ESRF	**Experiment title:** Chemical Structure of Magnetite Precursor Clusters Formed under Biomimetic and Biological Conditions	**Experiment number**: SC-3451
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Report:

Here we report on our X-ray spectroscopy (XAS) experiments at ID26 on magnetite precursor structures in magnetotactic bacteria and a biomimetic synthetic system. In this study we measured quasi-time-resolved XANES, pre-edge and EXAFS spectra of cryo-quenched synthetic as well as biogenic magnetite and its precursors.

Experimental Procedure

Synthesis. Magnetite time-course synthesis samples were precipitated on site at pH=9. Samples (100 µL) were taken at consecutive time intervals, mixed with 25 µL glycerol and frozen in liquid nitrogen on sample holders.

Bacteria. Magnetotactic bacteria were sampled at consecutive time points and centrifuged.after induction of magnetite biomineralization. The pellets were re-suspended in 100 µL TBS plus 25 µL glycerol and frozen in liquid nitrogen on sample holders with Kapton film support. Samples were stored at -80°C, sent to ESRF on dry ice where they were stored at -80°C until measurement.

XAS. Fe K-edge X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) were recorded in fluorescence mode at beamline ID26 of the European Synchrotron Radiation Facility (ESRF). During measurements samples were cooled to 20 K using a liquid He cryostat. XANES spectra and pre-edge spectra were recorded with 0.1 eV resolution from 7100 to 7200 eV and from 7103 to 7122 eV, respectively. EXAFS spectra were recorded up to maximum $k \approx 12$ Å^{-1}. Recorded spectra were averaged using PyMca 4.6.0 after evaluation for photo-reduction and other artefacts. Averaged XAS spectra were then normalized using Athena 0.8.059. Linear combination fitting of XANES data as well as EXAFS data extraction were performed with the respective functions of Athena.

Results

Figure 1 shows a selection of data recorded by our team at ID26 in June 2012. Figure 1A shows Fe K-edge raw XANES spectra (before normalization) of biogenic and synthetic time series samples. Linear combination fitting (LCF) with reference standards of the complete series of obtained spectra for the biogenic time-series samples revealed that they contain likely three major different pools of iron, which change their spectral contributions over the time-course of biomineralization (Figure 1B): basal Fe in the cells before induction, a precursor phase and the growing magnetite phase. Unfortunately, the ESRF-provided sample holders contained trace-amounts of iron, which led to contamination of the very dilute early stage spectra (Figure 1A). Due to preparative constrains with the bacteria, this issue could not be fully resolved during our beamtime for the biogenic samples.
Finally, we could obtain good quality EXAFS up to $k \approx 12$ Å^{-1} for bacterial samples and late stage synthetic samples (Figure 1C,D) (higher k were not accessible due to instabilities above 7650 eV). Only the early stage synthetic samples were of too dilute nature (80 µM $\approx$ 4 ppm wt. Fe) to obtain spectra with usable signal-to-noise ratio (Figure 1C, Fe3O4 1min). Also EXAFS data confirms the disorder-order transition in the biogenic system as no clear major second shell (Fe-Fe) contribution is visible in the radial structure function of early stage bacterial samples (Figure 1C, MTB 30min), whereas EXAFS of late stage bacteria resemble synthetic magnetite (Figure 1E and F, MTB 46h and Fe3O4 60min).

Conclusions and Outlook

XANES of the Fe-induction time series in bacterial cells shows that (i) the Fe concentrations even in maximally iron-depleted cells are sufficiently high to obtain good quality spectra, (ii) the Fe-compound mixture in the cells seems to comprise only two major significant species relevant in the biomineralization, which (iii) thus allows determination of full phase transformation kinetics.
XANES of the synthetic system evidences characteristic differences to the biogenic system in terms of structure and iron oxidation state. EXAFS confirms that the early stage precursors are strongly disordered. Further detailed EXAFS data analysis is currently in progress; however, precise identification and characterization of the precursor species will require further high quality measurements beyond $k \approx 12$ Å^{-1} for biogenic samples and at least to $k = 12$ Å^{-1} for the synthetic abiotic system.

