Magnetite as a prokaryotic biomarker: A review

Concepcion Jimenez-Lopez,[1] Christopher S. Romanek,[2] and Dennis A. Bazylinski[3]

Received 9 September 2009; revised 9 November 2009; accepted 20 November 2009; published 22 April 2010.

[1] Over the years, nanometer-sized magnetite (Fe_3O_4) crystals have been recovered from many modern and ancient environments including sediments and soils and even meteorites. In some cases these crystals have been used as "magnetofossils" for evidence of the past presence of specific microbes. Magnetite nanocrystals can be formed by a number of different biological and inorganic mechanisms resulting in crystals with different physical and magnetic characteristics. Prokaryotes (bacteria) biomineralize magnetite through two methods that differ mechanistically, including: biologically induced mineralization (BIM) and biologically controlled mineralization (BCM). Magnetite nanocrystals produced by BIM are known to be synthesized by the dissimilatory iron-reducing bacteria, are deposited external to the cell, and generally are physically indistinguishable from magnetite particles formed inorganically. BCM magnetites, in contrast, are synthesized by the magnetotactic bacteria and some higher organisms and are precipitated intracellularly as membrane-bounded structures called magnetosomes. These magnetites appear to have unique crystal morphologies and a narrow size range leading to their original use as magnetofossils. Because of the discovery of nanometer-sized crystals of magnetite in the Martian meteorite ALH84001, the use of these criteria for the determination of whether magnetite crystals could constitute a prokaryotic biomarker was questioned. Thus, there is currently great debate over what criteria to use in the determination of whether specific magnetite crystals are biogenic or not. In the last decade, additional criteria have been established (e.g., the Magnetite Assay for Biogenicity), and new tools and technologies have been developed to determine the origin of specific types of magnetite crystals.

Citation: Jimenez-Lopez, C., C. S. Romanek, and D. A. Bazylinski (2010), Magnetite as a prokaryotic biomarker: A review, *J. Geophys. Res.*, *115*, G00G03, doi:10.1029/2009JG001152.

1. Introduction

[2] A major problem for understanding the origin of life and the evolutionary origin and phylogeny of prokaryotes is the lack of unambiguous, reliable microbial fossils. The search for reliable biomarkers is not only crucial to understanding early evolution on our planet, but appropriate biomarkers might also help to reveal traces of ancient or current biological activity on extraterrestrial bodies such as Mars. Although there are numerous organic compounds (e.g., hopanoids, a class of pentacyclic triterpenoid lipids [*Peters et al.*, 2005]) that are often preserved in the fossil record and can and/or have been used as molecular biosignatures, mineral biomarkers are preferred by many because of their perceived longer persistence in nature. The rationale for this use of minerals is based on the fact that organisms that have the capability of precipitating minerals are present in all the major biological groups, from prokaryotes to eukaryotes including humans. However, a marked difference exists among the different taxonomic groups concerning their mechanisms of biomineral synthesis [*Simkiss and Wilbur*, 1989].

[3] Many, if not most, biominerals contain one or more metals. The most numerous metal-containing biominerals are phosphates followed by oxides and carbonates. Roughly 25% of these biominerals are hydrated and are not well crystallized [*Simkiss and Wilbur*, 1989]. In the past decade there has been increasing interest in the biogeochemical transformations of Fe(III)-bearing precursors and processes leading to the biomineralization of Fe minerals [*Dong et al.*, 2000]. This is partially due to the fact that iron is the fourth most abundant element in the Earth's crust and also because many believe that Fe(III) was the first external electron acceptor of global significance in microbial respiration [*Walker*, 1987; *Cairns-Smith et al.*, 1992; *de Duve*, 1995a, 1995b].

2. Importance of Magnetite as a Biomarker

[4] Magnetite, ferrous-ferric oxide (Fe_3O_4), is a commonly occurring mineral on Earth usually found in natural

[1]Departamento de Microbiología, Universidad de Granada, Granada, Spain.
[2]NASA Astrobiology Institute and Department of Earth and Environmental Sciences, University of Kentucky, Lexington, Kentucky, USA.
[3]School of Life Sciences, University of Nevada, Las Vegas, Nevada, USA.

0148-0227/10/2009JG001152

terrestrial environments ranging from igneous and metamorphic rocks to all kinds of sedimentary environments [*Thomas-Keprta et al.*, 2000]. It has also been found in extraterrestrial materials (e.g., the Martian meteorite ALH84001 [*Thomas-Keprta et al.*, 2000]). Magnetite can be produced either inorganically or biologically, through both biologically induced mineralization (BIM) [*Frankel and Bazylinski*, 2003] and biologically controlled mineralization (BCM) [*Bazylinski and Frankel*, 2003]. Magnetite has been found to be a byproduct of microbial Fe(III) respiration [e.g., *Lovley et al.*, 1987] and therefore the presence of magnetite has been used as possible evidence for respiratory processes in the early evolution of the Earth [*Walker*, 1987; *Vargas et al.*, 1998], as evidence for a vast terrestrial subsurface biosphere [*Gold*, 1992], and even for evidence of life on ancient Mars [*McKay et al.*, 1996].

3. Synthesis and Features of Abiotic and Biogenic Magnetites

3.1. Inorganically Produced Magnetites

[5] The inorganic formation of magnetite as a primary mineral phase can be achieved through a variety of methods including homogeneous and/or heterogeneous precipitation from bulk solution. It can also be formed as a secondary phase by thermal decomposition of Fe-bearing carbonates such as siderite and by the recrystallization of ferrihydrite, green rust and/or ferric oxyhydroxides.

3.1.1. Magnetite Precipitation as a Primary Mineral Phase

[6] The best developed techniques for the synthesis of inorganic magnetite as a primary mineral phase are based on the precipitation of magnetite from bulk solution since large quantities of the mineral can be produced. There are a number of different specific methods to do this, some of which are detailed below, that mainly differ in how Fe(II) is introduced in the solution. All these methods, however, are dependent on controlling the conditions consistent with the thermodynamic stability field for magnetite, which include Eh, pH and alkalinity/pCO_2 [*Garrels and Christ*, 1990] as shown in Figure 1.

[7] Most of the procedures involving the precipitation of magnetite from bulk solution follow the so-called "coprecipitation" method, in which Fe(II) and Fe(III) mixtures are introduced as starting solutions under anoxic conditions. In order to maintain the conditions necessary for the thermodynamic stability field for magnetite, different compounds are used to increase and maintain alkaline conditions during magnetite precipitation including the following: NH_3 at 85°C [*Vayssières et al.*, 1998; *Tseng et al.*, 2007], NaOH at 25°C in agarose gel [*Prozorov et al.*, 2007a, 2007b], NaOH at 25°C in solution [*Vayssières et al.*, 1998; *Perez-Gonzalez et al.*, 2010], NH_4OH at 25°C [*Arató et al.*, 2005], and $N(CH_3)_4OH$ [*Vayssières et al.*, 1998]. It is common for the magnetite crystals formed in this manner to be of several different morphologies (within the same reaction mixture) including cubic, rounded, octahedral and/or irregular (Figures 2a and 2b). However, different morphologies, sizes and size distributions can be obtained by varying the conditions in the reaction mixture [e.g., *Nyirö-Kósa et al.*, 2009]. For instance, the mean crystal size of the magnetite crystals formed can be adjusted over a large range at the nanometer scale (1.5–12.5 nm) by controlling the pH and the ionic strength of the iron solutions. Smaller magnetite particles can be obtained through higher pH values and higher ionic strengths of the iron solutions [*Vayssières et al.*, 1998]. *Nyirö-Kósa et al.* [2009] studied in great detail the influence of synthesis conditions on the size and shape of magnetite nanoparticles that were produced in inorganic coprecipitation processes. Parameters examined included the types of reagents used and their concentrations, pH, temperature (from 9 to 90°C) and the presence and absence of oxygen (oxic versus anoxic under N_2). The size of the magnetite nanoparticles produced ranged between ~11 and 120 nm and the mean size within this range was controllable by adjusting these parameters. The morphologies of the magnetite nanocrystals were also affected by the synthesis conditions and varied according to grain size. Crystals with diameters between 10 and 25 nm had irregular or rounded crystal morphologies, whereas those with diameters greater than 50 nm were octahedral.

[8] For those applications in which specific sizes and relatively narrow size distributions of the magnetite particles are desired, several modifications of the bulk "coprecipitation" technique have been developed. These modifications are mainly based on limiting the space available for crystal growth by precipitating magnetite in microemulsions, vesicles, polymer solutions, or gels [*Mann and Hannington*, 1988; *Ward and Friberg*, 1989; *Liu et al.*, 2004].

[9] Another method for inorganic precipitation of magnetite is the "reduction-precipitation" technique, in which the precipitation of magnetite occurs by the addition of iron only as an Fe(III) solution (mainly $FeCl_3$). The precipitation of spherical magnetite particles with mean diameter of about 10 nm or less has been shown to occur through the reduction of ferric ions (as $FeCl_3$) to ferrous ions by Na_2SO_3 followed by an increase in the pH of the system by the addition of ammonia, always under anoxic conditions [*Schwertmann and Murad*, 1990; *Qu et al.*, 1999]. The mineralogy of the product strongly depends on the initial $[Fe^{3+}]/[SO_3^{2-}]$ ratio.

[10] Another way to reduce Fe(III) ions and to obtain and maintain the necessary Eh value range to comply with the stability field for magnetite is by applying a constant voltage to the solution throughout the experiment (the "electrochemical" method). Using this method, cubic nanoparticles (45–80 nm) of magnetite were obtained from an iron-based electrode immersed in an alkaline aqueous medium containing Fe-complexing molecules [*Franger et al.*, 2004].

[11] Magnetite can also be precipitated at high temperatures, which results in the synthesis of larger, morphologically well defined crystals. Magnetite can be produced by the oxidation of a Fe(II) solution ($FeSO_4$) at 90°C by the addition of KNO_3 while maintaining a high pH using a solution of KOH. The resulting magnetite is close to stoichiometric and forms cubes varying in sizes between 0.05 and 0.20 μm [*Schwertmann and Cornell*, 2000]. Magnetite is also known to precipitate at high temperature via the mixing of solutions of $FeSO_4 \cdot 7H_2O$ and $N_2H_4 \cdot H_2O$ and the subsequent heating of the reaction mixture in an autoclave at 150°C for 8 h [*Zhu et al.*, 2007]. Using this method, well crystallized, cubic magnetite particles of about 1 μm in diameter were obtained.

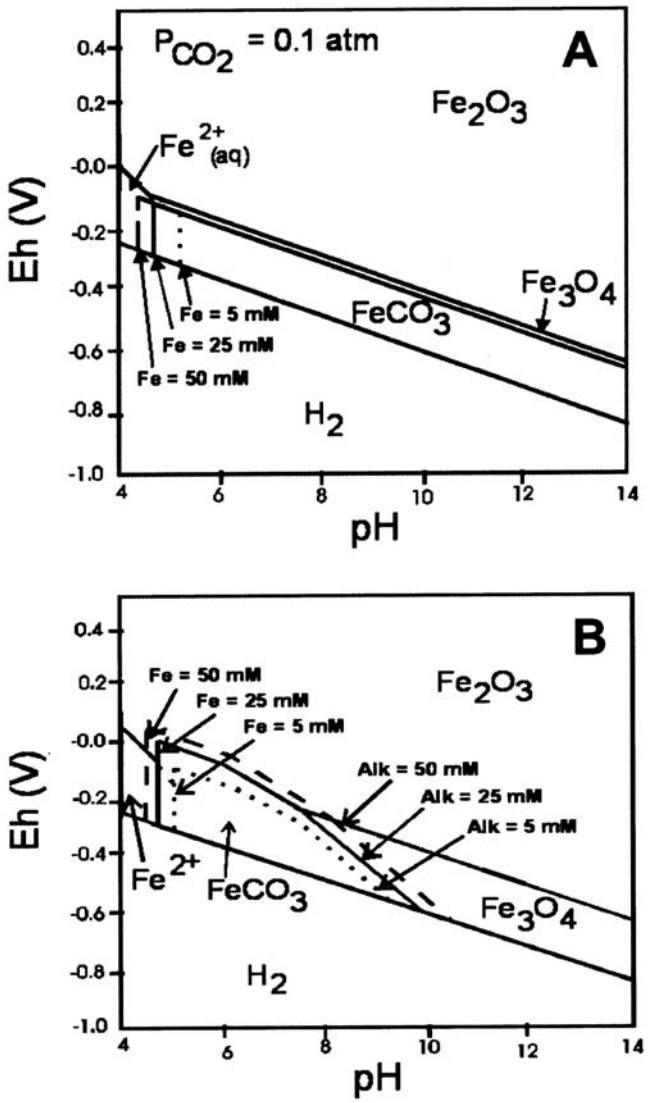

Figure 1. Eh–pH diagram showing the stability field for the mineral magnetite (Fe_3O_4): (a) in an open system at a constant pCO_2 (=0.1 atm) calculated from the equations of *Garrels and Christ* [1990]. The line that separates the stability field for magnetite and siderite ($FeCO_3$) shifts up at higher pCO_2 and down at lower pCO_2. The stability field for magnetite is extremely sensitive to Eh conditions (determined by the concentration of aqueous Fe^{2+} and Fe^{3+} and pO_2), pH and pCO_2; (b) in a closed system at a constant alkalinity, again calculated from the equations of *Garrels and Christ* [1990]. The line that separates the stability field for magnetite and siderite shifts left at lower alkalinities and right at higher alkalinities, increasing and decreasing, respectively, the magnetite stability field.

3.1.2. Magnetite Formation as a Secondary Mineral Phase

[12] Magnetite can be formed as a secondary phase, a term which implies that the magnetite forms through the transformation of certain iron-bearing mineral phases at high or at low temperatures. At low temperature (~25°C), magnetite forms by the transformation of ferrihydrite and/or green rust, both of which are thermodynamically unstable under anoxic conditions, once the pH, Eh, pCO_2, [Fe(II)] of the system meet the requirements necessary to reach the stability field for magnetite [*Zachara et al.*, 2002].

[13] Magnetite has also been shown to form through reactions between soluble $Fe(OH)_2$ and FeOOH at temperatures ranging from 25 to 100°C and pH values from 3 to 13 [*Ishikawa et al.*, 1998]. Well crystallized cubic magnetite particles were obtained using this reaction, mixed with spindle- and rod-shaped, needle-like and irregular crystals of FeOOH.

[14] The thermal decomposition of siderite or other Fe-rich carbonate phases at temperatures higher than 400°C in the absence of oxygen has been shown to result in the formation of magnetite (Figures 2c and 2d). This reaction has been explored in some detail [*Golden et al.*, 2001, 2004; *Thomas-Keprta et al.*, 2009; *Jimenez-Lopez et al.*, 2008] in connection with controversy over the origin of nanometer-sized magnetite crystals within the Martian mete-

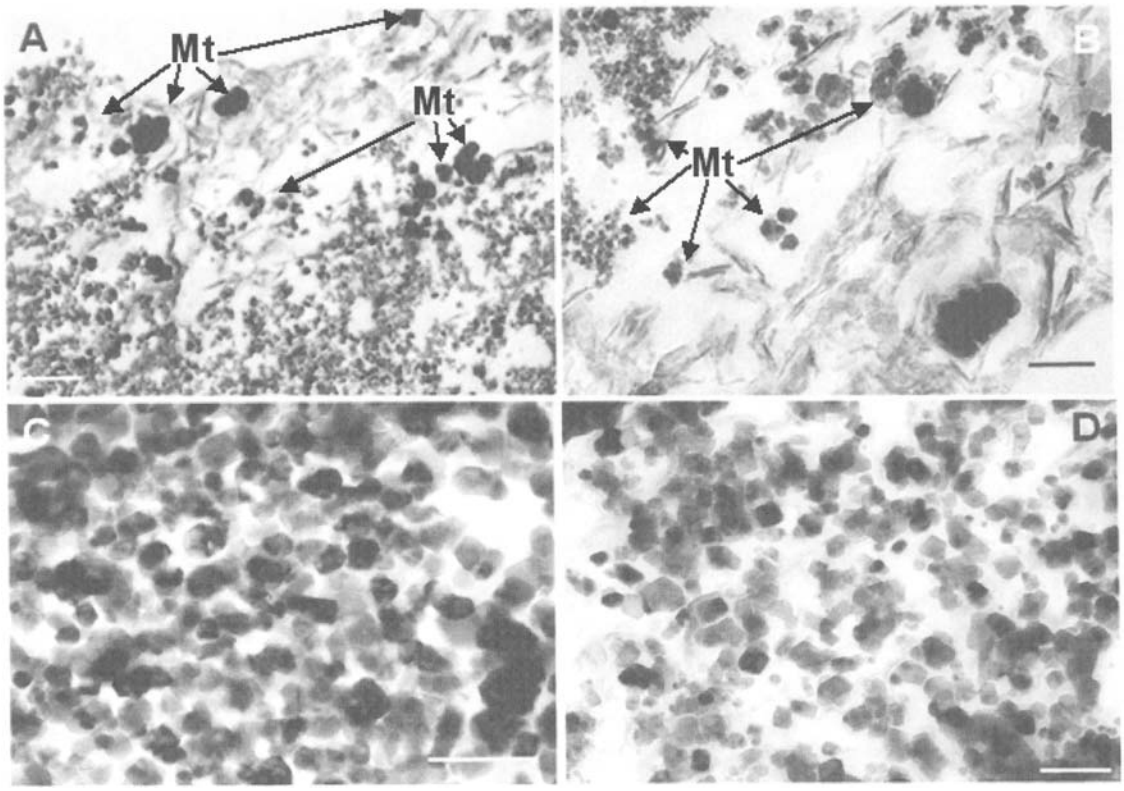

Figure 2. Transmission electron microscope (TEM) micrographs of thin-sectioned preparations of inorganic magnetites. (a, b) Magnetite synthesized by the coprecipitation method from $NaHCO_3$-Na_2CO_3-$Fe(ClO_4)_2$-$FeCl_3$ solution at 25°C. NaOH was added until a pH of 11 was reached. Arrows labeled Mt indicate examples of magnetite crystals. (c, d) Magnetite produced from the thermal decomposition of ankerites at 700°C and 1 atm pCO_2. In this case, all crystals consist of magnetite. Scale bars represent: Figure 2a, 400 nm; Figures 2b–2d, 200 nm.

orite ALH84001 (discussed in a later section) [*McKay et al.*, 1996].

