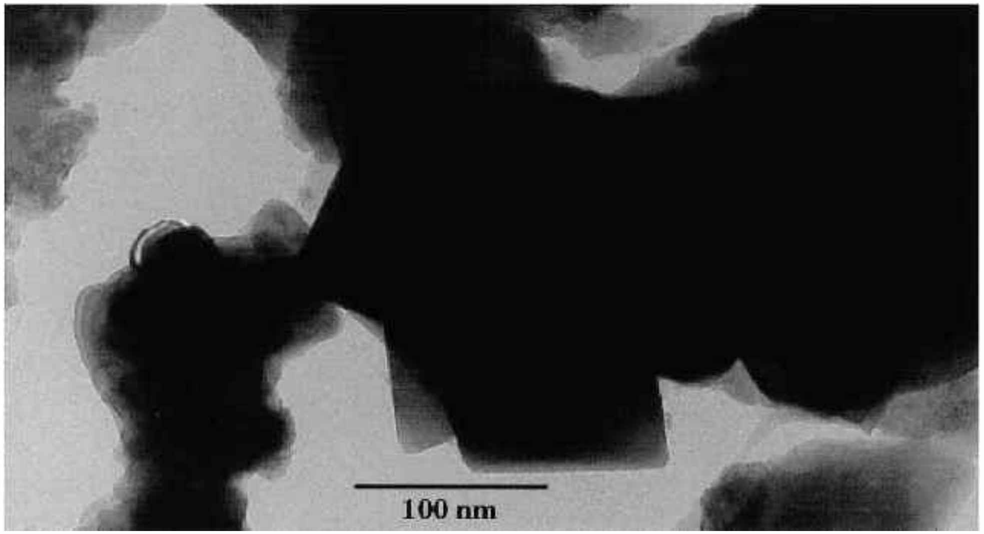


Carol E. Diebel, et al; NATURE, VOL 406, (20 JULY 2000) page 300

Biosynthesis: Magnetite Nanoparticles

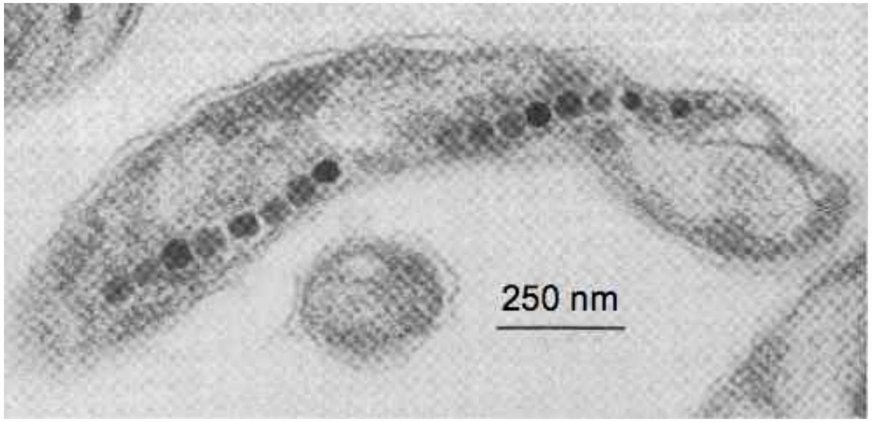
Degenerative diseases: Alhzeimer, Parkinson

Human brain



TEM micrograph of biogenetic **magnetite** extracted from the human **hippocampus**.
J. Dobson, FEBS Let 496 1 (2001)

Magnetotactic Bacteria



Electron micrograph of thin-sectioned magnetic cells of strain MS-1. Science 1978

Bellini, S. Thesis, Su di un Particolare Comportamento di Batteri d'Acqua Dolce. University of Pavia, Italy, 1963

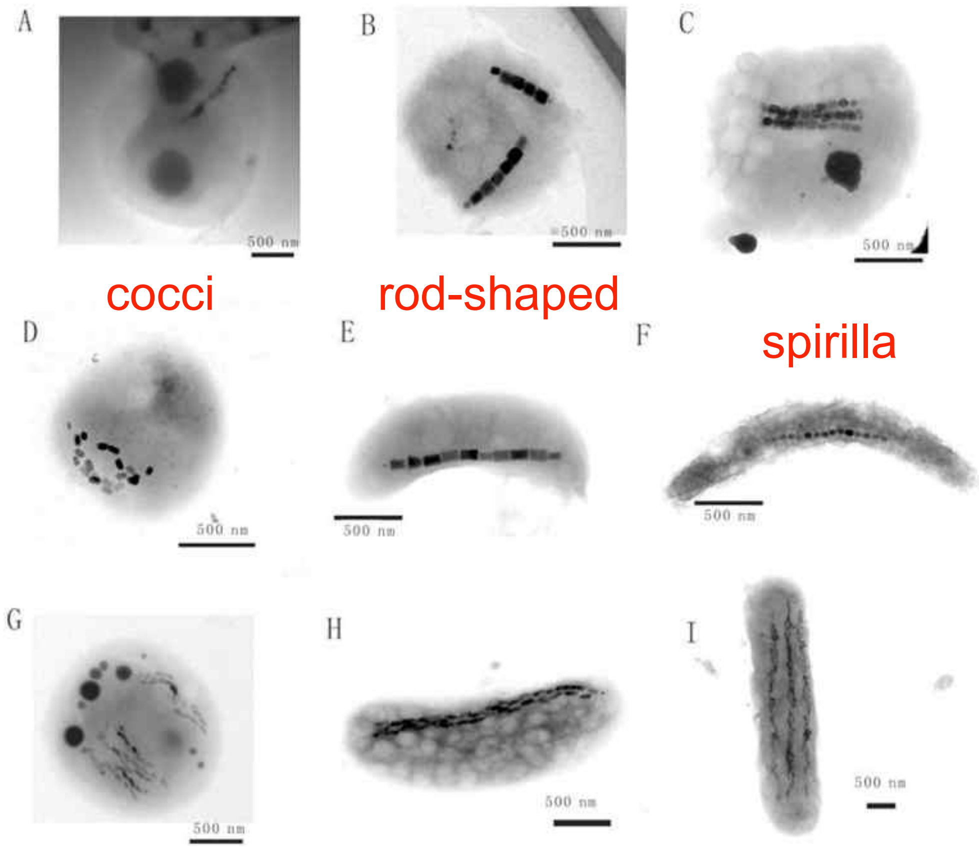
R. Blakemore, Science 1975 → Marine aquatic enviroment

R. Frankel, R. Blakemore,R. Wolfe, Science 1978 → Freshwater

Magnetotactic bacteria are a microorganism that can align and navigate along geomagnetic field lines. → Presence one or more **chains of magnetic nanoparticles.**

magnetic nanoparticles are surrounded by a lipid bilayer membrane: **Magnetosomes**

Magnetotactic bacteria



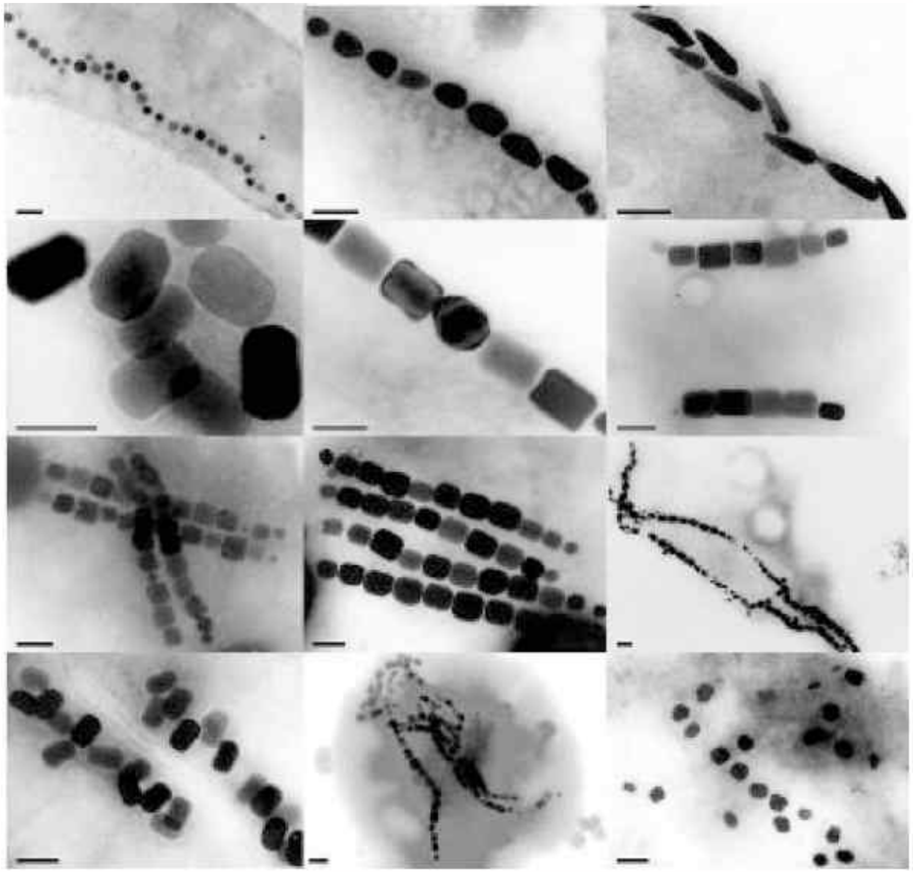
Magnetotactic bacteria

Different magnetotactic bacteria species

Shape, size, type of magnetosomes are specific of the bacteria species

W.E.G. Müller (ed.), Molecular Biomineralization

Magnetosomes



R. Frankel and R. Blakemore,
Phil. Trans. Roy. Soc. London B 304, 567–574 (1984).

magnetic nanoparticles surrounded by a lipid bilayer membrane: Magnetosomes

Shape, size, type of magnetosomes are specific of the bacteria species

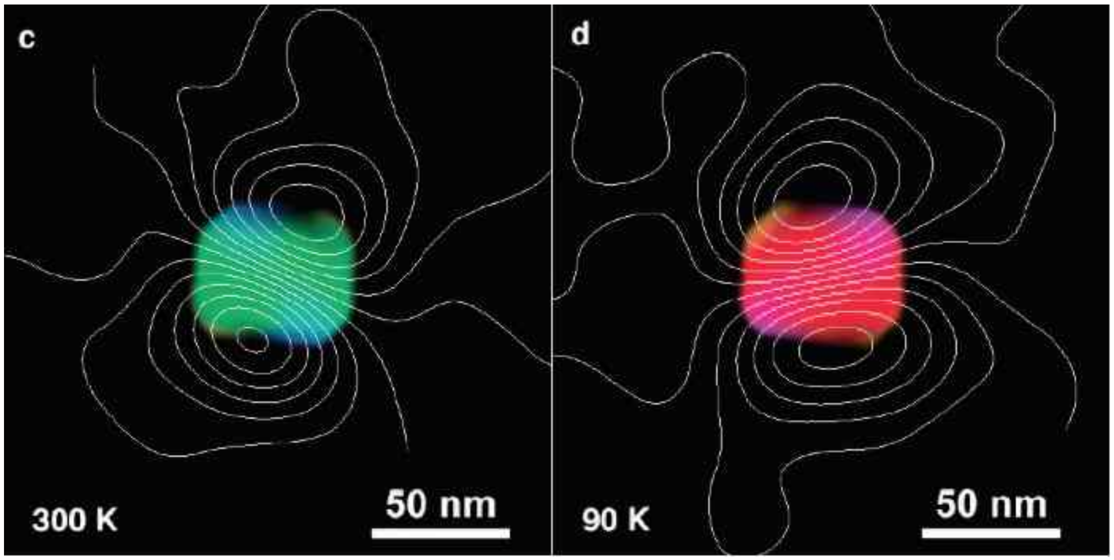
Magnetic nanoparticle:
Fe_3O_4 (magnetite), Fe_3S_4 (greigite)

Shape: cubooctahedral, prismatic, arrowhead

Size: 40 – 120 nm

Magnetosomes

T>T_{Verwey} T<T_{Verwey}



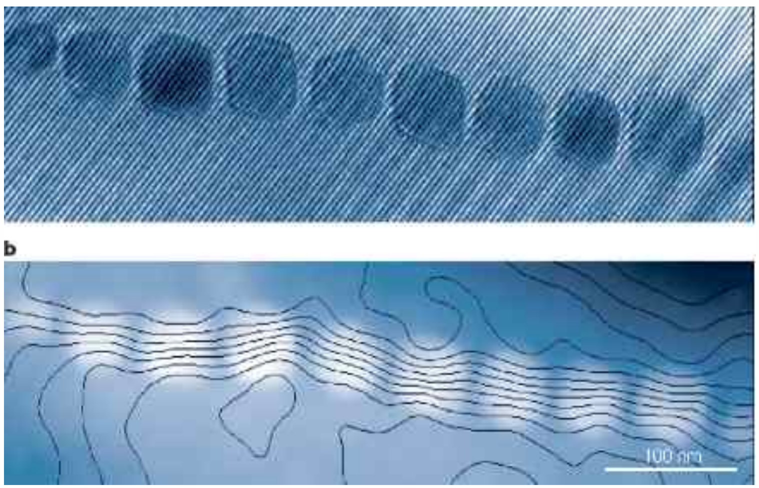
⟨111⟩ $_{cubic}$ [001]$_{monoclinic}$

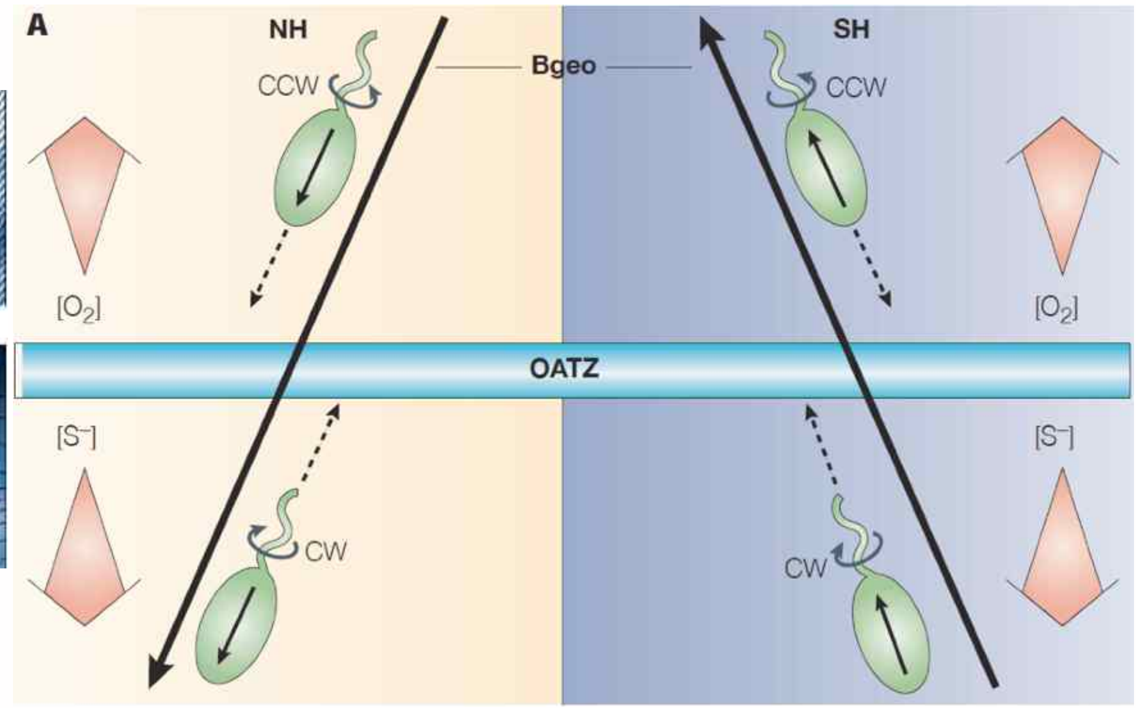
J. Meurig Thomas, ACCOUNTS OF CHEMICAL RESEARCH, 2008

Magnetic induction maps recorded using off-axis electron holography from the same particle, showing remanent magnetic states at 300K and at 90 K

