# Magnetite biomineralization in Magnetospirillum gryphiswaldense: a time resolved X-ray Absorption Spectroscopy study

**M. Luisa Fdez-Gubieda**[1], Alicia Muela[2], Javier Alonso[1,3], Ana García-Prieto[4], Luca Olivi[5], Rodrigo Fernandez-Pacheco[6], and José Manuel Barandiaran[1,3]

[1]Departamento de Electricidad y Electrónica, UPV/EHU, Barrio Sarriena s/n, 48940 Leioa (Spain)
[2]Departamento de Inmunología, Microbiología y Parasitología, UPV/EHU, Barrio Sarriena s/n, 48940 Leioa (Spain)
[3]BCMaterials, Camino de Ibaizabal, Building #500, Technological Park of Biscay, 48160 Derio (Spain)
[4]Departamento de Física Aplicada I, UPV/EHU, Rafael Moreno Pitxitxi, 2, 48013 Bilbao (Spain)
[5]XAFS Beamline, Elettra Sincrotrone, AREA Science Park 34149 Basovizza, Trieste (Italy)
[6]Laboratorio de Microscopías Avanzadas - Instituto de Nanociencia de Aragón (LMA-INA), Universidad de Zaragoza, C/ Mariano Esquillor s/n, 50018 Zaragoza (Spain)
javier.alonso@ehu.es

**Abstract**
Magnetotactic bacteria biosynthesize magnetite nanocrystals (magnetosomes) with high chemical purity, species-specific crystal morphology on shape and size, and narrow size distribution. These magnetosomes allow the bacteria to orient and navigate along the geomagnetic field lines. The interest in these bacteria has recently increased because of the potential biomedical applications of the magnetosomes [1].

A good understanding of the biomineralization process is key for addressing challenges as the design of new materials. In general, little is known about the biomineralization process of the bacteria, but several steps have been identified [2]: (i) magnetosome vesicles are formed, (ii) iron is taken up from the environment, (iii) iron is transported into the magnetosome vesicle, and finally (iv) iron is nucleated and precipitated in the form of magnetite.

Different models have been proposed regarding this last step: Frankel et al.[3] suggest that magnetite is formed from a ferrihydrite precursor, while Faivre et al. [4] do not find evidence of the existence of a mineral precursor, and suggest as a possible mechanism of magnetite biomineralization a fast co-precipitation of Fe2+ and Fe3+ ions within the magnetosome vesicle. A different mechanism is proposed by Staniland et al. [5], who found a shell of an Fe oxide, hematite (a-$Fe_2O_3$), around the magnetite particles, which they suggest acts as the precursor of magnetite.

In our case, we have studied the biomineralization process of the magnetotactic bacteria Magnetospirillum gryphiswaldense strain MSR-1 by the combination of magnetic and structural techniques in a time-resolved study. These techniques have allowed the identification of two Fe phases (magnetite and ferrihydrite) and, more importantly, measurement of the mass of each phase during the mineralization process. Besides more commonly used techniques like Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), magnetic analysis, and Transmission Electron Microscopy (TEM), we have performed Fe K-edge X-ray Absorption Near Edge Spectroscopy (XANES) and High resolution Transmission Electron Microscopy (HRTEM).

TEM images have revealed that at early stages, most of the bacteria do not present nanoparticles, and only occasionally some isolated nanoparticles can be observed. As the time after Fe incubation increases, these nanoparticles organize in small "subchains" containing around 3-6 nanoparticles. With increasing time, these "subchains" become more frequent and closer together, and the size of the nanoparticles steadily increases. From t = 240 min, some long chains of several nanoparticles (> 6) are formed by the union of these small "subchains".

