Iron-biomineralizing organelle in magnetotactic bacteria: function, synthesis and preservation in ancient rock samples

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Abstract

Magnetotactic bacteria (MTB) are ubiquitous aquatic microorganisms that incorporate iron from their environment to synthesize intracellular nanoparticles of magnetite (Fe_3O_4) or greigite (Fe_3S_4) in a genetically controlled manner. Magnetite and greigite magnetic phases that allow MTB to swim towards redox transitions zones where they thrive. MTB may represent some of the oldest microorganisms capable of synthesizing minerals on Earth, and have been proposed to significantly impact the iron biogeochemical cycle by immobilizing soluble iron into crystals that subsequently fossilize in sedimentary rocks. In the present article, we provide an overview of the mechanisms leading to iron mineralization in MTB. We then describe their distribution in the environment, and discuss the possible function of the magnetite and greigite nanoparticles. Finally, we update the methods used for the detection of MTB crystals in sedimentary rocks and present their occurrences in the geological record.

Introduction

Biomineralization corresponds to the capacity for living organisms to produce minerals such as carbonates, sulfides, phosphates and oxides. It can be an indirect process during which the metabolic activity of organisms transforms the physico-chemical conditions of their environment and induces the formation of mineral phases. Alternatively, biomineralization can be genetically controlled and serve biological functions. Biomineralization is widespread among living organisms, and can significantly impact biogeochemical cycles of elements (*e.g.* C, Ca, P, S, Fe) both qualitatively and quantitatively (*e.g.* Ridgwell and Zeebe, 2005).

Magnetotactic Bacteria (MTB) constitute an elegant example of controlled biomineralization (Fig. 1). They were first observed by Salvatore Bellini, a medical Doctor at the University of Pavia in Italia in the late 1950s and early 1960s (Bellini, 2009a,b). They were then described by Richard Blakemore from samples collected near Woods Hole, Massachusetts (Blakemore, 1975). They both noticed a group of bacteria whose swimming direction could be tuned using a magnet. Such magnetic behavior was explained by R. Blakemore, who observed intracellular crystals corresponding to either magnetite [$Fe(II)Fe(III)_2O_4$] or greigite [$Fe(II)Fe(III)_2S_4$] in these bacteria (Blakemore, 1975; Mann *et al.*, 1990).

Since their discovery, MTB have been found in a large variety of aquatic ecosystems including lakes, rivers, oceans, estuaries and lagoons (Lin *et al.*, 2014a). They incorporate a high amount of dissolved iron from their environment for magnetite or greigite synthesis, which corresponds to a mass of 10- to 100-fold higher than the one estimated in the bacterium *Escherichia coli* (Amor *et al.*, 2020a). When magnetotactic cells die, their crystals may be deposited in sediments and preserved during diagenesis (*i.e.* the physico-chemical transformation of sediments into sedimentary rock associated with natural burial), which effectively removes iron from the soluble pool (Chen *et al.*, 2014; Larrasoaña *et al.*, 2014). This

raised the hypothesis that MTB could significantly contribute to the iron biogeochemical cycle by sequestering a large amount of dissolved iron into insoluble crystals (Lin *et al.*, 2014a; Amor *et al.*, 2020a). MTB could have also impacted the ancient Earth surface, since they were proposed to have emerged during the Archean (Kopp and Kirschvink, 2008; Lin *et al.*, 2017a). In MTB, magnetite and greigite crystals are produced in organelles called magnetosomes. They correspond to prokaryotic organelles made of a bi-layered lipid membrane containing proteins and surrounding a single magnetite or greigite nanoparticle. Magnetosomes are aligned as chains, and provide the cell with a magnetic dipole. It is assumed that chains of iron nanoparticles are used as a compass, allowing the bacteria to passively align along the geomagnetic field's lines to efficiently reach the Oxic/Anoxic Transition Zone (OATZ) in water columns or sediments where they thrive (Frankel *et al.*, 1981). However, observation of MTB at the geomagnetic equator where the Earth's magnetic field has no vertical component (Frankel *et al.*, 1981) suggests that magnetite or greigite could have an additional function for the bacteria such as energy storage (Byrne *et al.*, 2015).

Here, we first describe the biodiversity, distribution and ecophysiology of MTB, and discuss the proposed function for magnetosomes in the environment. Then, we provide an overview of iron biomineralization in MTB. Mechanisms of iron incorporation and magnetite formation discovered in model magnetotactic strains cultivated in the laboratory are presented. Finally, we summarize occurrences of fossil MTB found in the geological record, as well as the methods used for identification of biological magnetite and greigite. Based on paleontological data, we speculate on the emergence of MTB and potential additional function(s) for magnetosomes over geological times.

1. Ecophysiology of magnetotactic bacteria

Biodiversity of magnetotactic bacteria

MTB diversity is reflected by a great variation of their shape, physiology and taxonomy. All known cultured and uncultured MTB are motile by means of flagella and have a cell wall characteristic of Gram-negative bacteria. The arrangement of flagella differs among MTB and can be either polar, bipolar or arranged in bundles. Another trait showing considerable diversity is the arrangement of magnetosomes within the cell (Fig. 1). In the majority of MTB, magnetosomes are aligned in one or more straight chains parallel to the cell's long axis, which maximizes the magnetic moment of the bacteria and reflects their evolutionary optimization for magnetic navigation. However, disordered magnetosomes located at one side of the cell occur in some magnetotactic cocci (Moench, 1988; Cox *et al.*, 2002; Freitas *et al.*, 2003) (Fig. 1C). Magnetosome mineral particles display diverse, well-defined and strain-specific morphologies (Bazylinski *et al.*, 1994). Four magnetite or greigite morphologies are found in MTB: cuboctahedral, elongated prismatic, tooth-shaped and bullet-shaped (Balkwill *et al.*, 1980; Mann *et al.*, 1984a, 1984b; Mann *et al.*, 1987a, 1987b; Heywood *et al.*, 1990; Devouard *et al.*, 1998; Pósfai *et al.*, 1998a, 1998b; Lefèvre *et al.*, 2011a) (Fig. 1). In addition to magnetosomes, other inclusions containing elemental sulfur, polyphosphate or poly-β-hydroxybutyrate (PHB) are common in MTB (Bazylinski *et al.*, 2004; Schultheiss *et al.*, 2005; Rivas-Lamelo *et al.*, 2017). A study of MTB from the Seine River (France) indicated that cells of uncultured MTB contain barium-rich and calcium oxide inclusions (Isambert *et al.*, 2007). Some MTB from the Nitrospirae and Omnitrophica phyla also contain numerous sulfur globules (Jogler *et al.*, 2010; Lefèvre *et al.*, 2011b; Kolinko *et al.*, 2012; Qian *et al.*, 2019). Another feature illustrating MTB diversity is their type of magnetotactic behavior. A study carried out on 12 cultured MTB from different taxa with various morphologies, physiologies, and flagellar apparatus showed six different magnetotactic behaviors described as a

96 combination of three distinct mechanisms, including dipolar, axial and unipolar mechanisms
97 (Lefèvre *et al.*, 2014).

98 A straightforward experiment to reveal the diversity of MTB consists in performing a
99 magnetic enrichment of an environmental sample containing sediment and water. In most
100 habitats the light microscope observation will allow the observation of various cell
101 morphotypes and behaviors. The main morphotypes include coccoid-to-ovoid cells (Fig. 1A-C),
102 rods (Fig. 1D), vibrios and spirilla (Fig. 1E) of various dimensions. Two unique morphotypes
103 include the multicellular magnetotactic prokaryotes (MMPs) (Keim *et al.*, 2007) (Fig. 1F), and
104 the very large rod *Candidatus* Magnetobacterium bavaricum (Jogler *et al.*, 2010) (Fig. 1G).
105 MTB do not represent a well-defined taxonomic group of prokaryotes: they are distributed
106 over several bacterial taxa containing non-magnetotactic species as well (Lefèvre and
107 Bazylinski, 2013) (Fig. 2). Most of our knowledge on MTB diversity is based on isolation,
108 cultivation, genomic, metagenomic and single-cell genomic studies (Jogler *et al.*, 2011; Kolinko
109 *et al.*, 2012; Lefèvre *et al.*, 2012; Lin *et al.*, 2018). The majority of MTB described so far belongs
110 to four subdivisions of Proteobacteria (Alpha, Beta, Gamma and Deltaproteobacteria classes),
111 and some are affiliated with the Nitrospirae and Omnitrophica phyla (Lefèvre and Bazylinski,
112 2013; Lin *et al.*, 2017b) Recent metagenomic data have suggested that even some lineages
113 within the deep-branching Latescibacteria and Planctomycetes phyla may also produce
114 magnetosomes (Lin *et al.*, 2018). With their deep-branched phylogenetic position, the latter
115 groups are particularly important to study since they would represent the earliest examples
116 of biomineralization on Earth (Parks *et al*., 2018). Magnetosome biogenesis is thus a
117 polyphyletic, possibly ancient trait associated with a large genetic, metabolic, and
118 morphological diversity. Interestingly, there is an obvious and apparently strong correlation
119 between the composition and morphology of magnetosome mineral crystals produced by

MTB and their phylogenetic affiliation (Lefèvre and Wu, 2013; Lefèvre *et al.*, 2013; Pósfai *et al.*, 2013) (Fig. 2).

The physiology of known MTB, either determined experimentally with cultured strains or inferred from genomic studies on uncultured strains, is also quite diverse. Cultured MTB belong to three classes of the Proteobacteria phylum (Alpha, Gamma and Deltaproteobacteria) (Fig. 2). They are obligate microaerophiles, anaerobes or both (Bazylinski and Williams, 2007). Those that tolerate relatively high concentrations of oxygen do not synthesize magnetite under these conditions. They are mesophilic with regard to growth temperature, and none grow at temperatures much higher than 30°C. Known MTB are chemoorganoheterotrophic and/or chemolithoautotrophic. In the model strains of the *Magnetospirillum* genus, heterotrophic growth occurs while cells are using organic acids as a source of carbon and electrons (Schleifer *et al.*, 1991). MSR-1 is also capable of autotrophic and mixotrophic growth using reduced sulfur compounds as a source of electrons (Geelhoed *et al.*, 2010). Species of the *Magnetococcus* (Lefèvre *et al.*, 2009; Bazylinski, Williams, Lefèvre, Berg, *et al.*, 2013; Morillo *et al.*, 2014) and *Magnetospira* genera (Zhu *et al.*, 2010; Williams *et al.*, 2012) as well as strains SS-5 and BW-2 of the Gammaproteobacteria class (Lefèvre *et al.*, 2012) are also growing autotrophically with reduced sulfur compounds. Species of the *Magnetovibrio* (Bazylinski *et al.*, 2013) and *Terasakiella* (Monteil *et al.*, 2018) genera are heterotrophic with *Magnetovibrio blakemorei* strain MV-1 that shows the greatest metabolic versatility regarding the compounds that can be used as potential electron donors and carbon sources for growth during microaerobic and anaerobic growth (Bazylinski *et al.*, 2013). Indeed, it has a respiratory metabolism, using oxygen, nitrate, and nitrous oxide (N_2O) as terminal electron acceptors (Bazylinski *et al.*, 1988). It grows chemoorganoheterotrophically and chemolithoautotrophically but also chemoorganoautotrophically with formate as the electron