Size: 40 – 120 nm **Single-domain** particles → **nano-magnet**

Magnetotaxis





$m \approx 3x10^{-14}$ Am^2

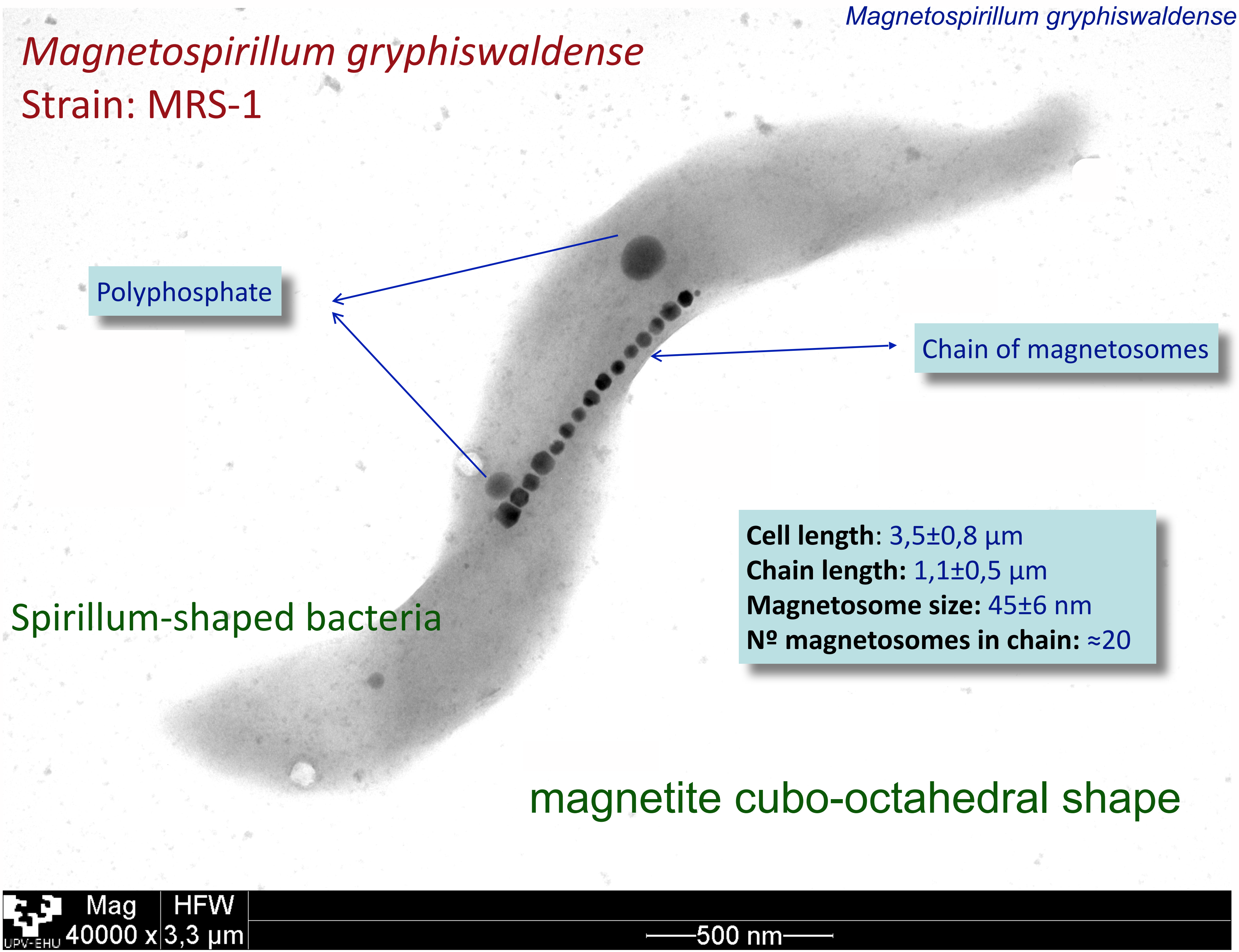
compass needle

D.A. Bazylinski and R. Frankel, Nature Reviews (2004)

Aim of the work

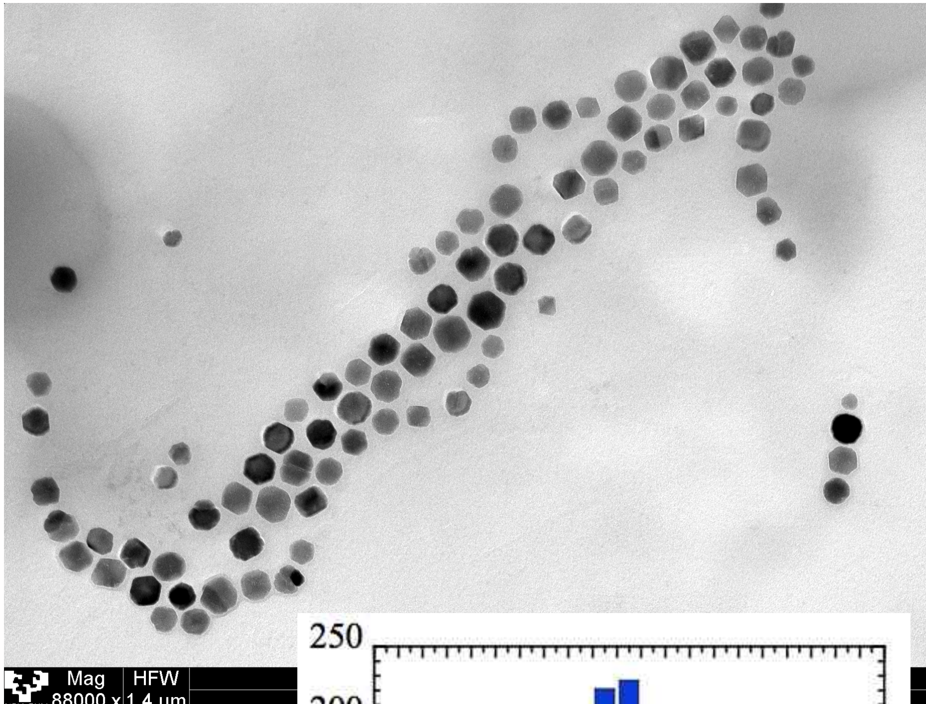
- Growth Magnetotactic bacteria: *Magnetospirillum gryphiswaldense* and isolated the magnetosomes

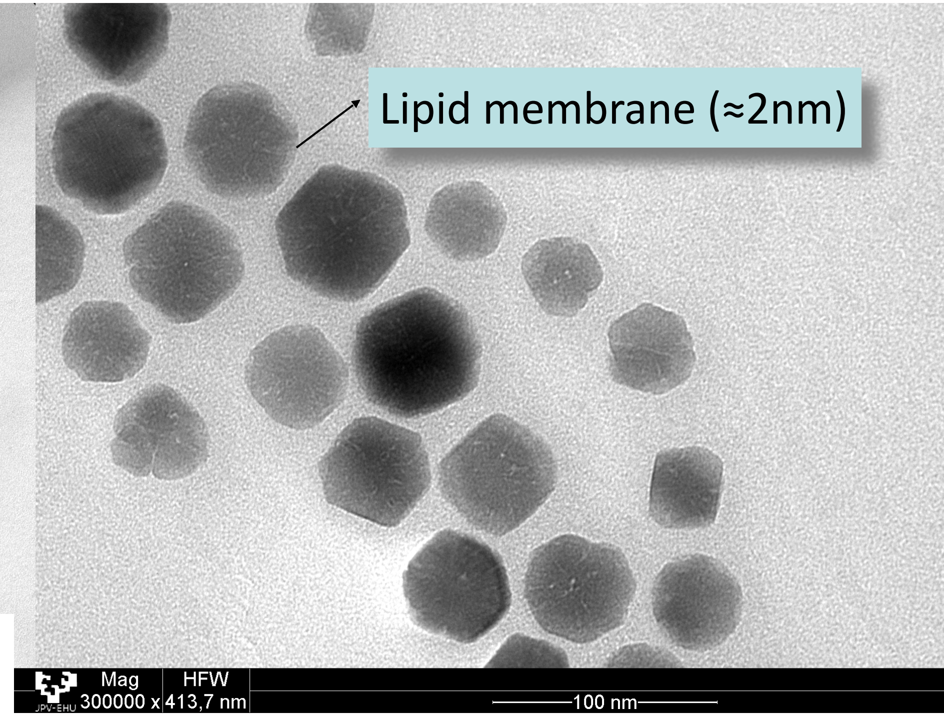
- Biomineralization process: is the controlled formation of solid inorganic compounds by biological system

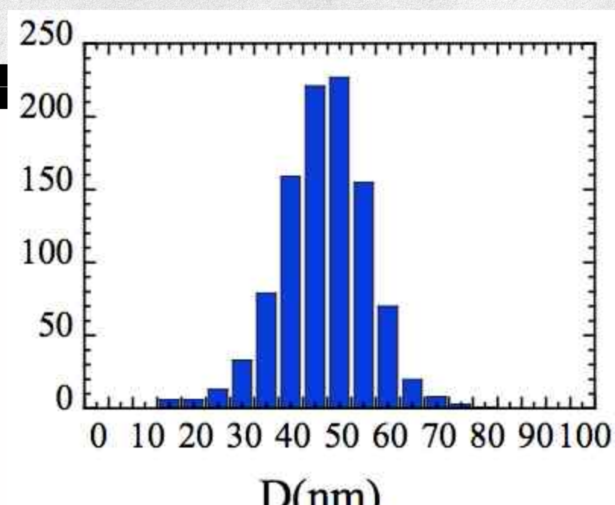

Magnetospirillum gryphiswaldense
Magnetospirillum gryphiswaldense
Strain: MRS-1
Polyphosphate
Chain of magnetosomes
Cell length: 3,5±0,8 µm
Chain length: 1,1±0,5 µm
Magnetosome size: 45±6 nm
Nº magnetosomes in chain: ≈20
Spirillum-shaped bacteria
magnetite cubo-octahedral shape
UPV-EHU
Mag
40000 x
HFW
3,3 µm
500 nm

