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# Controlled biomineralization of magnetite ($Fe_3O_4$) by *Magnetospirillum gryphiswaldense*

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**ABSTRACT**

Results from a study of the chemical composition and micro-structural characteristics of bacterial magnetosomes extracted from the magnetotactic bacterial strain *Magnetospirillum gryphiswaldense* are presented here. Using high-resolution transmission electron microscopy combined with selected-area electron diffraction and energy dispersive X-ray microanalysis, biogenic magnetite particles isolated from mature cultures were analysed for variations in crystallinity and particle size, as well as chain character and length. The analysed crystals showed a narrow size range (~14−67 nm) with an average diameter of 46±6.8 nm, cuboctahedral morphologies and typical *Gamma* type crystal size distributions. The magnetite particles exhibited a high chemical purity (exclusively $Fe_3O_4$) and the majority fall within the single-magnetic-domain range.

## Introduction

Biomineralization, the processes by which living organisms induce mineral formation, is widespread on Earth (e.g. Bäuerlein, 2000; Lowenstam and Weiner, 1989; Weiner and Dove, 2003). Among the organisms that form true biominerals, magnetotactic bacteria (or MTB) are considered to be model species for studying the controlled biomineralization of Fe oxide and sulphide nanocrystals in living organisms. The MTB are a fascinating group of microbes that have the peculiar ability to orient themselves and migrate along the magnetic field lines of Earth's magnetic field. This ability is based on unique nanosized organelles (magnetosomes), which are membrane-enclosed intracellular crystals of magnetic iron minerals (i.e. magnetite: $Fe_3O_4$ or greigite: $Fe_3S_4$) that are usually arranged into long intracellular chains (Bazylinski, 1999; Bazylinski *et al*., 1995; Pósfai *et al*., 1998). The biomineralization of magnetite and greigite by MTB plays a significant role in environmental iron cycling. The MTB magnetic minerals affect the magnetization of sediments and act as proxies for the geological record of orientation of the Earth's magnetic field. The crystallographic characteristics of magnetosome-derived magnetite crystals reveal very high quality magnetic properties and therefore magnetosome-bound magnetite crystals are increasingly sought after as novel nanoparticulate biomaterials for industrial and medical applications (e.g. Lang and Schüler, 2006). Yet, in the past decade, this also created the need for a better characterization of the particle quality (i.e. purity, single domain etc.). In this study, results of detailed mineralogical, chemical and microstructural characteristics of biogenic magnetite crystals produced by the magnetotactic bacterial strain *Magnetospirillum gryphiswaldense* are presented.

## Methods and materials

Magnetosomes of the *M. gryphiswaldense* strain (DSM 6361) were extracted from mature cultures with 1 M NaOH, for 10 min at 95ºC, followed by magnetic collection. The morphologies, size distributions and ultra-structural characteristics of the extracted magnetite particles were analysed by transmission electron microscopy (TEM)

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(JEOL 1200 EX TEM, operated at 80 kV and Philips CM200 FEG TEM equipped with a Gatan Imaging Filter, operated at 200 kV). Together with high-resolution TEM (HR-TEM) micrographs, the selected area electron diffraction (SAED) patterns and energy dispersive spectroscopy (EDS) spectra of the magnetite crystals were recorded and combined with standard powder X-ray diffraction (XRD), providing quantitative data on crystallinity, structure and composition of the crystals. In addition, non-extracted MTB samples were also studied with TEM in order to gain an *in situ* insight into magnetosome chain length and character in this strain. Finally, crystal outlines from the TEM micrographs were digitized and crystal dimensions were estimated by calculating the best fit of an ellipse to the contours of the crystals. The particle size (particle diameter) is reported as the average of length (L) and half-length width (W) of the measured ellipse (e.g. Devouard *et al*., 1998). The shape factors, describing the elongation of the crystals, were calculated from their width to length ratio, so that $W/L \leqslant 1$.

## Results and discussion

Species-specific morphologies and narrow size distributions of magnetosome in the MTB are characteristic features of highly-controlled biomineralization processes. In this study, the magnetite particles formed by *M. gryphiswaldense*, occurred mostly as single chains (although sometimes the chains were doubled at the central region, Fig. 1*a*) that usually contained up to 60 well-ordered magnetite crystals. The crystals exhibited a narrow size range (~14−67 nm) with an average diameter of 46±6.8 nm (Fig. 1*e*) and a cuboctahedral morphology (Fig. 1*c*). In agreement with previous results (e.g. Devouard *et al*., 1998; Meldrum *et al*., 1993; Pósfai and Arató, 2000), the crystal size distribution curves of magnetite magnetosomes formed by *M. gryphiswaldense* revealed an asymmetric and negatively skewed distribution with sharp cut-offs toward larger sizes typical of a Gamma type distribution (Fig. 1*e*). This type of distribution is due to the strict control of biomineralization by MTB, because the magnetosomes stop growing once they reach a