144 donor. The pathway of autotrophy is the Calvin-Benson-Bassham cycle since ribulose-1,5-
145 bisphosphate carboxylase/oxygenase (RubisCO) genes were found in the genome of most
146 autotrophic MTB of the Alpha and Gammaproteobacteria classes. Only *Magnetococcus*
147 species such as strain MC-1 appear to use the rTCA cycle for autotrophic carbon assimilation
148 (Bazylinski *et al.*, 2013). Cultured strains of the Deltaproteobacteria, including several species
149 of the Desulfovibrionales (Sakaguchi *et al.*, 2002; Lefèvre *et al.*, 2011c) and Desulfobacterales
150 (Abreu *et al.*, 2007; Descamps *et al.*, 2017) orders grow anaerobically while reducing sulfate.
151 Cells of some strains of the *Desulfovibrio* genus also present the potential to grow as a
152 microaerophilic band of cells at the oxic–anoxic interface in media lacking sulfate (Lefèvre *et*
153 *al.*, 2016). *Desulfovibrio magneticus* strain RS-1 is the only cultured magnetotactic bacterium
154 known to be capable of fermentation: pyruvate is fermented to acetate and hydrogen
155 (Sakaguchi *et al.*, 2002).
156 Genomic, environmental and ultrastructural evidences seem to indicate that the deep-
157 branched MTB including the Nitrospirae candidate genera *Magnetobacterium* (Jogler *et al.*,
158 2011; Lin *et al.*, 2014b; Kolinko *et al.*, 2016) and *Magnetoovum* (Lefèvre *et al.*, 2011; Kolinko
159 *et al.*, 2016) as well as some magnetotactic cocci of the Magnetococcaceae family isolated
160 from the water column of the Black Sea (Schulz-Vogt *et al.*, 2019) are growing anaerobically
161 using nitrate as respiratory electron acceptor and oxidizing reduced sulfur compounds.
162 Although some MTB have been shown to be sensitive to light (Frankel *et al.*, 1997; Chen *et al.*,
163 2011; Shapiro *et al.*, 2011) with genomic evidences showing the presence of phototrophic
164 genes in some *Magnetospirillum* strains (Wang *et al*., 2019), no photosynthetic MTB have
165 been found. So far, no MTB were shown to be able to use reduced or oxidized forms of iron
166 for their energetic metabolism.
167

Environments hosting magnetotactic bacteria

MTB have been described worldwide and found on all continents. They are ubiquitous in sediments of freshwater, brackish, marine, and hypersaline habitats (Lin *et al.*, 2017b). The occurrence of MTB appears to depend on the presence of an Oxic-Anoxic Transition Zone (OATZ) consisting of opposing gradients of oxygen from the surface and reduced compounds from the sediments (Frankel *et al.*, 1997). The largest numbers of MTB are typically found at or slightly below the OATZ (Jogler *et al.*, 2010; Lefèvre *et al.*, 2011b). The different species of MTB show specific distributions within the OATZ. Magnetite-producing MTB are generally found at or very close to the OATZ, while greigite producers are present in reducing biotopes, below the OATZ, in the sulfidic anoxic zone (Moskowitz *et al.*, 2008; Lefèvre *et al.*, 2011c). Although most environmental studies on MTB were conducted on sediments of aquatic environments, some freshwater and saline lakes with water column stratification, seasonally or permanently, were also found to be suitable to host diverse populations of MTB (Simmons *et al.*, 2004; Rivas-Lamelo *et al.*, 2017; Schulz-Vogt *et al.*, 2019).

Biogeochemical studies indicate that some environmental parameters such as salinity, temperature, nitrate, or sulfur compounds could explain MTB abundance or community differences (Martins *et al.*, 2009, 2012; Lin *et al.*, 2012, 2013). Even if the largest proportion of MTB is detected within the suboxic zone, a strict correlation between the distribution of MTB and specific geochemical parameters has never been demonstrated (Flies *et al.*, 2005). For example, the presence of MTB is not correlated with the concentration in dissolved iron. Ferrous or ferric salts in the micromolar concentrations range are sufficient for growth and magnetite production (Schüler and Baeuerlein, 1996, 1998; Amor *et al*, 2020a). Such concentrations were shown to be typical of the free soluble iron found in environmental

sediments where MTB are most abundant (Flies *et al.*, 2005). Thus, iron does not represent an element limiting growth and magnetosome formation of natural populations of MTB.

For years, MTB were thought to be restricted to habitats with pH values near neutral and ambient temperatures. However, moderate thermophilic, psychrophilic, alkaliphilic and acidophilic MTB were described including: an uncultured, moderately thermophilic bacterium found in hot springs located in northern Nevada with a likely upper growth limit of about 63 °C (Lefèvre *et al.*, 2010); a cocci isolated from the low-temperature (<1°C) Antarctic maritime region (Abreu *et al.*, 2016); several obligatory alkaliphilic strains isolated in pure culture from different aquatic habitats in California, including the hypersaline and hyperalkaline Mono Lake, with an optimal growth pH of >9.0 (Lefèvre *et al.*, 2011c); and various morphotypes of MTB isolated from an acidic freshwater lagoon in Brazil (pH ∼ 4.4) (Abreu *et al.*, 2018). Deep-sea sediments were also investigated for the presence of MTB. A study reported the presence of alive MTB of different morphologies at water depths up to 3,000 m in the African continental margin (Petermann and Bleil, 1993). Metagenomic analyses coupled to magnetic measurements and electron microscope observations evidenced the occurrence of MTB in deep-sea sediments from the eastern Pacific manganese nodule province at water depths of 4,970 - 5,620 m (Dong *et al.*, 2016). Given that MTB are markers of chemical and redox gradients in aquatic settings, all chemically stratified environments could potentially support populations of MTB.

Finally, magnetosome-producing cells were also found in association with other living organisms. The presence of magnetotactic symbionts was observed within the marine bivalve *Thyasira cf. gouldi* (Dufour *et al.*, 2014). This association is likely more related to predation since the integrity of magnetosome chains and possibly flagellum is lost in the host. Predation of MTB was also revealed in some protists such as bacterivorous ciliates that grazed MTB

(Bazylinski *et al.*, 2000; Monteil *et al.*, 2018). Interestingly, the accumulation of magnetite in these microeukaryotes makes them sensitive to magnetic fields. In marine anoxic sediments, another report evidenced a mutualistic symbiosis between excavate protists (Symbiontida, Euglenozoa) and ectosymbiotic Deltaproteobacteria producing magnetosomes (Monteil *et al.*, 2019).

Biological function of magnetosomes

The Earth's core generates a permanent magnetic dipole with field vectors having a non-zero vertical component at every location on the planet but at the magnetic equator (Guyodo and Valet, 1999). The magnetosome chain in MTB imparts a permanent magnetic moment to the cell, and acts as a compass needle that aligns along the geomagnetic field lines (Frankel, 1984). MTB can thus use the geomagnetic field to swim upward or downward along chemical gradients in a process called magnetotaxis. It represents a selective advantage for microorganisms living in spatially fluctuating niches such as those of chemically stratified environments, and limits environmental prospection for energy sources and electron acceptors to bidirectional transects instead of a volume (Frankel *et al.*, 1997). This trait compensates unstable disturbances of vertical chemical gradients and help MTB to move toward their optimal local niche. The geomagnetic field selects for a dominant cell polarity in each hemisphere by favoring cells whose polarity caused them to swim downward along the inclined field lines toward microaerobic/anaerobic sediments and away from high oxygen concentrations in surface waters. This is supported by the observation of north-seeking MTB that predominate in the Northern Hemisphere, and south-seeking cells predominating in the Southern Hemisphere (Blakemore *et al.*, 1980). At the magnetic equator, south-seeking and north-seeking MTB were found in equal concentrations (Frankel *et al.*, 1981). However,

magnetotaxis is not considered as a true taxis, but rather corresponds to a magnetically assisted chemo-aerotaxis, directing the cells towards attractants or away from repellents (Frankel *et al.*, 1997; Klumpp *et al.*, 2019). It is assumed that magnetic field passively orientates the cells, which in turn actively swim using their flagellar apparatus (Frankel and Bazylinski, 2006). In both hemispheres, magnetotaxis thus reduces the three-dimensional random swim associated with chemo-aerotaxis to a one-dimensional search, supposedly yielding an energy advantage over non-magnetotactic organisms (*Klumpp et al.*, 2019).

However, there are some exceptions that reported species of south-seeking MTB from the Northern Hemisphere and north-seeking MTB from the Southern Hemisphere (Simmons et al., 2006; Leão et al., 2016). The magnetotactic protists whose magnetotaxis originates from the interaction with ectosymbiotic magnetosome-producing bacteria were also described with an atypical orientation of the cells polarity regarding the magnetic moment (Monteil *et al.*, 2019). These specific behaviors, that could illustrate sensors having distinct response to chemical gradients, seem to be restricted to anaerobes swimming upward to the bottom limit of the OATZ. A variation in sensory response could also be at the origin of the different magneto-aerotactic behaviors observed in cultured MTB that formed aerotactic band under oxygen-gradient conditions (Lefèvre *et al.*, 2014). Indeed, it was suggested that the three magneto-aerotactic mechanisms (*i.e.* polar, dipolar, and axial) are related to distinct oxygen sensing mechanisms that regulate the direction of cells' motility in an oxygen gradient.

Thus, the most currently accepted hypothesis regarding the function of magnetosomes corresponds to the increase of efficiency in finding preferred biotope, but alternative biological functions were also proposed. Indeed, similar to other complex functions, magnetosome biomineralization requires the coordination of a complex set of interacting proteins with a large range of functions and domains (Uebe and Schüler, 2016; McCausland

and Komeili, 2020). This raises questions about the evolutionary steps and ancestral functions, as well as on the environmental conditions and selective pressures that led to the emergence of such a complexity. Several observations support that magnetosomes of modern lineages could be used for other purposes than cell orientation. For example, some MTB produce more magnetosomes than necessary for magnetotaxis (Spring *et al.*, 1993) (Figs. 1F and 1G), while others thrive at the geomagnetic equator, where the Earth's magnetic field has no vertical component (Frankel *et al.*, 1981). There, MTB cannot use the geomagnetic field to swim upward or downward to find their optimal environment following redox gradients. When grown in a metal chamber that cancels external magnetic field or without redox gradient, some MTB keep producing magnetosomes (unpublished observations). Alternative hypotheses for magnetosome function has thus been proposed.

It was originally speculated that magnetosomes could play a role in iron storage (Blakemore, 1982). Even though no evidence supports the role of magnetosomes as an iron-storage vesicle, they could be used to prevent accumulation of free iron in the cytoplasm that would be harmful to the cell (Imlay, 2003).

Alternatively, it has been proposed that magnetosomes could be used as energy-storing systems (Kopp *et al.*, 2004; Byrne *et al*., 2015). This could be achieved by oxidizing magnetite into maghemite under oxidative conditions, thus releasing electrons that could be used as a source of energy. Maghemite would then be reduced back to magnetite under reductive conditions.

In addition, it was shown in MSR-1 and AMB-1 that proteins contained in the magnetosome membrane exhibit a peroxidase-like activity (Guo *et al.*, 2012; Li *et al.*, 2015). Magnetosomes were thus proposed to decrease and eliminate reactive oxygen species in the cell. However, magnetite produces reactive oxygen species through Fenton reactions (He *et al.*, 2015).

Moreover, such hypothesis cannot explain the presence of crystal phases as only the proteins located in the magnetosome membrane would be needed to eliminate reactive oxygen species. Therefore, the peroxidase activity of magnetosomes may instead be used to prevent accumulation of free radicals generated by magnetite crystals in the bacteria.

2. Iron biomineralization in magnetotactic bacteria

Magnetosomes are produced from the invagination of the inner cell membrane (Komeili *et al.*, 2006). In magnetite-forming MTB, a set of ~30 genes located in a distinct portion of the genome, the Magnetosome Gene Cluster (MGC), is involved in the step-wise formation of magnetosomes (Murat *et al.*, 2010). Magnetosome genes are used for (*i*) the formation of magnetosome vesicles from invagination of the inner cell membrane, (*ii*) the alignment of magnetosome vesicles as a chain, (*iii*) iron trafficking to magnetosomes for magnetite precipitation, and (*iv*) crystal growth and maturation with controlled size and shape. Two recent review articles discussed in details the genetic factors leading to magnetosome formation (Uebe and Schüler, 2016; McCausland and Komeili, 2020). In the present contribution, we focus on the chemical reactions leading to iron incorporation and transformation in MTB during magnetosome formation. Iron biomineralization was mostly studied in magnetite-forming strains, and was extensively characterized in the three Alphaproteobacteria *Magnetospirillum magneticum* AMB-1, *Magnetospirillum magnetotacticum* MS-1, and *Magnetospirillum gryphiswaldense* MSR-1, as well as in the sulfate-reducing Deltaproteobacteria *Desulfovibrio magneticus* RS-1. A model compiling the proposed pathways for iron biomineralization in MTB is illustrated in Figure 3.