3.2. Biogenic Magnetites

[15] Magnetite is known or thought to be produced biologically by a number of organisms that range from prokaryotic microorganisms (includes the Bacteria and the Archaea) [*Bazylinski and Frankel*, 2003; *Frankel and Bazylinski*, 2003] to possibly humans [*Kirschvink et al.*, 1992]. However, as previously stated, organisms differ as to their mechanisms of magnetite biomineralization. In this section we focus on magnetite biomineralization by prokaryotic microorganisms.

[16] The biomineralization of magnetite by prokaryotes can be separated into two mechanistic modes: (1) biologically induced mineralization (BIM) [*Lowenstam*, 1981; *Lowenstam and Weiner*, 1989]; and (2) biologically controlled mineralization (BCM) [*Frankel and Bazylinski*, 2003]. Biologically controlled mineralization has also been referred to in the past as organic matrix-mediated mineralization [*Lowenstam*, 1981; *Lowenstam and Weiner*, 1989] and boundary-organized biomineralization [*Mann*, 1986] implying that membranes are important in the biomineralization process. There are several important marked differences between BIM and BCM and we will focus on these in the context of biomarkers.

[17] In general, BIM is a result of the metabolic activity of organisms and subsequent chemical reactions that involve metabolic byproducts and, for this reason, BIM minerals are virtually always deposited external to the cell. In some cases, organisms secrete one or more metabolic products that react with ions or compounds in the environment resulting in the subsequent precipitation of mineral particles [*Frankel and Bazylinski*, 2003] while in others, bacterial surfaces such as cell walls or polymeric materials (exopolymers) exuded by bacteria, act as important sites for the adsorption of ions and subsequent mineral nucleation and growth [*Beveridge and Murray*, 1980; *Konhauser*, 1998]. In essence, BIM is equivalent to inorganic mineralization under the same environmental conditions and the mineral particles are therefore likely to have crystallochemical features that are generally indistinguishable from those synthesized inorganically.

[18] In BCM, minerals usually form on or within organic matrices or vesicles within the cell suggesting that the organism exerts a significant degree of control over the nucleation and growth of the mineral crystals and thus over the composition, size, habit, and intracellular location of the particles [*Bazylinski and Frankel*, 2003]. Crystals formed by BCM are generally structurally well ordered with a narrow size distribution and species-specific, consistent, crystal

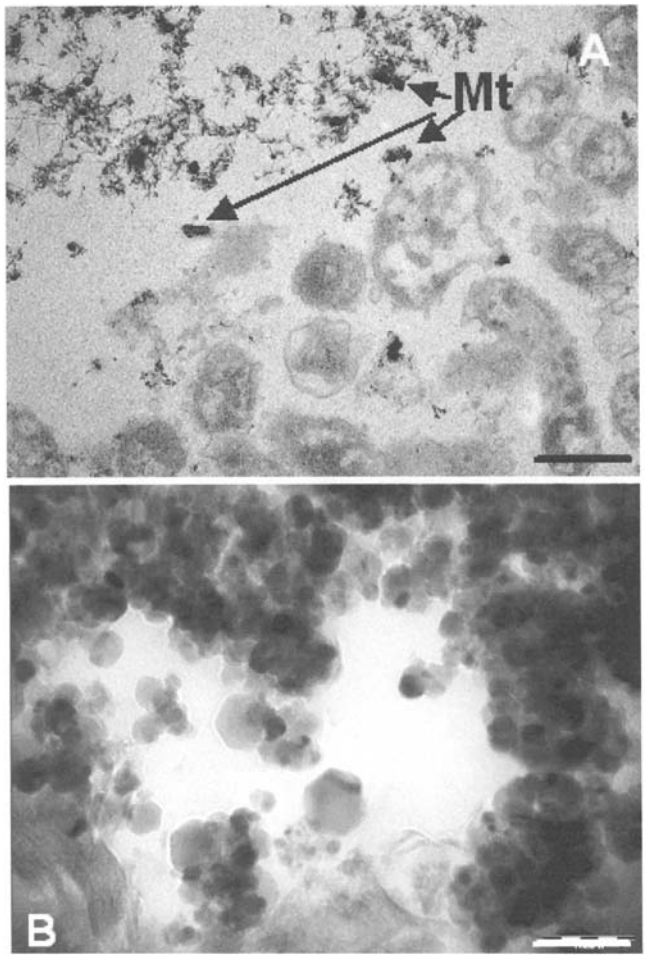

Figure 3. TEM micrographs of extracellular magnetite produced by biologically induced mineralization (BIM) by cells of *Shewanella oneidensis*. (a) Low-magnification TEM image of thin section of *Shewanella* cells and associated extracellular magnetite (indicated by arrows labeled Mt). Scale bar represents 500 nm. (b) High-magnification TEM image of thin-sectioned crystals of BIM magnetite produced by *Shewanella* cells. All crystals in Figure 3b consist of magnetite. Scale bar represents 100 nm.

habits. These qualities indicate that BCM processes are under metabolic and genetic control.

3.2.1. Magnetite Produced by BIM

[19] Magnetite produced through BIM is most commonly recognized and studied in the dissimilatory iron-reducing bacteria although other microorganisms may also be involved. These facultatively anaerobic to anaerobic microorganisms respire with Fe(III), reducing it to Fe(II), as a terminal electron acceptor under anaerobic conditions. These bacteria are ubiquitous and have been found in all kinds of fresh and marine aquatic habitats [*Lovley et al.*, 1990; *Caccavo et al.*, 1992; *Roh et al.*, 2006] including soda lakes [*Zavarzina et al.*, 2006], thermal springs [*Sokolova et al.*, 2007], leachate ponds [*Ye et al.*, 2004], mining-impacted lake sediments [*Cummings et al.*, 1999] and drainage waters [*Kusel*, 2003; *Johnson and Hallberg*, 2003], and oxic-anoxic interfaces [*Frankel and Bazylinski*, 2003].

[20] Magnetite formed by BIM has been found to be produced and studied in a number of iron-reducing prokaryotes including *Shewanella putrefaciens* strain CN32 [*Fredrickson et al.*, 1998; *Zachara et al.*, 2002], *Shewanella oneidensis* [*Perez-Gonzalez et al.*, 2010], *Shewanella* sp. PV-4 [*Roh et al.*, 2006] several species of *Geobacter* [*Lovley*, 1997; *Zachara et al.*, 2002], *Ferribacterium limneticum* [*Cummings et al.*, 1999], alkalophilic bacteria such as *Geoalkalibacter ferrihydriticus* [*Zavarzina et al.*, 2006] and *Alkaliphilus metalliredigens* [*Ye et al.*, 2004], and thermophilic bacteria such as TOR-39 [*Zhang et al.*, 1998], *Thermolithobacter ferrireducens* and *Thermolithobacter carboxydivorans* [*Sokolova et al.*, 2007]. Generally, unlike the case of BCM magnetite in the magnetotactic bacteria (discussed in the next section), there appears to be no known function that can be ascribed to these BIM particles except, perhaps, as a solid substrate for attachment (although their small size seems to preclude this notion).

[21] The mineralogy, morphology, composition and size of iron-bearing precipitates including magnetite formed through BIM by the dissimilatory iron-reducing bacteria depend strongly on: environmental conditions under which precipitation occurs (e.g., pH, pO_2, pCO_2, Eh, temperature [*Bell et al.*, 1987; *Kukkadapu et al.*, 2006; *Roh et al.*, 2006]); growth medium composition including the concentrations and chemical forms of electron donor and acceptor; and sorbed ions [*Zachara et al.*, 2002]. In the laboratory, microorganisms most frequently use poorly crystalline Fe(III) oxides as electron acceptor with ferrihydrite and nanogoethite the most easily used by cells. These minerals are common secondary weathering products in soils, unsaturated and saturated subsurface materials and aquatic sediments [*Zachara et al.*, 2002; *Van der Zee et al.*, 2003; *Kukkadapu et al.*, 2005]. Some dissimilatory iron-reducing bacteria also utilize and reduce the Fe(III) in phyllosilicates (e.g., montmorillonite, illite [*Kukkadapu et al.*, 2006]) and even magnetite [*Dong et al.*, 2000, 2003a, 2003b].

[22] Although the specific mechanism(s) of magnetite biomineralization by the dissimilatory iron-reducing bacteria have not been completely characterized chemically, *Zachara et al.* [2002] examined the production of magnetite by *Shewanella putrefaciens* when ferrihydrite was used as the source of Fe(III) as electron acceptor. These authors proposed that the precipitation of magnetite via BIM by *S. putrefaciens* occurred by topotactic conversion of 2-line ferrihydrite driven by Fe(II) sorption under specific chemical conditions in the growth medium which included: (1) intermediate flux of Fe(II) being released by cells; and (2) a gradient of increasing pH and other ions (e.g., PO_4^{3-}) occurring from the cell into the surrounding culture medium. The metabolic products of these reductions (Fe(II)) make the system supersaturated with respect to magnetite, thus allowing the precipitation of this mineral phase.

[23] Magnetite crystals produced by the dissimilatory iron-reducing bacteria have been characterized to some degree as to their morphology and size. Crystals have been reported to be globular by some while other studies report the synthesis of euhedral or irregularly shaped crystals with variable morphologies and sizes [*Sparks et al.*, 1990; *Zachara et al.*, 2002; *Kukkadapu et al.*, 2005; *Perez-Gonzalez et al.*, 2010] (Figure 3). The size of the magnetite crystals in these studies ranged from <35 nm, placing them in the super-

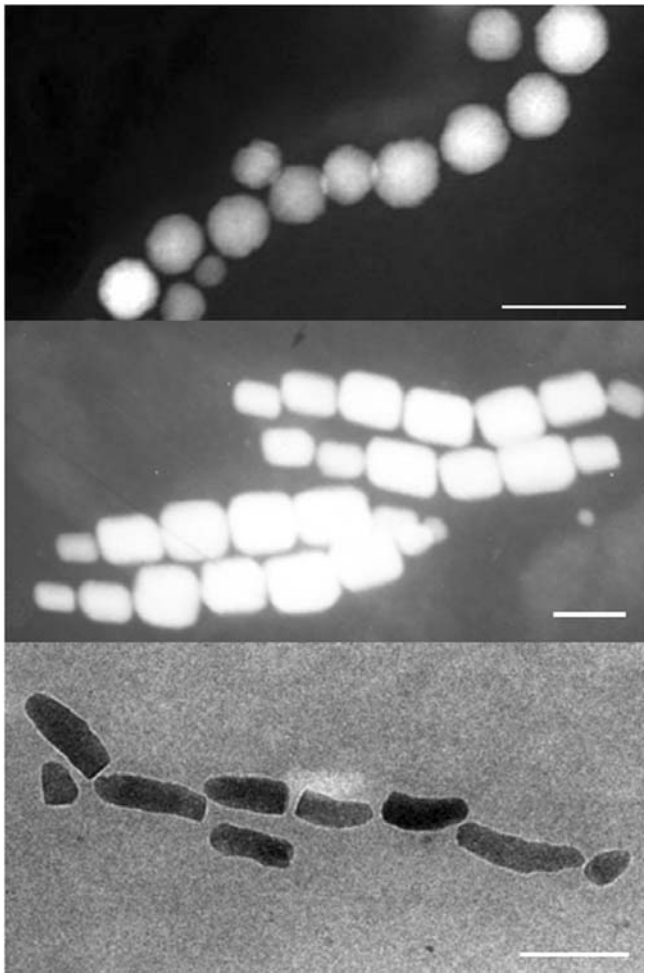

Figure 4. Examples of magnetite crystals in magnetosomes produced via biologically controlled mineralization (BCM) in cells of different magnetotactic bacteria. (top) Darkfield scanning transmission electron microscope (STEM) image of a chain of cubooctahedral crystals with rounded edges in a newly isolated magnetotactic spirillum. (middle) Darkfield STEM image of multiple chains of anisotropic elongated hexagonal prismatic crystals in an uncultured magnetotactic coccus collected from Morro Bay, California. Note that the smaller immature crystals are located at the ends of the chains. (bottom) Bright field STEM image of a chain of anisotropic tooth-shaped crystals in an uncultured magnetotactic spirillum collected from a hot spring in northern Nevada. In all cases, the bar represents 100 nm.

paramagnetic size range meaning that they would not be permanently magnetic at ambient temperature, to the single magnetic domain size range (~35–120 nm) in which the individual crystals each carry a permanent magnetic dipole moment at ambient temperature [*Vali et al.*, 2004; *Roh et al.*, 2006; *Perez-Gonzalez et al.*, 2010]. Under some experimental conditions, the small magnetite particles formed by *Shewanella putrefaciens* have been shown to contain an excess Fe(II)/Fe total ratio (compared to stoichiometric magnetite) and were chemically unstable transforming to ferrous hydroxyl carbonate over time [*Kukkadapu et al.*, 2005]. In almost all instances of BIM, unlike in BCM, it is clear that the organism has little, if any, control over the biomineralization process(es) and thus BIM minerals, including magnetite, are generally indistinguishable in morphology and size from minerals formed inorganically under the same chemical conditions; that is, these biominerals are generally characterized by poor crystallinity, broad particle-size distributions, lack of specific crystal morphologies, poor mineral specificity (mixed mineral compositions) and/or the inclusion of impurities in the mineral lattice [*Frankel and Bazylinski*, 2003; *Bazylinski et al.*, 2007]. This is why BIM minerals, including magnetite, are not commonly used as biomarkers at the present.

[24] However, there are some exceptions to the examples described above. A unique form of tabular, single-domain magnetite has been shown to be produced through BIM by cells of *Geobacter metallireducens* strain GS-15 under nontraditional (low-CO_2) culture conditions. This magnetite has a well defined crystal habit and magnetic properties and it has been proposed by *Vali et al.* [2004] that because of the uniqueness of this form of magnetite, its presence could be used as an indicator of ancient biological activity in terrestrial and extraterrestrial environments. Other distinctions between inorganically produced and BIM magnetites, not based on morphological criteria, have recently been determined that suggest that at least some BIM magnetites can be used as biosignatures. These are discussed in a later section.

3.2.2. Magnetite Produced Through BCM

[25] Magnetite is also produced by a group of prokaryotes known as the magnetotactic bacteria through BCM. These metabolically, morphologically, and phylogenetically diverse motile bacteria are ubiquitous in aquatic habitats and their swimming direction is influenced by the Earth's and external magnetic fields. This unusual behavior, termed magnetotaxis, is due to the presence of a number of unique intracellular organelles called magnetosomes which are membrane-encased crystals of a magnetic mineral, either magnetite and/or greigite (Fe_3S_4) in the case of many marine magnetotactic bacteria [*Bazylinski and Frankel*, 2004]. There is much evidence to show that there is functionality to magnetosomes and they are currently thought to aid cells in locating and maintaining an optimal position in vertical chemical concentration (e.g., oxygen) gradients in natural aquatic and sedimentary habitats [*Bazylinski and Frankel*, 2003; *Frankel et al.*, 1997]. Unlike the externally produced magnetite crystals synthesized by the dissimilatory iron-reducing bacteria through BIM, those produced by the magnetotactic bacteria are biomineralized intracellularly in vesicles that originate from invaginations of the cell membrane [*Komeili et al.*, 2004, 2006].

