

**IRON OXIDES AND SULFIDES IN MAGNETOTACTIC BACTERIA:
ELECTRON HOLOGRAPHY OF MAGNETIC MICROSTRUCTURE
AND ELECTRON TOMOGRAPHY OF CRYSTAL MORPHOLOGY**

M. PÓSFAI ⁽¹⁾, R.E. DUNIN-BORKOWSKI ^(2,3), T. KASAMA ^(3,2), R.K.K. CHONG ⁽²⁾,
E.T. SIMPSON ⁽²⁾, A. FINLAYSON ⁽²⁾, I. KÓSA ⁽¹⁾, Z. KRISTÓF ⁽⁴⁾, P.R. BUSECK ⁽⁵⁾,
R.B. FRANKEL ⁽⁶⁾, R.J. HARRISON ⁽⁷⁾

⁽¹⁾ Dept of Earth and Environmental Sciences, University of Veszprém, Hungary

⁽²⁾ Dept of Materials Science and Metallurgy, University of Cambridge, UK

⁽³⁾ Frontier Research System, The Institute of Physical and Chemical Research, Japan

⁽⁴⁾ Dept of Plant Anatomy, Eötvös University, Budapest, Hungary

⁽⁵⁾ Dept of Geological Sciences, Arizona State University, Tempe, Arizona, USA

⁽⁶⁾ Dept of Physics, California Polytechnic State University, San Luis Obispo, California, USA

⁽⁷⁾ Dept of Earth Sciences, University of Cambridge, UK

Magnetotactic bacteria contain magnetosomes, which are membrane-bound ferrimagnetic mineral grains of magnetite (Fe_3O_4) or greigite (Fe_3S_4). Their presence results in the orientation and migration of such cells along geomagnetic field lines in aquatic environments^[1]. Different strains of bacteria have different crystal habits and arrangements, which are in turn controlled by their biological environment.

Here, we use a combination of advanced transmission electron microscopy techniques to study the spatial arrangements and the physical, chemical and magnetic properties of crystals in magnetotactic bacteria collected from lakes and streams, in order to understand the mechanisms of biological control over crystal growth and orientation. We examine both air-dried cells and ultrathin sections of cells.

Figure 1 shows results obtained from a double chain of magnetite crystals in an air-dried cell. The morphologies and orientations of the crystals were measured using electron diffraction, high-resolution electron microscopy and high-angle annular dark field electron tomography. It was established that the [111] axes of most of the crystals are approximately parallel to the chain axis, as marked by the line of white arrows in Fig. 1a. In contrast, the crystallographic directions perpendicular to the chain axis are randomly distributed, as shown in the form of a stereographic projection in Fig. 1b. On the assumption that air drying has not affected the relative orientations of the crystals, the chain is therefore analogous to beads on a string, in which biological control appears to be stricter in setting the [111] magnetocrystalline easy axis of each crystal to be parallel to the chain axis than in constraining their orientation about this direction. The magnetosomes are structurally perfect, as indicated by HRTEM images such as that shown in Fig. 1c.

Figure 2 shows a stained ultrathin section of a coccus that contains disrupted chains of crystals scattered throughout the cell. The image shows that the magnetite crystals appear to be anchored to the inner cell membrane, and to be enveloped by stained material, apparently representing a magnetosome membrane. Chemical analysis shows that the cells do not contain detectable iron outside the magnetite crystals. It is likely that the organic membrane templates the oriented nucleation of the crystals, in this case setting their [111] axis perpendicular to the cell wall.