Iron uptake

311 MTB can take either dissolved Fe(II) or Fe(III) up from their environment. Incorporation of
312 reduced iron species occurs as a diffusion process (Schüler and Baeuerlein, 1996, 1998; Amor
313 *et al.*, 2018). Fe(III) assimilation requires energy-dependent mechanisms (Schüler and
314 Baeuerlein, 1996). In AMB-1 and MS-1, they involve biosynthesis of hydroxamate and catechol
315 siderophores, which are subsequently released to the external medium for Fe(III)
316 solubilization and incorporation (Paoletti and Blakemore, 1986; Calugay *et al.*, 2003, 2006).
317 Under iron-rich conditions, genes encoding for high-affinity Fe(II) transport are up-regulated,
318 while Fe(III) acquisition systems are down-regulated under the same conditions (Suzuki *et al.*,
319 2006). Contrastingly, siderophore-independent Fe(III) incorporation was observed for MSR-1
320 (Schüler and Baeuerlein, 1996). Finally, Fe(II) has been shown to be preferentially
321 incorporated by MTB when both oxidized and reduced species are available for the bacteria
322 (Amor *et al.*, 2018). This illustrates that iron assimilation favors energy-independent
323 mechanisms under sufficient iron conditions (Suzuki *et al.*, 2006).
324
325 ***Iron-storing mechanisms***
326 Once incorporated, iron is first stored into the cell as Fe(III) species (Amor *et al.*, 2018). In the
327 case of Fe(II) incorporation, iron is thus fully oxidized upon internalization (Amor *et al.*, 2018).
328 The subcellular location of these species remains debated. They could be contained in the
329 cytoplasm and/or periplasm. Several proteins are known to store iron, including ferritins,
330 bacterioferritins and DNA-binding proteins (Dps) (Arosio *et al.*, 2017; Uebe *et al.*, 2019).
331 Ferritin-like structures have been identified in AMB-1 (Baumgartner *et al.*, 2013), MSR-1
332 (Faivre *et al.*, 2007; Fdez-Gubieda *et al.*, 2013; Uebe *et al*, 2019) and MS-1 (Frankel *et al.*, 1983).
333 From Mössbauer characterization of iron in MS-1 and MSR-1, it was proposed that oxidized
334 iron present as ferritin-like structures is then partially reduced into ferrous iron for trafficking

335 to magnetosomes and precipitation as magnetite (Frankel *et al.*, 1983; Faivre *et al.*, 2007).
336 These results were confirmed by measurement of iron isotopes in AMB-1 (Amor *et al.*, 2016,
337 2018). RS-1 can also accumulate phosphate- and sulfur-associated Fe(II) species upon iron
338 assimilation (Baumgartner *et al.*, 2016).
339
340 ***Iron delivery to magnetosomes***
341 In contrast to ferric iron, ferrous iron is soluble at circumneutral pH, which can help iron
342 trafficking in the cell (Millero *et al.*, 1995). Fe(II) could be addressed to magnetosomes *via* the
343 periplasm when the magnetosome membrane is still invaginating from the cell's inner
344 membrane (Faivre *et al.*, 2007; Jones *et al.*, 2015). Alternatively, ferrous iron could be
345 transported to mature magnetosome vesicles across the magnetosome membrane. The latter
346 hypothesis was recently supported by two high-resolution electron microscopy and
347 fluorescence studies, which evidenced a layer of unmineralized ferrous ions surrounding
348 magnetite in the magnetosome membrane in both cultured and environmental bacteria
349 (Werckmann *et al.*, 2017; Amor *et al.*, 2020b). In that regard, genes encoding Fe(II)
350 transporters (*feoAB* operon) have been found in the vicinity of all MCGs of MTB with their
351 genome sequenced (Suzuki *et al.*, 2006; Rong *et al.*, 2008, 2012; Uebe and Schüler, 2016).
352 Deletion of these genes causes a strong impairment of magnetite biomineralization.
353
354 ***Magnetite formation and possible precursors***
355 Fe(II) / Fe(III) ratio at the magnetosome location needs to be tightly controlled for magnetite
356 or greigite formation. This ratio is 0.5 in both mineral phases, although slightly oxidized
357 magnetite in MTB [*i.e.* Fe(II) / Fe(III) ratios lower than 0.5] has been consistently reported
358 (Weiss *et al.*, 2004; Kopp *et al.*, 2006; Moskowitz *et al.*, 2008; Li *et al.*, 2009). Several groups

359 proposed that magnetite precursors are first precipitated. In AMB-1, MS-1, and MSR-1, these
360 precursors include oxidized phases such as ferrihydrite [$Fe(III)_2O_3.5H_2O$] (Frankel *et al.*, 1983;
361 Faivre *et al.*, 2007; Baumgartner *et al.*, 2013; Fdez-Gubieda *et al.*, 2013). One study also
362 suggested that hematite [α-$Fe(III)_2O_3$] could represent a magnetite precursor (Le Nagard *et*
363 *al.*, 2019). Fe(II) transported to magnetosomes would thus have to be oxidized for
364 precipitation of these precursors (Amor *et al.*, 2018). The candidate proteins for oxidation
365 reactions include MamP, which contains a c-type cytochrome domain (Siponen *et al.*, 2012,
366 2013; Jones *et al.*, 2015). It has been detected in the magnetosome membrane (Raschdorf *et*
367 *al.*, 2018). Fe(II) oxidation could thus happen during iron diffusion across the magnetosome
368 membrane or in the magnetosome lumen. Magnetite would then form upon further Fe(II)
369 addition in magnetosomes (Tronc *et al.*, 1992; Ahn *et al.*, 2012). This proposed
370 biomineralization pathway was recently challenged by Uebe and co-workers, who showed
371 that ferritins and ferritin-like structures (*i.e.* ferrihydrite) are not necessary for magnetite
372 precipitation in MSR-1 (Uebe *et al.*, 2019). They produced a mutant strain lacking iron-storing
373 proteins, and characterized iron species in these bacteria as well as in wild-type cells. They
374 found that the presence of ferrihydrite in MSR-1 is tied to the presence of iron-storing proteins.
375 However, magnetite biomineralization was not altered in the mutant strain. They rather
376 proposed a distinct function for these proteins, which protected the bacteria from oxidative
377 stress. From these results, the authors concluded that iron-storing proteins are not involved
378 in biomineralization and that ferrihydrite does not represent a magnetite precursor. However,
379 the possibility of alternative biomineralization pathways triggered by the absence of iron-
380 storing proteins cannot be completely ruled out. Moreover, the mutant strains lacking iron-
381 storing proteins were cultivated under a limited number of growth conditions. It is thus
382 difficult to give a definitive conclusion about the role of ferritin-like structures. Finally, green

rust [a mixed Fe(II)/Fe(III) hydroxide] was proposed as a potential magnetite precursor in RS-1, a strain genetically distant from species of the *Magnetospirillum* genus (Baumgartner *et al.*, 2016). Although the genetic content appears to be quite conserved among MTB, it is possible that they developed different strategies for magnetite biomineralization depending on their physiology and environment.

Iron distribution in magnetotactic bacteria

Physical and chemical characterizations of model magnetotactic strains demonstrated that different iron species can be present in MTB. Time-course experiments conducted with AMB-1 and MSR-1 observed that virtually all iron was contained in magnetite when biomineralization was complete (Baumgartner *et al.*, 2013; Fdez-Gubieda *et al.*, 2013). In contradiction with these findings, direct measurements of iron in wild-type AMB-1 and MSR-1, as well as an AMB-1 mutant strain unable to form magnetosomes, evidenced a large pool of iron distinct from magnetite (Amor *et al.*, 2016, 2018, 2020a; Berny *et al.*, 2020). Such additional pool of iron can represent half of the total intracellular iron in wild-type AMB-1 and MSR-1 (Amor *et al.*, 2020b; Berny *et al.*, 2020). The discrepancy between these findings and the time-course experiments was proposed to raise from experimental conditions. Indeed, time-course experiments require to first grow bacteria under iron-starving conditions (*i.e.* no iron provided to the growth medium), which trigger overexpression of iron transporters (Suzuki *et al.*, 2006; Wang *et al.*, 2017). This could optimize the transfer of iron to magnetosomes for magnetite precipitation, and prevent accumulation of iron in a reservoir distinct from magnetite. Interestingly, mutant AMB-1 strains unable to form magnetosomes also accumulated iron (Amor *et al.*, 2020a). However, the total mass of iron incorporated by these mutant bacteria was significantly lower than the one contained in wild-type bacteria.

Fe(II) dynamics in magnetotactic bacteria

Finally, iron isotope modeling during magnetite biomineralization in AMB-1 allowed the identification of Fe(II) diffusion from the cell's internal medium to the external growth medium (Amor *et al.*, 2016, 2018). A release of intracellular Fe(II) to the external medium could have resulted from cell lysis. However, monitoring of cell concentration during bacterial cultures ruled out such possibility. Bacterial Fe(II) outward diffusion thus rather reflects passive Fe(II) exchange with the external medium.

3. Tracking the footprint of MTB in the fossil record

Criteria for detection of MTB fossils

The specific mineralization pathway provides MTB magnetite with unique features that are used for the detection of MTB fossils (hereafter referred to as magnetofossils) in ancient rock samples. For several decades, magnetofossil identification relied on morphological, crystallographic and magnetic criteria, which are now completed by chemical and isotopic fingerprints, as well as additional magnetic methodologies. Thomas-Keprta and co-workers (2000) defined six robust criteria for magnetofossil identification (see also review and discussion in Jimenez-Lopez *et al.*, 2010; Li *et al.*, 2013). Here we summarize these criteria and update them using the most recent discoveries (Fig. 4)

Unlike abiotic magnetite, MTB crystals show blunt edges associated with typical shapes (see below) (Kopp and Kirschvink, 2008; Witt *et al.*, 2005). Their size ranges from less than 35 nm up to 120 nm (Konhauser *et al.*, 1998). Because crystal growth in MTB is tightly controlled by proteins, the particle sizes and shape factors (*i.e.* width/length ratios) in a given population

show a narrow skewed gaussian distribution (Devouard *et al.*, 1998) compared to their abiotic counterparts as well as magnetite produced through induced biomineralization (Loveley *et al.*, 1987; Sparks *et al.*, 1990; Arató *et al.*, 2005). However, this criterion is not sufficient to distinguish magnetofossil from abiotic magnetite in natural sedimentary rocks because abundance peaks of biotic and abiotic magnetite can overlap and create more complex distributions (Arató *et al.*, 2005).

The size and shape of MTB nanoparticles fall into the stable single magnetic domain, which maximizes the magnetic moment for each particle (Witt *et al.*, 2005; Muxworthy and Williams, 2008). The alignment of Stable Single-Domain (SSD) magnetite particles in the magnetosome chain imparts a strong magnetic moment to the bacteria. The unique chain arrangement of SSD nanoparticles provides MTB with distinct magnetic features characterized by a large remanent magnetization as well as a limited interaction field between particles (Li *et al.*, 2012). First Order Reversal Curves (FORC), a magnetic method based on the measurement of hysteresis curves, as well as Ferromagnetic Resonance (FMR) spectroscopy have successfully identified intact chains of fossilized magnetosomes (Roberts *et al.*, 2013; Heslop *et al.*, 2014; Roberts *et al.*, 2019). However, such magnetite alignment may be lost during sediment burial and fossilization processes owing to the degradation of proteins assembling magnetosome chains (Li *et al.*, 2012). In that case, the typical FORC signatures of magnetosome chains are lost. The Verwey temperature (*i.e.* the temperature below which magnetite undergoes a phase transition associated with a modification of surface charge configuration and a sharp change of susceptibility and remanent magnetization) has also been used to test the magnetotactic origin of fossil magnetite samples (Chang *et al.*, 2016). Finally, Isothermal Remanent Magnetization (IRM) and Anhysteretic Isothermal Remanent (ARM) analyses allowed to cluster the origins of magnetite and to estimate the proportion of biogenic

magnetite in a bulk sample using a non-negative matrix factorization (Egli, 2004; Heslop and Dillon, 2007; Roberts *et al.*, 2013). This method has been used efficiently on magnetite samples from Eocene sedimentary rocks that survived diagenetic modifications (Day *et al.*, 1977; Roberts *et al.*, 2013; Roberts *et al.*, 2019).