TRANSMISSION ELECTRON MICROSCOPY

Philips EM208S 120 kV

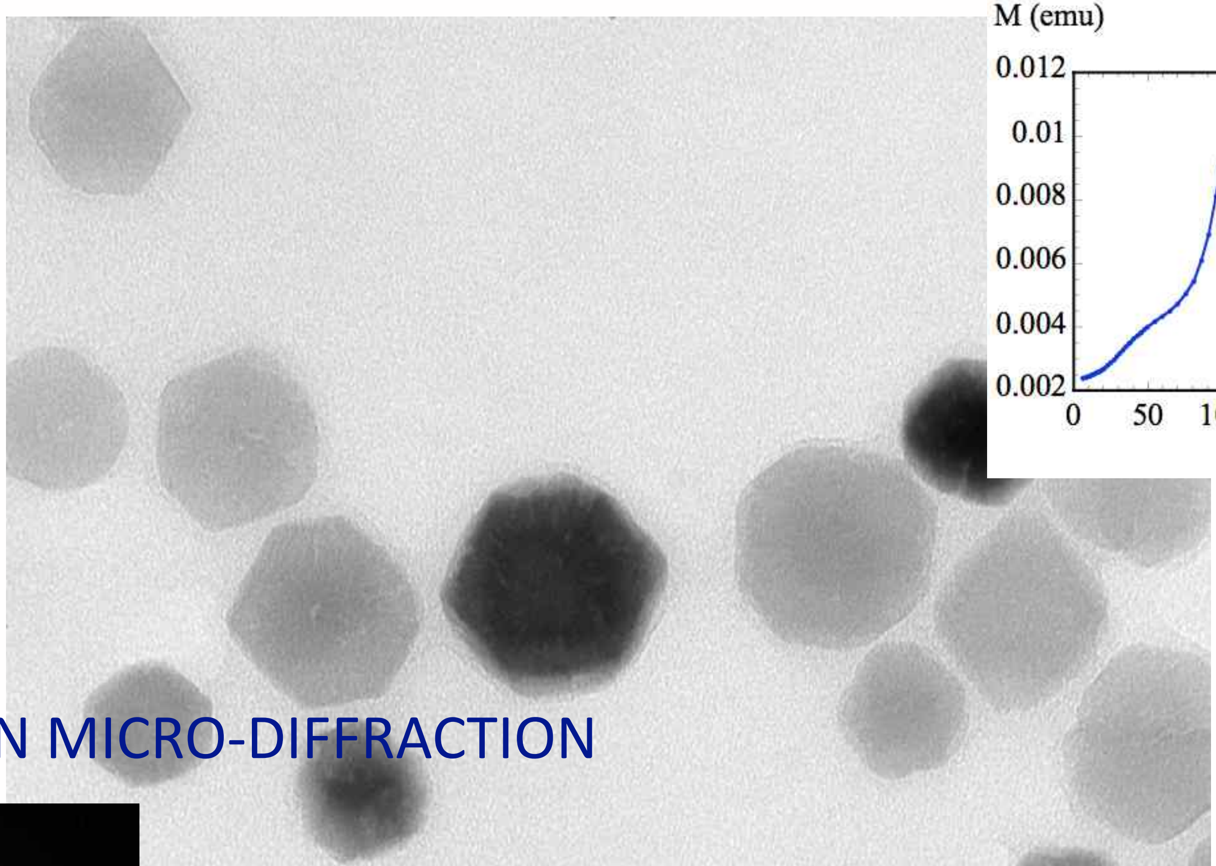


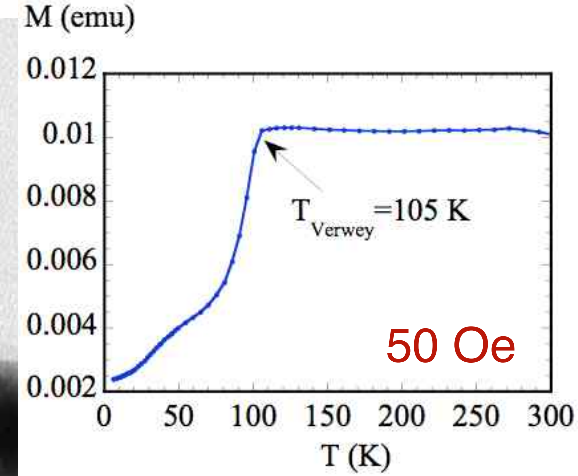




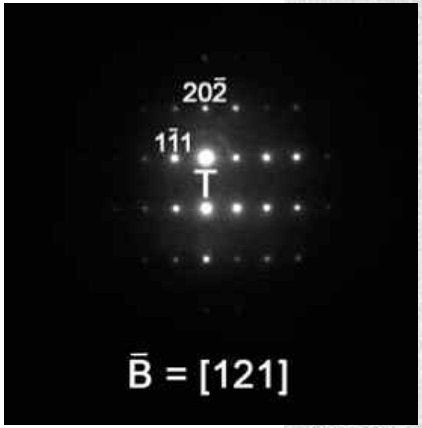
Mean 45.0 nm
σ=6.7 nm
N=1001

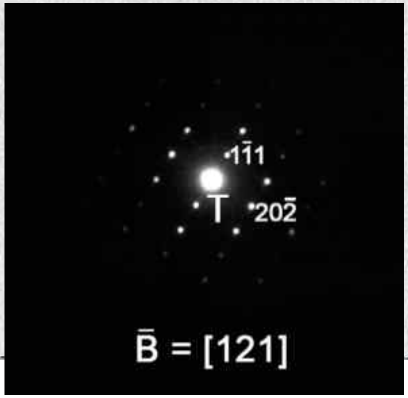
MAGNETIC CHARACTERIZATION





ELECTRON MICRO-DIFFRACTION





Spot size electron beam: 20-40nm

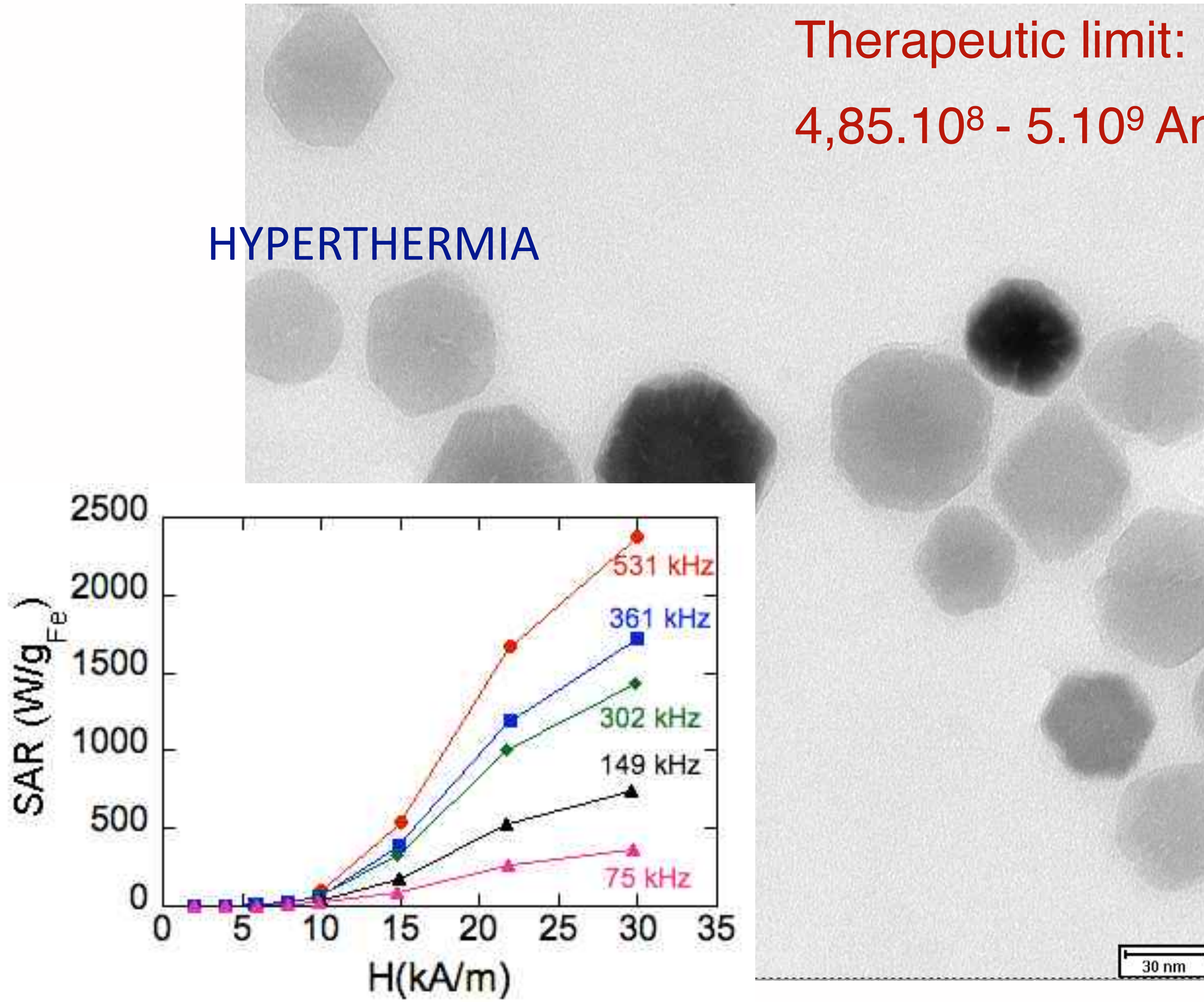
Index patterns: a=0.83969nm

BIOMEDICAL APPLICATION

Therapeutic limit:

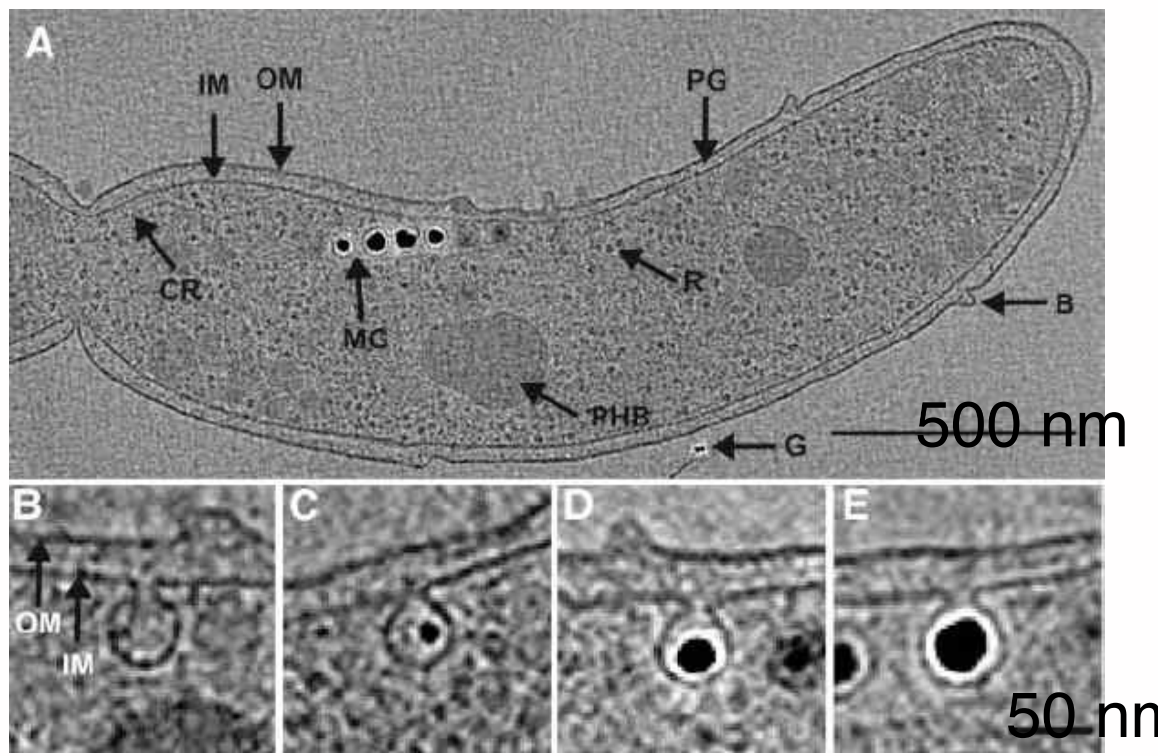
$4,85.10^{8}$ - 5.10^{9} $Am^{-1}s^{-1}$

HYPERTHERMIA



Biomineralization process

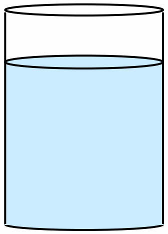
Magnetosome vesicle formed → **Fe taken** → **Transport** from the cell exterior into the magnetosome vesicles → **Precipitation** of Fe



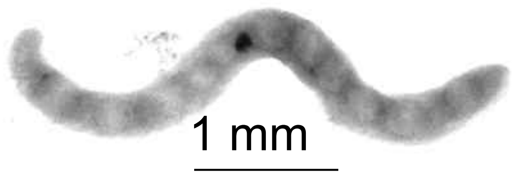
A. Komeili et al., Science 311 (2006) 242

Electron microtomography

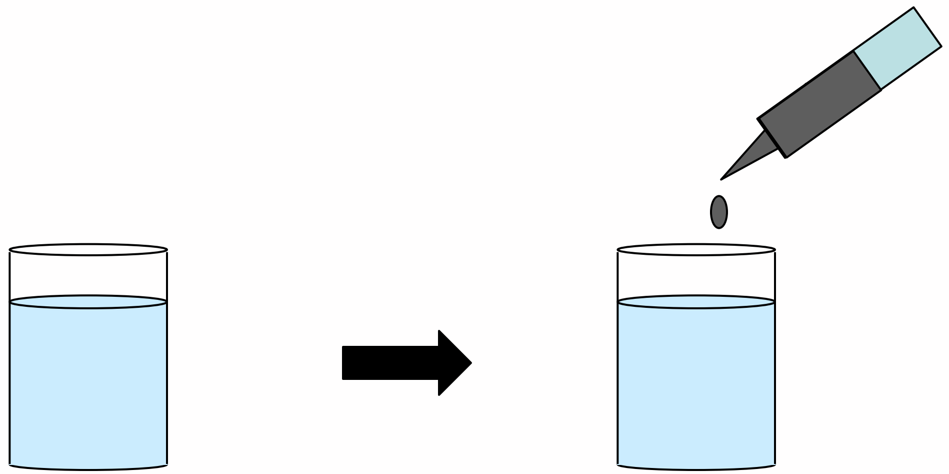
Time-resolved experiment



M. gryphiswaldense cultivated in iron-free medium
28ºC, aerobic condition, 17 rpm

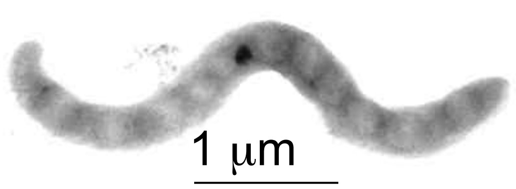


Time-resolved experiment

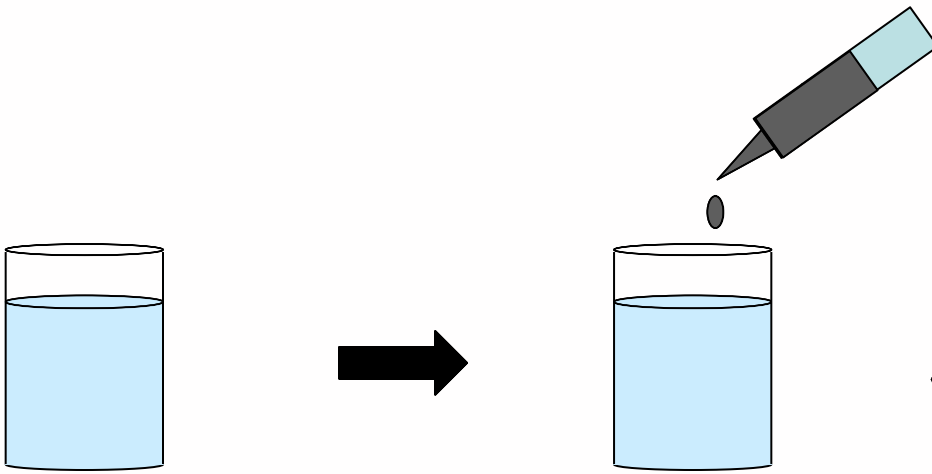


M. gryphiswaldense cultivated in iron-free medium
28ºC, aerobic condition, 17 rpm

Addition of Fe(III)-citrate 100 μM

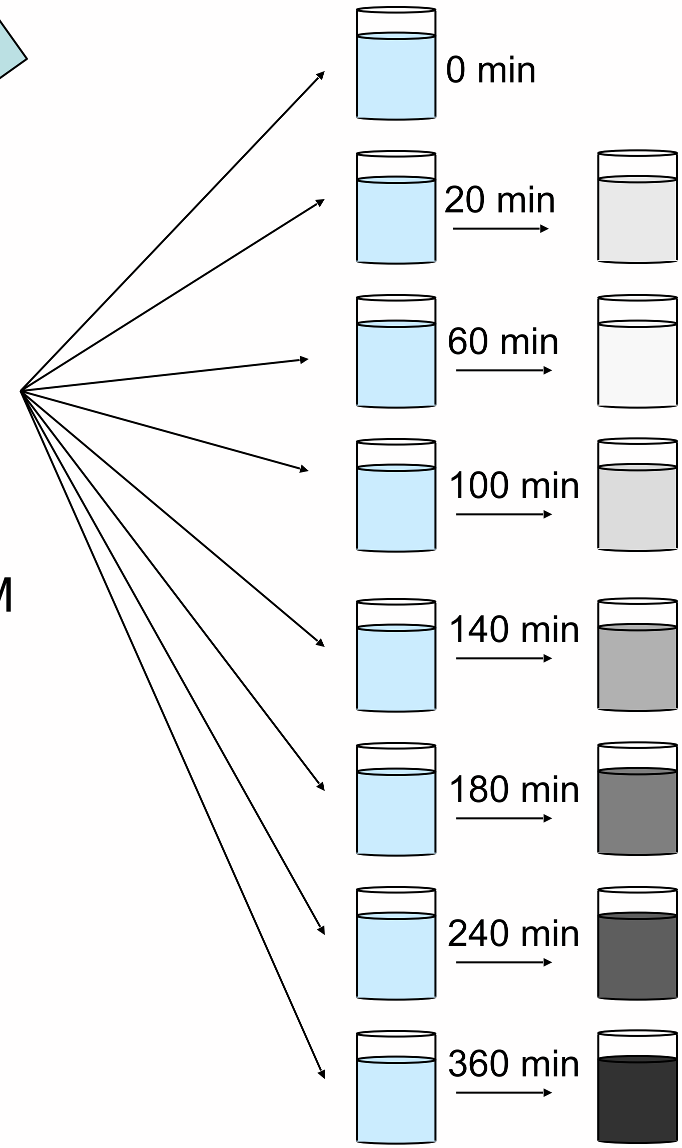


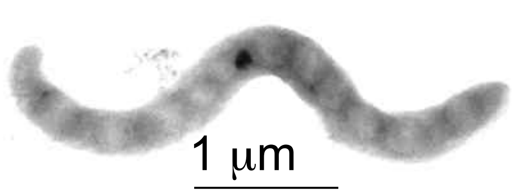
Time-resolved experiment



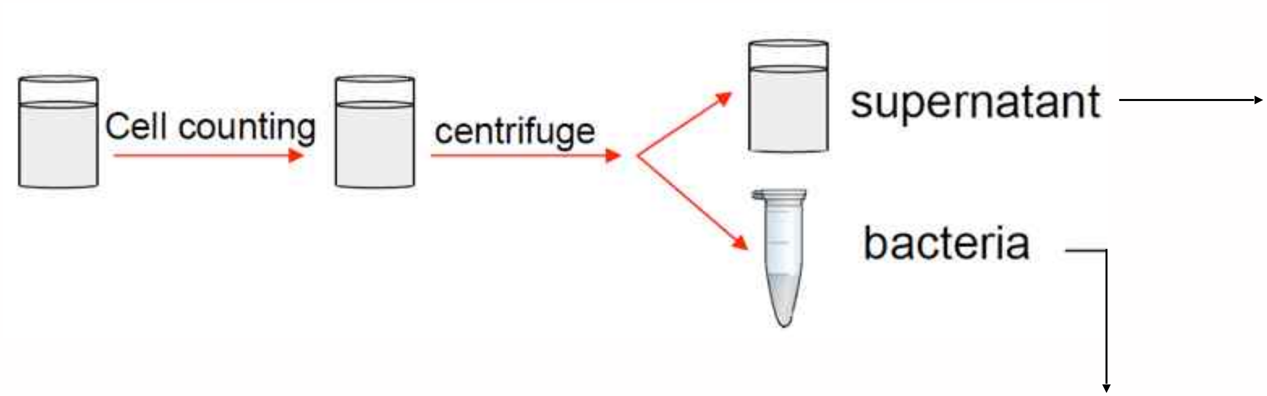
M. gryphiswaldense
cultivated in iron-free medium
28ºC, aerobic condition,
17 rpm