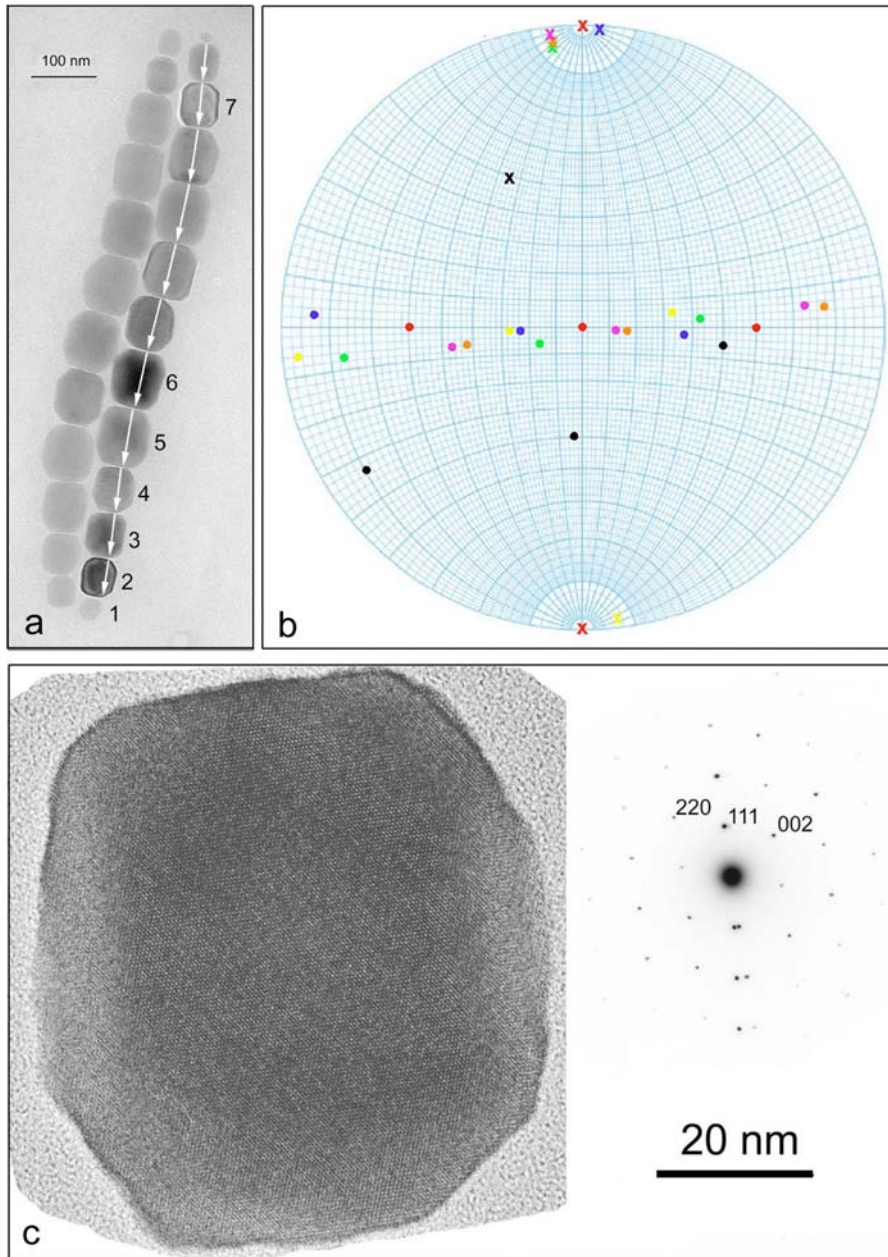


Figure 1. (a) Bright-field image of a double chain of magnetite magnetosomes from a single bacterial cell. The orientations of the crystals marked 1-7 were determined by using electron diffraction. The white arrows are approximately parallel to $[111]$ in each crystal. (b) Stereographic projection showing the orientations of the numbered crystals in (a). The crosses show the $[111]$ poles, which are almost all approximately parallel to the chain axis. The black cross corresponds to crystal 1. The dots show $\langle 110 \rangle$ directions. (c) High-resolution image and electron diffraction pattern of crystal 4 in (a).

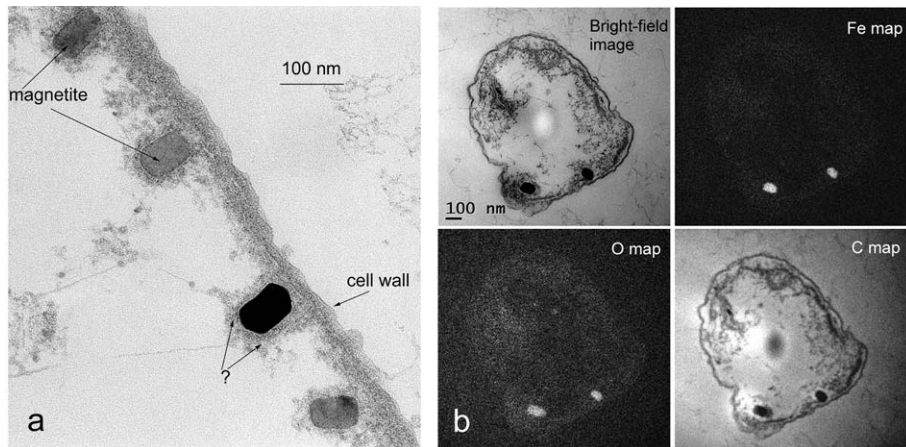


Figure 2. (a) Bright-field image and (b) energy-selected elemental maps of magnetite magnetosomes in ultrathin sections. Stained material surrounds each crystal in (a), as indicated by the question mark.

Crystals in magnetotactic bacteria also provide model systems for studying the fundamental effects that influence the magnetic properties of closely-spaced nanoscale magnets. The overall magnetic properties of such nanoparticle chains result from a delicate balance between the competing effects of crystal size, morphology, crystallography and spacing, as well as external factors such as temperature and applied magnetic field. We have used off-axis electron holography in the transmission electron microscope to image the magnetic induction in both isolated and closely-spaced magnetite crystals in magnetotactic bacteria, both at room temperature and at liquid nitrogen temperature, which is close to the Verwey transition for magnetite (116K)^[2]. At this temperature, magnetite undergoes a phase change from a cubic to a monoclinic structure, and the easy axis of magnetization changes. Representative results from our study are shown in Fig. 3. At room temperature, the magnetic signal in the magnetite crystals is observed to be dominated by interactions, with highly parallel and straight field lines closely following the axis of each chain of crystals. In contrast, at low temperature the magnetic induction undulates along the length of the chain. This behavior is thought to result from a competition between interparticle interactions and an easy axis of magnetization that is no longer parallel to the chain axis.