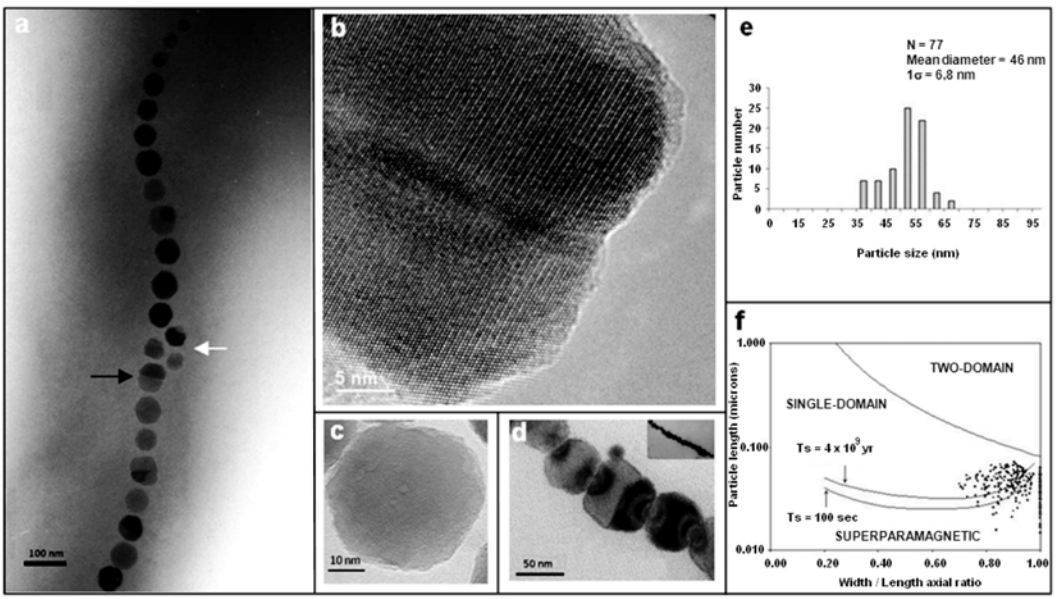


FIG. 1. Intracellular organization, crystal morphology and size distributions of magnetosomes from *M. gryphiswaldense*. (*a*) Low-resolution TEM image of a typical chain containing cuboctahedral crystals, twinned crystals (black arrow) and clusters of small crystals (white arrow); (*b*) HR-TEM image of a twining plane within a magnetite crystal; (*c*) HR-TEM image of a structurally perfect cuboctahedral magnetite crystal; (*d*) cuboctahedral crystals with thickness fringes; (*f*) size distribution for *M. gryphiswaldense* magnetite crystals; (*e*) crystal size distribution from this study plotted on a Butler-Banerjee diagram (Butler and Banerjee, 1975) with the boundaries between magnetic single-domain and superparamagnetic stability fields shown.

certain strain-specific size. The majority of the magnetite particles observed fall within the single-domain range (i.e. 35–120 nm, Fig. 1*f*, based on Butler and Banerjee, 1975). In this plot, smaller particles (20–30 nm) fall outside the single-domain field but within the superparamagnetic region, while perfectly isometric particles with an axial ratio close to or equal to 1.0 are at the very margin of the single-domain field and in the superparamagnetic region (Fig. 1*f*).

More than 90% of all crystals observed in this study were single crystals (Fig. 1*c*), only rarely were deviations from the ideal structure (i.e. spinel-law twins or defects that could be stacking faults or sub-grain boundaries) observed (Fig. 1*b*). The thickness fringes visible on some thicker crystals (Fig. 1*d*) can give indications about the 3D morphology of the crystal.

One of the prime characteristics of magnetite crystals synthesized by magnetotactic bacteria is the fact that each particle in a chain is a single crystal. Using high angular dark-field TEM (HADF-TEM) imaging, we can reveal information about possible lattice defects, grain boundaries, ordered domain structures etc. Figure 2*a–b* shows a cluster of typical biogenic magnetite single-crystals indicating that the particles extracted from *M. gryphiswaldense* were single crystallites of high perfection. In addition, the magnetite crystals extracted from the studied *M. gryphiswaldense* strain were particularly pure in chemical composition (EDS analysis revealed only Fe and O as constituent elements) and the SAED patterns obtained from the magnetite crystals were consistent with a face-centred-cubic spinel structure. The SAED patterns were indexed and all rings identified as those characteristic for magnetite (Fig. 2*c*). Finally, the particle size distributions and mineralogical purity of the isolated magnetosomes derived from the high-resolution image analyses were confirmed by powder XRD results which yielded an average particle size of 46.05±6 nm (evaluated from a calibrated XRD pattern of isolated magnetosomes) and the presence of only one type of Fe oxide, single-phase magnetite ($Fe_3O_4$), in *M. gryphiswaldense.*