Addition of
Fe(III)-citrate 100 μM





Time-resolved experiment

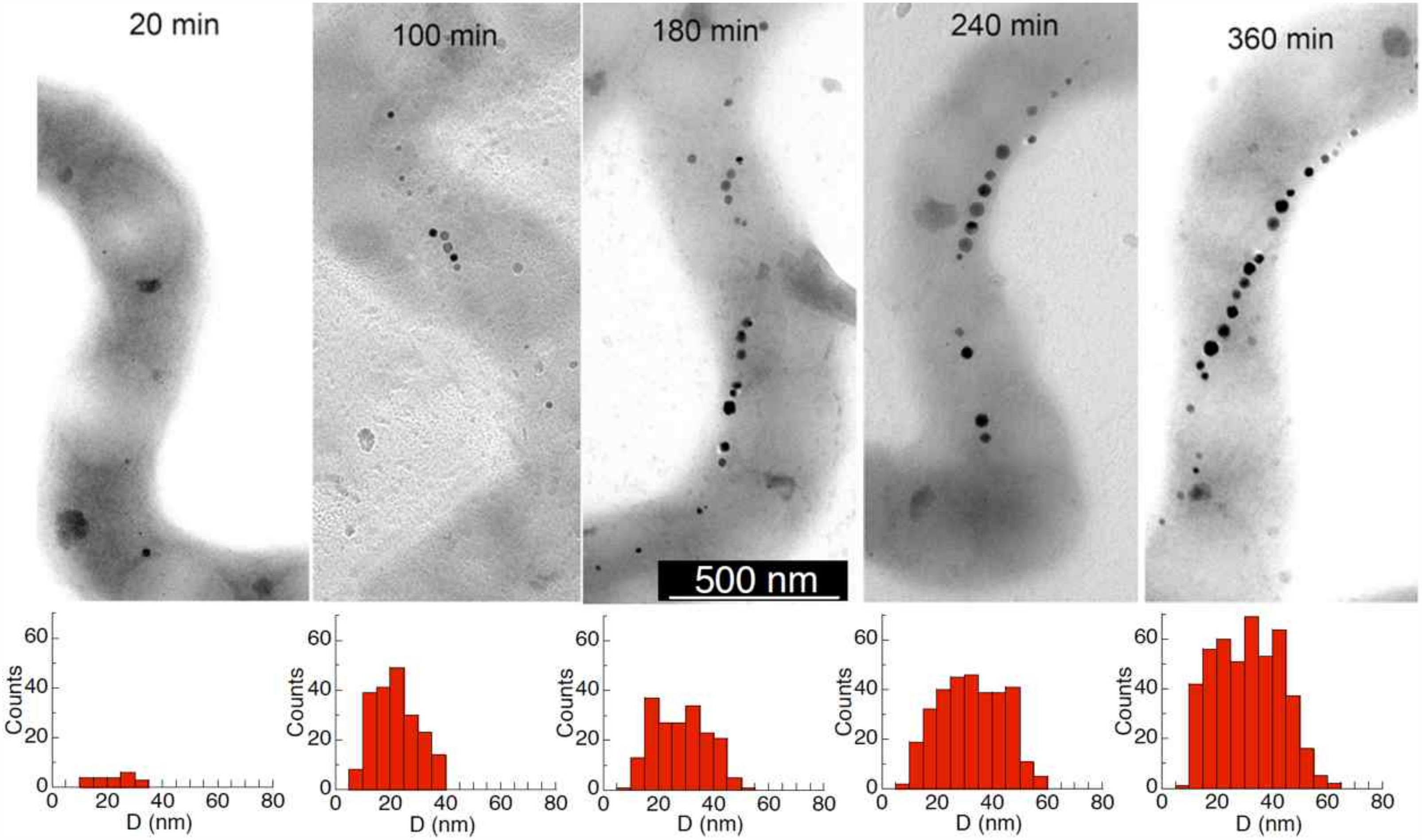


- Inductive Coupling Plasma spectroscopy (ICP-AES)

Fe in the supernatant

- TEM: to follow the formation of the magnetosomes chain
- Magnetic measurements: mass of magnetite
- X-ray absorption Near Edge Structure: Fe inorganic phases
- EXAFS spectroscopy: to follow the evolution of the structure

Transmission Electron Microscopy



1. At early stages most of the bacteria do not present nanoparticles

2. As time increases, the number of bacteria with nanoparticles increases, and they are organized in small subchains.

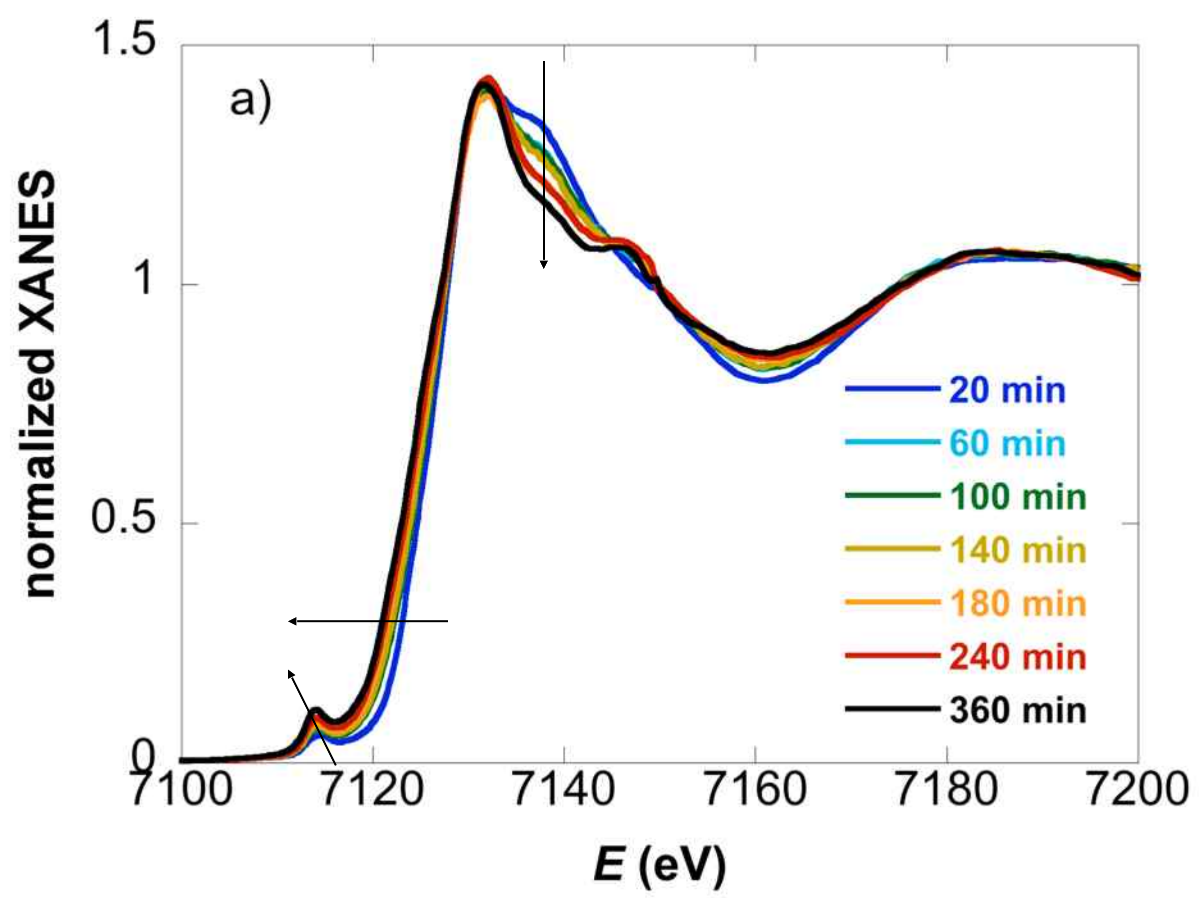
3. With increasing time this sub-chains become more frequent

4. From t = 240 min, longer chains are fomed by the union of the subchains.

5. From there on, the chains become longer and formed by increasingly bigger nanoparticles.

X- ray Absorption Near Egde Structure (XANES)

•Elettra Synchrotron (Trieste, Italy)

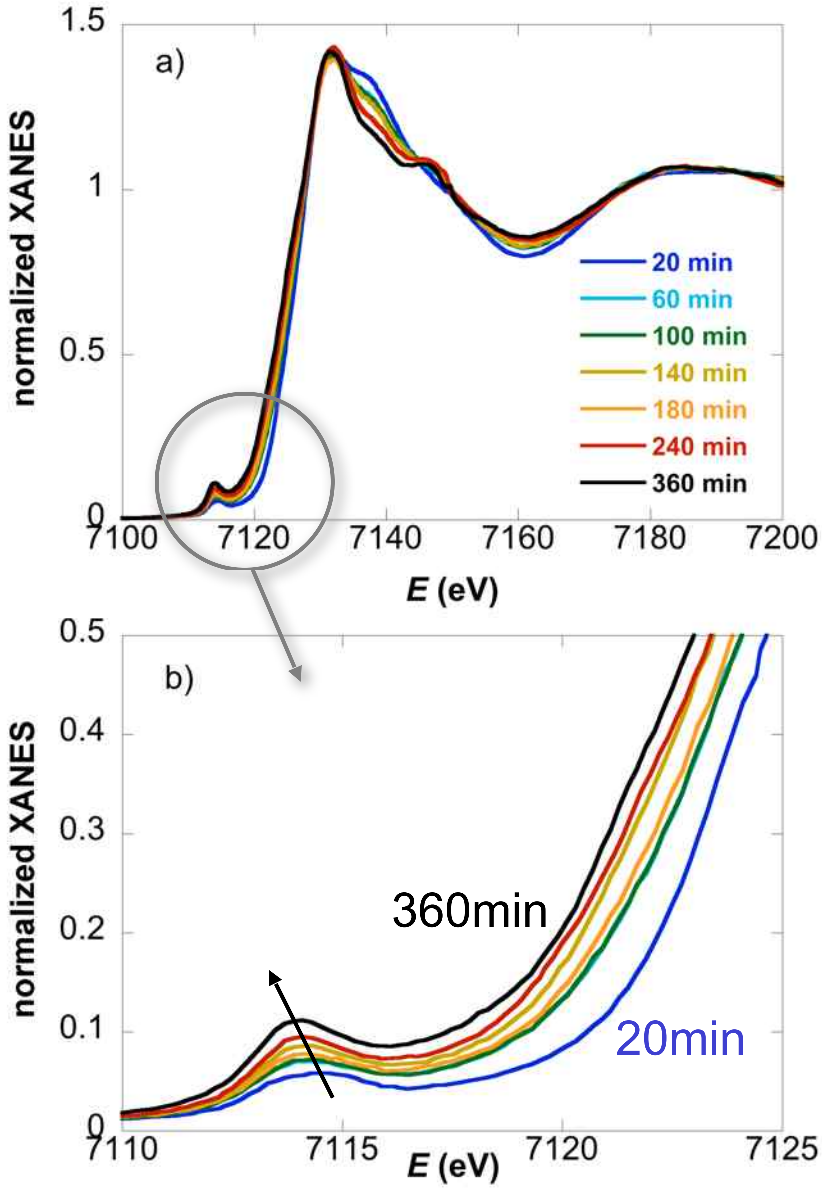


- Fe K-edge
- Fluorescence + transmission set-up
- Room Temperature

XANES:

- Valence state
- Structure

X- ray Absorption Near Egde Structure (XANES)

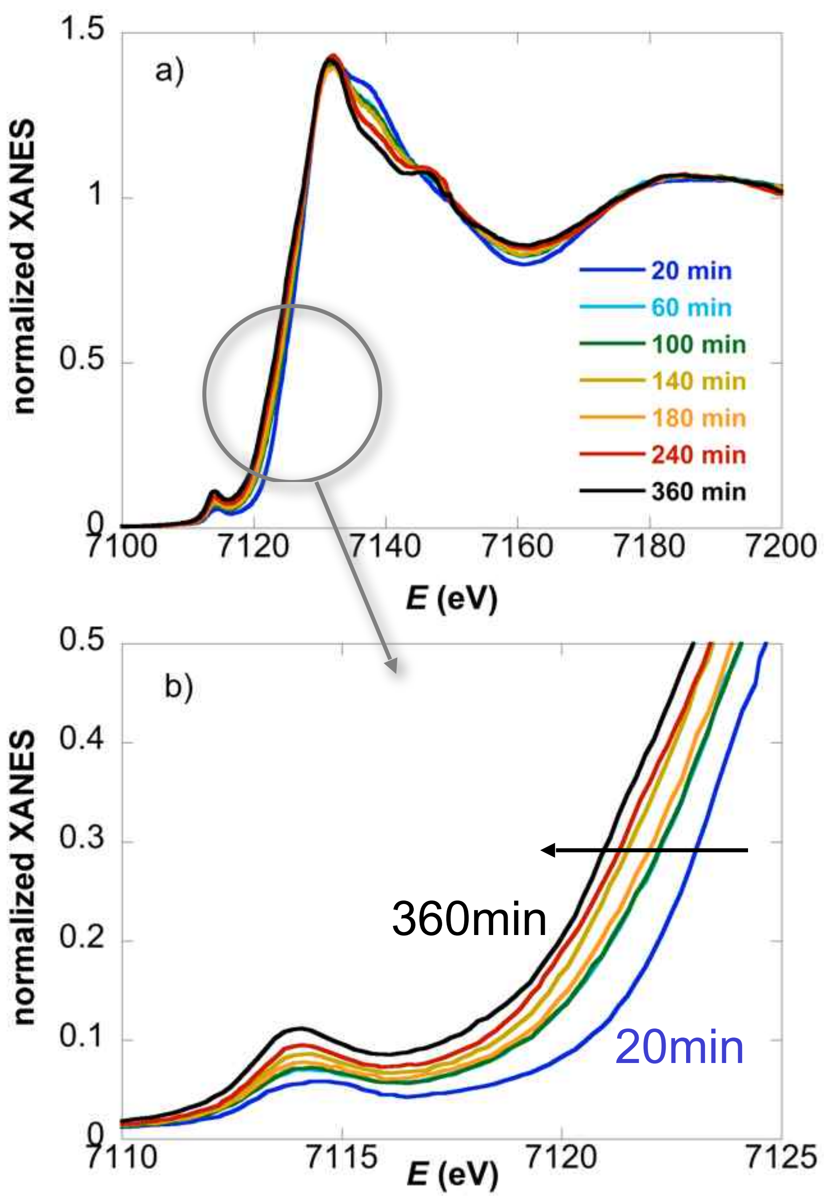


Pre-edge: from a shoulder to a well defined peak

Fe site

from a **centrosymmetric** (broad and low intensity pre-edge) to **non-centrosymmetric site** (narrow and more intense pre-edge peak)

X- ray Absorption Near Egde Structure (XANES)



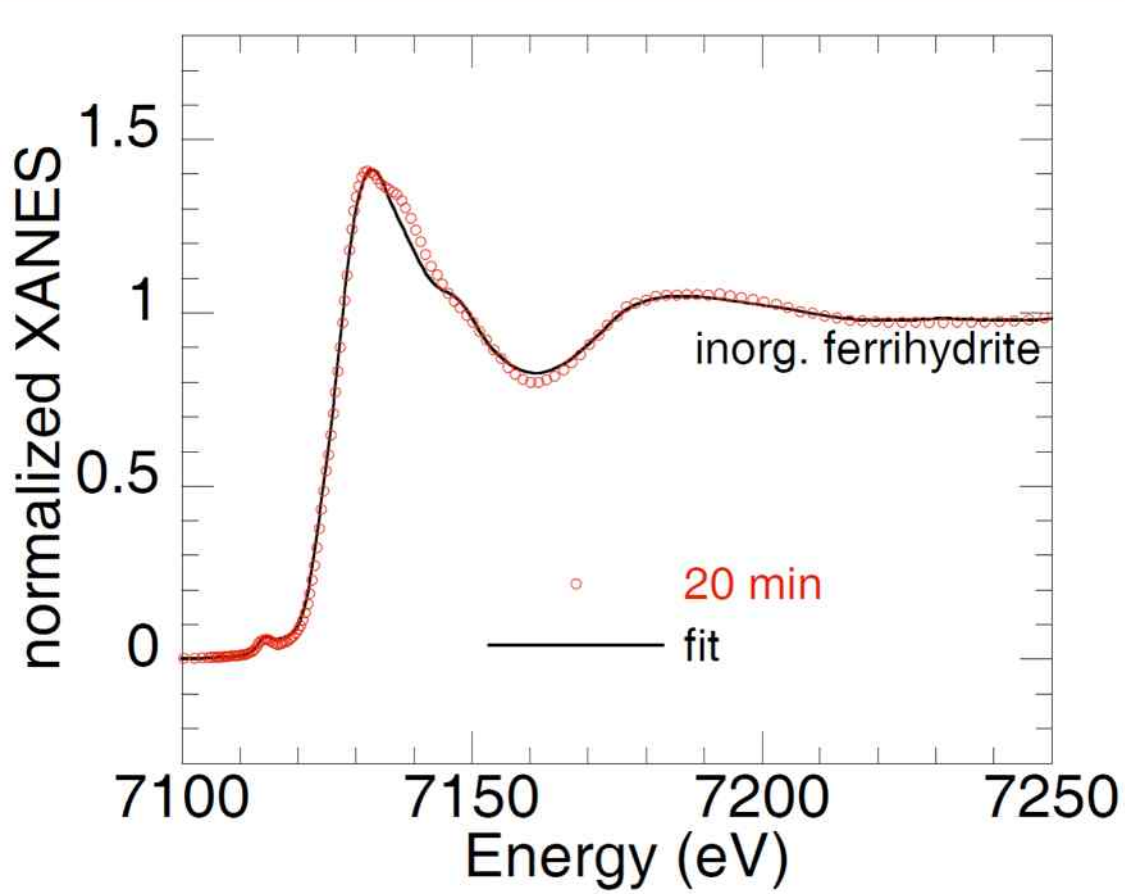
Edge position: move 2 eV

Change in the oxidation state of the Fe atom

X- ray Absorption Near Egde Structure (XANES)