[26] Features of BCM magnetites differ greatly from those produced through BIM. BCM magnetite crystals produced by the magnetotactic bacteria have the following characteristics: (1) high chemical purity; (2) high structural perfection; (3) consistent crystal habits within a given species or strain, most commonly equidimensional cubooctahedra or nonequidimensional, pseudohexagonal prisms with (110) side faces and truncated (111) end caps, elongated along the [111] axis perpendicular to the endcaps, or tooth- and bullet-shaped crystals with a pointed end (Figure 4); (4) a certain fraction (~10% but may differ with bacterial strain) of twinned crystals characterized by rotations of 180 degrees around [111] axis with a common (111) contact plane and to a lesser degree multiple twinned crystals; (5) a

consistent width to length ratio; and (6) an asymmetric crystal-size distribution with a sharp cutoff for larger sizes within the single magnetic domain size range [e.g., *Sparks et al.*, 1990; *Bazylinski et al.*, 1994; *Devouard et al.*, 1998]. In addition, BCM magnetite crystals have novel magnetic properties that differ from those produced through BIM [*Moskowitz et al.*, 1989, 1993]. The features, taken together, show that magnetite synthesis in the magnetotactic bacteria is a result of an exquisitely controlled biomineralization process probably regulated at gene level by the bacteria [*Bazylinski and Schübbe*, 2007]. In addition, magnetosomes are usually arranged in a chain motif within the cell [*Bazylinski et al.*, 1994], an arrangement in which the total magnetic dipole moment of the cell is simply the sum of the permanent dipole moments of the individual, single-magnetic-domain magnetite particles [e.g., *Penninga et al.*, 1995; *Proksch et al.*, 1995; *Suzuki et al.*, 1998; *Dunin-Borkowski et al.*, 1998, 2001]. The result is that the chain of magnetosomes in a magnetotactic cell functions like a single magnetic dipole and causes the cell to behave similarly [*Bazylinski and Frankel*, 2003]. The development and construction of the magnetosome chain in some magnetotactic bacteria has been shown clearly to be under genetic control and relies on the MamK and MamJ proteins [*Komeili et al.*, 2006; *Scheffel et al.*, 2006].

4. Criteria Used to Distinguish Between Inorganically Produced and Biogenic Magnetite

[27] Since *McKay et al.* [1996], *Clemett et al.* [2002], and *Thomas-Keprta et al.* [2000, 2001] reported the morphological similarity of some nanometer-scale magnetite crystals in the rims of carbonate inclusions in the Martian meteorite ALH84001, to BCM magnetite in terrestrial magnetotactic bacteria, as part of the evidence for the presence of life on ancient Mars, much scientific debate has been focused on the criteria that can and must be used to distinguish between the biological and inorganic origins of magnetite crystals. At present, six criteria, when considered collectively, are used by many as a means of determining whether magnetite crystals found in the environment are biogenic and can therefore be used as a biomarker. Although these criteria were first proposed by *Thomas-Keprta et al.* [2000] and referred to as the magnetite assay for biogenicity (MAB), some have been used informally for nearly 30 years to identify the putative fossil remnants of bacterial magnetosomes (magnetofossils) in the sedimentary rock record on Earth [e.g., *Petersen et al.*, 1986; *Chang and Kirschvink*, 1989; *Chang et al.*, 1989; *Stolz et al.*, 1989; *Hesse*, 1994; *Akai et al.*, 1997; *Schwartz et al.*, 1997]. We stress here that these criteria are specifically aimed at identifying certain types of BCM magnetite and are not applicable for BIM magnetite. They include specific physical and chemical characteristics of magnetite crystals formed through BCM that, when considered collectively, are not observed in any known population of inorganic magnetite crystals.

4.1. Magnetite Assay for Biogenicity

4.1.1. Narrow Size Range

[28] Mature magnetosomes contain magnetite or greigite crystals that occur in a narrow size range, ~35–120 nm, indicating that the crystals are stable single magnetic domains [e.g., *Bazylinski*, 1995; *Bazylinski and Moskowitz*, 1997; *Sparks et al.*, 1990; *Thomas-Keprta et al.*, 2000]. This fact has physical significance for the cell because smaller particles would be superparamagnetic at ambient temperature and would not have stable remanent magnetization and multiple domains would form in larger particles thereby lowering the remanent magnetization [*Bazylinski and Frankel*, 2003]. Thus, superparamagnetic or multidomain magnetite crystals are of little or no value for magnetotaxis [*Kirschvink and Lowenstam*, 1979]. By synthesizing single magnetic domains, the cell has maximized the magnetic remanence of its magnetosome crystals. Magnetite crystals from a single species of magnetotactic bacterium exhibit a nonlognormal size distribution with the mode above the median in the single-magnetic-domain-size range and a sharp cutoff for larger sizes [*Devouard et al.*, 1998].

4.1.2. Restricted Anisotropic Width/Length Ratios

[29] Magnetosome magnetite crystals show very consistent width/length ratios within the same species of bacterium (as well as a consistent crystal habit).

4.1.3. Chemical Purity

[30] Magnetite produced by magnetotactic bacteria is generally considered to be pure stoichiometric Fe_3O_4, lacking other metal contaminants [e.g., *Mann and Frankel*, 1989; *Sparks et al.*, 1990; *Meldrum et al.*, 1993a; *Bazylinski*, 1995; *Bazylinski and Moskowitz*, 1997; *Thomas-Keprta et al.*, 2000] although some exceptions have been reported. Trace amounts of titanium have been found in magnetite from uncultured bacteria from unamended natural sediments [*Towe and Moench*, 1981]. In addition, some uncultured magnetotactic bacteria appear to incorporate manganese in their magnetosomes when $MnCl_2$ was added to the microcosms containing the cells [*Keim et al.*, 2009] while cells of three species of *Magnetospirillum* in culture have been shown to incorporate cobalt present in the growth medium in magnetosome magnetite, most probably in the surface layers of the crystals [*Staniland et al.*, 2008].

4.1.4. Crystallographic Perfection

[31] Magnetite crystals formed by magnetotactic bacteria (studied by high-resolution transmission electron microscopy, HRTEM) indicate that they are structurally perfect (i.e., free of internal defects), with the minor exception of occasional twinning usually perpendicular to the [111] axis of elongation [*Vali et al.*, 1987; *Mann et al.*, 1988a, 1988b; *Vali and Kirschvink*, 1991; *Devouard et al.*, 1998; *Thomas-Keprta et al.*, 2000]. Lack of lattice defects ensures no loss in the net magnetic moment of the particle.

4.1.5. Unusual Crystal Morphology

[32] Inorganically produced magnetite crystals less than 1 μm generally adopt isotropic forms (e.g., cubooctahedral, the equilibrium form of magnetite) in which the surface free energy is minimized [*Ichinose et al.*, 1992]. Growth of this type of crystal is centrosymmetric. Although magnetite crystals produced by some magnetotactic bacteria are cubooctahedral in morphology (e.g., *Magnetospirillum* species [e.g., *Mann et al.*, 1984; *Moisescu et al.*, 2008]), many magnetotactic bacteria biomineralize unusual anisotropic particles including elongated hexagonal prisms and tooth- or bullet-shaped crystals whose crystal growth cannot be centrosymmetric. These elongated, pseudoprismatic structures, corresponding to the unequal growth of some symmetry related faces, might occur either because of anisotropy in the

environment surrounding the crystal (e.g., chemical concentration, temperature and/or pH gradients) or the growth sites [*Mann and Frankel*, 1989]. Anisotropy might originate from an anisotropic flux of ions through the magnetosome membrane surrounding the crystal, or from anisotropic interactions of the magnetosome membrane with the growing crystal [*Mann and Frankel*, 1989].

[33] Although the information in the previous paragraph suggests that there is no significant variance to the morphologies of magnetite crystals produced by a specific species of magnetotactic bacterium, there is evidence to the contrary. *Faivre et al.* [2008] showed that biological control over magnetite biomineralization by magnetotactic bacteria can be affected by environmental parameters. More specifically, they found that the morphology of magnetite crystals (in a single species of magnetotactic bacterium, *Magnetospirillum gryphiswaldense*, which synthesizes cubooctahedral crystals) was not exclusively determined by biological intervention through vectorial regulation at organic boundaries or by molecular interaction with the magnetosome membrane but also by the rates of cellular Fe uptake. Moreover, not only morphologies affected but also crystal aspect ratios and crystal-size distributions. The authors suggest that the expression of different faces is favored for different growth conditions.

[34] *Thomas-Keprta et al.* [2002] are more stringent in their definition of unusual magnetite crystal morphology in their description of the MAB; they describe an unusual morphology based on the magnetosome crystals of marine magnetotactic bacterium strain MV-1 and their similarity to a population of presumably biogenic magnetite crystals in the ALH84001 meteorite. This elongated crystal geometry is referred to as truncated hexa-octahedral and consists of eight {111} octahedral faces, divided into two end faces and six small truncation faces, six {110} dodecahedral faces, and six {100} cubic truncation faces [*Thomas-Keprta et al.*, 2002]. The remaining six {110} faces, required by the face-centered cubic crystal structure, are presumably truncation faces of vanishingly small dimension. This is the most commonly observed crystal morphology of magnetite crystals in strain MV-1 and approximately 25% of the total magnetite crystals examined from Martian meteorite ALH84001 carbonates [*Thomas-Keprta et al.*, 2002].

4.1.6. Crystallographic Direction of Elongation

[35] For those biogenic magnetite crystals that are elongated, it is characteristic for these crystals to be elongated along one of the [111] directions, that is, one of the possible four threefold rotation axes of a regular octahedron [*Mann et al.*, 1988a, 1988b; *Vali et al.*, 1987; *Vali and Kirschvink*, 1991; *Thomas-Keprta et al.*, 2000, 2002]. Recently, however, it has been questioned whether or not the [111] crystallographic direction of elongation of BCM magnetite crystals should be used as a criterion for magnetite biogenicity since it has been found that not all BCM magnetites display crystal elongation along this direction [*Isambert et al.*, 2007].

4.2. Other Features Used to Distinguish Biogenic From Inorganic Magnetite

4.2.1. Crystal Size and Shape Factor Distributions

[36] *Buseck et al.* [2001] and *Faivre and Zuddas* [2006] suggested that the shape of the crystal size distribution (CSD; commonly shown as a plot of size along the long axes of the crystals versus frequency) could be diagnostic for BCM magnetite. For example, most CSDs of magnetite from magnetotactic bacteria studied to date are asymmetric and negatively skewed, and some have sharp cutoffs toward larger sizes [*Meldrum et al.*, 1993a, 1993b; *Devouard et al.*, 1998]. CSDs of inorganically produced magnetites are typically lognormal and tail off toward larger sizes [*Buseck et al.*, 2001]. *Arató et al.* [2005] extended these analyses by combining the use of the CSD in combination with a shape-factor distribution (SFD; represents the distribution of crystals in the shape factor which is width/length) of magnetite populations to determine whether environmental magnetite crystals are biogenic, more specifically whether they were those of magnetotactic bacteria. They found that if the SFDs of distinct magnetosome magnetite types occurring in the same sample differ, the CSDs of individual magnetosome types can be retrieved from bulk data and used to show that this specific magnetite crystal population was from magnetotactic bacteria. Interestingly, they also found that one strain of magnetotactic bacterium produced magnetite crystals with a Gaussian size distribution. *Faivre and Zuddas* [2006] combined the use of the CSD with oxygen isotope determinations (discussed in a later section) to distinguish biogenic magnetite.

4.2.2. Presence of Chains of Magnetite Crystals

[37] *Magnetospirillum* species align their magnetosomes in a linear chain within the cell through the actions of the actin-like protein MamK and the acidic protein Mam J [*Scheffel et al.*, 2006; *Komeili et al.*, 2006]. These proteins have been shown to be required for magnetosome chain assembly. Magnetosomes are attached by the protein MamJ to a series of cytoskeletal filaments that traverse the cell along its long axis and are composed of MamK. In the chain arrangement, the total magnetic moment of the chain and the cell is the sum of the individual crystals so the bacterium behaves as a single magnet. Thus, by arranging the magnetosomes in chains, the cell has maximized its possible magnetic dipole moment. The presence of magnetite chains in sediments and rocks along with morphology of the particles has been used as an indicator of the past presence of magnetotactic bacteria [*Kopp and Kirschvink*, 2008]. Chains of structures resembling magnetite crystals have even been observed in Martian meteorite ALH84001 and interpreted similarly although the structures have never been unequivocally identified as magnetite [*Friedmann et al.*, 2001]. However, the reliability of this criterion is in doubt by many since magnetosome magnetite crystals may not remain in chains after the bacterium dies and lyses [*Thomas-Keprta et al.*, 2000, 2002]. In addition, chain formation can be induced in collections of magnetic particles when strong magnets are used to separate the particles from sediments, which is often the case.

4.2.3. Magnetic Property Measurements

[38] *Kopp and Kirschvink* [2008] recently suggested the use of six criteria for the identification of biogenic magnetites based on the quality of the geologic, magnetic, and electron microscopic evidence. These criteria are mainly based on those discussed earlier (e.g., crystal morphology) but, unlike some of the criteria used in the past, also rely on a number of magnetic property determinations. Magnetosome magnetite crystals have been shown in the past to have novel magnetic properties [e.g., *Moskowitz et al.*, 1989,

1993] and several researchers have tried to exploit these properties as a means of distinguishing whether magnetite crystals are biogenic or not.

[39] One of the early magnetic techniques used to detect biogenic magnetite, now referred to the Moskowitz test [*Weiss et al.*, 2004a, 2004b], is based on work by *Moskowitz et al.* [1993] that detects the Verway transition. In 1939, Verwey reported an unusual feature of magnetite, a discontinuous drop in the conductance (an increase in resistivity) on cooling magnetite crystals below 122 K [*Verway*, 1939]. This temperature, T_V, is known as the Verwey temperature and is dependent on the chemical purity of magnetite and to a lesser extent crystal size. The Moskowitz test is based on a number of low-temperature magnetic measurements, the most useful being: (1) acquisition and demagnetization of isothermal remanent magnetization (IRM) using static, pulse and alternating magnetic fields; (2) acquisition of anhysteretic remanent magnetization (ARM); and (3) thermal dependence of low-temperature (20 K) saturation IRM (SIRM) after cooling in a zero magnetic field (ZFC) or in a 2.5 T field (FC) from 300 K [*Moskowitz et al.*, 1993]. The most diagnostic magnetic parameter for magnetosome chain identification in bulk sediment is related to the difference between low-temperature zero field and field cooled SIRMs on warming through the Verwey transition (T ~ 100 K). Intact chains of unoxidized magnetite (magnetite oxidizes to maghemite over time in air) magnetosomes have ratios of $\delta_{FC}/\delta_{ZFC} > 2$, where δ is a measure of the amount of magnetic remanence lost by warming through the Verwey transition. Oxidation of the magnetite to maghemite or disruption of the chain arrangement reduces the δ_{FC}/δ_{ZFC} ratio to about 1, a value typically observed for some inorganically produced magnetite, maghemite, greigite and BIM magnetite particles produced by dissimilatory Fe(III)-reducing bacteria [*Moskowitz et al.*, 1993]. Numerical simulations of δ_{FC}/δ_{ZFC} ratios for simple binary mixtures of magnetosome chains and inorganic magnetic fractions suggest that δ_{FC}/δ_{ZFC} can be a sensitive indicator of the presence of biogenic magnetite in the form of intact chains of magnetite magnetosomes and can be a useful magnetic technique for detecting them in bulk sediment samples or rocks [*Moskowitz et al.*, 1993; *Weiss et al.*, 2004a, 2004b].

[40] A more recently developed type of magnetic determination is through ferromagnetic resonance spectroscopy (FMR) which can be used to measure the effective magnetic field within a sample and includes contributions from both magnetic anisotropy and magnetostatic interactions [*Weiss et al.*, 2004a, 2004b; *Kopp et al.*, 2006]. FMR has been used to determine the presence of BCM magnetite produced by magnetotactic bacteria since the technique can detect many important features of this magnetite such as they are single-magnetic-domain crystals with narrow size and shape distributions that are often elongated and generally arranged in chains [*Kopp et al.*, 2006].

[41] The criteria of *Kopp and Kirschvink* [2008] extensively utilize magnetic measurements and include the following: (1) whether the geological context of where the magnetofossils were found is well understood stratigraphically, geochemically, and paleomagnetically (whether robust paleomagnetic evidence is available that suggest a primary magnetization is present); (2) whether magnetic or microscopic evidence support the presence of significant single-domain magnetite; (3) whether magnetic or FMR evidence indicates narrow size and shape distributions, and whether microscopic evidence reveals single-domain particles with truncated edges, elongate single-domain particles, and/or narrow size and shape distributions; (4) whether FMR, low-temperature magnetic, or electron microscopic evidence reveals the presence of chains of magnetosome crystals; (5) whether low-temperature magnetometry, energy dispersive X-ray spectroscopy, or other techniques demonstrate the high chemical purity of the particles (lacking metal ions other than Fe, and, in particular, the absence of Ti); and (6) whether high-resolution TEM (transmission electron microscopy) indicates crystallographic perfection (the near absence of crystallographic defects). These authors use a score system to determine whether a magnetofossil identification is robust, using the first criterion to set the threshold.