Transmission Electron Microscopy (TEM) analyses also showed that magnetite produced by MTB usually grows along the [111] crystalline axis without lattice defects (Mann *et al.*, 1987a; Vali *et al.*, 1987; Devouard *et al.*, 1998). However, evidences of elongation anomalies along the [110] or [100] axes were reported (Isambert *et al.*, 2007; Benzerara and Menguy, 2009; Li *et al.*, 2015). So far, the crystal growth along the [111] axis still seems to be congruent for prismatic magnetite.

Laboratory experiments demonstrated a low incorporation of trace elements contained in the bacterial growth medium into AMB-1 magnetite (Amor *et al.*, 2015). For most tested elements, such incorporation was at least 100 times lower compared to abiotic magnetite samples synthesized from co-precipitation of Fe(II) and Fe(III) ions in aqueous solutions (Amor *et al.*, 2015). Chemical purity can thus be used as a chemical fingerprint of MTB magnetite. However, a few elements such as molybdenum and tin showed preferential incorporation into AMB-1 magnetite. They could reflect metabolic reactions in which these two elements are involved, and thus represent biological signatures of MTB activity recorded in magnetite. We note that several publications reported improved incorporation of elements from the iron series such as cobalt and manganese into MTB magnetite for biotechnology purposes (*e.g.* Staniland *et al.*, 2008; Keim *et al.*, 2009). Trace elements incorporation was not compared with abiotic conditions under such high concentrations. Therefore, they have limited paleontological applications.

Finally, isotopic tools were used to search for magnetofossils in ancient rock samples. Oxygen isotope composition of magnetite produced by the strains MS-1 and MV-1 has been investigated, and showed a temperature dependency (Mandernack *et al.*, 1999). However, no difference was found between MTB magnetite and magnetite formed extracellularly by Dissimilatory-Iron Reducing Bacteria (DIRB), a group of iron-metabolizing microorganisms that perform magnetite induced biomineralization. In the same study, no specific iron isotope signatures were evidenced in the magnetite produced by the magnetotactic strains MS-1 and MV-1 (Mandernack *et al.*, 1999). Contrastingly, magnetite produced by AMB-1 demonstrated a significant depletion in heavy iron isotopes relative to the growth medium and the organic fraction of the cell (Amor *et al.*, 2016). Additionally, the odd iron isotope (^{57}Fe) showed a specific incorporation into AMB-1 magnetite. Such isotopic anomaly with preferential incorporation of ^{57}Fe compared to the three other isotopes (^{54}Fe, ^{56}Fe and ^{58}Fe) has never been observed before and may thus constitute a unique biosignature of magnetite produced by MTB. However, the origin of this isotopic signature remains unclear and needs to be investigated further on other MTB strains and natural samples.

So far, it can be highlighted that neither a single nor couples of criteria are sufficient to attest for magnetite biogenicity in the past sedimentary record. A whole body of evidences based on mineralogical, magnetic, chemical and isotopic tools is required for a firm identification of magnetofossils (Kopp and Kirschvink, 2008). Such difficulty raises from the limited number of magnetotactic strains tested for their morphologic, magnetic, chemical, and isotopic specificities. Finally, additional work should be carried out to determine whether these criteria can survive sediment burial and fossilization processes (see below).

Magnetofossil occurrences in the rock record

Magnetofossils were identified in sedimentary rocks from various ages, most of which were observed in quaternary sediments from lakes, carbonate platforms, pelagic or hemi-pelagic environments (*e.g.* Mc Neill *et al.*, 1988; Yamazaki and Ioka, 1998). The oldest observation during the current geologic eon Phanerozoic (0-540 Ma) was reported in Late Cretaceous chalks from Southern England that were deposited about 85 Ma ago (Montgomery *et al.*, 1998). This study reported detailed paleomagnetic data based on SSD magnetite-bearing samples. Their observation using TEM demonstrated the presence of well-preserved chains of magnetosomes, constituted mainly of prismatic magnetite although cuboctahedral and bullet-shaped particles were also present.

In contrast, scarce traces of magnetofossils were reported in rock records of the Precambrian representing the earliest and longest period of Earth's history, from 3.8 Ga to 540 Ma (Fig. 5). In the 1980s, Chang and co-workers examined a number of Precambrian stromatolithic limestones and cherts using rock magnetic methods and high-resolution electron microscopy on magnetic extracts (Chang *et al.*, 1989). They described several potential magnetofossils, among which the most robust and ancient traces including magnetosome chains were recorded in the 2.0 Ga-old Gunflint Formation, Western Australia. Several years later, Akai and coworkers (1997) provided evidence for the oldest putative magnetofossils ever reported in terrestrial samples in stromatolites from the Tumbiana Formation, Western Australia, formed in a marine basin 2.7 Ga ago. Although very promising, these results rely only on the size (in the SSD) and shape of magnetite crystals determined by TEM analyses (Akai *et al.*, 1997); they thus require to be validated by further investigations.

The presence of magnetofossils in ancient extraterrestrial material was also suggested, as illustrated by the controversial studies of the Martian meteorite ALH84001 (McKay *et al.*, 1996; Thomas-Keprta *et al.*, 2000, 2009; Brearley, 2003; Treiman, 2003; Jimenez-Lopez *et al.*,

2012). This sample might represent the oldest evidence for magnetofossils in the solar system. The meteorite ALH84001 is an orthopyroxenite that crystallized 4.1 Ga ago (Lapen *et al.*, 2010). It contains carbonate globules formed in a late stage about 3.9 Ga ago, likely from low-temperature hydrothermal fluids. Nano-sized magnetite crystals with cuboctahedral, teardrop, prismatic and irregular shapes were identified in the carbonate globules (McKay *et al.*, 1996; Thomas-Keprta *et al.*, 2009). Large statistical TEM analyses indicate that 30% of the nanomagnetites were elongated prisms showing a striking similarity with those formed on Earth by the magnetotactic strain MV-1 (Thomas-Keprta *et al.*, 2000). In addition, extraterrestrial SSD nanomagnetites exhibited restricted anisotropy, crystallographic perfection (*i.e.* free of internal defects), elongation along the [111] axis and chemical purity, but no chain arrangement (Thomas-Keprta *et al.*, 2000, 2009). It should be noted that chemical purity was assessed from energy dispersive X-ray spectroscopy, a method that is not suitable to strictly evaluate the biogenicity of magnetite based on its trace element content (Amor *et al.*, 2015). The nanomagnetite biogenicity in the ALH84001 meteorite is still a matter of debate because alternative hypotheses have been proposed to explain their formation. These crystals could also have been formed from shock-induced thermal decomposition and metamorphism of Fe carbonates (Brearley, 2003; Treiman, 2003), even though chemically-pure magnetite can hardly be produced from impure carbonates (Jimenez-Lopez *et al.*, 2012). These last ten years, new magnetofossil criteria were identified such as trace element and iron isotope compositions, which could help to further decipher the origin of nanomagnetites in the ALH84001 meteorite.

Alteration vs preservation of magnetofossils in sediments

549 As stated in the introduction, magnetite can be degraded during diagenesis. Three main
550 diagenetic processes can alter magnetite and prevent the identification of primary
551 magnetosomes: (*i*) oxidation, (*ii*) chain breakup, and/or (*iii*) reductive dissolution (see review
552 of Roberts, 2015). The first process happens when magnetite experiences oxidizing conditions,
553 due to diffusion of molecular oxygen in sediment or late exposition of sedimentary rock to the
554 atmosphere. This leads to partial or total transformation of magnetite to maghemite [γ-
555 $Fe(III)_2O_3$] (Chang *et al.*, 2013). In the second and third processes, one must consider that all
556 sediments contain a fraction of organic matter used as electron donor for aerobic or anaerobic
557 respiration processes. Various oxidants are utilized as electron acceptors following a well-
558 established sequence depending on the free energy of each respiration pathway: O_2, NO_3^-,
559 manganese oxides, iron oxides, and SO_4^{2-} (Froelich *et al.*, 1979). The magnetosome
560 membranes of MTB are mostly made of lipids, which can be degraded by microbial oxidative
561 process. The chain of magnetosomes is hold by a filament mainly constituted by the actin-like
562 MamK protein (Komeili, 2012; Uebe and Schuler, 2016), and can thus also be degraded by
563 diagenetic microbial processes. The last stage of anaerobic respiration during sediment burial
564 is based on microbial SO_4^{2-} reduction that releases H_2S in porewater. Many studies of marine
565 sediments demonstrated that H_2S reduces and dissolves magnetite (*e.g.* Canfield and Berner,
566 1987; Mohamed *et al.*, 2011), with magnetite half-life ranging from 5 to 1000 years (Canfield
567 and Berner, 1987; Emiroglu *et al.*, 2004). If SO_4^{2-} and organic matter are abundant in sediments,
568 one might expect magnetite to be mostly consumed and not preserved in rocks (Roberts,
569 2015). What might thus be a factor of magnetofossil preservation in sediments? SO_4^{2-}-poor
570 environments such as modern lake and river systems provide potentially favorable conditions
571 for preservation. Indeed, only a small amount of H_2S can be produced in SO_4^{2-}-poor sediments
572 and reductive dissolution of magnetite may be limited. Significant amount of magnetofossils

could also be present in Archean sedimentary rocks (3.8 to 2.5 Ga old) since SO_4^{2-} concentrations dissolved in the ocean at that time were extremely low (< 2.5 μM; Crowe *et al.*, 2014), and organic matter supply to the sediments was likely weak due to low biological productivity (Canfield, 2005). It can be noted that the biggest natural reservoir of magnetite in the Earth's crust is from the Archean banded iron formation, termed BIF (Konhauser *et al.*, 2017). This indirectly confirms the high capacity of magnetite preservation in Archean sediments even though BIFs have never been supposed to be formed from MTB remaining.

Magnetofossil preservation in sediments can also be inferred from data available for Phanerozoic sedimentary rocks, in which a striking relationship was observed between magnetofossil features and glacial-interglacial cycles (Paasche *et al.*, 2004, 2011). Analysis of sediments covering the last million years showed that glacial stages are associated with an enrichment of organic matter but a decrease in magnetofossil abundance (Lean and McCave, 1998; Hesse, 1994; Yamazaki and Kawahata, 1998). These variations are interpreted as an increase of biological productivity leading to higher organic matter flux and stronger reductive dissolution of magnetite during sediment diagenesis. In contrast, interglacial periods correspond to lower biological productivity, thus lowering organic carbon flux. The O_2 diffusion from bottom water to sediment extended deeper in the sediment and increased the size of the OATZ, where MTB thrive, up to tens of meters. This resulted in (*i*) a decrease of organic matter content (inducing a lower H_2S production and reductive dissolution) and (*ii*) an increase of MTB in the larger OATZ (Roberts, 2015). Both effects globally increase the capacity of magnetofossil preservation in sediments. Another scenario has also been suggested to explain abundant magnetofossils in Phanerozoic sediments. Several authors proposed that the amount of iron-rich dust could control the production and preservation of MTB (Roberts *et al.*, 2011; Yamazaki and Ikehara, 2012; Savian *et al.*, 2014). Indeed, iron-dust particles

increase the primary productivity in the water column but can also be delivered to the sediments and be used for anaerobic microbial oxidation of organic matter. This latter process would increase the availability of dissolved Fe(II) in sediment porewater (used by MTB for magnetite production), and decrease organic matter content, which in turn would decrease the relative production of H_2S (and dissolution of magnetite) by sulfate-reducing bacteria. Finally, magnetofossil spikes were also evidenced during a global warming event ~55 Ma ago known as the Paleocene-Eocene Thermal Maximum (PETM) (Kopp *et al.*, 2007, 2009). Unusually large magnetite crystals sharing morphological and crystallographic features typical of MTB were observed during the PETM (Schumann *et al.*, 2008). As suggested by the glacial/interglacial cycles, warmer climate conditions could thus favor MTB magnetite formation and/or preservation.