20 min

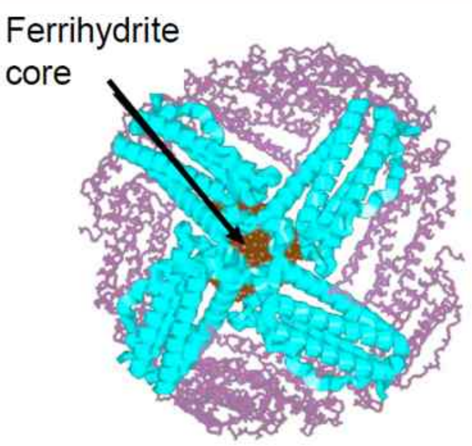
- •Pure Fe^{3+} compound
- •Fits with inorganic ferrihydrite

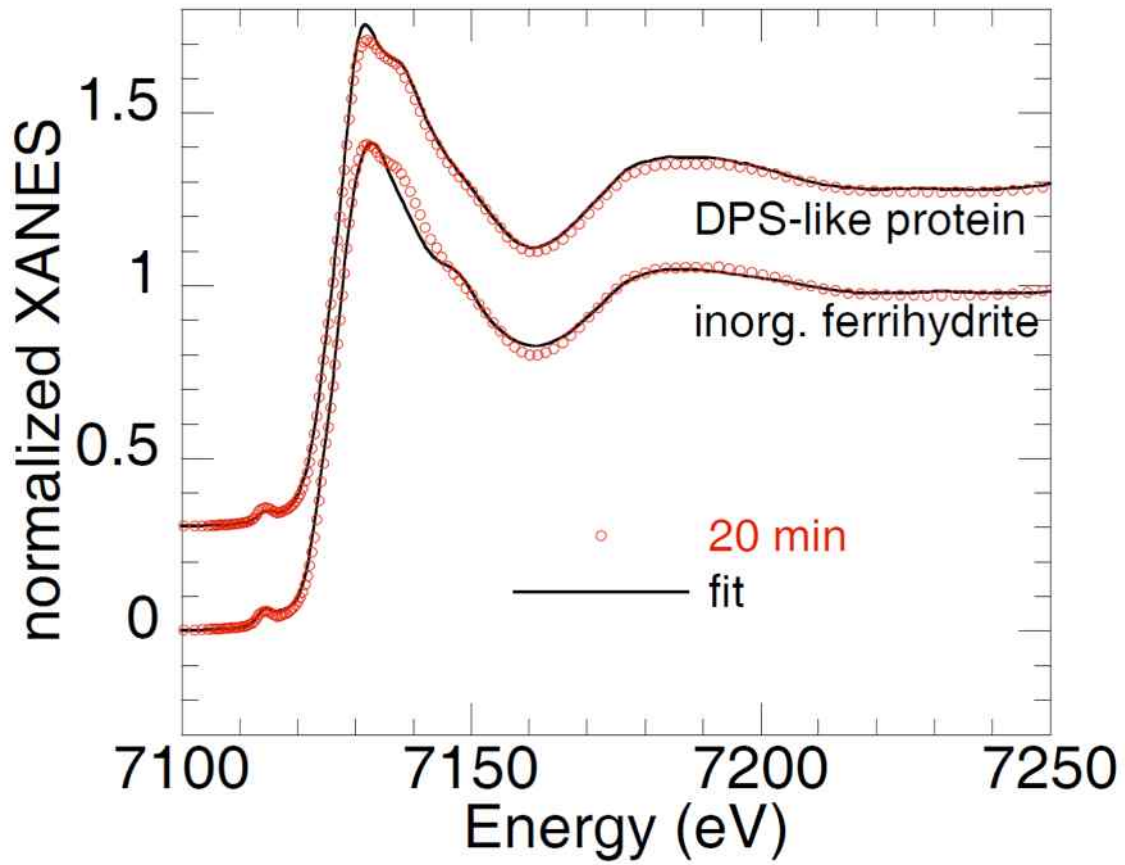


X- ray Absorption Near Egde Structure (XANES)

20 min

- Pure Fe^{3+} compound
- Fits with inorganic ferrihydrite
- But better with bioferrihydrite from ferritin-like protein cores



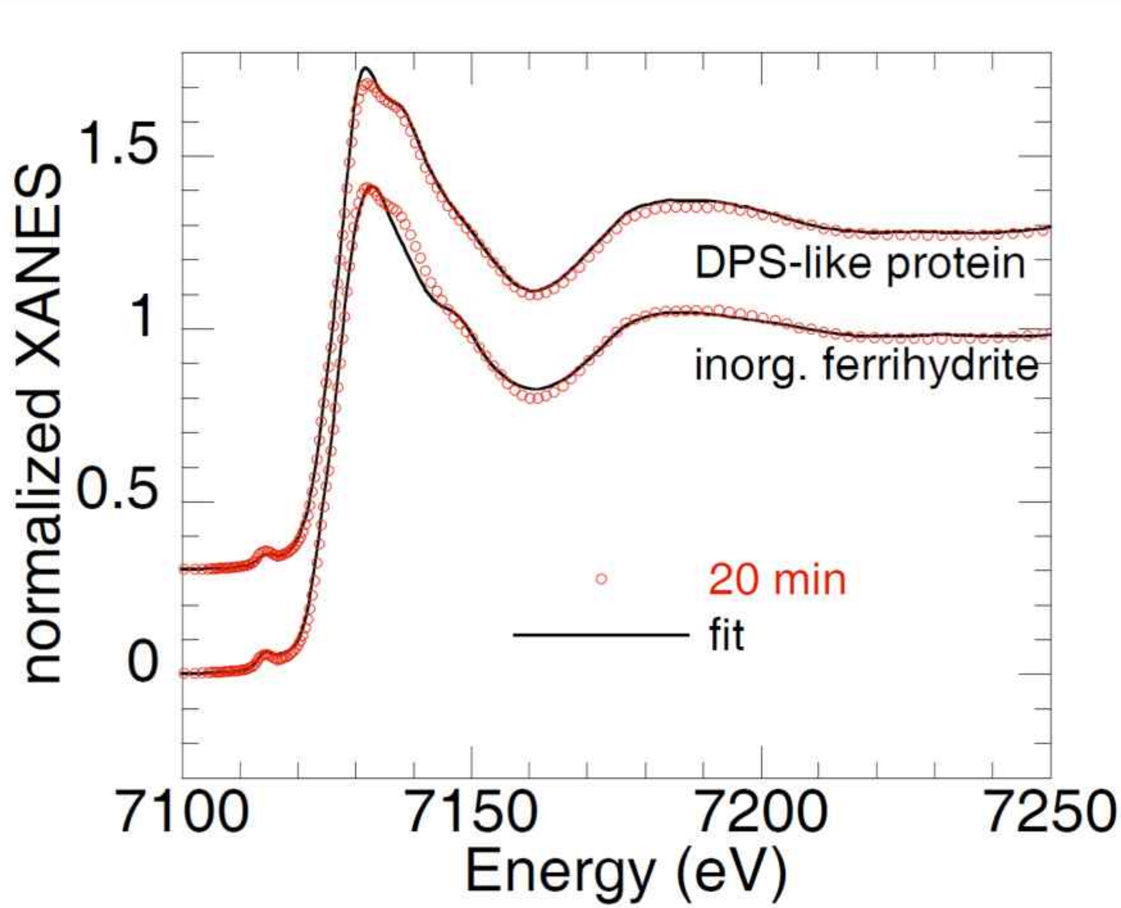


Ferritin protein: spherical protein shell, 12 nm in diameter, encapsulating a nanoparticle-sized core of ferrihydrite with a diameter of up to 8-9 nm

X- ray Absorption Near Egde Structure (XANES)

20 min

- Pure Fe^{3+} compound
- Fits with inorganic ferrihydrite
- But better with bioferrihydrite from ferritin-like protein cores

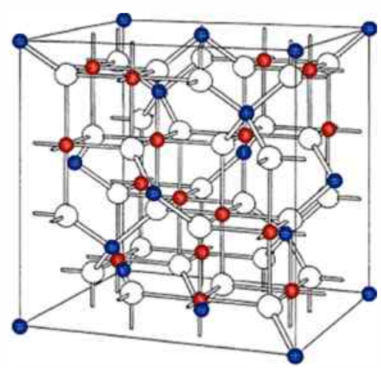


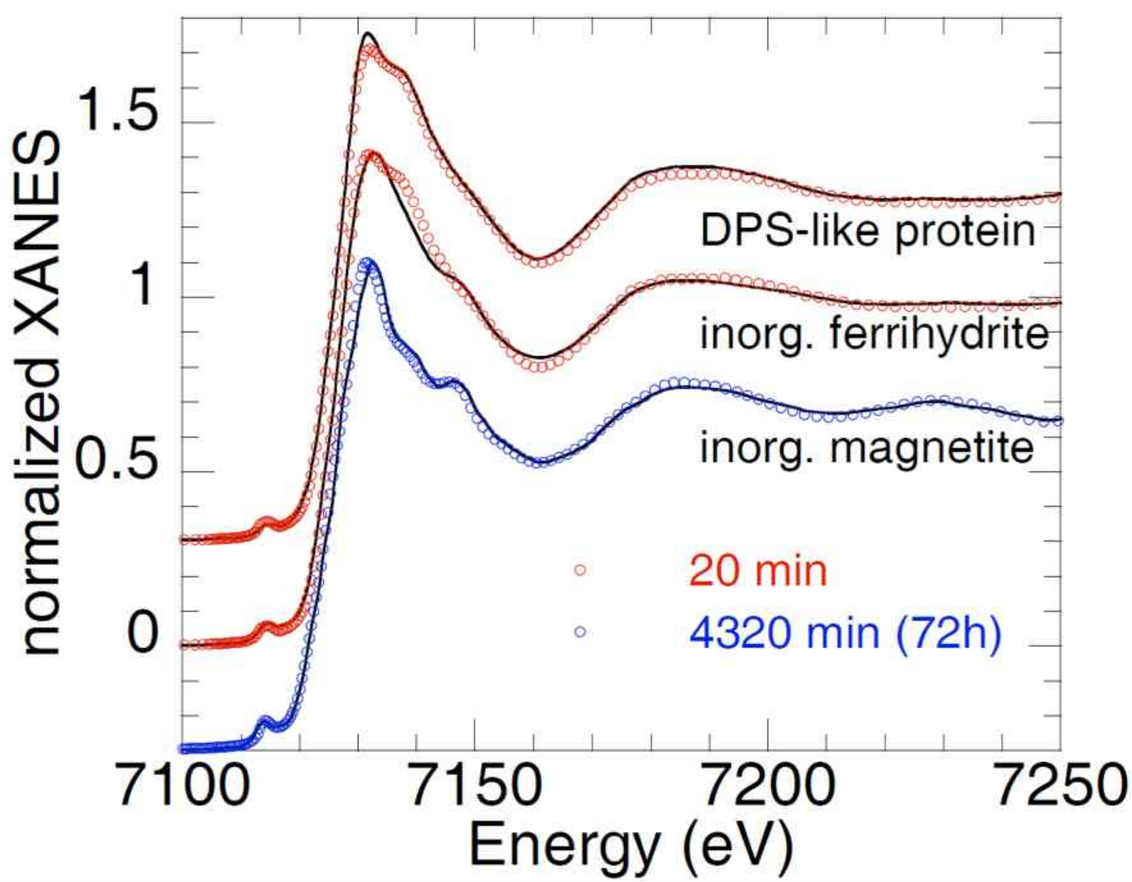
Magnetite biomineralization starts from bacterial ferrihydrite

X- ray Absorption Near Egde Structure (XANES)

4320 min (72 h)

•Pure magnetite (16 Fe^{3+}, 8Fe^{2+})





- 8 Fe^{3+} tetrahedral sites
- 8 Fe^{3+} octahedral sites
- 8 Fe^{2+} octahedral sites

X- ray Absorption Near Egde Structure (XANES)

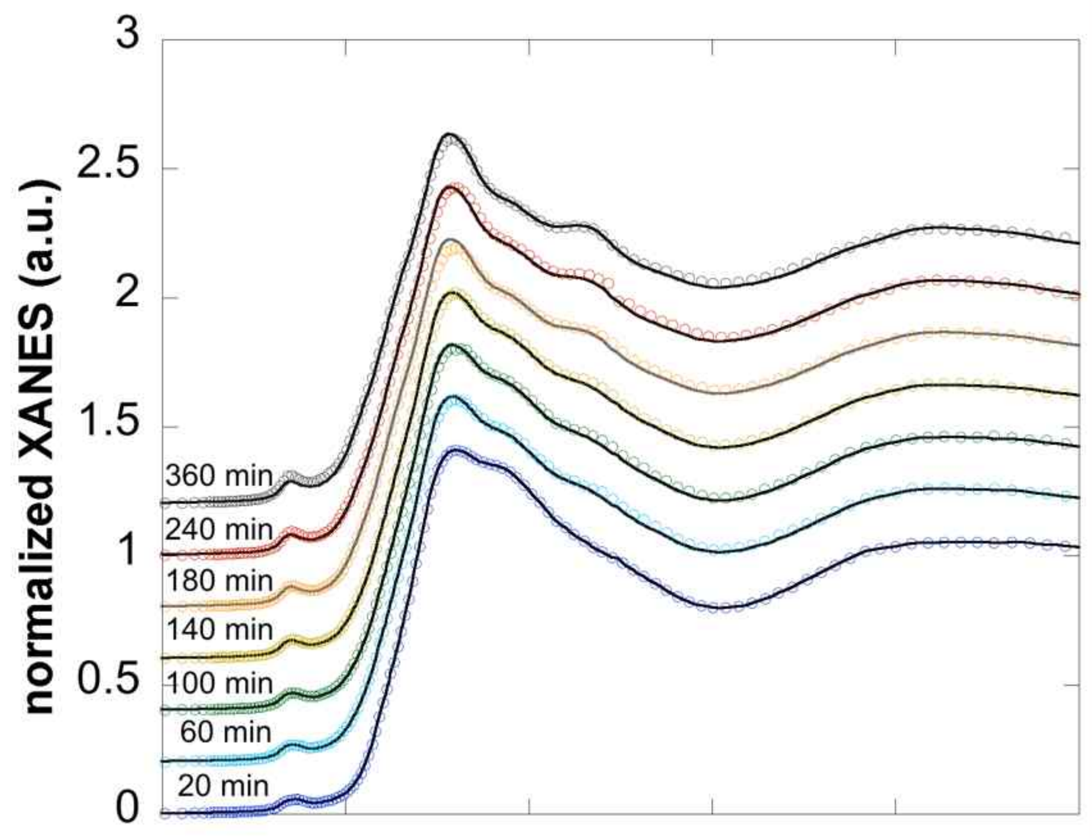
$$\alpha \times \text{bio-magnetite (4320 min)}$$