5. Potential Problems, New Techniques, and Methodologies

[42] In spite of the considerable effort to define criteria to distinguish whether magnetite crystals found in natural environments are biogenic in origin, the use of some of these criteria has been questioned and debated by many for a number of reasons [e.g., *Buseck et al.*, 2001]. We pointed out several problems with the use of the separate criteria in the MAB in the previous section and will address others here. We remind the reader, however, that satisfying a sole criterion is not considered enough to claim a biological origin of magnetite. The fulfillment of all the criteria has been proposed as a robust signature for biogenic magnetite [*Thomas-Keprta et al.*, 2000, 2002].

5.1. Issues and Problems Using Crystal Morphology and Size as an Indicator of Biogenicity

[43] The problem of using crystal morphology as a criterion to recognize the biological origin of minerals is an old one and is not limited to magnetite. The authenticity of many ancient fossils of prokaryotes, once accepted by the scientific community as valid, is currently under great scrutiny [*Dalton*, 2002]. An illustration of this point concerns the putative microbial fossils supposedly representing cyanobacterial species from 3.5 billion year old cherts from the Precambrian Warrawoona formation in Western Australia [*Schopf and Packer*, 1987; *Schopf*, 1993]. *Brasier et al.* [2002] reexamined the putative fossils and offered an alternative explanation for their formation, i.e., the structures are secondary artifacts formed from amorphous graphite within multiple generations of metalliferous hydrothermal vein chert and volcanic glass while *Dalton* [2002] described these putative fossils as "carbonaceous blobs, probably formed by the action of scalding water on minerals." *García-Ruiz et al.* [2003, 2009] were able to synthesize inorganic micron-sized filaments of silica-coated nanometer-sized carbonate crystals, arranged with strong orientational order that exhibit noncrystallographic morphologies (curved, helical) reminiscent of biological forms. The morphology of those filaments (so-called biomorphs) is very similar to the putative cyanobacterial microfossils in the cherts. These authors propose an inorganic model for the formation of those biomorphs involving the participation of simple

organic hydrocarbons, whose sources may also be inorganic. The authors conclude "Our results demonstrate that abiotic and morphologically complex microstructures that are identical to currently accepted biogenic materials can be synthesized inorganically" [*García-Ruiz et al.*, 2003, 2009].

[44] Much attention has been focused on the use of crystal morphologies as indicators of magnetite biogenicity since it was the first criteria used historically for this purpose. Again, this was based on the fact that magnetotactic bacteria biomineralize crystals with morphologies not observed in inorganically synthesized particles. One of the major criticisms in using morphology as a criterion is based on the methodology used in the determination of the three-dimensional (3-D) structures of nanometer-sized crystals. Such particles are most often analyzed by some form of electron microscopy, and the difficulty arises when 3-D shapes are inferred from 2-D images. One method used by *Thomas-Keprta et al.* [2001] and *Golden et al.* [2004] to minimize this problem was to reconstruct the 3-D shapes of magnetite crystals by multiple 2-D TEM bright field images taken at different tilting angles. The best method, however, in determining the 3-D structure of small crystals is the use of electron tomography which has been used successfully for this purpose on magnetite crystals from a magnetotactic bacterium and from ALH84001 [*Clemett et al.*, 2002]. These techniques involve the examination of a statistically significant number of crystals and thus a large amount of labor intensive work is required.

[45] With the exception of octahedral magnetite crystals, we are unaware of any abiotic procedure that has exactly reproduced the morphologies and physical and magnetic features of BCM anisotropic magnetite produced by the magnetotactic bacteria with the possible exception of magnetite crystals synthesized in the study by *Golden et al.* [2004]. The key to synthesizing these types of magnetites may lie in understanding how the magnetotactic bacteria and other organisms biomineralize such particles. This information, in turn, might prove valuable in determining whether crystal morphology is useful in determining magnetite biogenicity.

[46] Although some of the proteins and molecular events involved in the construction of the magnetosome chain have been recently characterized [*Komeili et al.*, 2004, 2006; *Scheffel et al.*, 2006], little is known regarding the actual biochemical and biomineralization processes leading to synthesis of magnetite by magnetotactic bacteria. Synthesis of the bacterial magnetosome chain involves several discrete steps including magnetosome vesicle formation, assembly of the vesicles in chains, iron uptake by the cell, iron transport into the magnetosome vesicle and controlled Fe_3O_4 (or Fe_3S_4) biomineralization within the magnetosome vesicle [*Bazylinski and Frankel*, 2004; *Frankel and Bazylinski*, 2006].

[47] The magnetosome membrane vesicle was thought to be of prime importance in the process as it was assumed that the vesicle controlled the chemical conditions (e.g., pH, Eh) that result in making magnetite the most stable Fe-bearing mineral phase as well as the size and shape of the magnetite crystal [*Gorby et al.*, 1988]. It is now clear that the magnetosome membrane vesicle originates from an invagination of the cell (plasma) membrane and that the vesicle forms prior to magnetite biomineralization [*Komeili et al.*, 2006]. The magnetosome membrane contains proteins that are unique to this structure and that are not found in other parts of the cell [*Bazylinski and Frankel*, 2004]. For this reason, these proteins are those thought to be involved in the controlled biomineralization of magnetite which has led to a number of studies where magnetite is chemically precipitated in the presence of specific magnetosome membrane proteins.

[48] *Arakaki et al.* [2003] examined a number of magnetosome membrane proteins in *Magnetospirillum magneticum* strain AMB-1 and found that when magnetite was precipitated chemically in the presence of one of them, Mms6, the morphology of the crystals was affected and slightly approached that of mature magnetosome crystals in intact cells. Others [*Amemiya et al.*, 2007; *Prozorov et al.*, 2007a, 2007b] performed modified versions of this experiment and confirmed the results of *Arakaki et al.* [2003]. Mms6 is an amphiphilic protein consisting of an N-terminal LG-rich hydrophobic region and a C-terminal hydrophilic region containing repeats of acidic amino acids that might suggest that cations such as Fe might bind to this latter region of the protein. *Amemiya et al.* [2007] concluded that the Mms6 protein: (1) acts as a template/scaffold for magnetite precipitation; (2) regulates the size of the magnetite crystals to approximately 20 nm; and (3) restricts the shape of the magnetite crystals to the cubooctahedral habit by association with specific crystal faces. It would be interesting to examine the morphology of the crystals using some of the high-resolution techniques discussed earlier to obtain an accurate reconstruction of the crystals' 3-D morphology.

[49] There are few in vivo studies involving the role specific magnetosome proteins in the BCM process of magnetite in magnetotactic bacteria. The MamGFDC proteins are highly conserved among magnetotactic bacteria and make up about 35% of the protein associated with the magnetosome membrane in *Magnetospirillum gryphiswaldense* [*Scheffel et al.*, 2008]. These proteins have been found to regulate the size of magnetosome magnetite crystals as mutants that lack these genes produced magnetite crystals that were only about 75% of the size of the crystals produced by cells of the wild-type. The proteins appear to be at least partially functionally redundant and act synergistically in controlling the size of magnetite crystals in *M. gryphiswaldense* [*Scheffel et al.*, 2008].

[50] The key to understanding the biomineralization of the unusual morphologies of BCM magnetites, protein-mineral interactions must be explored to a much greater extent, in both in vitro and in vivo experiments. It seems unlikely that, given the relatively large number of different proteins present in the magnetosome membrane, that only one acts as a template/scaffold for magnetite precipitation and is responsible for the size and shape of the crystal. Other parameters that should be investigated are the effects of protein concentration, pH, and so on. It is noteworthy that the experiments conducted by *Amemiya et al.* [2007] were performed at unusually high temperatures (90°C) at which the bacterium is incapable of growth. Because it is difficult to distinguish between magnetite and maghemite using X-ray diffraction (XRD) and selected area electron diffraction (SAED), it is crucial to ensure that the final product of these types of reactions is indeed magnetite. These minerals can be differentiated using Mossbauer spectroscopy [*Schwertmann*

and Cornell, 2000] and Raman spectroscopy [*Hanesch*, 2009].

[51] Another approach to understand the role(s) of specific magnetosome membrane proteins in the biomineralization of magnetite is the use of mutants in which specific magnetosome membrane protein genes have been inactivated (knockout mutants). After inactivation of specific genes, the organism can be grown under normal conditions where normal cells produce magnetosomes, and then examined for effects on magnetosome synthesis. This is a commonly used strategy in microbiology to understand and elucidate the processes involved in many physiologic and genetic functions and was used successfully in demonstrating the roles of the proteins MamJ and MamK in construction of the magnetosome chain [*Komeili et al.*, 2006; *Scheffel et al.*, 2006] and the roles of the MamGFDC proteins in regulating the size of magnetosome magnetite crystals [*Scheffel et al.*, 2008].

[52] The last point we present here regarding crystal morphology is that it must be considered that organisms other than prokaryotes biomineralize single-magnetic-domain crystals of magnetite that have similar morphologies to the BCM crystals of the magnetotactic bacteria. These organisms include single-celled eukaryotes such as protists [*Bazylinski et al.*, 2000] and algae [*Torres de Araujo et al.*, 1985] and higher organisms such as some fish (e.g., sockeye salmon [*Mann et al.*, 1988a, 1988b]) and even some plants [*Gajdardziska-Josifovska et al.*, 2001]. Although some of these organisms biomineralize magnetite crystals with a cubooctahedral morphology like sockeye salmon [*Mann et al.*, 1988a, 1988b], others synthesize elongated anisotropic particles (tooth-shaped in the algae [*Torres de Araujo et al.*, 1985] and a percentage of hexahedral prisms as well as octahedra in grass cells [*Gajdardziska-Josifovska et al.*, 2001]). Recently, exceptionally large biogenic magnetite crystals were discovered in clay-rich sediments spanning the Paleocene-Eocene Thermal Maximum in a borehole at Ancora, NJ [*Schumann et al.*, 2008]. These crystals exhibited novel spearhead-like and spindle-like morphologies with sizes up to 4 μm long and hexaoctahedral prisms up to 1.4 μm long. Like the BCM magnetite crystals of magnetotactic bacteria, these single-crystal particles exhibit chemical composition, lattice perfection, and oxygen isotopes consistent with an aquatic origin. Electron holography demonstrates single-domain magnetization despite their large crystal size. The large size of these crystals appears to preclude them from being synthesized internally in prokaryotes and therefore it seems likely that if they represent true examples of BCM then it would be by some type of eukaryote. The contribution of eukaryotes to the magnetization of sediments, soils and other natural habitats is unknown.

5.2. Issues and Problems With Chemical Purity

[53] Although most early high-resolution studies of magnetosome magnetite resulted in the conclusion that this magnetite is essentially pure enough to be considered stoichiometric magnetite [e.g., *Sparks et al.*, 1990; *Meldrum et al.*, 1993a, 1993b; *Thomas-Keprta et al.*, 2000], some recent studies show that metals other than iron can be incorporated into magnetosome magnetite [*Towe and Moench*, 1981; *Staniland et al.*, 2008; *Keim et al.*, 2009] as discussed in a previous section. Two of these studies [*Towe and Moench*, 1981; *Keim et al.*, 2009] deal with uncultured cells collected from natural environments indicating that the incorporation of contaminating metals in magnetosome magnetite can occur in nature and not just in culture.

[54] Key questions regarding these studies is whether the metal cation is actually incorporated within the mineral structure, and, if so, is the resulting crystal a solid solution where the metal cation replaces some of the Fe atoms in the crystal lattice or is the crystal composed of one or more mixed mineral phases. The situation for the examples provided above is unclear although in the case of cobalt incorporation by cells of *Magnetospirillum* species, the cobalt appears to be confined to surface layers of the magnetite [*Staniland et al.*, 2008]. Several techniques can be used for this determination including XRD and Raman spectroscopy. A thorough and careful analysis of XRD spectra of magnetite might reveal peak shifts indicating a solid solution or peak shoulders which would indicate a mixture of mineral phases. Analyzing shifts in Raman spectra or changes in magnetic properties or using X-ray absorption fine structure spectroscopy (XAFS) to determine cation composition and coordination might also prove very helpful here. Raman spectroscopy is nonintrusive and nondestructive and has also been used in the form of laser-Raman spectroscopic imagery to examine the putative ancient filamentous cyanobacterial microbial fossils [*Schopf et al.*, 2002] and thus could prove a valuable tool in the armamentaria used in the evaluation and assessment of structures that could represent microbial fossils.

[55] The high purity criterion has recently gained more importance in the debate whether there is a subset of biogenic magnetite crystals in the ALH84001 meteorite. *Golden et al.* [2004] claimed that chemically pure magnetites can be obtained from the thermal decomposition of magnesium siderites at 470°C under anoxic conditions and proposed an inorganic mechanism for the formation of the chemically pure magnetites in ALH84001. *Thomas-Keprta et al.* [2009], performing similar experiments, obtained a strikingly different result. They could not produce chemically pure magnetites from thermally decomposed sideritic carbonates that contained Mg, Mn and Ca and reported only finding magnetites containing several mol % Mg and Mn. This result is consistent with those of many other similar studies [e.g., *Gallagher and Warne*, 1981; *Dubrawski*, 1991; *Gotor et al.*, 2000; *Isambert et al.*, 2006]. Recently, *Jimenez-Lopez et al.* [2008] reported that the thermal decomposition of (Ca, Mg, Fe)CO_3 at 700°C under 1 atm CO_2 yielded magnetites with different amounts of Mg and Ca incorporated into the magnetite crystal structure. This result is interesting because of the difficulty of integrating the Ca(II) cation into the magnetite structure due to its size while Mg and Mn are more easily incorporated into the magnetite structure [*Thomas-Keprta et al.*, 2009]. It appears that only *Golden et al.* [2004] were able to obtain chemically pure magnetite from the thermal decomposition of mixed cation siderite (in this case from Copper Lake). However, *Bell* [2007] showed that the Copper Lake siderite used by *Golden et al.* [2004] was a finely intermixed sample of nearly pure siderite embedded within impure siderite and, during heating as described by *Golden et al.* [2004] only the most Fe-rich portion of the siderite would have decomposed forming nearly pure magnetite [*Thomas-Keprta et al.*, 2009].

Thus it seems that *Golden et al.* [2004] did not produce chemically pure magnetite from mixed composition siderite since the temperature was too low to decompose the mixed cation carbonate and only the decomposition threshold for nearly pure siderite was reached [*Thomas-Keprta et al.*, 2009].

[56] Before the "chemically pure magnetite" criterion can be used reliably, it is clearly important to determine whether or not cations other that iron can get incorporated into the structure of BCM magnetites.

6. Other Options, Possibilities, and Considerations

6.1. Use of Isotopes

[57] Living organisms are known to fractionate the stable isotopes of some elements that are incorporated in biominerals because the biochemical pathway(s) used to produce biominerals may be influenced by kinetic isotope effect [*O-Neil*, 1986]. If characteristic isotope fractionations are expressed for iron (Fe) and/or oxygen (O), this would provide a valuable biosignature for the origin of Fe-oxides (e.g., magnetite) that are produced biogenically. Delta notation is a common convention used to report isotope compositions and it is expressed here as δ^{56}Fe for Fe and δ^{18}O for O where:

$$\delta = \left[R_{sample}/R_{standard} - 1\right] \times 1000 \text{ in per mill (‰) units;}$$
$$R = {}^{56}Fe/{}^{54}Fe \text{ or } {}^{18}O/{}^{16}O$$

[58] *Johnson et al.* [2004] recognized three principal pathways through which Fe isotopes may be fractionated by microorganisms: (1) assimilatory Fe metabolism (iron incorporated into cell material; e.g., through the action of siderophores [*Brantley et al.*, 2001] or biologically controlled mineralization of magnetosomes [*Bazylinski and Moskowitz*, 1997]), (2) lithotrophic or phototrophic Fe(II) oxidation [*Widdel et al.*, 1993; *Heising and Schink*, 1998; *Heising et al.*, 1999; *Emerson*, 2000; *Straub et al.*, 2001; *Ehrlich and Newman*, 2008], and (3) dissimilatory Fe(III) reduction [*Lovley*, 1987; *Nealson and Myers*, 1990]; each of these pathways is briefly discussed below.

[59] Little work has been conducted on Fe isotope fractionation during assimilatory Fe metabolism. A single prominent study by *Mandernack et al.* [1999] suggests there is little to no measurable Fe isotope fractionation during the production of intracellular magnetosomes by magnetotactic bacteria.