Tracking the age of the last common MTB ancestor

Resolving the evolution of controlled iron biomineralization in MTB is a major challenge in evolutionary biology because this trait is polyphyletically distributed in taxa that are separated by billion years of evolution from their most recent common ancestor. This intriguing phylogenetic distribution of MTB raised three main questions tackled by the scientific community over the last three decades: (*i*) has magnetosome formation a single genetic origin?, (*ii*) was it an ancestral character shared by the ancestors of all phyla, genera and species including MTB lineages?, and (*iii*) when this function emerged during the evolution of life on Earth? To figure this out, several research groups performed whole genome sequencing and inferred molecular phylogenies of MTB species and magnetosome genes (Abreu *et al.*, 2011; Lefèvre *et al.*, 2013; Lin *et al.*, 2017a, 2018; Monteil *et al.*, 2018). Over the years, datasets got progressively stronger and stronger by including a higher taxonomic sampling

621 and a higher degree of phylogenetic signal thanks to whole genome datasets. Together with
622 molecular genetics, they provided evidence of a single genetic origin followed by a duplication
623 event that led to the emergence of the modern greigite and magnetite Magnetosome Gene
624 Clusters (MGCs) at some point in the MTB diversification. Two scenarios are currently
625 proposed: (*i*) a duplication of an unknown form of the MGCs that occurred well before the
626 emergence of the deep-branched PVC group (*i.e.* the superphylum gathering the
627 Planctomycetes, Verrucomicrobia and Chlamydiae phyla) that contain MTB, and (*ii*) a much
628 more recent duplication of the magnetite MGC in the Deltaproteobacteria that was
629 neofunctionalized to biomineralize greigite (Lin *et al.*, 2018). Whenever this duplication
630 occurred, the distribution of magnetotaxis in the different bacterial phyla was mainly
631 explained by the vertical inheritance of the MGCs followed by numerous independent gene
632 losses in the non-magnetotactic lineages over phyla diversification (Lefèvre *et al.*, 2013; Lin *et*
633 *al.*, 2017a). Accordingly, controlled iron biomineralization emerged just before the emergence
634 of Nitrospirae and Proteobacteria phyla, which was estimated to have occurred during the
635 Archean over a timescale between 3.21 to 3.38 Ga ago with 95% confidence intervals ranging
636 over approximately 500 million years (Lin *et al.*, 2017a). As a consequence, the ancestor of a
637 tremendous amount of modern bacterial lineages, but not all, could have performed
638 controlled iron biomineralization.
639 However, the above-mentioned phylogenies and the use of molecular clocks for prokaryotic
640 organisms were discussed by other research groups. Tackling MTB evolution is equivalent to
641 inferring the timing of the explosive radiation that led to the emergence of all major
642 Eubacteria phyla billions of years ago (Battistuzzi *et al.*, 2004; Betts *et al.*, 2018). At such
643 evolution timescale, methods in molecular phylogeny faces numerous limitations that
644 introduces uncertainties in reconstruction of tree topologies and estimation of event timing

[*e.g.* long branch attraction artifacts (Bergsten, 2005), impracticable and miscalibrated molecular clocks (Graur *et al.*, 2004), effect of gene flow blurred over timescales and site saturation (Duchêne *et al.*, 2016)]. According to some authors (Wang and Chen, 2017), the current proposed scenario may not be parsimonious as long as it involves too many loss events and rely on an uncertain tree topology. Additional investigations will help to refine these inferences, and in the case of controlled iron biomineralization, magnetofossils could represent a major asset to calibrate evolutionary history of life.

Predicting MTB evolution in relation to the Precambrian ocean chemistry

Geochemical traces of biogenic reduced carbon in turbiditic and pelagic sedimentary rocks from the Isua supracrustal belt in west Greenland suggested that early life emerged long before 3.7 Ga (Rosing, 1999). Molecular clock analyses later confirmed that the last universal common ancestor of cellular life emerged ~4 Ga ago followed by an evolutionary burst of all Eubacteria phyla that likely occurred all over the Archean eon (Battistuzzi *et al.*, 2004; Betts *et al.*, 2018). Do current knowledge of MTB evolution and magnetofossils allows to estimate the time of emergence of controlled iron biomineralization among prokaryotes and its role in Earth's evolution?

Although the presence of magnetofossils in the geological record before the end of the Paleoproterozoic needs to be further investigated, controlled iron biomineralization could have evolved before -2.7 Ga. From the angle of molecular phylogeny, this event could have occurred a lot earlier. Indeed, the age of the last common MTB ancestor was estimated between the Paleo- and Mesoarchean era, which corresponds to the timescale of the radiative evolution at the origin of all the major Eubacteria phyla. On such evolutionary timescale, deepest branching relationships of the tree of life are extremely difficult to reconstruct and

669 interpret because of the paucity of fossils and the tremendous uncertainty of phylogenetic
670 reconstructions. However, given the currently known MTB phylogenetic distribution, it seems
671 very unlikely that controlled iron biomineralization emerged before the radiation of the
672 Terrabacteria, Actinobacteria, Cyanobacteria, Firmicutes, and all candidate phyla radiations
673 revealed in the dark microbial matter (Hug *et al.*, 2016). Although no geological data have ever
674 supported these inferences yet, paleontology may represent a major ally by reconstructing
675 the global chemical environment over time scales and help to determine if both biology and
676 environmental conditions were suitable for magnetosome biogenesis.
677 In the Archean eon (-4 to -2.5 Ga), early life undoubtedly thrived in the oceans that were
678 dominantly anoxic and ferruginous [rich in dissolved Fe(II)], with limited zones of surface
679 waters being oxygenated by photosynthetic activity (Lyons *et al.*, 2014). During this period,
680 other strategies than photosynthesis emerged to exploit energy sources in anoxic conditions
681 including methanogenesis and methanotrophy (Rosing, 1999). Anoxic and ferruginous waters
682 of the Archean ocean, with local redox gradients such as those in which modern MTB are
683 observed, could have represented ideal conditions for the development and evolution of MTB.
684 Unlike modern oceans, redox boundary of the Archean ocean was likely located in the water
685 column (not in the sediment), and possibly extended in the photic zone (exposed to sunlight)
686 since O_2 was produced in shallow water by photosynthesis. As mentioned above, MTB can
687 respire oxygen but can also get energy anaerobically by respiring nitrate, nitrous oxide or
688 sulfate. However, it is still unclear whether they can oxidize hydrogen, sulfur or iron, or even
689 reduce Fe(III), metabolism that would be more suitable in Archean environments. This gap is
690 mainly due to the lack of representative deep-branching MTB for which full genomes and
691 cultures are available, especially those from ferruginous environments (Rivas-Lamelo *et al*.,
692 2017).

693 After 2.4 Ga, a progressive surface ocean oxygenation occurred in the early Paleoproterozoic
694 and diffused into the atmosphere, which is a stage known as the great oxidation event (GOE)
695 (Holland, 2002). As a consequence of atmospheric oxygenation, continental sulfides were
696 oxidized into highly soluble sulfates that were massively transferred to the ocean by river
697 systems (Canfield, 1998). From a paleontological perspective, it is widely accepted that
698 environmental conditions strongly influenced the evolution of life on Earth, and life in turn
699 impacted environmental conditions (Anbar and Knoll, 2002). It is likely that dissimilatory
700 sulfate-reducing prokaryotes have progressively reduced sulfates into sulfides in deep anoxic
701 ocean waters that precipitated as Fe sulfides. Such geochemical processes changed ocean
702 chemistry by decreasing the dissolved Fe concentrations. While dominated by iron in the early
703 Precambrian, deep ocean became S-dominated for a period that persisted at least from -1.8
704 Ga to -700 Ma (Canfield, 1998). Complete oxygenation of the oceans similarly to current state
705 occurred only between -700 and -600 Ma. The evolution of ocean chemistry towards more
706 sulfidic conditions after -2.4 to -1.8 Ga (*i.e.* Paleoproterozoic) (Canfield, 1998) could thus have
707 conducted to the development of other magnetotactic Deltaproteobacteria forming greigite
708 rather than magnetite. Although more speculative, this assertion could be tested by looking
709 in detail the magnetofossil record and crossing these data with phylogenetic reconstructions.
710 Overall, available data indicate that redox gradients generated by oxygen diffusion and
711 sustaining modern MTB could have existed since the Archean. This is critical to discuss the
712 evolution of MTB, since the magnetosome chains is thought to be used for navigation toward
713 optimal redox conditions in the water column or sediments. In the case of distinct function(s),
714 one cannot rule out the possibility that MTB magnetosomes evolved for purposes other than
715 magnetotaxis. Thus, first magnetosomes and MTB could have appeared even if no redox
716 gradients were established. Future studies will have to elucidate these points.

Concluding remarks

More than 40 years after the discovery of the first iron-biomineralizing magnetotactic bacterium, technological advances in cell-sorting, genome sequencing, microscopy and chemical analyses keep revealing the magnetosome complexity at the genetic, biological and chemical levels. Magnetosome properties and its preservation in the geological record make this organelle as one of the most promising tool to calibrate early Earth's chemistry and life evolution. We believe that interdisciplinary collaborations between microbiologists, paleobiologists, bioinformaticians and geochemists will foster innovative researches that will answer to major questions in their fields and determine the origin of biomineralization and its contribution to Earth.

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Conflicts of interest

The authors declare no conflict of interest.

References

Abreu F, Leão P, Vargas G, Cypriano J, Figueiredo V, Enrich-Prast A, Bazylinski DA, Lins U (2018) Culture-independent characterization of a novel magnetotactic member affiliated to the

741 Beta class of the Proteobacteria phylum from an acidic lagoon. *Environ. Microbiol.* 20,
742 2615-2624.
743 Abreu F, Carolina A, Araujo V, Leão P, Silva KT, Carvalho FM, Cunha OL, Almeida LG, Geurink
744 C, Farina M, Rodelli D, Jovane L, Pellizari VH, Vasconcelos AT, Bazylinski DA, Lins U (2016)
745 Culture-independent characterization of novel psychrophilic magnetotactic cocci from
746 Antarctic marine sediments. *Environ. Microbiol.* 18, 4426-4441.
747 Abreu F, Cantão ME, Nicolás MF, Barcellos FG, Morillo V, Almeida LGP, do Nascimento FF,
748 Lefèvre CT, Bazylinski DA, de Vasconcelos ATR, Lins U (2011) Common ancestry of iron
749 oxide- and iron-sulfide-based biomineralization in magnetotactic bacteria. *ISME J.* 5, 1634–
750 1640.
751 Abreu F, Martins JL, Silveira TS, Keim CN, de Barros HGPL, Filho FJG, Lins, U (2007) "Candidatus
752 Magnetoglobus multicellularis", a multicellular, magnetotactic prokaryote from a
753 hypersaline environment. *Int. J. Syst. Evol. Microbiol.* 57, 1318-1322.
754 Ahn T, Kim JH, Yang H-M, Lee JW, Kim J-D (2012) Formation pathways of magnetite
755 nanoparticles by coprecipitation method. J. Phys. Chem. C. 116, 6069-6076.
756 Akai J, Iida A, Akai K, Chiba A (1997) Mn and Fe minerals of possible biogenic origin from two
757 Precambrian stromatolites in western Australia. *Jour. Geol. Soc. Japan* 103, 484–488.
758 Amor M, Tharaud M, Gélabert A, Komeili A (2020a) Single-cell determination of iron content
759 in magnetotactic bacteria: implications for the iron biogeochemical cycle. *Environ.*
760 *Microbiol.* 22, 823-831.
761 Amor M, Ceballos A, Wan J, Simon CP, Aron AT, Chang CJ, Hellman F, Komeili A (2020b)
762 Magnetotactic bacteria accumulate a large pool of iron distinct from their magnetite
763 crystals. *Biorxiv*. Doi: 10.1101/2020.03.10.986679.