$$+$$

$$(1-\alpha) \times \text{bio-ferrihydrite (20 min)}$$

↓

α: atomic % of the phase



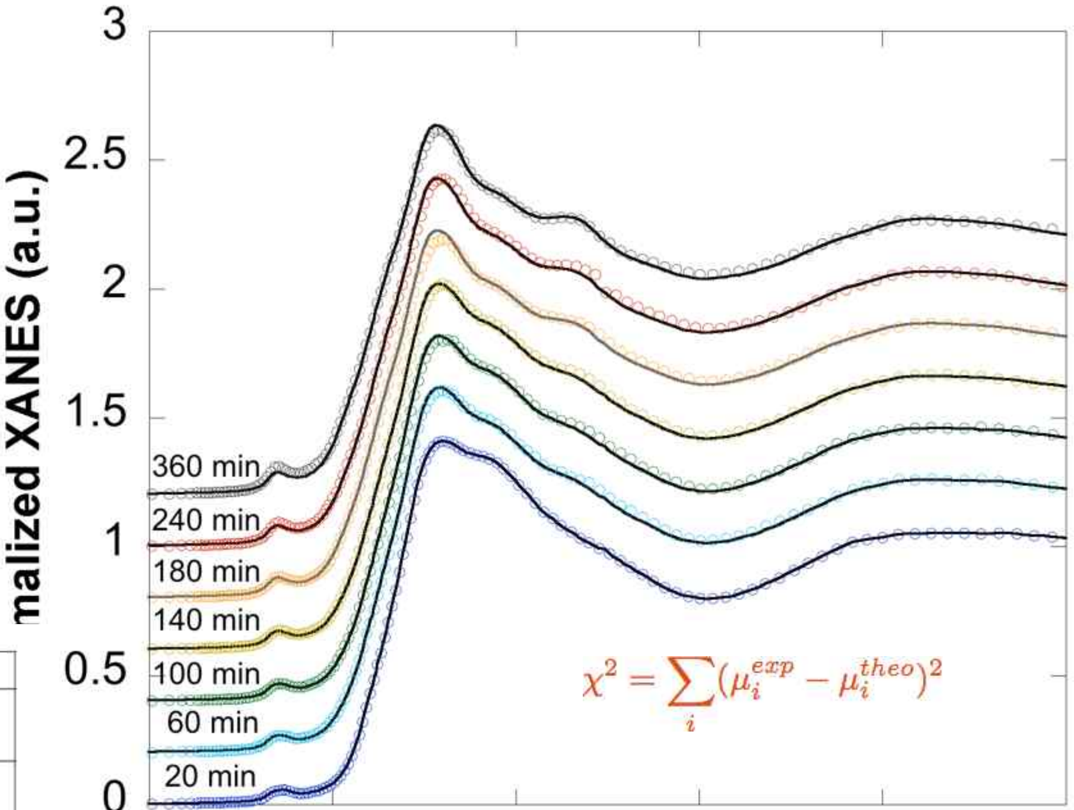
Use bacteria at **20 min (bio-ferrihydrite)** and at **72 h (biomagnetite)** as models to fit the rest of the samples:

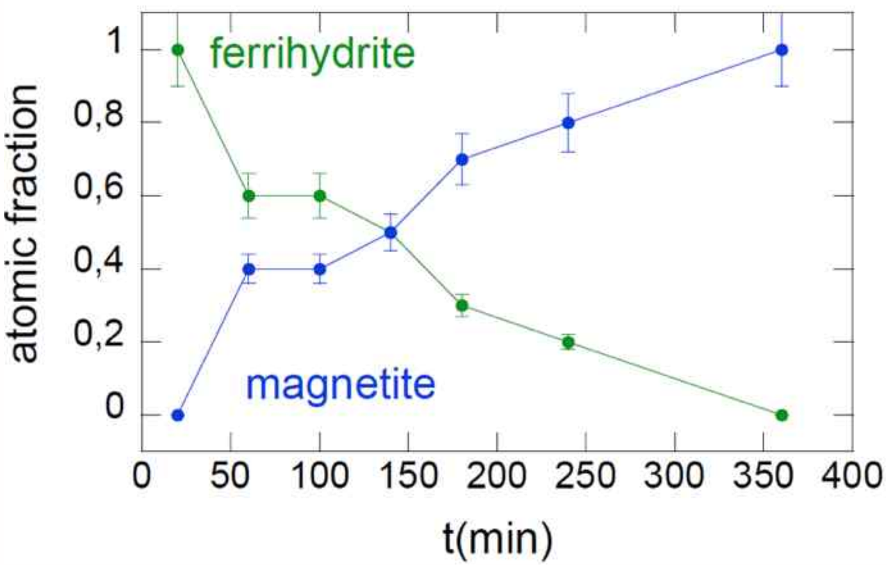
X- ray Absorption Near Egde Structure (XANES)

$$\alpha \times \text{bio-magnetite (4320 min)}$$

+

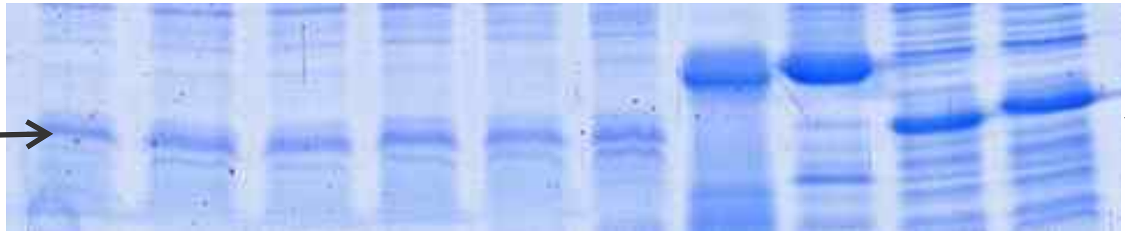
$$(1-\alpha) \times \text{bio-ferrihydrite (20 min)}$$