[60] Two prominent studies have been conducted on Fe isotope fractionation during Fe oxidation by bacteria under circum-neutral [*Croal et al.*, 2004] and acidic [*Balci et al.*, 2006] conditions. These experiments showed a 1 to 3‰ increase in δ^{56}Fe in the precipitated ferric oxide, regardless of whether the reaction occurred under sterile or microbiologically active conditions. This is not to say that biological fractionation does not occur during Fe oxidation as isotope fractionations are best expressed in systems where the exchange kinetics proceed more slowly that the precipitation rate of the solid. *Beard and Johnson* [2004] proposed a conceptual model of iron oxidation that predicts the net Fe isotope fractionation between soluble Fe and iron oxide as a combination of the equilibrium fractionation between soluble Fe(II) and Fe(III) (2.9‰ at 25°C [*Welch et al.*, 2003]) and a kinetic fractionation between aqueous Fe(III) and iron oxide (≤1.3‰ [*Skulan et al.*, 2002]). The model predicts an isotope fractionation between soluble Fe(II) and ferric oxide up to ~3‰, with lower values if a strong kinetic fractionation exists between Fe(III) and iron oxide. This conceptual framework was validated experimentally by *Balci et al.* [2006] who showed that acidophilic bacteria are capable of fractionating Fe isotopes at low pH when the precipitation rate of ferric oxide is relatively low. They found that the difference in isotope composition between soluble Fe(III) and Fe(II) was approximately 3‰, and that ferric oxide had an Fe isotope composition that was equal to or less than soluble Fe(III).

[61] Most studies of microbiological Fe isotope fractionation are conducted with microorganisms that conserve energy through dissimilatory Fe(III) reduction. Experimental studies have shown that a variety of substrates can act as electron acceptors including dissolved Fe(III), hydrous ferric oxide (HFO), goethite, hematite, magnetite, and Fe(III)-clay minerals [*Lovley*, 1991; *Gates et al.*, 1993; *Kostka and Nealson*, 1995; *Kostka et al.*, 1996; *Neal et al.*, 2003; *Kim et al.*, 2004]. The end product of dissimilatory Fe(III) reduction is Fe(II), which commonly precipitates as magnetite and siderite under the appropriate environmental conditions [*Lovley and Phillips*, 1988; *Roden and Lovley*, 1993; *Fredrickson et al.*, 1998; *Zhang et al.*, 1998, 2001; *Roden et al.*, 2002; *Zachara et al.*, 2002]. Based on batch culture experiments, the aqueous Fe(II) produced by dissimilatory Fe(III) reduction has an Fe isotope composition that is 0.5 to 2‰ lower than the ferric substrate from which it is derived [*Beard et al.*, 1999, 2003; *Icopini et al.*, 2004; *Johnson et al.*, 2005; *Crosby et al.*, 2005, 2007]. As previous studies suggest, the key step to understanding Fe isotope fractionation during dissimilatory Fe(III) reduction is the transformation reaction that occurs during electron transfer. Based on culture experiments with cells of *Geobacter sulfurreducens* and *Shewanella putrefaciens* that used goethite or hematite as the ferric substrate, *Crosby et al.* [2005, 2007] demonstrated there was a 3‰ isotope fractionation between aqueous Fe(II) and the outermost layer of ferric iron on the oxide surface, which was explained by equilibrium isotope fractionation between aqueous Fe(II) and the solid reactive substrate. Leaches of the solid revealed that sorbed Fe(II) had a Fe isotope composition that was 0.4‰ (hematite) or 0.8‰ (goethite) higher than aqueous Fe(II). Although sorbed Fe(II) had a slightly different Fe isotope composition compared to soluble Fe(II), sorption alone was insufficient to produce Fe(II) with the relatively low Fe isotope composition measured in these experiments. Instead, it appears the fractionation step requires electron and atom exchange between Fe(II) and Fe(III) iron within a reactive surface layer of the iron oxide.

[62] As stated above, the end product of dissimilatory Fe(III) reduction is soluble Fe(II), that could be incorporated in siderite and/or magnetite. These solid minerals may preserve an Fe isotope signature of the processes responsible for their formation. *Johnson et al.* [2005] reported little difference between the Fe isotope composition of pure siderite and soluble Fe(II) in dissimilatory Fe(III) reduction experiments

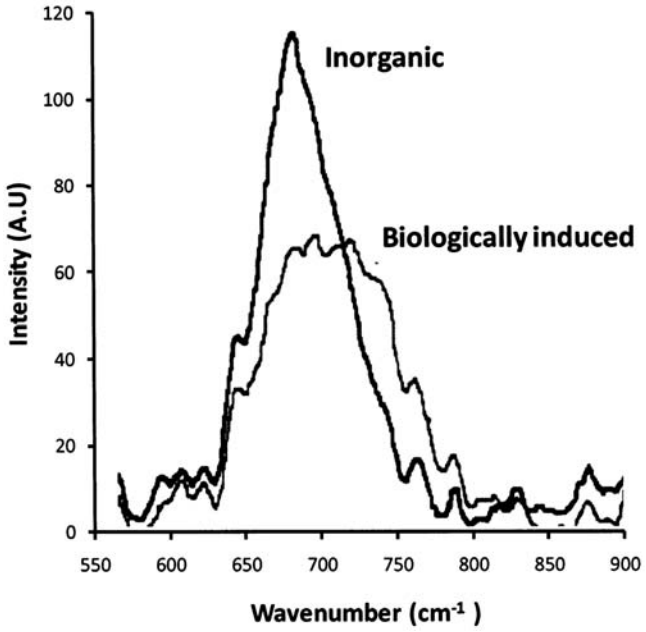

Figure 5. Raman spectra of inorganically produced magnetite and biologically induced mineralized (BIM) magnetite produced by cells of *Shewanella oneidensis*. The inorganic magnetite was synthesized using the coprecipitation method as described in the caption of Figure 2. Note that the oxide peak of magnetite is shifted toward higher wave numbers in the BIM magnetite (700 cm^{-1}) compared to that of the inorganically produced magnetite (670 cm^{-1}).

when these phases reached isotope equilibrium, while siderite was ~1.0‰ higher than soluble Fe(II) for experiments where kinetic fractionation was expressed. In addition, substitution of foreign ions into the siderite structure strongly influences the Fe isotope composition of the solid, with Ca-substituted siderite being ~1.0‰ greater than pure $FeCO_3$.

[63] Calculated and measured Fe isotope fractionations for the microbial magnetite–soluble Fe(II) system range from 4.2 to 0.0‰ [*Mandernack et al.*, 1999; *Polyakov and Mineev*, 2000; *Schauble et al.*, 2001; *Johnson et al.*, 2003, 2004], which is nearly as broad as the entire range of terrestrial Fe isotope compositions observed in nature. Based on long-term (~1 year) dissimilatory Fe(III) experiments where magnetite was produced through the reaction between ferrihydrite and soluble Fe(II), *Johnson et al.* [2005] inferred that the equilibrium fractionation between Fe(II) and biological magnetite is −1.3‰. However, isotope fractionation of Fe has been observed in abiotic systems [*Bullen et al.*, 2001; *Johnson et al.*, 2002; *Roe et al.*, 2003], so it is still unclear whether the observed fractionations are due to biological or abiotic processes. Thus additional studies are required to definitively determine whether Fe isotopic fractionation can be used reliably as a fingerprint of bacterial activity.

[64] Potential isotope biosignatures have also been investigated for magnetite using oxygen isotope systematics. For instance, *Mandernack et al.* [1999] determined the O isotope composition of BCM magnetite produced by two species of magnetotactic bacteria grown at temperatures between 4°C and 35°C under microaerobic and anaerobic conditions. Their data indicate a temperature-dependent fractionation for the magnetite-water system that is consistent with the BIM magnetite-water fractionation determined by *Zhang et al.* [1997] using thermophilic Fe(III)-reducing bacteria (BIM magnetite-water system decreased from −0.09‰ at 50°C to −1.08‰ at 70°C).

[65] *Faivre and Zuddas* [2006] determined the fractionation of O isotopes for the magnetite-water system under a wide range of conditions. Their data showed marked differences in the fractionation of oxygen isotopes for biologically and inorganically produced magnetite compared to *Mandernack et al.* [1999] and *Zhang et al.* [1997]. While the fractionation factor for the inorganic magnetite–water system increased by ~2.5‰ between 5° and 69°C, the biological magnetite–water system factor decreased ~3.5‰, with a crossover point at about 43°C [*Faivre et al.*, 2004]. These results suggest that the oxygen isotope composition of magnetite could be a suitable criterion to identify biogenic magnetite if the formation temperature of the mineral or the oxygen isotope composition of water is known. Because many bacteria are known to synthesize magnetite near the crossover point, more work should be performed at these temperatures with both Fe(III)-reducing and magnetotactic bacteria.

6.2. Organic Compounds and Changes in the Crystal Structure

[66] In many cases, organic compounds have an essential role on mineral formation. They can: (1) template the nucleation of crystals [*Pentecost*, 1985; *Mann et al.*, 1988a, 1988b; *Dupraz and Visscher*, 2005]; (2) concentrate positively charged cations at the negatively charged areas of the cell wall, membranes, debris or even byproducts of bacterial metabolic activity [*Fowle and Fein*, 2001; *Rodríguez-Navarro et al.*, 2007; *Neal et al.*, 2007]; (3) favor the oriented aggregation of homogenously nucleated crystals by electrostatic affinity between negatively charged ions and specific atomic planes of the crystals (ionotropic effect [*Rodríguez-Navarro et al.*, 2007]); and (4) trap seed crystals which act as nuclei for heterogeneous precipitation [*Knorre and Krumbein*, 2000; *Turner and Jones*, 2005].

[67] Organic compounds have been shown on occasion to become incorporated within the crystal structure of a mineral, modifying and sometimes even stabilizing the mineral. This has been shown to be true for BIM vaterite ($CaCO_3$) produced by *Myxococcus xanthus* [*Rodríguez-Navarro et al.*, 2007] and BIM magnetite produced by *Shewanella oneidensis* [*Perez-Gonzalez et al.*, 2010]. There is a visible shift to higher frequencies of the Raman spectrum of the *Shewanella* BIM magnetite compared to that of magnetite produced inorganically (Figure 5). More studies are needed to confirm such differences in other BIM magnetites. This may be true of some BCM magnetites although there is currently no evidence for the presence of even trace amounts of organics in the mineral structure of BCM magnetite. If these structural differences due to the presence of organics are maintained over significant periods of time, their presence could constitute a biosignature for BIM magnetite if other characteristics of BIM magnetite are present. Raman spectrometry should be considered now as a valuable tool for detecting changes in magnetite structure

caused by the incorporation of both organics and "foreign" cations.

6.3. Structural Relationships Between Crystals and Topotaxy

[68] Because of the controversy regarding the possible thermal decomposition origin of the magnetites in ALH84001, it is important to determine whether or not magnetites found in other geological samples originated via thermal decomposition of a carbonate precursor. There is an increasing amount of evidence that the endothermic decomposition reactions of a solid phase A into two phases B (solid) + C (gas) are topotactic. This means that the product crystals of the reaction maintain the orientation of the precursor mineral. Structural features of the precursor, frequently the packing of the bulkiest ions, survive relatively unchanged in the product crystals [*Dasgupta*, 1961]. Topotaxy has been found to occur in the decomposition of many hydroxides and oxy-hydroxides [e.g., *Chaix-Pluchery et al.*, 1983; *Kim et al.*, 1987; *Figlarz et al.*, 1990], dolomite ($MgCa(CO_3)_2$) [*Carter and Buseck*, 1985], ankerite ($MgFe(CO_3)_2$) [*Dasgupta*, 1965], siderite ($FeCO_3$) [*Dasgupta*, 1961], magnesite ($MgCO_3$) [*Dasgupta*, 1964; *Kim et al.*, 1987] and calcite ($CaCO_3$) [*Rodríguez-Navarro et al.*, 2009].

[69] Therefore, magnetites formed by the thermal decomposition of a Fe-bearing carbonate phase (e.g., siderite), would maintain a topotactic relationship with the mineral precursor. Studies involving structural relations within crystals and their relative orientations may help identify whether or not such crystals were produced by a thermal decomposition of a carbonate precursor.

[70] *Barber and Scott* [2002] examined magnetite and periclase (MgO) crystals in the Fe-Mg-Ca carbonates in the Martian meteorite ALH84001 using transmission electron microscopy, to evaluate whether there was any kind of structural relationship with the carbonate matrix that might indicate whether the crystals originated from thermal decomposition of the carbonates. They found that magnetite crystals growing in small voids and in microfractures occasionally had epitaxial relationships with the carbonate, while those nanocrystals fully embedded in the ferroan carbonate showed topotaxy (3-D lattice continuity) with the carbonate matrix rather than epitaxy. The structural relationship between the two minerals (magnetite and carbonate matrix) was $\{111\}_{mag}$// (i.e., parallel) $(0001)_{carb}$ and $\{110\}_{mag}//\{11\bar{2}0\}_{carb}$. Based on these results, *Barber and Scott* [2002] concluded that the mineral crystals resulted from the thermal decomposition of the carbonates. However, there were thousands of magnetite crystals that in ALH84001 that show no structural relationship with the ankerite matrix. *Barber and Scott* [2002] also assumed that the thermal decomposition of the ankerite matrix would result in pure oxides (MgO, Fe_3O_4 and CaO), a supposition that is in sharp contrast with the findings of *Thomas-Keprta et al.* [2009] who demonstrated that thermal decomposition of the ankerite matrix would result in the formation of magnetites with Mg substitutions. If *Barber and Scott*'s [2002] assumption was true, periclase (MgO) would probably be present in relatively large amounts in ALH84001 and yet very little was found. Nonetheless, others [e.g., *Treiman*, 2003] agree with the conclusions of *Barber and Scott*'s [2002] and believe the heat for the ankerite decomposition originated from thermal shock. It seems obvious that more work is required to better understand the chemistry and structural changes following the thermal decomposition of ankerites.

7. Conclusions and Challenges for the Future

[71] A major problem for understanding the origin of life and the evolutionary origin and phylogeny of prokaryotes and eukaryotic microbes is the general lack of reliable microbial fossils. As we illustrated in an earlier section, many of the structures assumed to be fossilized microbes, much of which are at least partially mineralogical, is subject to alternate interpretations in which abiotic, inorganic reactions have been implicated.

[72] In the last several decades, much attention has been focused on magnetite synthesis by prokaryotes including both the dissimilatory iron-reducing and the magnetotactic bacteria, and the subsequent use of nanometer-sized crystals of magnetite as magnetofossils, a biosignature of the past presence of these organisms. Although much has been learned as to how these organisms biomineralize magnetite and how magnetite is formed inorganically, there are still many parameters that affect the chemical and isotopic composition, crystal morphology and mineral structure of magnetite that still cause many to doubt the reliability of magnetite nanocrystals as magnetofossils. Others are convinced and the debate continues. In this paper, we tried to present a nonbiased viewpoint on this issue by describing studies that have data and conclusions that represent both sides of the issue. At this point in time based on the data we currently have, it seems unlikely that a consensus of opinion on the issue will be reached. The good news is that it appears that there are a number of new and old promising technologies and techniques, some of which we describe here, that might be applied in novel ways to studies of magnetite and other potential microbial fossils.

[73] **Acknowledgments.** We thank R. B. Frankel for critically reviewing this manuscript and for helpful suggestions and comments and C. Lefevre, T. Perez-Gonzalez, and T. Prozorov for use of figures and for technical assistance. We gratefully acknowledge support from U.S. National Science Foundation grant EAR-0715492 (D.A.B.) and from Spanish Ministry of Science (MEC) grant CGL2007–63859 (C.J.-L.). This work was also supported in part by NASA's Astrobiology Institute for C.S.R.. Finally, we are grateful to three anonymous reviewers whose suggestions and comments, we believe, greatly improved this paper.

References

Akai, J., A. Iida, K. Akai, and A. Chiba (1997), Mn and Fe minerals of possible biogenic origin from two Precambrian stromatolites in western Australia, *J. Geol. Soc. Jpn.*, *103*, 484–488.

Amemiya, Y., A. Arakaki, S. S. Staniland, T. Tanaka, and T. Matsunaga (2007), Controlled formation of magnetite crystal by partial oxidation of ferrous hydroxide in the presence of recombinant magnetotactic bacterial protein Mms6, *Biomaterials*, *28*, 5381–5389, doi:10.1016/j.biomaterials.2007.07.051.

Arakaki, A., J. Webb, and T. Matsunaga (2003), A novel protein tightly bound to bacterial magnetic particles in *Magnetospirillum magneticum* strain AMB-1, *J. Biol. Chem.*, *278*, 8745–8750, doi:10.1074/jbc.M211729200.