764 Amor M, Busigny V, Louvat P, Tharaud M, Gélabert A, Cartigny P, Carlut J, Isambert A, Durand-
765 Dubief M, Ona-Nguema G, Alphandéry E, Chebbi I, Guyot F (2018) Iron uptake and
766 magnetite biomineralization in the magnetotactic bacterium *Magnetospirillum*
767 *magneticum* strain AMB-1: an iron isotope study. *Geochim. Cosmochim. Acta*. 232, 225-
768 243.
769 Amor M, Busigny V, Louvat P, Gélabert A, Cartigny P, Durand-Dubief M, Ona-Nguema G,
770 Alphandéry E, Chebbi I, Guyot F (2016) Mass-independent and -independent signature of
771 Fe isotopes in magnetotactic batceria. *Science* 352, 705-708.
772 Amor M, Busigny V, Durand-Dubief M, Tharaud M, Ona-Nguema G, Gélabert A, Alphandéry E,
773 et al. (2015) Chemical Signature of Magnetotactic Bacteria. *Proc. Natl. Acad. Sci. U.S.A*. 6,
774 1699 1703.
775 Anbar AD, Knoll AH (2002) Proterozoic ocean chemistry and evolution: a bioinorganic bridge?
776 *Science* 297, 1137-1142.
777 Andrews SC, Robinson AK, Rodríguez-Quiñones F (2003) Bacterial iron homeostasis. *FEMS*
778 *Microbiol. Rev.* 27, 215-237.
779 Arato B (2005) Crystal-Size and Shape Distributions of Magnetite from Uncultured
780 Magnetotactic Bacteria as a Potential Biomarker. *Am. Mineral.* 90,1233–1240.
781 Arosio P, Elia L, Poli M (2017) Ferritins, cellular iron storage and regulation. *IUBMB Life* 69,
782 414-422.
783 Balkwill D, Maratea D, Blakemore R (1980) Ultrastructure of a Magnetotactic Spirillum. *J.*
784 *Bacteriol*. 141, 1399-1408.
785 Battistuzzi FU, Feijao A, Hedges SB (2004) A genomic timescale of prokaryote evolution:
786 insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BMC*
787 *Evol Biol* 4, 44.

788 Baumgartner J, Menguy N, Perez-Gonzalez T, Morin G, Widdrat M, Faivre D (2016) Elongated
789 magnetite nanoparticle formation from a solid ferrous precursor in a magnetotactic
790 bacterium. *J. R. Soc. Interface*. 13, 20160665.
791 Baumgartner J, Morin G, Menguy N, Perez Gonzalez T, Widdrat M, Cosmidis J, Faivre D (2013)
792 Magnetotactic bacteria form magnetite from a phosphate-rich ferric hydroxide via
793 nanometric (oxyhydr)oxide intermediates. *Proc. Natl. Acad. Sci. U.S.A*. 110, 14883-14888.
794 Bazylinski DA, Williams TJ, Lefèvre CT, Berg RJ, Zhang CL, Bowser SS, Dean AJ, Beveridge TJ
795 (2013a) *Magnetococcus marinus* gen. nov., sp. nov., a marine, magnetotactic bacterium
796 that represents a novel lineage (Magnetococcaceae fam. nov.; Magnetococcales ord. nov.)
797 at the base of the Alphaproteobacteria. *Int. J. Syst. Evol. Microbiol.* 63, 801-808.
798 Bazylinski DA, Williams TJ, Lefèvre CT, Trubitsyn D, Fang J, Beveridge TJ, Moskowitz BM, Ward
799 B, Schübbe S, Dubbels BL, Simpson B (2013b) *Magnetovibrio blakemorei*, gen. nov. sp. nov.,
800 a new magnetotactic bacterium (Alphaproteobacteria: Rhodospirillaceae) isolated from a
801 salt marsh. *Int. J. Syst. Evol. Microbiol.* 65, 1824-833.
802 Bazylinski D, Williams T (2007) Ecophysiology of Magnetotactic Bacteria. In Magnetoreception
803 and Magnetosomes in Bacteria. *Microbiol. Monogr.* Schüler, D. (ed). Springer Berlin /
804 Heidelberg, pp. 37-75.
805 Bazylinski DA, Dean AJ, Williams TJ, Long LK, Middleton SL, Dubbels BL (2004)
806 Chemolithoautotrophy in the marine, magnetotactic bacterial strains MV-1 and MV-2. *Arch.*
807 *Microbiol.* 182, 373-387.
808 Bazylinski DA, Schlezinger DR, Howes BH, Frankel RB, Epstein SS (2000) Occurrence and
809 distribution of diverse populations of magnetic protists in a chemically stratified coastal
810 salt pond. *Chem. Geol.* 169, 319-328.

811 Bazylinski D, Garrattreed A, Frankel R. (1994) Electron-microscopic studies of magnetosomes
812 in magnetotactic bacteria. *Microsc. Res. Tech.* 27, 389-401.
813 Bazylinski DA, Frankel RB, Jannasch HW (1988) Anaerobic magnetite production by a marine,
814 magnetotactic bacterium. *Nature* 334, 518-519.
815 Bellini S (2009a) On a unique behavior of freshwater bacteria. *Chin. J. Oceanol. Limnol.* 27, 3-
816 5.
817 Bellini S (2009b) Further studies on ‘‘magnetosensitive bacteria’’. *Chin. J. Oceanol. Limnol.* 27,
818 6-12.
819 Benzerara K, Menguy N (2009) Looking for Traces of Life in Minerals. *Comptes Rendus Palevol.*
820 7, 617-628.
821 Bergsten J (2005) A review of long-branch attraction. *Cladistics* 21, 163–193.
822 Betts HC, Puttick MN, Clark JW, Williams TA, Donoghue PCJ, Pisani D (2018) Integrated
823 genomic and fossil evidence illuminates life’s early evolution and eukaryote origin. *Nat.*
824 *Ecol. Evol.* 2, 1556–1562.
825 Berny C, Le Fèvre R, Guyot F, Blondeau K, Guizonne C, Rousseau E, Bayan N, Alphandéry E
826 (2020) A method for producing highly pure magnetosomes in large quantity for medical
827 applications using *Magnetospirillum gryphiswaldense* MSR-1 magnetotactic bacteria
828 amplified in minimal growth media. *Front. Bioeng. Biotechnol*. 8, 16.
829 Blakemore RP (1982) Magnetotactic bacteria. *Annu. Rev. Microbiol.* 36, 217-238.
830 Blakemore RP, Frankel RB, Kalmijn AJ (1980) South-seeking magnetotactic bacteria in the
831 Southern Hemisphere. *Nature* 286, 384-385.
832 Blakemore RP (1975) Magnetotactic bacteria. *Science* 190, 377-379.
833 Brearley AJ (2003) Magnetite in ALH 84001: an origin by shock-induced thermal
834 decomposition of iron carbonate. *Meteorit. Planet. Sci.* 38, 849–870.

Byrne JM, Klueglein N, Pearce C, Rosso KM, Appel E, Kappler A (2015) Redox cycling of Fe(II) and Fe(III) in magnetite by Fe-metabolizing bacteria. *Science* 347, 1473-1476.

Calugay RJ, Takeyama H, Mukoyama D, Fukuda Y, Suzuki T, Kanoh K, Matsunaga T (2006) Catechol siderophores excretion by magnetotactic bacterium *Magnetospirillum magneticum* AMB-1. J. Biosci. Bioeng. 101, 445-447.

Calugay RJ, Miyashita H, Okamura Y, Matsunaga T (2003) Siderophore production by the magnetotactic bacterium Magnetospirillum magneticum AMB-1. *FEMS Microbiol. Lett*. 218, 371-375.

Canfield DE (2005) The early history of atmospheric oxygen: Homage to Robert M. Garrels. *Annu. Rev. Earth Planet. Sci.* 33, 1–36.

Canfield DE (1998) A new model for Proterozoic ocean chemistry. *Nature* 396, 450-453.

Canfield DE, Berner RA (1987) Dissolution and pyritization of magnetite in anoxic marine sediments. *Geochim. Cosmochim. Acta* 51, 645–659.

Chang L, Heslop D, Roberts AP, Rey D, Mohamed KJ (2016) Discrimination of biogenic and detrital magnetite through a double Verwey transition temperature. *J. Geophys. Res. Solid Earth* 121, 3-14.

Chang L, Winklhofer M, Roberts AP, Heslop D, Florindo F, Dekkers MJ, Krijgsman W, Kodama K, Yamamoto Y (2013) Low-temperature magnetic properties of pelagic carbonates: oxidation of biogenic magnetite and identification of magnetosome chains. *J. Geophys. Res.* 118, 6049–6065.

Chang SBR, Stolz JF, Kirschvink JL, Awramik SM (1989) Biogenic magnetite in stromatolites. 2. Occurrence In ancient sedimentary environments. *Precambrian Res.* 43, 305-315.

Chen AP, Berounsky VM, Chan MK, Blackford MG, Cady C, Moskowitz BM, Kraal P, Lima EA, Kopp RE, Lumpkin GR, Weiss BP, Hesse P, Vella NGF (2014) Magnetic properties of

uncultivated magnetotactic bacteria and their contribution to a stratified estuary iron cycle. *Nat. Commun.* 5, 4797.

Chen C, Ma Q, Jiang W, Song T (2011) Phototaxis in the magnetotactic bacterium Magnetospirillum magneticum strain AMB-1 is independent of magnetic fields. *Appl. Microbiol. Biotechnol.* 90, 269-275.

Cox BL, Popa R, Bazylinski DA, Lanoil B, Douglas S, Belz A, Engler DL, Nealson KH (2002) Organization and elemental analysis of P-, S-, and Fe-rich inclusions in a population of freshwater magnetococci. *Geomicrobiol. J.* 19, 387-406.

Crowe SA, Paris G, Katsev S, Jones C, Kim S-T, Zerkle AL, Nomosatryo S, Fowle DA, Adkins JF, Sessions AL, Farquhar J, Canfield DE (2014) Sulfate was a trace constituent of Archean seawater. *Science* 346, 735-739.

Day R, Fuller M, Schmidt VA (1977) Hysteresis properties of titanomagnetites: grain-size and compositional dependence . *Phys. Earth Planet. Inter*. 4, 260-267.

Descamps ECT, Monteil CL, Menguy N, Ginet N, Pignol D, Bazylinski DA, Lefèvre CT (2017) Desulfamplus magnetovallimortis gen. nov., sp. nov., a magnetotactic bacterium from a brackish desert spring able to biomineralize greigite and magnetite, that represents a novel lineage in the Desulfobacteraceae. *Syst. Appl. Microbiol.* 40, 280-289.

Devouard B, Pósfai M, Hua X, Bazylinski DA, Frankel RB, Buseck PR (1998) Magnetite from Magnetotactic Bacteria; Size Distributions and Twinning. *Am. Mineral.* 83, 1387–98.

Dong Y, Li J, Zhang W, Zhang W, Zhao Y, Xiao T, Wu F, Pan H (2016) The detection of magnetotactic bacteria in deep sea sediments from the east Pacific Manganese Nodule Province. *Environ. Microbiol. Rep.* 8, 239–249.