## Conclusion

In this study, various high-resolution imaging and X-ray techniques were used to characterize the size, shape, crystal structure, crystallographic orientation and defect structures of ferrimagnetic Fe oxide crystals in MTB. The results showed that the ferrimagnetic crystals produced by the magnetotactic bacterium *M. gryphiswaldense* are crystallographically perfect and morphologically analogous to each other. The only deviation from an ideal magnetite structure of the crystals produced by this bacterial strain seems to be the occurrence of spinel-law twins or stacking-fault defects. The studied strain showed narrow, asymmetric size distributions and width/length ratios which fell within the permanent single-magnetic-domain size range. These results reflect, in addition to the roughly equidimensional shapes interpreted as cuboctahedral habits, the control of the bacteria, which limits the growth of the magnetite crystals to specific sizes and morphol-

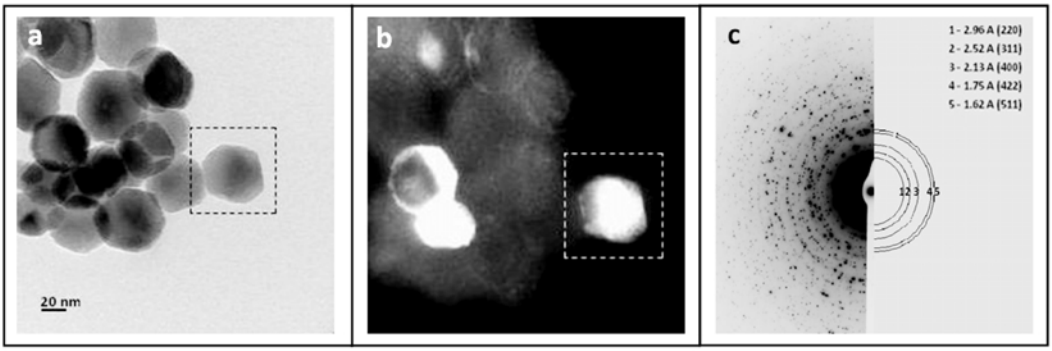


FIG. 2. (*a*) Bright-field; and (*b*) corresponding dark-field images of single-crystals of *M. gryphiswaldense* magnetite. (*c*) SAED pattern of a group of magnetite crystals. Distances from the centre to the various reflections were converted into a *d*-spacing (camera length of 24.1 mm). The *d* spacings are consistent with the standard magnetite electron diffraction pattern (PDF-19-629).

ogies, as marked by the asymmetry of the size distribution of magnetite crystals. All in all, this dataset helps to improve our knowledge of the potential of this novel class of magnetic nanoparticles for various biomedical and technological applications.

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## References

Bäuerlein, E. (2000) *Biomineralization: From Biology to Biotechnology and Medical Application*. Wiley-VCH, Weinheim, Germany, 294 pp.

Bazylinski, D.A. (1999) Synthesis of the bacterial magnetosome: the making of a magnetic personality. *International Microbiology*, **2**, 71–80.

Bazylinski, D.A., Frankel, R.B., Heywood, B.R., Mann, S., King, J.W., Donaghay, P.L. and Hanson, A.K. (1995) Controlled biomineralization of magnetite ($Fe_3O_4$) and greigite ($Fe_3S_4$) in a magnetotactic bacterium. *Applied Environmental Microbiology*, **61**, 3232–3239.

Butler, R.F. and Banerjee, S.K. (1975) Theoretical single-domain grain size range in magnetite and titanomagnetite. *Journal of Geophysical Research*, **80**, 252–259.

Devouard, B., Pósfai, M., Hua, X., Bazylinski, D.A., Frankel, R.B. and Buseck, P.B. (1998) Magnetite from magnetotactic bacteria: Size distributions and twinning. *American Mineralogist*, **83**, 1387–1398.

Lang, C. and Schüler, D. (2006) Biogenic nanoparticles: production, characterization and application of bacterial magnetosomes. *Journal of Physics: Condensed Matter*, **18**, 2815–2828.

Lowenstam, H.A. and Weiner, S. (1989) *On biomineralization*. Oxford University Press, Oxford, New York, 324 pp.

Meldrum, F.C., Mann, S., Heywood, B.R., Frankel, R.B. and Bazylinski, D.A. (1993) Electron microscopy study of magnetosomes in a cultured coccoid magnetotactic bacterium. *Proceedings of the Royal Society of London B*, **251**, 231–236.

Pósfai, M. and Arató, B. (2000) Magnetotactic bacteria and their mineral inclusions from Hungarian freshwater sediments. *Acta Geologica Hungarica*, **43**, 463–476.

Pósfai, M., Buseck, P.R., Bazylinski, D.A. and Frankel, R.B. (1998) Iron sulfides from magnetotactic bacteria: structure, composition, and phase transitions. *American Mineralogist*, **83**, 1469–1481.

Weiner, S. and Dove, P.M. (2003) An overview of biomineralization processes and the problem of the vital effect. Pp. 1–26 in: *Biomineralization* (P.M. Dove, J.J. De Yoreo and S. Weiner, editors). Reviews in Mineralogy and Geochemistry **54**, Mineralogical Society of America, Washington, D.C.