Arató, B., Z. Szányi, C. Flies, D. Schüler, R. B. Frankel, P. R. Buseck, and M. Pósfai (2005), Crystal-size and shape distributions of magnetite from uncultured magnetotactic bacteria as a potential biomarker, *Am. Mineral.*, *90*, 1233–1241, doi:10.2138/am.2005.1778.

Balci, N., T. D. Bullen, K. Witte-Lien, W. C. Shanks, M. Motelica, and K. W. Mandernack (2006), Iron isotope fractionation during microbially

stimulated Fe(II) oxidation and Fe(III) precipitation, *Geochim. Cosmochim. Acta*, *70*, 622–639, doi:10.1016/j.gca.2005.09.025.
Barber, D. J., and E. R. D. Scott (2002), Origin of supposedly biogenic magnetite in the Martian meteorite Allan Hills 84001, *Proc. Natl. Acad. Sci. U. S. A.*, *99*, 6556–6561, doi:10.1073/pnas.102045799.
Bazylinski, D. A. (1995), Structure and function of the bacterial magnetosome, *ASM News*, *61*, 337–343.
Bazylinski, D. A., and R. B. Frankel (2003), Biologically controlled mineralization in prokaryotes, *Rev. Mineral. Geochem.*, *54*, 95–114, doi:10.2113/0540217.
Bazylinski, D. A., and R. B. Frankel (2004), Magnetosome formation in prokaryotes, *Nat. Rev. Microbiol.*, *2*, 217–230, doi:10.1038/nrmicro842.
Bazylinski, D. A., and B. M. Moskowitz (1997), Microbial biomineralization of magnetic iron minerals: Microbiology, magnetism and environmental significance, *Rev. Mineral.*, *35*, 181–223.
Bazylinski, D. A., and S. Schübbe (2007), Controlled biomineralization by and applications of magnetotactic bacteria, *Adv. Appl. Microbiol.*, *62*, 21–62, doi:10.1016/S0065-2164(07)62002-4.
Bazylinski, D. A., A. J. Garratt-Reed, and R. B. Frankel (1994), Electron microscopic studies of magnetosomes in magnetotactic bacteria, *Microsc. Res. Tech.*, *27*, 389–401, doi:10.1002/jemt.1070270505.
Bazylinski, D. A., D. R. Schlezinger, B. H. Howes, R. B. Frankel, and S. S. Epstein (2000), Occurrence and distribution of diverse populations of magnetic protists in a chemically stratified coastal salt pond, *Chem. Geol.*, *169*, 319–328, doi:10.1016/S0009-2541(00)00211-4.
Bazylinski, D. A., R. B. Frankel, and K. O. Konhauser (2007), Modes of biomineralization of magnetite by microbes, *Geomicrobiol. J.*, *24*, 465–475, doi:10.1080/01490450701572259.
Beard, B. L., and C. M. Johnson (2004), Fe isotope variations in the modern and ancient earth and other planetary bodies, *Rev. Mineral. Geochem.*, *55*, 319–357, doi:10.2138/gsrmg.55.1.319.
Beard, B. L., C. M. Johnson, L. Cox, H. Sun, K. H. Nealson, and C. Aguilar (1999), Iron isotope biosignatures, *Science*, *285*, 1889–1892, doi:10.1126/science.285.5435.1889.
Beard, B. L., C. M. Johnson, J. L. Skulan, K. H. Nealson, L. Cox, and H. Sun (2003), Application of Fe isotopes to tracing the geochemical and biological cycling of Fe, *Chem. Geol.*, *195*, 87–117, doi:10.1016/S0009-2541(02)00390-X.
Bell, M. S. (2007), Experimental shock decomposition of siderite and the origin of magnetite in Martian meteorite ALH84001, *Meteorit. Planet. Sci.*, *42*, 935–949.
Bell, P. E., A. L. Mills, and J. S. Herman (1987), Biogeochemical conditions favoring magnetite formation during anaerobic iron reduction, *Appl. Environ. Microbiol.*, *53*, 181–224.
Beveridge, T. J., and R. G. E. Murray (1980), Sites of metal deposition in the cell wall of *Bacillus subtilis*, *J. Bacteriol.*, *141*, 876–887.
Brantley, S. L., L. Liermann, and T. D. Bullen (2001), Fractionation of Fe isotopes by soil microbes and organic acids, *Geology*, *29*, 535–538, doi:10.1130/0091-7613(2001)029<0535:FOFIBS>2.0.CO;2.
Brasier, M. D., O. R. Green, A. P. Jephcoat, A. K. Kleppe, M. J. Van Kranendonk, J. F. Lindsay, A. Steele, and N. V. Grassineau (2002), Questioning the evidence for Earth's oldest fossils, *Nature*, *416*, 76–81, doi:10.1038/416076a.
Bullen, T. D., A. F. White, C. W. Childs, D. V. Vivit, and M. S. Schultz (2001), Demonstration of significant abiotic iron isotope fractionation in nature, *Geology*, *29*, 699–702, doi:10.1130/0091-7613(2001)029<0699:DOSAII>2.0.CO;2.
Buseck, P. R., R. E. Dunin-Borkowski, B. Devouard, R. B. Frankel, M. R. McCartney, P. A. Midgley, M. Pósfai, and M. Weyland (2001), Magnetite morphology and life on Mars, *Proc. Natl. Acad. Sci. U. S. A.*, *98*, 13,490–13,495, doi:10.1073/pnas.241387898.
Caccavo, F., Jr., R. P. Blakemore, and D. R. Lovley (1992), A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire, *Appl. Environ. Microbiol.*, *58*, 3211–3216.
Cairns-Smith, A. G., A. J. Hall, and M. J. Russell (1992), Mineral theories of the origin of life and an iron sulfide example, *Origins Life Evol. B.*, *22*, 161–180, doi:10.1007/BF01808023.
Carter, E. D., and P. R. Buseck (1985), Mechanisms of decomposition of dolomite $Ca_{0.5}Mg_{0.5}CO_3$ in the electron microscope, *Ultramicroscopy*, *18*, 241–252, doi:10.1016/0304-3991(85)90141-X.
Chaix-Pluchery, O., J. Bouillot, D. Ciosmak, J. C. Niepce, and F. Freund (1983), Calcium hydroxide dehydration early precursor states, *J. Solid State Chem.*, *50*, 247–255, doi:10.1016/0022-4596(83)90194-9.
Chang, S.-B. R., and J. L. Kirschvink (1989), Magnetofossils, the magnetization of sediments and the evolution of magnetite biomineralization, *Annu. Rev. Earth Planet. Sci.*, *17*, 169–195, doi:10.1146/annurev.ea.17.050189.001125.
Chang, S.-B. R., J. F. Stolz, J. L. Kirschvink, and S. M. Awramik (1989), Biogenic magnetite in stromatolites II: Occurrence in ancient sedimentary environments, *Precambrian Res.*, *43*, 305–315, doi:10.1016/0301-9268(89)90062-4.
Clemett, S. J., et al. (2002), Crystal morphology of MV-1 magnetite, *Am. Mineral.*, *87*, 1727–1730.
Croal, L. R., C. M. Johnson, B. L. Beard, and D. K. Newman (2004), Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria, *Geochim. Cosmochim. Acta*, *68*, 1227–1242, doi:10.1016/j.gca.2003.09.011.
Crosby, H. A., C. M. Johnson, E. E. Roden, and B. L. Beard (2005), Fe(II)-Fe(III) electron/atom exchange as a mechanism for Fe isotope fractionation during dissimilatory iron oxide reduction, *Environ. Sci. Technol.*, *39*, 6698–6704, doi:10.1021/es0505346.
Crosby, H. A., E. E. Roden, C. M. Johnson, and B. L. Beard (2007), The mechanisms of iron isotope fractionation produced during dissimilatory Fe(III) reduction by *Shewanella putrefaciens* and *Geobacter sulfurreducens*, *Geobiology*, *5*, 169–189, doi:10.1111/j.1472-4669.2007.00103.x.
Cummings, D. E., F. Caccavo Jr., S. Spring, and R. F. Rosenzweig (1999), *Ferribacterium limneticum*, gen. nov., sp. nov., a Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments, *Arch. Microbiol.*, *171*, 183–188, doi:10.1007/s002030050697.
Dalton, R. (2002), Squaring up over ancient life, *Nature*, *417*, 782–784, doi:10.1038/417782a.
Dasgupta, D. R. (1961), Topotactic transformations in iron oxides and oxyhydroxides, *Indian J. Phys.*, *35*, 401–409.
Dasgupta, D. R. (1964), Oriented transformation of magnesite, *Indian J. Phys.*, *38*, 623–626.
Dasgupta, D. R. (1965), The transformation of ankerite during thermal treatment, *Mineral. Mag.*, *35*, 634–639, doi:10.1180/minmag.1965.035.272.07.
de Duve, C. (1995a), The beginnings of life on Earth, *Am. Sci.*, *38*, 428–437.
de Duve, C. (1995b), *Vital Dust: Life as a Cosmic Imperative*, 362 pp., Basic, New York.
Devouard, B., M. Pósfai, X. Hua, D. A. Bazylinski, R. B. Frankel, and P. R. Buseck (1998), Magnetite from magnetotactic bacteria: Size distributions and twinning, *Am. Mineral.*, *83*, 1387–1398.
Dong, H., J. K. Fredrickson, D. W. Kennedy, J. M. Zachara, R. K. Kukkadapu, and T. C. Onstott (2000), Mineral transformation associated with the microbial reduction of magnetite, *Chem. Geol.*, *169*, 299–318, doi:10.1016/S0009-2541(00)00210-2.
Dong, H., J. E. Kostka, and J. Kim (2003a), Microscopic evidence for microbial dissolution of smectite, *Clays Clay Miner.*, *51*, 502–512, doi:10.1346/CCMN.2003.0510504.
Dong, H., R. K. Kukkadapu, J. K. Fredrickson, J. M. Zachara, D. W. Kennedy, and H. M. Kostandarithes (2003b), Microbial reduction of structural Fe(III) in illite and goethite, *Environ. Sci. Technol.*, *37*, 1268–1276, doi:10.1021/es020919d.
Dubrawski, J. V. (1991), Thermal decomposition of some siderite-magnesite minerals using DSC, *J. Therm. Anal.*, *37*, 1213–1221, doi:10.1007/BF01913855.
Dunin-Borkowski, R. E., M. R. McCartney, R. B. Frankel, D. A. Bazylinski, M. Pósfai, and P. R. Buseck (1998), Magnetic microstructure of magnetotactic bacteria by electron holography, *Science*, *282*, 1868–1870, doi:10.1126/science.282.5395.1868.
Dunin-Borkowski, R. E., M. R. McCartney, M. Pósfai, R. B. Frankel, D. A. Bazylinski, and P. R. Buseck (2001), Off axis electron holography of magnetotactic bacteria: Magnetic microstructure of strains MV-1 and MS-1, *Eur. J. Mineral.*, *13*, 671–684, doi:10.1127/0935-1221/2001/0013-0671.
Dupraz, C., and P. T. Visscher (2005), Microbial lithification in marine stromatolites and hypersaline mats, *Trends Microbiol.*, *13*, 429–436, doi:10.1016/j.tim.2005.07.008.
Ehrlich, H. L., and D. K. Newman (2008), *Geomicrobiology*, 5th ed., 628 pp., CRC Press, Boca Raton, Fla.
Emerson, D. (2000), Microbial oxidation of Fe(II) and Mn(II) at circumneutral pH, in *Environmental Metal-Microbe Interactions*, edited by D. R. Lovley, pp. 31–52, ASM Press, Washington, D. C.
Faivre, D., and P. Zuddas (2006), An integrated approach for determining the origin of magnetite nanoparticles, *Earth Planet. Sci. Lett.*, *243*, 53–60, doi:10.1016/j.epsl.2006.01.012.
Faivre, D., P. Agrinier, N. Menguy, P. Zuddas, K. Pachana, A. Gloter, J.-Y. Laval, and F. Guyot (2004), Mineralogical and isotopic properties of inorganic nanocrystalline magnetites, *Geochim. Cosmochim. Acta*, *68*, 4395–4403, doi:10.1016/j.gca.2004.03.016.
Faivre, D., N. Menguy, M. Pósfai, and D. Schüler (2008), Environmental parameters affect the physical properties of fast-growing magnetosomes, *Am. Mineral.*, *93*, 463–469, doi:10.2138/am.2008.2678.
Figlarz, M., B. Gérand, A. Delahaye-Vidal, B. Dumont, F. Harb, A. Coucou, and F. Fievet (1990), Topotaxy, nucleation and growth, *Solid State Ion.*, *43*, 143–170, doi:10.1016/0167-2738(90)90480-F.
Fowle, D. A., and J. B. Fein (2001), Quantifying the effects of *Bacillus subtilis* cell walls on the precipitation of copper hydroxide from aque-

ous solution, *Geomicrobiol. J.*, *18*, 77–91, doi:10.1080/01490450151079815.

Franger, S., P. Berthet, and J. Berthon (2004), Electrochemical synthesis of Fe_3O_4 nanoparticles in alkaline aqueous solutions containing complexing agents, *J. Solid State Electrochem.*, *8*, 218–223, doi:10.1007/s10008-003-0469-6.

Frankel, R. B., and D. A. Bazylinski (2003), Biologically induced mineralization by bacteria, *Rev. Mineral. Geochem.*, *54*, 217–247, doi:10.2113/0540095.

Frankel, R. B., and D. A. Bazylinski (2006), How magnetotactic bacteria make magnetosomes queue up, *Trends Microbiol.*, *14*, 329–331, doi:10.1016/j.tim.2006.06.004.

Frankel, R. B., D. A. Bazylinski, M. Johnson, and B. L. Taylor (1997), Magneto-aerotaxis in marine, coccoid bacteria, *Biophys. J.*, *73*, 994–1000, doi:10.1016/S0006-3495(97)78132-3.

Fredrickson, J. K., J. M. Zachara, D. W. Kennedy, H. Dong, T. C. Onstott, N. W. Hinman, and S.-M. Li (1998), Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium, *Geochim. Cosmochim. Acta*, *62*, 3239–3257, doi:10.1016/S0016-7037(98)00243-9.

Friedmann, E. I., J. Wierzchos, C. Ascaso, and M. Winklhofer (2001), Chains of magnetite crystals in the meteorite ALH84001: Evidence of biological origin, *Proc. Natl. Acad. Sci. U. S. A.*, *98*, 2176–2181, doi:10.1073/pnas.051514698.

Gajdardziska-Josifovska, M., R. G. McClean, M. A. Schofield, C. V. Sommer, and W. F. Kean (2001), Discovery of nanocrystalline botanical magnetite, *Eur. J. Mineral.*, *13*, 863–870, doi:10.1127/0935-1221/2001/0013/0863.

Gallagher, P. K., and S. S. J. Warne (1981), Thermomagnetometry and thermal decomposition of siderite, *Thermochim. Acta*, *43*, 253–267, doi:10.1016/0040-6031(81)85183-0.

García-Ruiz, J. M., S. T. Hyde, A. M. Carnerup, A. G. Christy, M. J. van Kranendonk, and N. J. Welham (2003), Self-assembled silica-carbonate structures and detection of ancient microfossils, *Science*, *302*, 1194–1197, doi:10.1126/science.1090163.

García-Ruiz, J. M., E. Melero-García, and S. T. Hyde (2009), Morphogenesis of self-assembled nanocrystalline materials of barium carbonate and silica, *Science*, *323*, 362–365, doi:10.1126/science.1165349.

Garrels, R. M., and C. L. Christ (1990), *Solutions, Minerals and Equilibria*, 450 pp., Jones and Bartlett, Boston, Mass.

Gates, W. P., H. T. Wilkinson, and J. W. Stucki (1993), Swelling properties of microbially reduced ferruginous smectite, *Clays Clay Miner.*, *41*, 360–364, doi:10.1346/CCMN.1993.0410312.

Gold, T. (1992), The deep, hot biosphere, *Proc. Natl. Acad. Sci. U. S. A.*, *89*, 6045–6049, doi:10.1073/pnas.89.13.6045.

Golden, D. C., D. W. Ming, C. S. Schwandt, H. V. Lauer, R. A. Socki, R. V. Morris, G. E. Lofgren, and G. A. McKay (2001), A simple inorganic process for formation of carbonates, magnetite and sulfides in Martian meteorite ALH84001, *Am. Mineral.*, *86*, 370–375.

Golden, D. C., D. W. Ming, R. V. Morris, A. Brearley, H. V. Lauer, A. H. Treiman, M. E. Zolensky, C. S. Schwandt, G. E. Lofgren, and G. A. McKay (2004), Evidence for exclusively inorganic formation of magnetite in Martian meteorite ALH84001, *Am. Mineral.*, *89*, 681–695.

Gorby, Y. A., T. J. Beveridge, and R. P. Blakemore (1988), Characterization of the bacterial magnetosome membrane, *J. Bacteriol.*, *170*, 834–841.