Atomic fraction of each phase

Gel electrophoresis



19 kDa

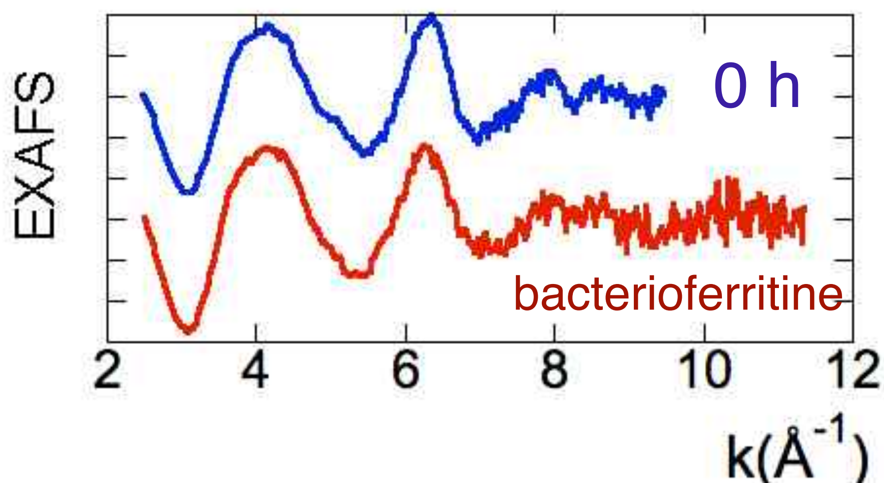
0h 1h 2h 4h 6h 24h

Horse spleen ferritin

E. coli overexpressing (genetically modified)

- archaeon ferritin
- bacterial ferritin
- bacterioferritin

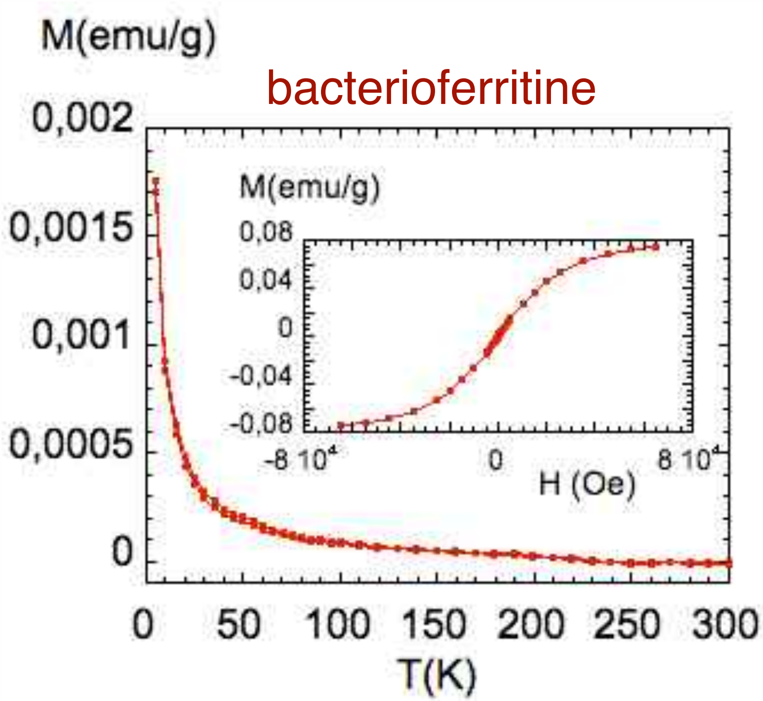
Fe K-edge



Amorphous P-content Fe oxy - hydroxide phase

Fe-Fe: N=3.96 R=1.97Å
Fe-P: N=0.89 R=2.33Å
Fe-O: N= 3.91 R=2.96Å

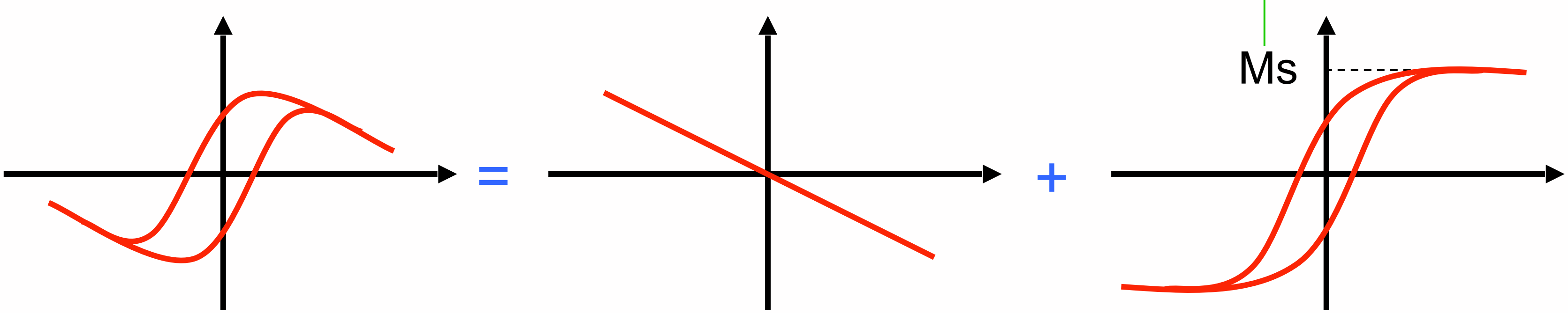
Magnetic measurements



Contribution: (Organic parts, holder) + ferrihydrite+ Magnetite particles

No magnetic order down 5K

Mass of magnetite per cell

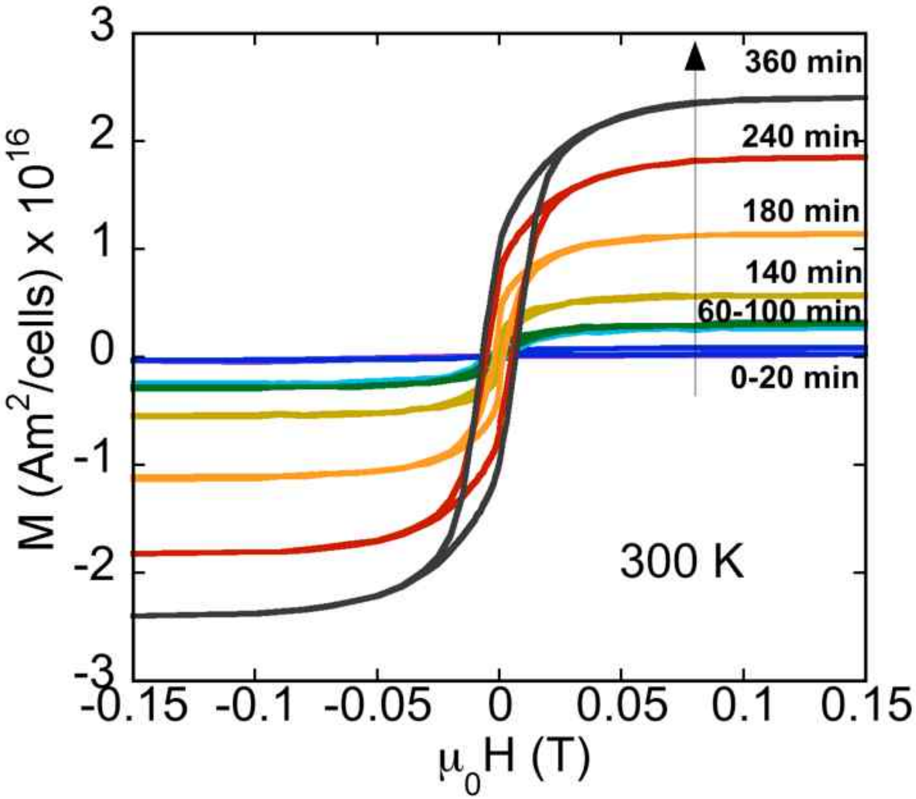


pellet

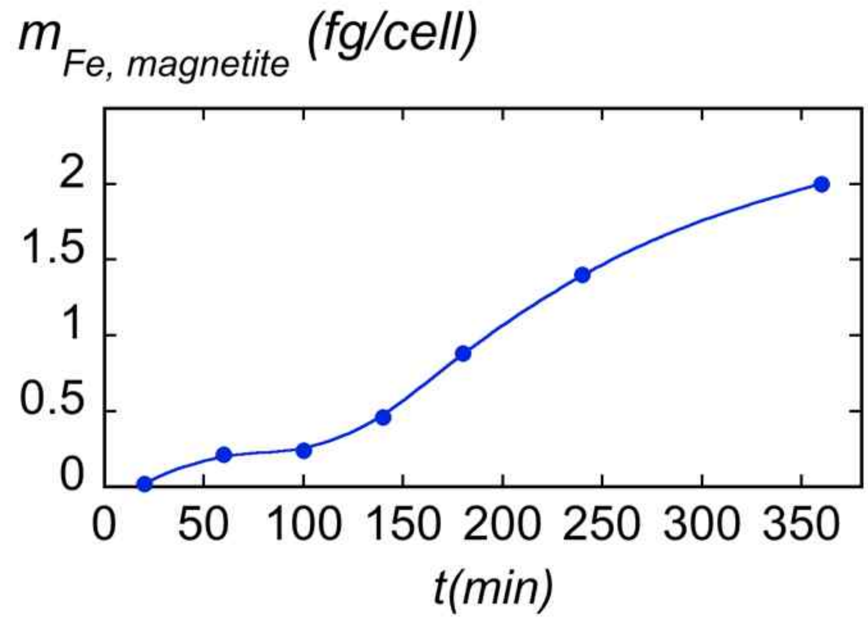
linear signal

Ferrimagnetic (magnetite) signal

Magnetic characterization



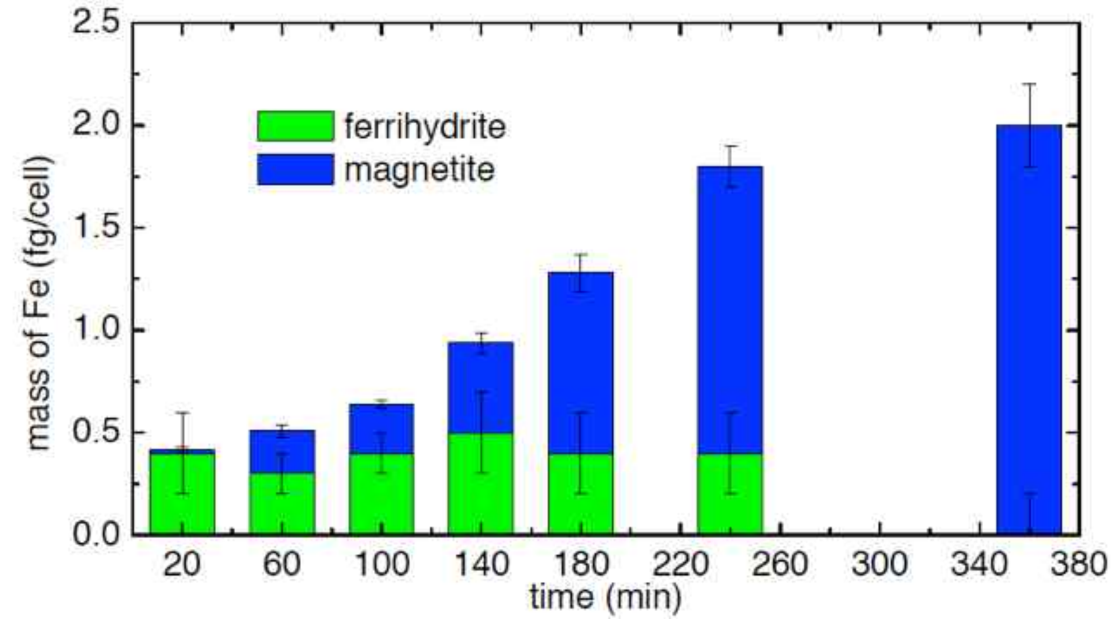
Fe_3O_4 M_s= 92 Am^2/kg



The amount of magnetite increase with the Fe incubation-time

Not all the Fe is in magnetite

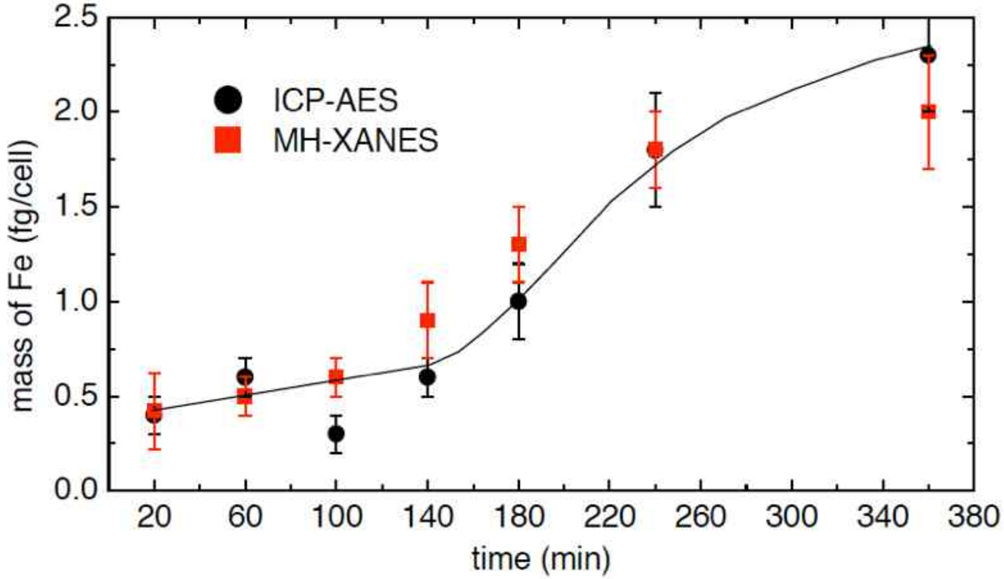
X- ray Absorption Near Egde Structure (XANES)
+
Magnetic analysis



Magnetic analysis: mass of magnetite

XANES: atomic fraction of each phase, α

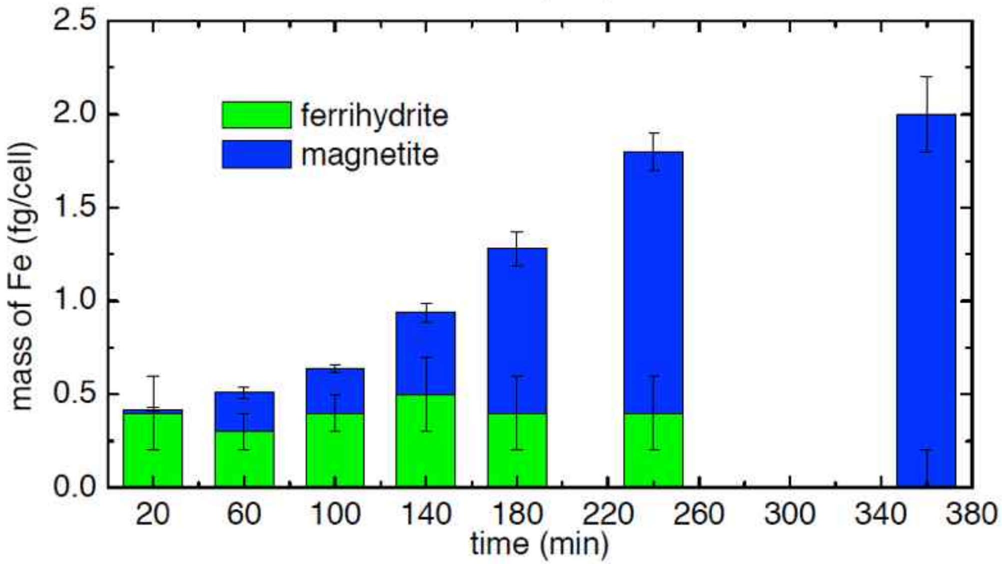
$$m_{Fe}(\text{ferrihydrite}) = \frac{1-\alpha}{\alpha}\, m_{Fe}(\text{magnetite})$$



total mass of Fe in the cell:
ICP-AES

total mass of Fe in the cell:
MH+XANES

•Similar evolution from two different techniques

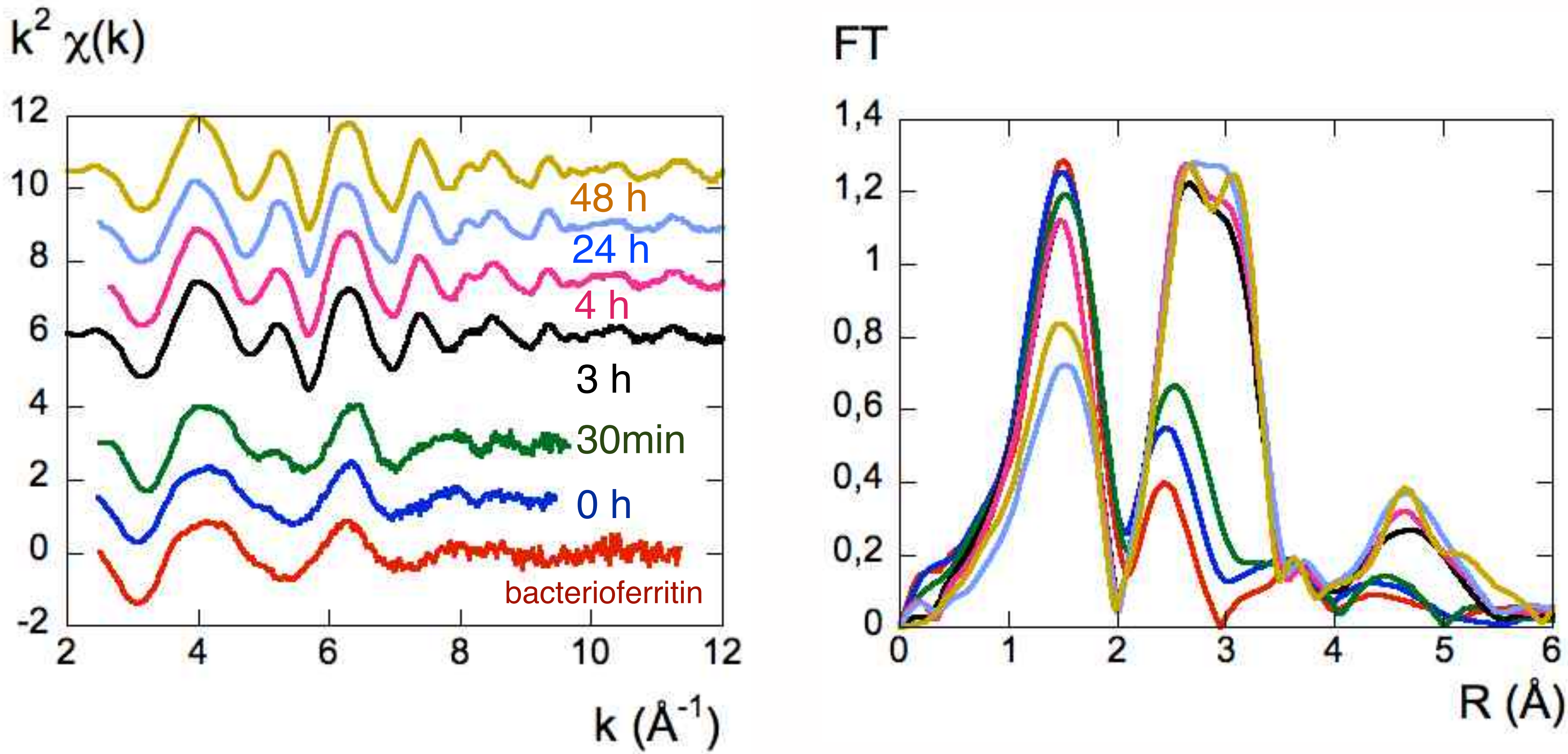


•Two steps:

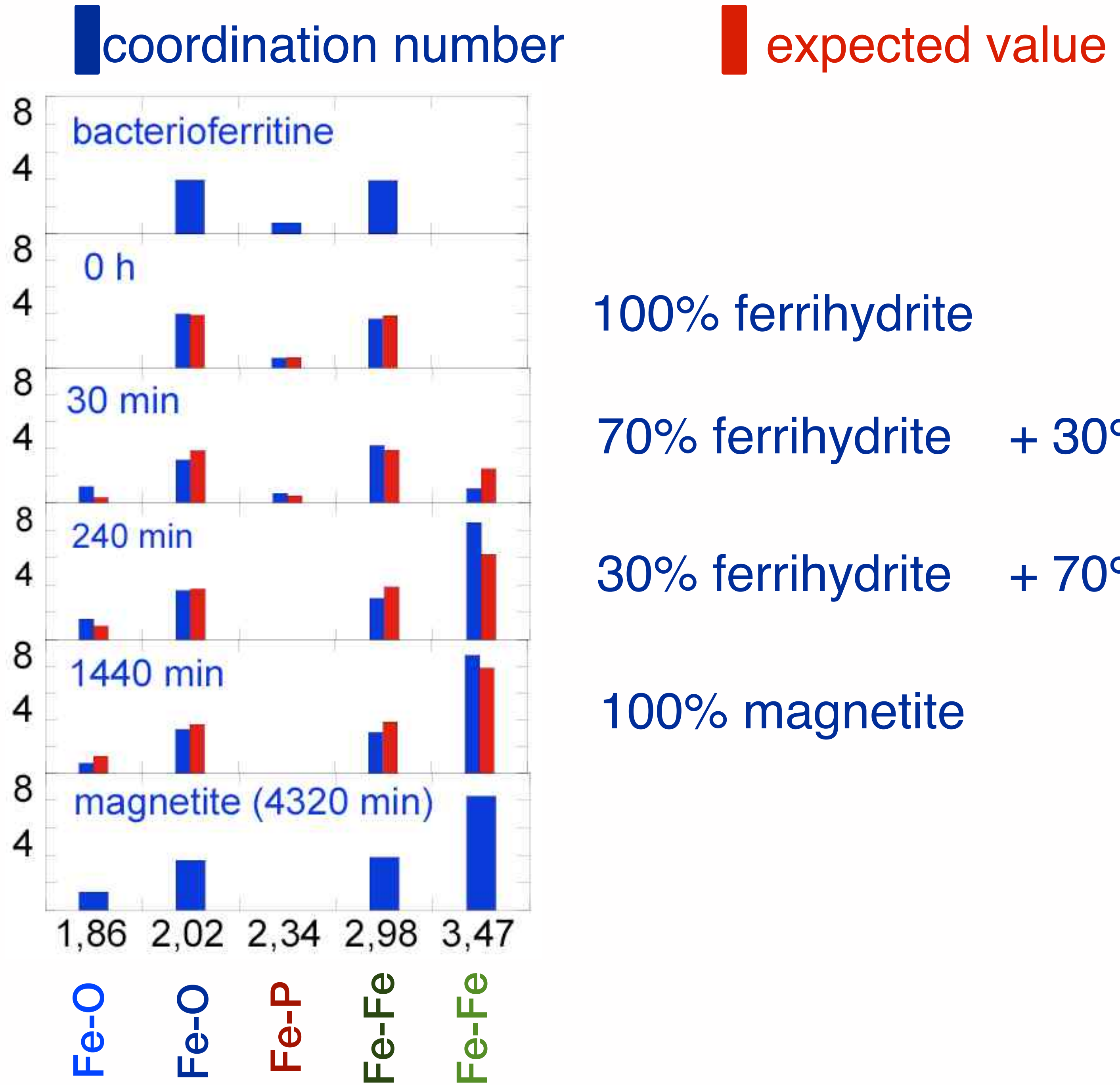
- •Before 140 min – slow – accumulation of bioferrihydrite
- •After 140 min – fast – magnetite biomineralization