In order to identify the different Fe phases present in the bacteria at specific times after Fe incubation, we have performed Fe K-edge X-ray Absorption Near Edge Structure (XANES) experiments at the XAFS beamline of the Elettra Synchrotron (Italy). Figure 1 a) shows an evolution of the XANES spectra during the biomineralization process.
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The edge position of these spectra is a clear-cut indication of the oxidation state of Fe. The edge position of the first bacterial sample, t = 20 min, is coincident with a pure $Fe^{3+}$ compound. As the biomineralization process evolves, the edge displaces 2 eV to lower energies, indicating that the Fe is reducing as the process evolves, from $Fe^{3+}$ to the presence of both $Fe^{3+}$, $Fe^{2+}$ oxidation states whose

relative amounts change with the time elapsed after Fe incubation. We found that the XANES spectrum of first bacterial sample, t = 20 min, can be clearly identified as bacterial ferrihydrite, and thereby it was chosen as a reference compound for biogenic bacterial ferrihydrite for the rest of the samples.

The spectra of the bacteria at specific times after Fe incubation were then fitted to a linear combination of both biogenic references, ferrihydrite (t = 20 min) and magnetite (t = 4320 min (72 hours)), as shown in Figure 1 b). As a result of these fits we have been able to quantify the atomic fraction of ferrihydrite and magnetite at each time after Fe induction incubation, as indicated in Figure 2. From these data it can be readily seen that at the beginning of the biomineralization process mostly ferrihydrite is present in the cells, which transforms progressively to magnetite as the process evolves.

**References**


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**Figures**

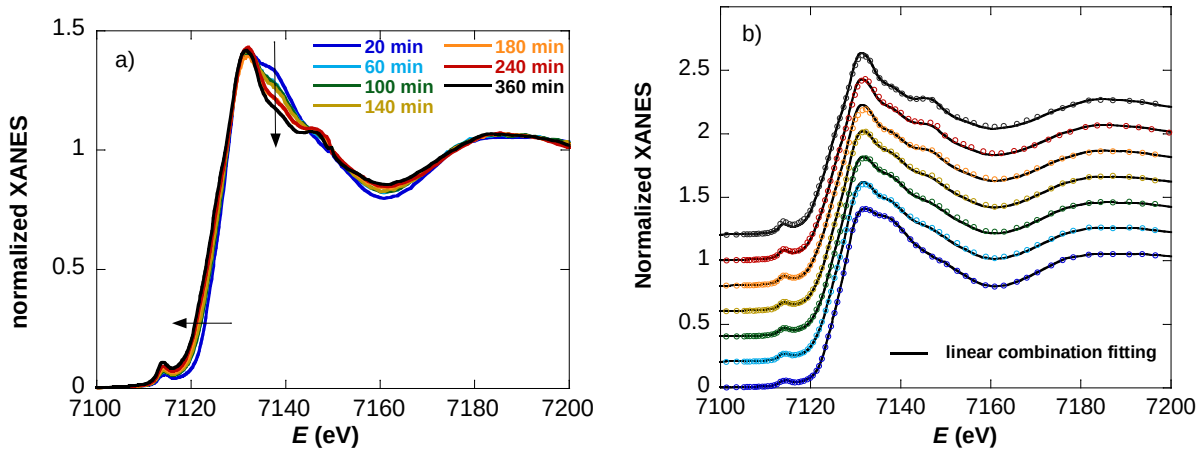


**Figure 1**: a) Normalized XANES spectra at Fe K-edge obtained for the samples at specific times after Fe incubation. b) XANES spectra and their corresponding fits for the bacteria obtained at every time after Fe induction.

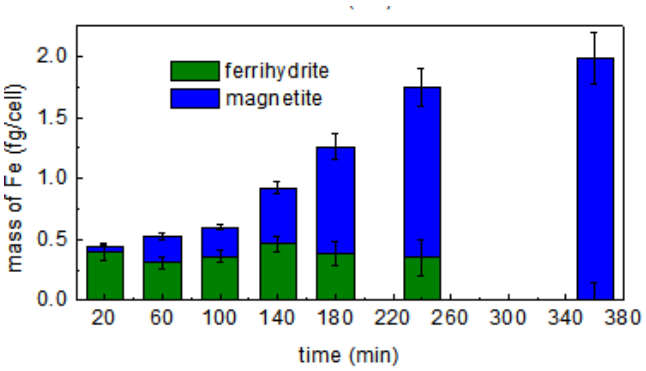


**Figure 2:** Distribution of the mass of Fe per cell in the ferrihydrite and magnetite phases as determined by the combination of magnetic and XANES results.