Gotor, F. J., M. Macias, A. Ortega, and J. M. Criado (2000), Comparative study of the kinetics of the thermal decomposition of synthetic and natural siderite samples, *Phys. Chem. Miner.*, *27*, 495–503, doi:10.1007/s002690000093.

Hanesch, M. (2009), Raman spectroscopy of iron oxides and (oxy)hydroxides at low laser power and possible applications in environmental magnetic studies, *Geophys. J. Int.*, *177*, 941–948, doi:10.1111/j.1365-246X.2009.04122.x.

Heising, S., and B. Schink (1998), Phototrophic oxidation of ferrous iron by a *Rhodomicrobium vannielii* strain, *Microbiology*, *144*, 2263–2269, doi:10.1099/00221287-144-8-2263.

Heising, S., L. Richter, W. Ludwig, and B. Schink (1999), *Chlorobium ferrooxidans* sp nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a "*Geospirillum*" sp strain, *Arch. Microbiol.*, *172*, 116–124, doi:10.1007/s002030050748.

Hesse, P. P. (1994), Evidence for bacterial palaeoecological origin or mineral magnetic cycles in oxic and sub-oxic Tasman Sea sediments, *Mar. Geol.*, *117*, 1–17, doi:10.1016/0025-3227(94)90003-5.

Ichinose, N., Y. Ozaki, and S. Kashu (1992), *Superfine Particle Technology*, 223 pp., Springer, London.

Icopini, G. A., A. D. Anbar, S. S. Ruebush, M. Tien, and S. L. Brantley (2004), Iron isotope fractionation during microbial reduction of iron: The importance of adsorption, *Geology*, *32*, 205–208, doi:10.1130/G20184.1.

Isambert, A., T. De Resseguier, A. Gloter, B. Reynard, F. Guyot, and J.-P. Valet (2006), Magnetite-like nanocrystals formed by laser-driven shocks in siderite, *Earth Planet. Sci. Lett.*, *243*, 820–827, doi:10.1016/j.epsl.2006.01.060.

Isambert, A., N. Menguy, E. Larquet, F. Guyot, and J.-P. Valet (2007), Transmission electron microscopy study of magnetites in a freshwater population of magnetotactic bacteria, *Am. Mineral.*, *92*, 621–630, doi:10.2138/am.2007.2278.

Ishikawa, T., Y. Kondo, A. Yasukawa, and K. Kandori (1998), Formation of magnetite in the presence of ferric oxyhydroxides, *Corros. Sci.*, *40*, 1239–1251, doi:10.1016/S0010-938X(98)00045-6.

Jimenez-Lopez, C., et al. (2008), Cation incorporation in magnetites formed upon thermal decomposition of ankerites, paper presented at General Assembly of the European Geosciences Union, Vienna, Austria.

Johnson, C. M., B. L. Beard, N. J. Beukes, C. Klein, and J. M. O'Leary (2002), Isotopic fractionation between Fe(III) and Fe(II) in aqueous solution, *Earth Planet. Sci. Lett.*, *195*, 141–153, doi:10.1016/S0012-821X(01)00581-7.

Johnson, C. M., B. L. Beard, N. J. Beukes, C. Klein, and J. M. O'Leary (2003), Ancient geochemical cycling in the Earth as inferred from Fe isotope studies of banded iron formations from the Transvaal Craton, *Contrib. Mineral. Petrol.*, *144*, 523–547, doi:10.1007/s00410-002-0418-x.

Johnson, C. M., B. L. Beard, E. E. Roden, D. K. Newman, and K. H. Nealson (2004), Isotopic constraints on biogeochemical cycling of Fe, *Rev. Mineral. Geochem.*, *55*, 359–408, doi:10.2138/gsrmg.55.1.359.

Johnson, C. M., E. E. Roden, S. A. Welch, and B. L. Beard (2005), Experimental constraints on Fe isotope fractionation during magnetite and Fe carbonate formation coupled to dissimilatory hydrous ferric oxide reduction, *Geochim. Cosmochim. Acta*, *69*, 963–993, doi:10.1016/j.gca.2004.06.043.

Johnson, D. B., and K. B. Hallberg (2003), The microbiology of acidic mine waters, *Res. Microbiol.*, *154*, 466–473, doi:10.1016/S0923-2508(03)00114-1.

Keim, C. M., U. Lins, and M. Farina (2009), Manganese in biogenic magnetite crystals from magnetotactic bacteria, *FEMS Microbiol. Lett.*, *292*, 250–253, doi:10.1111/j.1574-6968.2009.01499.x.

Kim, J., H. L. Dong, J. Seabaugh, S. W. Newell, and D. D. Eberl (2004), Role of microbes in the smectite-to-illite reaction, *Science*, *303*, 830–832, doi:10.1126/science.1093245.

Kim, M. G., U. Dahmen, and A. W. Searcy (1987), Structural transformations in the decomposition of $Mg(OH)_2$ and $MgCO_3$, *J. Am. Ceram. Soc.*, *70*, 146–154, doi:10.1111/j.1151-2916.1987.tb04949.x.

Kirschvink, J. L., and H. A. Lowenstam (1979), Mineralization and magnetization of chiton teeth: Paleomagnetic, sedimentologic, and biologic implications of organic magnetite, *Earth Planet. Sci. Lett.*, *44*, 193–204, doi:10.1016/0012-821X(79)90168-7.

Kirschvink, J. L., A. Kobayashi-Kirschvink, and B. J. Woodford (1992), Magnetite biomineralization in the human brain, *Proc. Natl. Acad. Sci. U. S. A.*, *89*, 7683–7687, doi:10.1073/pnas.89.16.7683.

Knorre, H. V., and W. E. Krumbein (2000), Bacterial calcification, in *Microbial Sediments*, edited by R. A. Riding and S. M. Awranik, pp. 25–31, Springer, Berlin.

Komeili, A., H. Vali, T. J. Beveridge, and D. K. Newman (2004), Magnetosome vesicles are present before magnetite formation, and MamA is required for their activation, *Proc. Natl. Acad. Sci. U. S. A.*, *101*, 3839–3844, doi:10.1073/pnas.0400391101.

Komeili, A., Z. Li, D. K. Newman, and G. J. Jensen (2006), Magnetosomes are invaginations organized by the actin-like protein MamK, *Science*, *311*, 242–245, doi:10.1126/science.1123231.

Konhauser, K. O. (1998), Diversity of bacterial iron mineralization, *Earth Sci. Rev.*, *43*, 91–121, doi:10.1016/S0012-8252(97)00036-6.

Kopp, R. E., and J. L. Kirschvink (2008), The identification and biogeochemical interpretation of fossil magnetotactic bacteria, *Earth Sci. Rev.*, *86*, 42–61, doi:10.1016/j.earscirev.2007.08.001.

Kopp, R. E., C. Z. Nash, A. Kobayashi, B. P. Weiss, D. A. Bazylinski, and J. L. Kirschvink (2006), Ferromagnetic resonance spectroscopy for assessment of magnetic anisotropy and magnetostatic interactions: A case study of mutant magnetotactic bacteria, *J. Geophys. Res.*, *111*, B12S25, doi:10.1029/2006JB004529.

Kostka, J. E., and K. H. Nealson (1995), Dissolution and reduction of magnetite by bacteria, *Environ. Sci. Technol.*, *29*, 2535–2540, doi:10.1021/es00010a012.

Kostka, J. E., J. W. Stucki, K. H. Nealson, and J. Wu (1996), Reduction of structural Fe(III) in smectite by a pure culture of *Shewanella putrefaciens* strain MR-1, *Clays Clay Miner.*, *44*, 522–529, doi:10.1346/CCMN.1996.0440411.

Kukkadapu, R. K., J. M. Zachara, J. K. Fredrickson, D. W. Kennedy, A. C. Dohnalkova, and D. E. McCready (2005), Ferrous hydroxy carbonate is a stable transformation product of biogenic magnetite, *Am. Mineral.*, *90*, 510–515, doi:10.2138/am.2005.1727.

Kukkadapu, R. K., J. M. Zachara, J. K. Fredrickson, J. P. McKinley, D. W. Kennedy, S. C. Smith, and H. Dong (2006), Reductive biotransformation of Fe in shale-limestone saprolite containing Fe(III) oxides and Fe(II)/Fe (III) phyllosilicates, *Geochim. Cosmochim. Acta*, *70*, 3662–3676, doi:10.1016/j.gca.2006.05.004.

Kusel, K. (2003), Microbial cycling of iron and sulfur in acidic coal mining lake sediments, *Water Air Soil Pollut.*, *3*, 67–90, doi:10.1023/A:1022103419928.

Liu, Z. L., et al. (2004), Synthesis of magnetite nanoparticles in W/O microemulsion, *J. Mater. Sci.*, *39*, 2633–2636, doi:10.1023/B:JMSC.0000020046.68106.22.

Lovley, D. R. (1987), Organic-matter mineralization with the reduction of ferric iron- a review, *Geomicrobiol. J.*, *5*, 375–399, doi:10.1080/01490458709385975.

Lovley, D. R. (1991), Dissimilatory Fe(III) and Mn(IV) reduction, *Microbiol. Rev.*, *55*, 259–287.

Lovley, D. R. (1997), Microbial Fe(III) reduction in subsurface environments, *FEMS Microbial. Rev.*, *20*, 305–313, doi:10.1111/j.1574-6976.1997.tb00316.x.

Lovley, D. R., and E. J. P. Phillips (1988), Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese, *Appl. Environ. Microbiol.*, *54*, 1472–1480.

Lovley, D. R., J. F. Stolz, G. L. Nord Jr., and E. J. P. Phillips (1987), Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism, *Nature*, *330*, 252–254, doi:10.1038/330252a0.

Lovley, D. R., F. H. Chapelle, and E. J. P. Phillips (1990), Fe(III)-reducing bacteria in deeply buried sediments of the Atlantic Coastal Plain, *Geology*, *18*, 954–957, doi:10.1130/0091-7613(1990)018<0954:FIRBID>2.3.CO;2.

Lowenstam, H. A. (1981), Minerals formed by organisms, *Science*, *211*, 1126–1131, doi:10.1126/science.7008198.

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

Mandernack, K. W., D. A. Bazylinski, W. C. Shanks, and T. D. Bullen (1999), Oxygen and iron isotope studies of magnetite produced by magnetotactic bacteria, *Science*, *285*, 1892–1896, doi:10.1126/science.285.5435.1892.

Mann, S. (1986), On the nature of boundary-organized biomineralization, *J. Inorg. Chem.*, *28*, 363–371.

Mann, S., and R. B. Frankel (1989), Magnetite biomineralization in unicellular organisms, in *Biomineralization: Chemical and Biochemical Perspectives*, edited by S. Mann, J. Webb, and R. J. P. Williams, pp. 389–426, VCH, New York.

Mann, S., and J. P. Hannington (1988), Formation of iron oxides in unilamellar vesicles, *J. Colloid Interface Sci.*, *122*, 326–335, doi:10.1016/0021-9797(88)90368-2.

Mann, S., R. B. Frankel, and R. P. Blakemore (1984), Structure, morphology and crystal growth of bacterial magnetite, *Nature*, *310*, 405–407, doi:10.1038/310405a0.

Mann, S., B. R. Heywood, S. Rajam, and J. D. Birchall (1988a), Controlled crystallization of $CaCO_3$ under stearic-acid monolayers, *Nature*, *334*, 692–695, doi:10.1038/334692a0.

Mann, S., N. H. C. Sparks, M. M. Walker, and J. L. Kirschvink (1988b), Ultrastructure, morphology and organization of biogenic magnetite from sockeye salmon, *Oncorhynchus nerka*: Implications for magnetoreception, *J. Exp. Biol.*, *140*, 35–49.

McKay, D. S., E. K. Gibson Jr., K. L. Thomas-Keptra, H. Vali, C. S. Romanek, S. J. Clemett, X. D. F. Chillier, C. R. Maechling, and R. N. Zare (1996), Search for past life on Mars: Possible relic biogenic activity in Martian meteorite ALH84001, *Science*, *273*, 924–930, doi:10.1126/science.273.5277.924.

Meldrum, F. C., S. Mann, B. R. Heywood, R. B. Frankel, and D. A. Bazylinski (1993a), Electron microscopy study of magnetosomes in a cultured coccoid magnetotactic bacterium, *Proc. R. Soc. London, Ser. B*, *251*, 231–236, doi:10.1098/rspb.1993.0034.

Meldrum, F. C., S. Mann, B. R. Heywood, R. B. Frankel, and D. A. Bazylinski (1993b), Electron microscope study of magnetosomes in two cultured vibrioid magnetotactic bacteria, *Proc. R. Soc. London, Ser. B*, *251*, 237–242, doi:10.1098/rspb.1993.0035.

Moisescu, C., S. Bonneville, D. Tobler, I. Ardelean, and L. G. Benning (2008), Controlled biomineralization of magnetite (Fe_3O_4) by *Magnetospirillum gryphiswaldense*, *Mineral. Mag.*, *72*, 333–336, doi:10.1180/minmag.2008.072.1.333.

Moskowitz, B. M., R. B. Frankel, D. A. Bazylinski, H. W. Jannasch, and D. R. Lovley (1989), A comparison of magnetite particles produced anaerobically by magnetotactic and dissimilatory iron-reducing bacteria, *Geophys. Res. Lett.*, *16*, 665–668, doi:10.1029/GL016i007p00665.

Moskowitz, B. M., R. B. Frankel, and D. A. Bazylinski (1993), Rock magnetic criteria for the detection of biogenic magnetite, *Earth Planet. Sci. Lett.*, *120*, 283–300, doi:10.1016/0012-821X(93)90245-5.

Neal, A. L., K. M. Rosso, G. G. Geesey, Y. A. Gorby, and B. J. Little (2003), Surface structure effects on direct reduction of iron oxides by *Shewanella oneidensis*, *Geochim. Cosmochim. Acta*, *67*, 4489–4503, doi:10.1016/S0016-7037(03)00386-7.

Neal, A. L., S. N. Dublin, J. Taylor, D. J. Bates, J. L. Burns, R. Apkarian, and T. J. DiChristina (2007), Terminal electron acceptors influence the quantity and chemical composition of capsular exopolysaccharides produced by anaerobically growing *Shewanella* spp, *Biomacromolecules*, *8*, 166–174, doi:10.1021/bm060826e.

Nealson, K. H., and C. R. Myers (1990), Iron reduction by bacteria - a potential role in the genesis of banded iron formations, *Am. J. Sci.*, *290A*, 35–45.

Nyirö-Kósa, I., D. C. Nagy, and M. Pósfai (2009), Size and shape control of precipitated magnetite nanoparticles, *Eur. J. Mineral.*, *21*, 293–302, doi:10.1127/0935-1221/2009/0021-1920.

O'Neil, J. R. (1986), Theoretical and experimental aspects of isotopic fractionation, *Rev. Mineral.*, *16*, 1–41.

Penninga, I., H. de Waard, B. M. Moskowitz, D. A. Bazylinski, and R. B. Frankel (1995), Remanence curves for individual magnetotactic bacteria using a pulsed magnetic field, *J. Magn. Magn. Mater.*, *149*, 279–286, doi:10.1016/0304-8853(95)00078-X.

Pentecost, A. (1985), Association of cyanobacteria with tufa deposits: Identity, enumeration and nature of the sheath material revealed by histochemistry, *Geomicrobiol. J.*, *4*, 285–298, doi:10.1080/01490458509385936.

Perez-Gonzalez, T., C. Jimenez-Lopez, A. L. Neal, F. Rull-Perez, A. Rodríguez-Navarro, A. Fernandez-Vivas, and E. Iañez-Pareja (2010), Magnetite biomineralization induced by *Shewanella oneidensis*, *Geochim. Cosmochim. Acta*, doi:10.1016/j.gca.2009.10.035, in press.

Peters, K. E., C. C. Walters, and J. M. Moldowan (2005), *Biomarkers and Isotopes in the Petroleum Exploration and Earth History*, vol. 2, 2nd ed., 700 pp., Oxford Univ. Press, New York.

Petersen, N., T. von Dobeneck, and H. Vali (1986), Fossil bacterial magnetite in deep-sea sediments from the South-Atlantic Ocean, *Nature*, *320*, 611–615, doi:10.1038/320611a0.

Polyakov, V. B., and S. D. Mineev (2000), The use of Mössbauer spectroscopy in stable isotope geochemistry, *Geochim. Cosmochim. Acta*, *64*, 849–865, doi:10.1016/S0016-7037(99)00329-4.

Proksch, R. B., B. M. Moskowitz, E. D. Dahlberg, T. Schaeffer, D. A. Bazylinski, and R. B. Frankel (1995), Magnetic force microscopy of the submicron magnetic assembly in a magnetotactic bacterium, *Appl. Phys. Lett.*, *66*, 2582–2584, doi:10.1063/1.113508.