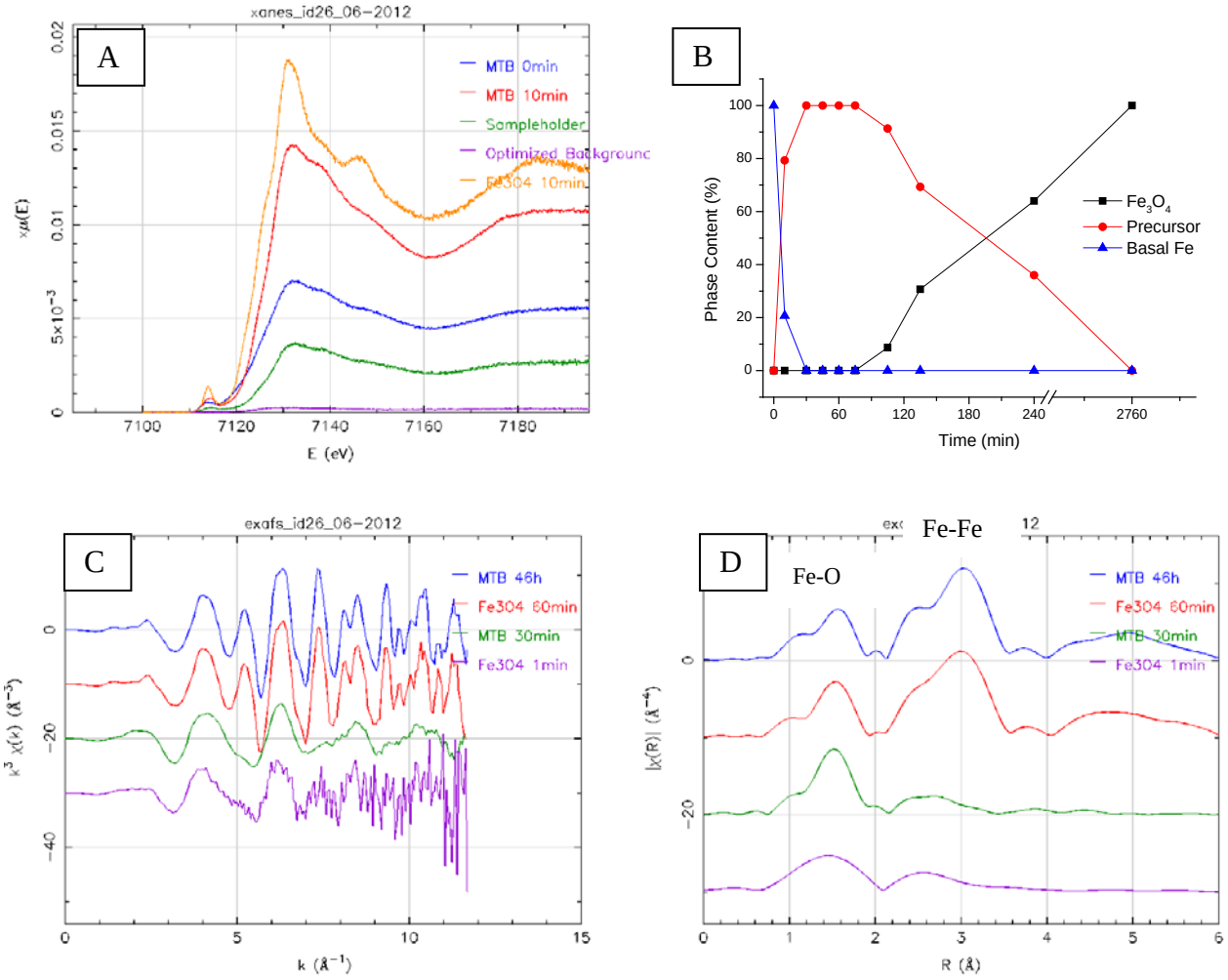

Figure 1. Selected Fe K-edge XANES and EXAFS data. (A) Examples of XANES data before normalization to an edge-jump of 1 illustrating the possible intensity-contribution of the sample holder to early stage spectra (MTB 0 min and 10 min) as well as the ideally achievable background (optimized background) as obtained in the synthetic experiment (B) phase development over Fe induction time in the bacteria as determined by LCF, (C) (D) Selected EXAFS data in k-space and R-space.

References

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