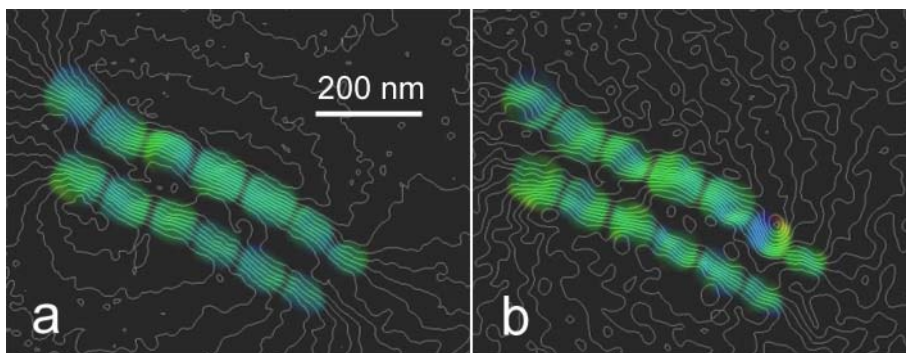


Figure 3. Magnetic induction maps acquired from bacterial magnetite chains at (a) 293 and (b) 116 K. The contour spacing is 0.25 radians in (a) and 0.31 radians in (b).

Whereas the structural and magnetic properties of magnetite magnetosomes have been studied extensively, this is not the case for greigite magnetosomes^[3]. We have used energy-selected imaging, electron tomography and off-axis electron holography to study the composition, three-dimensional arrangement, crystallography, morphology and magnetic microstructure of greigite crystals in uncultured bacterial cells collected from a sulfidic salt marsh pool near Morro Bay, California. Figure 4 shows results obtained from an air-dried cell that had been undergoing division, in which the crystals are arranged in a multiple chain-like structure. The morphologies and orientations of the crystals are less regular than in magnetite-containing bacteria. The contours, whose spacing is inversely proportional to the in-plane magnetic induction in the sample integrated in the electron beam direction, suggest that the crystals that lie along the axis of the chain appear to be more strongly magnetic than those at the ends and further from the chain axis. Work is in progress to establish whether this observation may result from the three-dimensional arrangements of the crystals in the cell, coupled with the insensitivity of electron holography to components of the magnetic induction parallel to the electron beam direction.

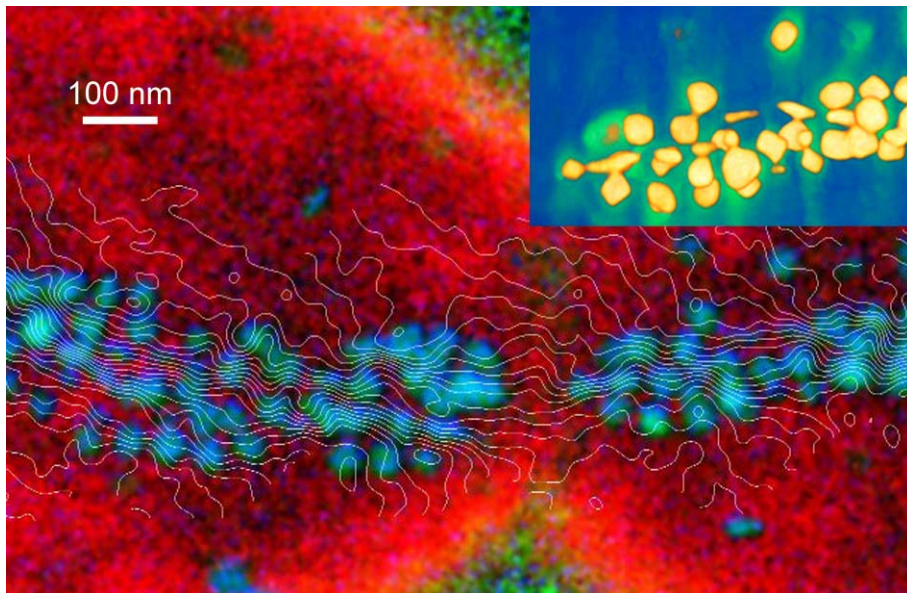


Figure 4. Greigite crystals in a magnetotactic bacterial cell that is undergoing division. The figure shows a composite of energy-selected chemical maps and contours formed from the magnetic contribution to the electron holographic phase shift. Blue corresponds to iron, red to carbon and green to sulfur. The insert in the upper right corner shows an electron tomographic reconstruction of the morphologies of the crystals in the chain fragment on the right side of the main image.

REFERENCES

- [1] Bazylinski, D.A. and Frankel, R.B. (2004) Magnetosome formation in prokaryotes. *Nature Reviews Microbiology* 2, 217-230.
- [2] Verwey, E.J.W. (1939) Electronic conduction of magnetite (Fe_3O_4) and its transition point at low-temperature. *Nature* 44, 327-328.
- [3] Pósfai, M., Buseck, P.R., Bazylinski, D.A. and Frankel, R.B. (1998) Reaction sequence of iron sulfide minerals in bacteria and their use as biomarkers. *Science* 280, 880-883.

posfaim@almos.vein.hu