881 Duchêne S, Holt KE, Weill F-X, Le Hello S, Hawkey J, Edwards DJ, Fourment M, Holmes EC
882 (2016) Genome-scale rates of evolutionary change in bacteria. *Microb. Genomics* 2,
883 e000094.
884 Dufour SC, Laurich JR, Batstone RT, McCuaig B, Elliott A, Poduska KM (2014) Magnetosome-
885 containing bacteria living as symbionts of bivalves. *ISME J.* 8, 2453-2462.
886 Egli R (2004) Characterization of Individual Rock Magnetic Components by Analysis of
887 Remanence Curves. 3. Bacterial Magnetite and Natural Processes in Lakes ». *Phys. Chem.*
888 *Earth,* Parts A/B/C 29, 13-14, 869 84.
889 Emiroglu S, Rey D, Petersen N (2004) Magnetic properties of sediment in the Ria de Arousa
890 (Spain): dissolution of iron oxides and formation of iron sulphides. *Phys. Chem. Earth* 29,
891 947–959.
892 Faivre D, Böttger LH, Matzanke BF, Schüler D (2007) Intracellular magnetite biomineralization
893 in bacteria proceeds by a distinct pathway involving membrane-bound ferritin and iron(II)
894 species. *Angew. Chem. Int. Ed.* 46, 8495-8499.
895 Fdez-Gubieda ML, Muela A, Alonso J, García-Prieto A, Olivi L, Fernández-Pacheco, Barandiarán
896 JM (2013) Magnetite biomineralization in *Magnetospirillum gryphiswaldense*: time-
897 resolved magnetic magnetic and structural studies. *ACS Nano.* 7, 3297-3305.
898 Flies CB, Jonkers HM, de Beer D, Bosselmann K, Bottcher ME, Schüler D. (2005) Diversity and
899 vertical distribution of magnetotactic bacteria along chemical gradients in freshwater
900 microcosms. *FEMS Microbiol. Ecol.* 52, 185-195.
901 Frankel RB, Bazylinski DA (2006) How magnetotactic bacteria make magnetosomes queue up.
902 *Trends Microbiol.* 14, 329-331.
903 Frankel RB, Bazylinski DA, Johnson MS, Taylor BL (1997) Magneto-aerotaxis in marine coccoid
904 bacteria. *Biophys. J.* 73, 994-1000.

905 Frankel RB (1984) Magnetic guidance of organisms. *Annu. Rev. Biophys. Bioeng.* 13, 85-103.
906 Frankel RB, Papaefthymiou GC, Blakemore RP, O'Brien W (1983) Fe_3O_4 precipitation in
907 magnetotactic bacteria. *Biochim. Biophys. Acta.* 763, 147-159.
908 Frankel RB, Blakemore RP, Torres De Araujo FF, Esquivel DMS, Danon J (1981) Magnetotactic
909 bacteria at the geomagnetic equator. *Science* 212, 1269-1270.
910 Freitas F, Keim CN, Kachar B, Farina M, Lins U (2003) Envelope ultrastructure of uncultured
911 naturally occurring magnetotactic cocci. *FEMS Microbiol. Lett.* 219, 33-38.
912 Frierdich AJ, Beard BL, Scherer MM, Johnson CM (2014) Determination of the $Fe(II)_{(aq)}$-
913 magnetite equilibrium iron isotope fractionation factor using the three-isotope method
914 and a multi-direction approach to equilibrium. *Earth Planet. Sci. Lett.* 391, 77-86.
915 Froelich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D, Dauphin P,
916 Hammond D, Hartman B, Maynard V (1979) Early oxidation of organic matter in pelagic
917 sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim. Cosmochim.*
918 *Acta* 43, 1075–1090.
919 Geelhoed JS, Kleerebezem R, Sorokin DY, Stams AJM, van Loosdrecht MCM (2010) Reduced
920 inorganic sulfur oxidation supports autotrophic and mixotrophic growth of
921 *Magnetospirillum* strain J10 and *Magnetospirillum gryphiswaldense. Environ. Microbiol.* 12,
922 1031-1040.
923 Gehring AU, Kind J, Charilaou M, García-Rubio I (2013) S-Band Ferromagnetic Resonance
924 Spectroscopy and the Detection of Magnetofossils. *J. R. Soc. Interface* 10, 20120790. Doi:
925 10.1098/rsif.2012.0790.
926 Graur D, Martin W (2004) Reading the entrails of chickens: molecular timescales of evolution
927 and the illusion of precision. *Trends Genet. TIG* 20, 80–86.

928 Guo FF, Yang W, Jiang W, Geng S, Peng T, Li JL (2012) Magnetosomes eliminate intracellular
929 reactive oxygen species in *Magnetospirillum gryphiswaldense* MSR-1. *Environ. Microbiol.*
930 14, 1722–1729.
931 Guyodo Y, Valet J-P (1999) Global changes in intensity of the Earth's magnetic field during the
932 past 800 kyr. *Nature* 399, 249-252.
933 Heslop D, Dillon M (2007) Unmixing Magnetic Remanence Curves without a Priori Knowledge.
934 *Geophys. J. Int*. 2, 556-566.
935 Heslop D, Roberts AP, Chang L (2014) Characterizing Magnetofossils from First-Order Reversal
936 Curve (FORC) Central Ridge Signatures. *Geochem. Geophys. Geosyst*. 6, 2170-2179.
937 Hesse PP (1994) Evidence for bacterial palaeoecological origin of magnetic mineral cycles in
938 oxic and sub-oxic Tasman Sea sediments. *Mar. Geol.* 117, 1–17.
939 Heywood BR, Bazylinski DA, Garrattreed A, Mann S, Frankel RB (1990) Controlled biosynthesis
940 of greigite (Fe_3S_4) in magnetotactic bacteria. *Naturwissenschaften* 77, 536-538.
941 Holland HD (2002) Volcanic gases, black smokers, and the Great Oxidation Event. *Geochim.*
942 *Cosmochim. Acta* 66, 3811-3826.
943 Hug LA, Baker BJ, Anantharaman K, Brown CT, Probst AJ, Castelle CJ, Butterfield CN, Hernsdorf
944 AW, Amano Y, Ise K, Suzuki Y, Dudek N, Relman DA, Finstad KM, Amundson R, Thomas BC,
945 Banfield JF (2016) A new view of the tree of life. *Nat. Microbiol.* 1, 16048.
946 Imlay JA (2003) Pathways of oxidative damage. *Annu. Rev. Microbiol.* 57, 395-418.
947 Isambert A, Menguy N, Larquet E, Guyot F, Valet J-P (2007) Transmission electron microscopy
948 study of magnetites in a freshwater population of magnetotactic bacteria. *Am. Mineral.*
949 92, 621–630.
950 Jimenez-Lopez C, Rodriguez-Navarro C, Rodriguez-Navarro A, Perez-Gonzalez T, Bazylinski DA,
951 Lauer Jr HV, Romanek CS (2012) Signatures in magnetites formed by (Ca,Mg,Fe)CO3

thermal decomposition: Terrestrial and extraterrestrial implications. *Geochim. Cosmochim. Acta* 87, 69-80.

Jimenez-Lopez C, Romanek CS, Bazylinski DA (2010) Magnetite as a Prokaryotic Biomarker: A Review. *J. Geophys. Res. – Biogeo.* 115, G2

Jogler C, Wanner G, Kolinko S, Niebler M, Amann R, Petersen N, Kube M, Reinhardt R, Schüler D (2011) Conservation of proteobacterial magnetosome genes and structures in an uncultivated member of the deep-branching Nitrospira phylum. *Proc. Natl. Acad. Sci. U.S.A* 108, 1134-1139.

Jogler C, Niebler M, Lin W, Kube M, Wanner G, Kolinko S, Stief P, Beck AJ, De Beer D, Petersen N, Pan Y, Amann R, Reinhardt R, Schüler D (2010) Cultivation-independent characterization of "Candidatus Magnetobacterium bavaricum" via ultrastructural, geochemical, ecological and metagenomic methods. *Environ. Microbiol.* 12, 2466-2478.

Jones SR, Wilson TD, Brown ME, Rahn-Lee L, Yu Y., Fredriksen LL, Ozyamak E, Komeili A, Chang MCY (2015) Genetic and biochemical investigations of the role of MamP in redox control of iron biomineralization in *Magnetospirillum magneticum. Proc. Natl. Acad. Sci.* U.S.A. 112, 3904-3909.

Keim CN, Martins JL, de Barros HL, Lins U, Farina M (2007) Structure, behavior, ecology and diversity of multicellular magnetotactic prokaryotes. In Magnetoreception and Magnetosomes in Bacteria. *Microbiol. Monogr.* Schüler, D. (ed). Springer Berlin Heidelberg, pp. 103–132.

Keim CN, Lins U, Farina (2009) Manganese in Biogenic Magnetite Crystals from Magnetotactic Bacteria. *FEMS Microbiol. Let*. 2, 250-253

Klumpp S, Lefèvre CT, Bennet M, Faivre D (2019) Swimming with magnets: from biological organisms to synthetic devices. *Phys. Rep.* 789, 1-54.

976 Kolinko S, Richter M, Glöckner F-O, Brachmann A, Schüler D (2016) Single-cell genomics of
977 uncultivated deep-branching magnetotactic bacteria reveals a conserved set of
978 magnetosome genes. *Environ. Microbiol.* 18, 21–37.
979 Kolinko S, Jogler C, Katzmann E, Wanner G, Peplies J, Schüler D (2012) Single-cell analysis
980 reveals a novel uncultivated magnetotactic bacterium within the candidate division OP3.
981 *Environ. Microbiol.* 14, 1709-1721.
982 Komeili A (2012) Molecular mechanisms of compartmentalization and biomineralization in
983 magnetotactic bacteria. *FEMS Microbiol. Rev*. 36, 232-255.
984 Komeili A, Li Z, Newmann DK, Jensen GJ (2006) Magnetosomes are cell membrane
985 invaginations organized by the actin-like protein MamK. *Science* 311, 242-245.
986 Konhauser K, Planavsky NJ, Hardistyc DS, Robbinsa LJ, Warcholaa TJ, Haugaarda R, Lalonde SV,
987 Partin CA, Oonkg PBH, Tsikos H, Lyons TW, Bekker A, Johnson CM (2017) Iron formations:
988 A global record of Neoarchaean to Palaeoproterozoic environmental history. *Earth-Sci. Rev.*
989 172, 140-177.
990 Konhauser KO (1998) Diversity of Bacterial Iron Mineralization. *Earth-Sci. Rev.* 43, 91–121.
991 Kopp RE, Schumann D, Raub TD, Powars DS, Godfrey LV, Swanson-Hysell NL, Maloof AC, Vali
992 H (2009) An Appalachian Amazon? Magnetofossil evidence for the development of a
993 tropical river-like system in the mid-Atlantic United States during the Paleocene-Eocene
994 maximum. *Paleoceanograph.* 24, PA4211.
995 Kopp RE, Kirschvink JL (2008) The Identification and Biogeochemical Interpretation of Fossil
996 Magnetotactic Bacteria. *Earth-Sci. Rev.* 86, 42–61.
997 Kopp RE, Raub TD, Schumann D, Vali H, Smirnov AV, Kirschvink JL (2007) Magnetofossil spike
998 during the Paleocene-Eocene thermal maximum: ferromagnetic resonance, rock magnetic,

999 and electron microscopy evidence from Ancora, New Jersey, United States.
1000 *Paleoceanography* 22, PA4103.
1001 Kopp RE, Weiss BP, Maloof AC, Vali H, Nash CZ, Kirschvink JL (2006) Chains, clumps, and
1002 strings: magnetofossil taphonomy with ferromagnetic resonance spectroscopy. *Earth*
1003 *Planet. Sci. Lett.* 247, 10-25.
1004 Kopp RE, Nash CZ, Kirschvink, JL, and Leadbetter JR (2004) A Possible Magnetite/Maghemite
1005 Electrochemical Battery in the Magnetotactic Bacteria. *AGUFM* 2004, GP34A–06–06.
1006 Lapen T J, Righter M, Brandon AD, Debaille V, Beard BL, Shafer JT, Peslier AH (2010) A younger
1007 age for ALH84001 and its geochemical link to shergottite sources in Mars. *Science* 328, 347-
1008 351.
1009 Larrasoaña JC, Liu Q, Hu P, Roberts AP, Mata P, Civis J, Sierro FJ, Pérez-Asensio JN (2014)
1010 Paleomagnetic and paleoenvironmental implications of magnetofossil occurences in late
1011 Miocene marine sediments from the Guadalquivir basin, SW Spain. *Front. Microbiol.* 5, 71.
1012 Lascu I, Harrison RJ, Li Y,. Muraszko JR, Channell JET, Piotrowski AM, Hodell DA (2015)
1013 Magnetic Unmixing of First-Order Reversal Curve Diagrams Using Principal Component
1014 Analysis: FORC UNMIXING USING PCA. *Geochem. Geophys. Geosyst.* 9, 2900-2915.
1015 Leão P, Teixeira LCRS, Cypriano J, Farina M, Abreu F, Bazylinski DA, Lins U (2016) North-Seeking
1016 Magnetotactic Gammaproteobacteria in the Southern Hemisphere. *Appl. Environ.*
1017 *Microbiol.* 82, 5595-5602.
1018 Lean CMB, McCave IN (1998) Glacial to interglacial mineral magnetic and palaeoceanographic
1019 changes at Chatham Rise, SW Pacific Ocean. *Earth Planet. Sci. Lett.* 163, 247–260.
1020 Lefèvre CT, Howse PA, Schmidt ML, Sabaty M, Menguy N, Luther GW, Bazylinski, DA (2016)
1021 Growth of magnetotactic sulfate-reducing bacteria in oxygen concentration gradient
1022 medium. *Environ. Microbiol. Rep.* 8, 1003-1015.