Evolution of the structure

EXAFS on the FeK-edge



Fluorescence and Transmission set up. T=100K



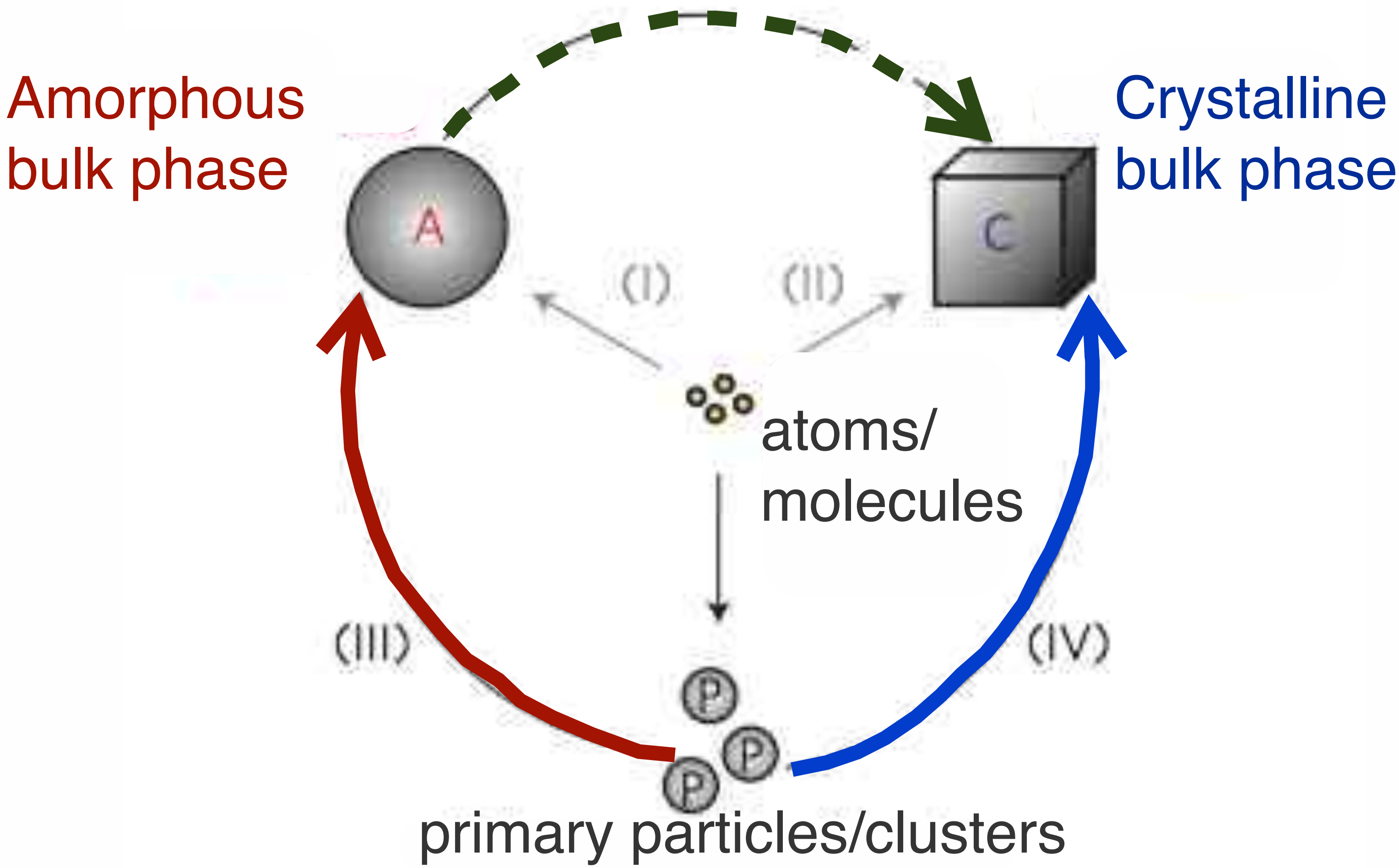
100% ferrihydrite

70% ferrihydrite + 30% magnetite

30% ferrihydrite + 70% magnetite

100% magnetite

Crystallization scenarios

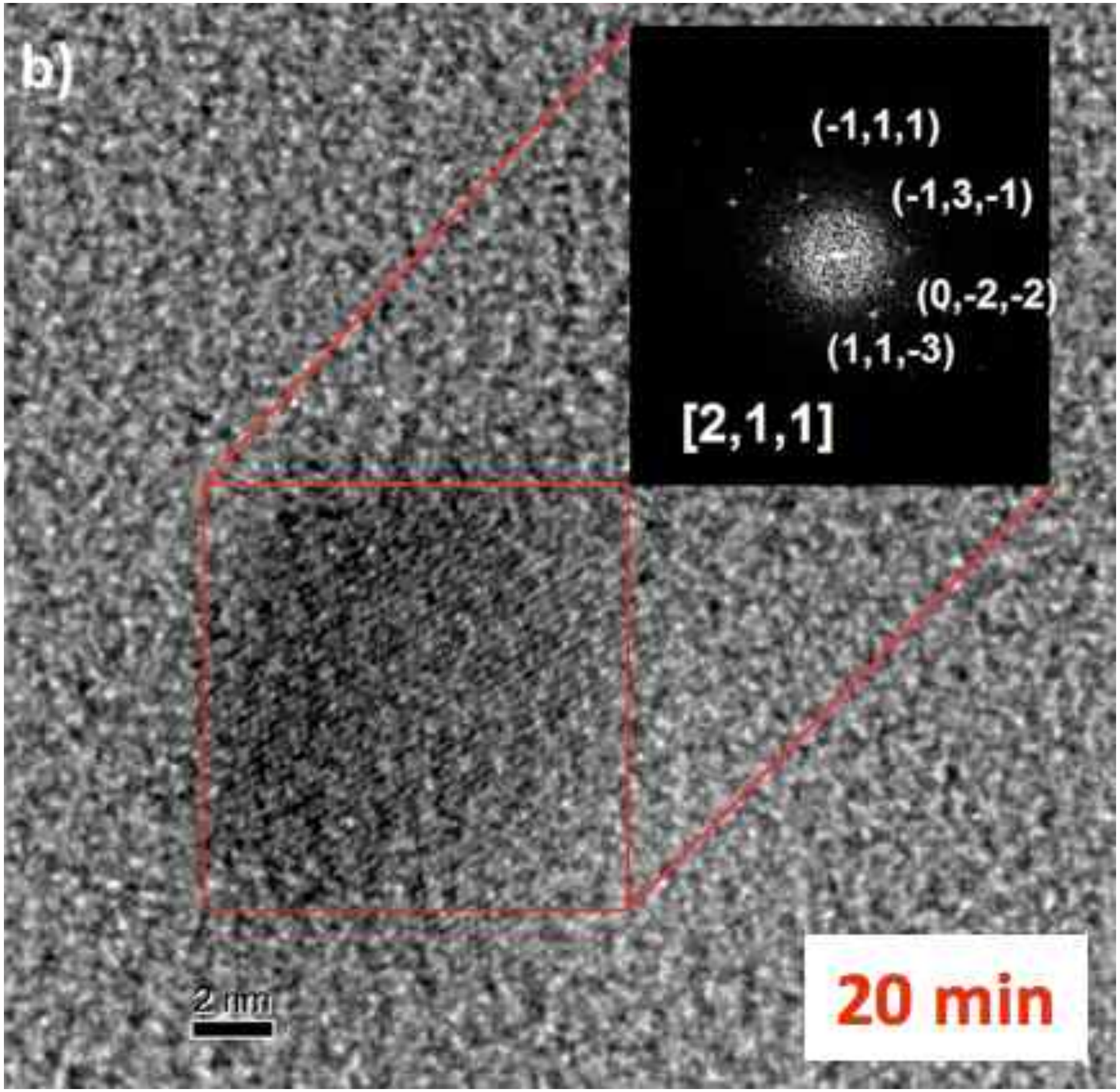


J. F. Banfield et al., Science (2000) **289** 751

J. Baumgartner et al, Nature Materials (2013): agglomeration of primary particles

High-Resolution TEM (FEI Titan)

Where is the ferrihydrite?

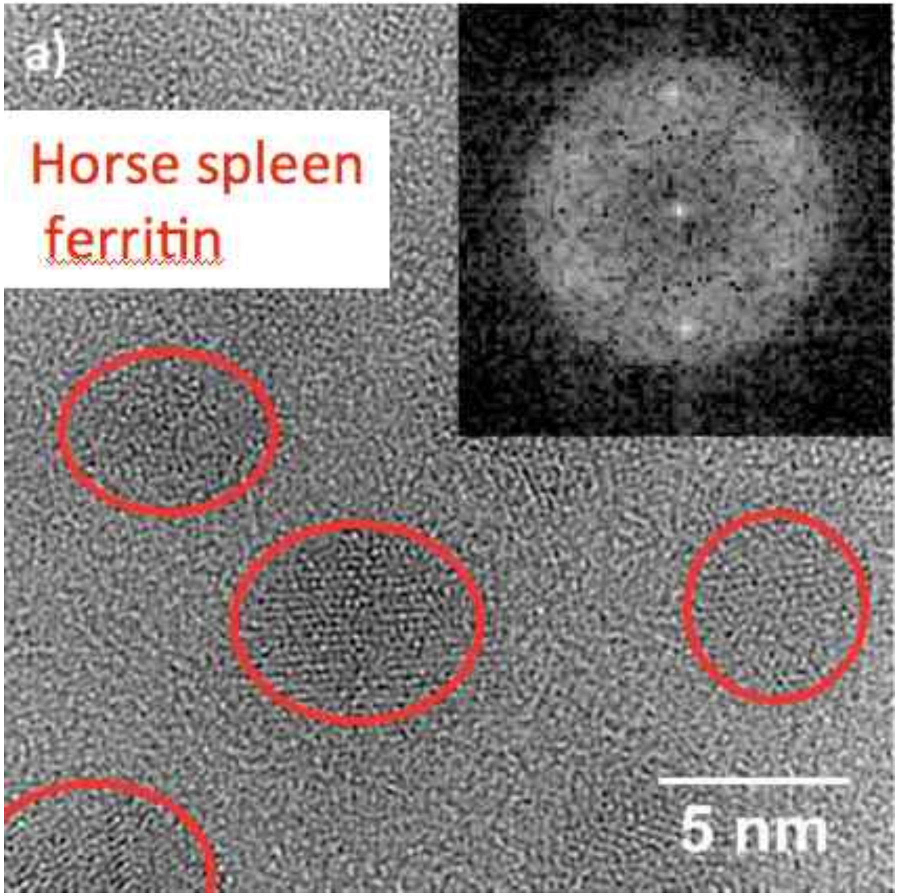


20min: the sample with the highest proportion of ferrihydrate. We only observed few and small magnetite nanoparticle, ≈ 9nm.

No amorphous bulk precursor

High-Resolution TEM (FEI Titan)

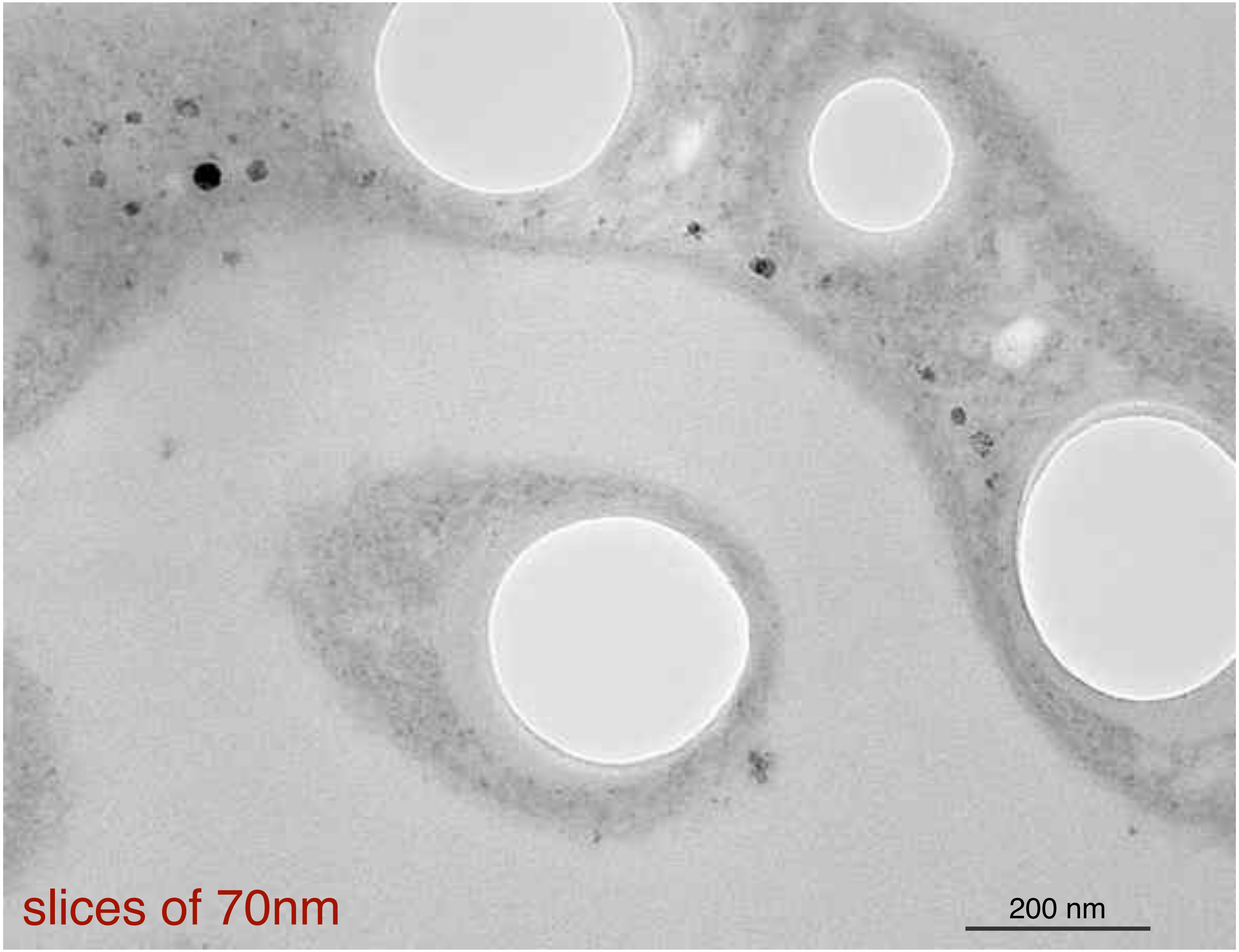
Where is the ferrihydrite?



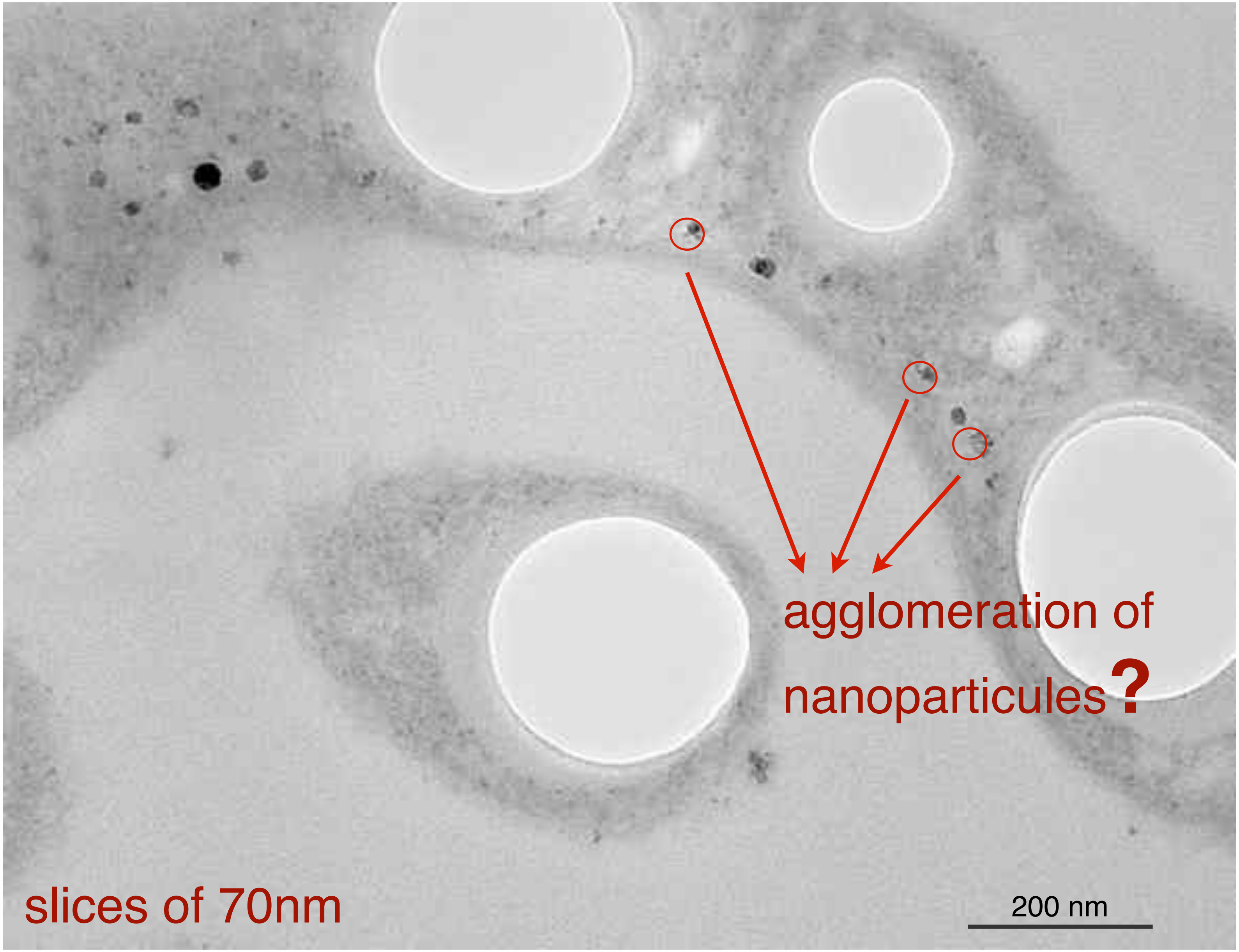
Mammalian ferritin: ferrihydrite core of 5nm

bacterioferritin: ferrihydrite core less dense than mammalian ferritins because of their larger phosphorous content.
Lower degree of crystalinity

micro-tomo



micro-tomo



Conclusions

- **Magnetosomes** are magnetic nanoparticles of high structural and chemical purity:

  - **Single domain (≈45nm)**
  - **Well defined shape**
  - **Narrow distribution size**
  - **Covered by lipid bilayer membrane:**

    - Biocompatible
    - Easy to functionalize
    - Avoid aggregation

→ **Biomedical applications: hyperthermia, drug delivery**

Biomineralization process: Ferrihydrite with the same structure of bacterioferritin cores

↓

magnetite

Two steps:

1. Fe accumulated in the form of ferrihydrite
2. Magnetite is rapidly biomineralize from ferrihydrite

M. Luisa Fdez-Gubieda et al, ACS Nano vol. 7 n.4 3297 (2013)

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Thanks for your attention

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