Prozorov, T., S. K. Mallapragada, B. Narasimhan, L. Wang, P. Palo, M. Nilsen-Hamilton, T. J. Williams, D. A. Bazylinski, R. Prozorov, and P. C. Canfield (2007a), Protein-mediated synthesis of uniform superparamagnetic magnetite nanocrystals, *Adv. Funct. Mater.*, *17*, 951–957, doi:10.1002/adfm.200600448.

Prozorov, R., T. Prozorov, S. K. Mallapragada, B. Narasimhan, T. J. Williams, and D. A. Bazylinski (2007b), Magnetic irreversibility and the Verwey transition in nanocrystalline bacterial magnetite, *Phys. Rev. B*, *76*, 054406 1–10, doi:10.1103/PhysRevB.76.054406.

Qu, S., H. Yang, D. Ren, S. Kan, G. Zou, D. Li, and M. Li (1999), Magnetite nanoparticles prepared by precipitation from partially reduced ferric chloride aqueous solutions, *J. Colloid Interface Sci.*, *215*, 190–192, doi:10.1006/jcis.1999.6185.

Roden, E. E., and D. R. Lovley (1993), Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*, *Appl. Environ. Microbiol.*, *59*, 734–742.

Roden, E. E., M. R. Leonardo, and F. G. Ferris (2002), Immobilization of strontium during iron biomineralization coupled to dissimilatory hydrous ferric oxide reduction, *Geochim. Cosmochim. Acta*, *66*, 2823–2839, doi:10.1016/S0016-7037(02)00878-5.

Rodríguez-Navarro, C., C. Jimenez-Lopez, A. Rodríguez-Navarro, M. T. González-Muñoz, and M. Rodríguez-Gallego (2007), Bacterially mediated mineralization of vaterite, *Geochim. Cosmochim. Acta*, *71*, 1197–1213, doi:10.1016/j.gca.2006.11.031.

Rodríguez-Navarro, C., E. Ruiz-Agudo, A. Luque, A. B. Rodríguez-Navarro, and M. Ortega-Huertas (2009), Thermal decomposition of calcite: Mechanisms of formation and textural evolution of CaO nanocrystals, *Am. Mineral.*, *94*, 578–593, doi:10.2138/am.2009.3021.

Roe, J. E., A. D. Anbar, and J. Barling (2003), Nonbiological fractionation of Fe isotopes: Evidence of an equilibrium isotope effect, *Chem. Geol.*, *195*, 69–85, doi:10.1016/S0009-2541(02)00389-3.

Roh, Y., et al. (2006), Metal reduction and iron biomineralization by a psychrotolerant Fe(III)-reducing bacterium, *Shewanella* sp. Strain PV-4,

Appl. *Environ. Microbiol.*, *72*, 3236–3244, doi:10.1128/AEM.72.5.3236-3244.2006.

Schauble, E. A., G. R. Rossman, and H. P. Taylor Jr. (2001), Theoretical estimates of equilibrium Fe-isotope fractionations from vibrational spectroscopy, *Geochim. Cosmochim. Acta*, *65*, 2487–2497, doi:10.1016/S0016-7037(01)00600-7.

Scheffel, A., M. Gruska, D. Faivre, A. Linaroudis, J. M. Jürgen, and D. Schüler (2006), An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria, *Nature*, *440*, 110–114, doi:10.1038/nature04382.

Scheffel, A., A. Gärdes, K. Grünberg, G. Wanner, and D. Schüler (2008), The major magnetosome proteins MamGFDC are not essential for magnetite biomineralization in *Magnetospirillum gryphiswaldense* but regulate the size of magnetosome crystals, *J. Bacteriol.*, *190*, 377–386, doi:10.1128/JB.01371-07.

Schopf, J. W. (1993), Microfossils of the Early Archean Apex chert: New evidence of the antiquity of life, *Science*, *260*, 640–646, doi:10.1126/science.260.5108.640.

Schopf, J. W., and B. M. Packer (1987), Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia, *Science*, *237*, 70–73, doi:10.1126/science.11539686.

Schopf, J. W., A. B. Kudryavtsev, D. G. Agresti, T. J. Wdowlak, and A. D. Czaja (2002), Laser-Raman imagery of Earth's earliest fossils, *Nature*, *416*, 73–76, doi:10.1038/416073a.

Schumann, D., et al. (2008), Gigantism in unique biogenic magnetite at the Paleocene-Eocene thermal maximum, *Proc. Natl. Acad. Sci. U. S. A.*, *105*, 17,648–17,653, doi:10.1073/pnas.0803634105.

Schwartz, M., S. P. Lund, D. E. Hammond, R. Schwartz, and K. Wong (1997), Early sediment diagenesis on the Blake/Bahama outer ridge, North Atlantic ocean and its effects on sediment magnetism, *J. Geophys. Res.*, *102*, 7903–7914, doi:10.1029/96JB03218.

Schwertmann, U., and R. M. Cornell (2000), *Iron Oxides in the Laboratory: Preparation and Characterization*, 2nd ed., 188 pp., Wiley-VCH, Weinheim, Germany.

Schwertmann, U., and E. Murad (1990), The influence of aluminum on iron oxides: XIV. aluminum substituted magnetite synthesized at ambient temperatures, *Clays Clay Miner.*, *38*, 196–202, doi:10.1346/CCMN.1990.0380211.

Simkiss, K., and K. M. Wilbur (1989), *Biomineralization. Cell Biology and Mineral Deposition*, 337 pp., Academic, San Diego, Calif.

Skulan, J. L., B. L. Beard, and C. M. Johnson (2002), Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(III) and hematite, *Geochim. Cosmochim. Acta*, *66*, 2995–3015, doi:10.1016/S0016-7037(02)00902-X.

Sokolova, T., J. Hanel, R. U. Onyenwoke, A.-L. Reysenbach, A. Banta, R. Geyer, J. M. González, W. B. Whitman, and J. Wiegel (2007), Novel chemolithotrophic, thermophilic, anaerobic bacteria *Thermolithobacter carboxydivorans* sp. nov, *Extremophiles*, *11*, 145–147, doi:10.1007/s00792-006-0022-5.

Sparks, N. H. C., S. Mann, D. A. Bazylinski, D. R. Lovley, H. W. Jannasch, and R. B. Frankel (1990), Structure and morphology of magnetite anaerobically produced by a marine magnetotactic bacterium and a dissimilatory iron-reducing bacterium, *Earth Planet. Sci. Lett.*, *98*, 14–22, doi:10.1016/0012-821X(90)90084-B.

Staniland, S., W. Williams, N. Telling, G. Van Der Laan, A. Harrison, and B. Ward (2008), Controlled cobalt doping of magnetosomes in vivo, *Nat. Nanotechnol.*, *3*, 158–162, doi:10.1038/nnano.2008.35.

Stolz, J. F., S.-B. R. Chang, and J. L. Kirschvink (1989), Biogenic magnetite in stromatolites I: Occurrence in modern sedimentary environments, *Precambrian Res.*, *43*, 295–304, doi:10.1016/0301-9268(89)90061-2.

Straub, K. L., M. Benz, and B. Schink (2001), Iron metabolism in anoxic environments at near neutral pH, *FEMS Microbiol. Ecol.*, *34*, 181–186, doi:10.1111/j.1574-6941.2001.tb00768.x.

Suzuki, H., T. Tanaka, T. Sasaki, N. Nakamura, T. Matsunaga, and S. Mashiko (1998), High resolution magnetic force microscope images of a magnetic particle chain extracted from magnetic bacteria AMB-1, *Jpn. J. Appl. Phys.*, *2*(37), L1343–L1345, doi:10.1143/JJAP.37.L1343.

Thomas-Keprta, K. L., D. A. Bazylinski, J. L. Kirschvink, S. J. Clemett, D. S. McKay, S. J. Wentworth, H. Vali, E. K. Gibson Jr., and C. S. Romanek (2000), Elongated prismatic magnetite crystals in ALH84001 carbonate globules: Potential Martian magnetofossils, *Geochim. Cosmochim. Acta*, *64*, 4049–4081, doi:10.1016/S0016-7037(00)00481-6.

Thomas-Keprta, K. L., S. J. Clemett, D. A. Bazylinski, J. L. Kirschvink, D. S. McKay, S. J. Wentworth, H. Vali, E. K. Gibson Jr., M. F. McKay, and C. S. Romanek (2001), Truncated hexa-octahedral magnetite crystals in ALH84001: Presumptive biosignatures, *Proc. Natl. Acad. Sci. U. S. A.*, *98*, 2164–2169, doi:10.1073/pnas.051500898.

Thomas-Keprta, K. L., S. J. Clemett, D. A. Bazylinski, J. L. Kirschvink, D. S. McKay, S. J. Wentworth, H. Vali, E. K. Gibson Jr., M. F. McKay, and C. S. Romanek (2002), Magnetofossils from ancient Mars: A robust biosignature in the Martian meteorite ALH84001, *Appl. Environ. Microbiol.*, *68*, 3663–3672, doi:10.1128/AEM.68.8.3663-3672.2002.

Thomas-Keprta, K. L., S. J. Clemett, D. S. McKay, E. K. Gibson Jr., and S. J. Wentworth (2009), Origins of magnetite nanocrystals in Martian meteorite ALH84001, *Geochim. Cosmochim. Acta*, *73*, 6631–6677, doi:10.1016/j.gca.2009.05.064.

Torres de Araujo, F. F., M. A. Pires, R. B. Frankel, and C. E. M. Bicudo (1985), Magnetite and magnetotaxis in algae, *Biophys. J.*, *50*, 375–378.

Towe, K. M., and T. T. Moench (1981), Electron-optical characterization of bacterial magnetite, *Earth Planet. Sci. Lett.*, *52*, 213–220, doi:10.1016/0012-821X(81)90222-3.

Treiman, A. H. (2003), Submicrometer magnetite grains and carbon compounds in Martian meteorite ALH84001: Inorganic abiotic formation by shock and thermal metamorphism, *Astrobiology*, *3*, 369–392, doi:10.1089/153110703769016451.

Tseng, J.-Y., C.-W. Chang, Y.-H. Chen, C.-F. Chang, and P.-C. Chiang (2007), Synthesis of micro-size magnetic polymer adsorbent and its application for the removal of Cu(II) ion, *Colloids Surf. A*, *295*, 209–216, doi:10.1016/j.colsurfa.2006.09.001.

Turner, E. C., and B. Jones (2005), Microscopic calcite dendrites in cold-water tufa: Implications for nucleation of micrite and cement, *Sedimentology*, *52*, 1043–1066, doi:10.1111/j.1365-3091.2005.00741.x.

Vali, H., and J. L. Kirschvink (1991), Observation of magnetosome organization surface structure and iron biomineralization of undescribed magnetic bacteria; evolutionary implications, in *Iron Biominerals*, edited by R. B. Frankel and R. P. Blakemore, pp. 97–115, Plenum, New York.

Vali, H., O. Förster, G. Amarantidis, and N. Petersen (1987), Magnetotactic bacteria and their magnetofossils in sediments, *Earth Planet. Sci. Lett.*, *86*, 389–400, doi:10.1016/0012-821X(87)90235-4.

Vali, H., B. Weiss, Y.-L. Li, S. K. Sears, S. K. Soon, J. L. Kirschvink, and C. L. Zhang (2004), Formation of tabular single-domain magnetite induced by *Geobacter metallireducens* GS-15, *Proc. Natl. Acad. Sci. U. S. A.*, *101*, 16,121–16,126, doi:10.1073/pnas.0404040101.

Van der Zee, C., D. R. Roberts, D. G. Rancourt, and C. P. Slomp (2003), Nanogoethite is the dominant reactive oxyhydroxides phase in lake and marine sediments, *Geology*, *31*, 993–996, doi:10.1130/G19924.1.

Vargas, M., K. Kashefi, E. L. Blunt-Harris, and D. R. Lovley (1998), Microbiological evidence for Fe(III) reduction on early Earth, *Nature*, *395*, 65–67, doi:10.1038/25720.

Vayssières, L., C. Chanèac, E. Tronc, and J. P. Jolivet (1998), Size tailoring of magnetite particles formed by aqueous precipitation: An example of thermodynamic stability of nanometric oxide particles, *J. Colloid Interface Sci.*, *205*, 205–212, doi:10.1006/jcis.1998.5614.

Verway, E. J. W. (1939), Electronic conduction of magnetite (Fe_3O_4) and its transition point at low temperatures, *Nature*, *144*, 327–328, doi:10.1038/144327b0.

Walker, J. C. G. (1987), Was the Archaean biosphere upside down?, *Nature*, *329*, 710–712, doi:10.1038/329710a0.

Ward, A. J. I., and S. Friberg (1989), Preparing narrow size distribution particles from amphiphilic association structure, *MRS Bull.*, *14*, 41.

Weiss, B. P., S. S. Kim, J. L. Kirschvink, R. E. Kopp, M. Sankaran, A. Kobayashi, and A. Komeili (2004a), Ferromagnetic resonance and low temperature magnetic tests for biogenic magnetite, *Earth Planet. Sci. Lett.*, *224*, 73–89, doi:10.1016/j.epsl.2004.04.024.

Weiss, B. P., S. S. Kim, J. L. Kirschvink, R. E. Kopp, M. Sankaran, A. Kobayashi, and A. Komeili (2004b), Magnetic tests for magnetosome chains in Martian meteorite ALH84001, *Proc. Natl. Acad. Sci. U. S. A.*, *101*, 8281–8284, doi:10.1073/pnas.0402292101.

Welch, S. A., B. L. Beard, C. M. Johnson, and P. S. Braterman (2003), Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(II) and Fe(III), *Geochim. Cosmochim. Acta*, *67*, 4231–4250, doi:10.1016/S0016-7037(03)00266-7.

Widdel, F., S. Schnell, S. Heising, A. Ehrenreich, B. Assmus, and B. Schink (1993), Ferrous iron oxidation by anoxygenic phototrophic bacteria, *Nature*, *362*, 834–836, doi:10.1038/362834a0.

Ye, Q., Y. Roh, S. L. Carroll, B. Blair, J. Zhou, C. L. Zhang, and M. W. Fields (2004), Alkaline anaerobic respiration: Isolation and characterization of a novel alkaliphilic and metal-reducing bacterium, *Appl. Environ. Microbiol.*, *70*, 5595–5602, doi:10.1128/AEM.70.9.5595-5602.2004.

Zachara, J. M., R. K. Kukkadapu, J. K. Fredrickson, Y. A. Gorby, and S. C. Smith (2002), Biomineralization of poorly crystalline Fe(III) oxides by dissimilatory metal reducing bacteria (DMRB), *Geomicrobiol. J.*, *19*, 179–207, doi:10.1080/01490450252864271.

Zavarzina, D. G., T. V. Kolganova, E. S. Boulygina, N. A. Kostrikina, T. P. Tourova, and G. A. Zavarzin (2006), *Geoalkalibacter ferrihydriticus* gen. nov. sp. nov., the first alkaliphic representative of the family *Geobacteraceae,* isolated from a soda lake, *Microbiology (Mikrobiologiia)*, *75*, 673–682, doi:10.1134/S0026261706060099.

Zhang, C. L., S. Liu, T. J. Phelps, D. R. Cole, J. Horita, S. M. Fortier, M. Elless, and J. W. Valley (1997), Physiochemical, mineralogical, and isotopic characterization of magnetite-rich iron oxides formed by thermophilic iron-reducing bacteria, *Geochim. Cosmochim. Acta*, *61*, 4621–4632, doi:10.1016/S0016-7037(97)00257-3.

Zhang, C., H. Vali, C. S. Romanek, T. J. Phelps, and S. V. Liu (1998), Formation of single-domain magnetite by a thermophilic bacterium, *Am. Mineral.*, *83*, 1409–1418.

Zhang, C. L., J. Horita, D. R. Cole, J. Z. Zhou, D. R. Lovley, and T. J. Phelps (2001), Temperature-dependent oxygen and carbon isotope fractionations of biogenic siderite, *Geochim. Cosmochim. Acta*, *65*, 2257–2271, doi:10.1016/S0016-7037(01)00596-8.

Zhu, H., D. Yang, and L. Zhu (2007), Hydrothermal growth and characterization of magnetite (Fe_3O_4) thin films, *Surf. Coat. Tech.*, *201*, 5870–5874, doi:10.1016/j.surfcoat.2006.10.037.

D. A. Bazylinski, School of Life Sciences, University of Nevada, 4505 Maryland Pkwy., Las Vegas, NV 89154-4004, USA. (dennis.bazylinski@unlv.edu)

C. Jimenez-Lopez, Departamento de Microbiología, Universidad de Granada, E-18071 Granada, Spain.

C. S. Romanek, Department of Earth and Environmental Sciences, University of Kentucky, Lexington, KY 40506, USA.