1023 Lefèvre CT, Bennet M, Landau L, Vach P, Pignol D, Bazylinski DA, Frankel RB, Klumpp S, Faivre
1024 D (2014) Diversity of magneto-aerotactic behaviors and oxygen sensing mechanisms in
1025 cultured magnetotactic bacteria. *Biophys. J.* 107, 527-538.
1026 Lefèvre CT, Wu L-F (2013) Evolution of the bacterial organelle responsible for magnetotaxis.
1027 *Trends Microbiol.* 21, 534,543.
1028 Lefèvre CT, Trubitsyn D, Abreu F, Kolinko S, Paula de Almeida LG, de Vasconcelos ATR, Lins U,
1029 Schüler D, Ginet N, Pignol D, Bazylinski DA (2013) Monophyletic origin of magnetotaxis and
1030 the first magnetosomes. *Environ. Microbiol.* 15, 2267-2274.
1031 Lefèvre CT, Bazylinski DA (2013) Ecology, diversity, and evolution of magnetotactic bacteria.
1032 *Microbiol. Mol. Biol. Rev.* 77, 497-526.
1033 Lefèvre CT, Viloria N, Schmidt ML, Pósfai M, Frankel RB, Bazylinski DA (2012) Novel magnetite-
1034 producing magnetotactic bacteria belonging to the Gammaproteobacteria. *ISME J.* 6, 440-
1035 450.
1036 Lefèvre CT, Pósfai M, Abreu F, Lins U, Frankel RB, Bazylinski DA (2011a) Morphological features
1037 of elongated-anisotropic magnetosome crystals in magnetotactic bacteria of the
1038 Nitrospirae phylum and the Deltaproteobacteria class. *Earth Planet. Sci. Lett.* 312, 194-200.
1039 Lefèvre CT, Frankel RB, Abreu F, Lins U, Bazylinski DA (2011b) Culture-independent
1040 characterization of a novel, uncultivated magnetotactic member of the Nitrospirae phylum.
1041 *Environ. Microbiol.* 13, 538-549.
1042 Lefèvre CT, Menguy N, Abreu F, Lins U, Pósfai M, Prozorov T, Pignol D, Frankel RB, Bazylinski
1043 DA (2011c) A cultured greigite-producing magnetotactic bacterium in a novel group of
1044 sulfate-reducing bacteria. *Science* 334, 1720-1723.

1045 Lefèvre CT, Frankel RB, Pósfai M, Prozorov T, Bazylinski DA (2011d) Isolation of obligately
1046 alkaliphilic magnetotactic bacteria from extremely alkaline environments. *Environ.*
1047 *Microbiol.* 13, 2342-2350.
1048 Lefèvre CT, Abreu F, Schmidt ML, Lins U, Frankel RB, Hedlund BP, Bazylinski DA (2010)
1049 Moderately thermophilic magnetotactic bacteria from hot springs in Nevada. *Appl. Environ.*
1050 *Microbiol.* 76, 3740-3743.
1051 Lefèvre CT, Bernadac A, Yu-Zhang K, Pradel N, Wu L-F (2009) Isolation and characterization of
1052 a magnetotactic bacterial culture from the Mediterranean Sea. *Environ. Microbiol.* 11,
1053 1646-1657.
1054 Le Nagard L, Zhu X, Yuan H, Benzerara K, Bazylinski DA, Fradin C, Besson A, Swaraj S, Stanescu
1055 S, Belkhou R, Hitchcock AP (2019) Magnetite magnetosome biomineralization in
1056 *Magnetospirillum magneticum* strain AMB-1: a time-course study. *Chem. Geol.* 530,
1057 119348.
1058 Li K, Chen C, Chen C, Wang Y, Wei Z, Pan W, Song T (2015) Magnetosomes extracted from
1059 Magnetospirillum magneticum strain AMB-1 showed enhanced peroxidase-like activity
1060 under visible-light irradiation. *Enzyme Microb. Technol.* 72, 72-78.
1061 Li J, Benzerara K, Bernard S, Beyssac O (2013) The Link between Biomineralization and
1062 Fossilization of Bacteria: Insights from Field and Experimental Studies. *Chem. Geol.* 359,
1063 49–69.
1064 Li J, Wu W, Liu Q, Pan Y (2012) Magnetic anisotropy, magnetostatic interactions and
1065 identification of magnetofossils. *Geochem. Geophys. Geosyst.* 13, Q10Z51.
1066 Li J, Pan Y, Chen G, Liu Q, Tian L, Lin W (2009) Magnetite magnetosome and fragmental chain
1067 formation of *Magnetospirillum magneticum* AMB-1: transmission electron microscopy and
1068 magnetic observations. *Geophys. J. Int.* 177, 33-42.

1069 Li J, Menguy N, Gatel C, Boureau V, Snoeck E, Patriarche G, Leroy E, Pan Y (2015) Crystal
1070 Growth of Bullet-Shaped Magnetite in Magnetotactic Bacteria of the Nitrospirae Phylum ».
1071 *J. R. Soc. Interface* 103, 20141288.
1072 Li YL, Konhauser KO, Zhai M (2017) The Formation of Magnetite in the Early Archean Oceans.
1073 *Earth Planet. Sci. Lett.* 466, 103-114.
1074 Lin W, Zhang W, Zhao X, Roberts AP, Paterson GA, Bazylinski DA, Pan Y. (2018) Genomic
1075 expansion of magnetotactic bacteria reveals an early common origin of magnetotaxis with
1076 lineage-specific evolution. *ISME J.* 12, 1508–1519.
1077 Lin W, Paterson GA, Zhu Q, Wang Y, Kopylova E, Li Y, Knight R, Bazylinski DA, Zhu R, Kirschvink
1078 JL, Pan Y (2017a) Origin of microbial biomineralization and magnetotaxis during the
1079 Archean. *Proc. Natl. Acad. Sci. U.S.A.* 114, 2171-2176.
1080 Lin W, Pan Y, Bazylinski DA (2017b) Diversity and ecology of and biomineralization by
1081 magnetotactic bacteria. *Environ. Microbiol. Rep.* 9, 345-356.
1082 Lin W, Bazylinski DA, Xiao T, Wu L-F, Pan Y (2014a) Life with compass: diversity and
1083 biogeography of magnetotactic bacteria. *Environ. Microbiol.* 16, 2646-2658.
1084 Lin W, Deng A, Wang Z, Li Y, Wen T, Wu L-F, Wu M, Pan Y (2014b) Genomic insights into the
1085 uncultured genus "*Candidatus* Magnetobacterium" in the phylum Nitrospirae. *ISME J*. 8,
1086 2463-2477.
1087 Lin W, Wang Y, Gorby Y, Nealson K, Pan Y (2013) Integrating niche-based process and spatial
1088 process in biogeography of magnetotactic bacteria. *Sci. Rep.* 3, 1643.
1089 Lin W, Wang Y, Li B, Pan Y (2012) A biogeographic distribution of magnetotactic bacteria
1090 influenced by salinity. *ISME J.* 6, 475-479.
1091 Lovley DR, Stolz JF, Nord GL, Phillips EJP (1987) Anaerobic Production of Magnetite by a
1092 Dissimilatory Iron-Reducing Microorganism. *Nature* 330, 252–54. Doi: 10.1038/330252a0.

1093 Lyons TW, Reinhard CT, Planavsky NJ (2014) The rise of oxygen in Earth's early ocean and
1094 atmosphere. *Nature* 506, 307-315.
1095 Mandernack KW (1999) Oxygen and Iron Isotope Studies of Magnetite Produced by
1096 Magnetotactic Bacteria. *Science* 5435, 1892-1896.
1097 Mann S, Sparks NHC, Frankel RB, Bazylinski DA, Jannasch HW (1990) Biomineralization of
1098 ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium. *Nature*
1099 343, 258-261.
1100 Mann S, Sparks NHC, Blakemore RP (1987a) Structure, morphology and crystal-growth of
1101 anisotropic magnetite crystals in magnetotactic bacteria. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*
1102 231, 477-487.
1103 Mann S, Sparks NHC, Blakemore RP (1987b) Ultrastructure and characterization of anisotropic
1104 magnetic inclusions. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 231, 469-476.
1105 Mann S, Frankel RB, Blakemore RP (1984a) Structure, morphology and crystal-growth of
1106 bacterial magnetite. *Nature* 310, 405-407.
1107 Mann S, Moench T, Williams RJP (1984b) A high resolution electron microscopic investigation
1108 of bacterial magnetite. Implications for crystal growth. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*
1109 221, 385-393.
1110 Martins JL, Silveira TS, Abreu F, de Almeida FP, Rosado AS, Lins U (2012) Spatiotemporal
1111 distribution of the magnetotactic multicellular prokaryote *Candidatus* Magnetoglobus
1112 multicellularis in a Brazilian hypersaline lagoon and in microcosms. *Int. Microbiol.* 15, 141-
1113 149.
1114 Martins JL, Silveira TS, Silva KT, Lins U (2009) Salinity dependence of the distribution of
1115 multicellular magnetotactic prokaryotes in a hypersaline lagoon. *Int. Microbiol. Off. J. Span.*
1116 *Soc. Microbiol.* 12, 193-201.

McCartney MR, Lins U, Farina M, Buseck PR, Frankel RB (2001) Magnetic Microstructure of Bacterial Magnetite by Electron Holography ». *Eur. J. Mineral*. 4, 685-689.

McCausland HC, Komeili A (2020) Magnetic genes: studying the genetics of biomineralization in magnetotactic bacteria". *PLoS Genet.* 16, e1008499.

McNeill DF, Ginsburg RN, Chang SBR, Kirschvink JL (1988) Magnetostratigraphic dating Of Shallow-Water Carbonates From San-Salvador, Bahamas. *Geology* 16, 8-12.

Millero FJ, Yao W, Aicher J (1995) The speciation of Fe(II) and Fe(III) in natural waters. *Mar. Chem.* 50, 21-39.

Miot J, Li J, Benzerara K, Tahar Sougrati M, Ona-Nguema G, Bernard S, Jumas JC, Guyot F (2014) Formation of Single Domain Magnetite by Green Rust Oxidation Promoted by Microbial Anaerobic Nitrate-Dependent Iron Oxidation ». *Geoch. Cosmoch. Acta* 139, 327-343.

Moench T (1988) Bilophococcus-Magnetotacticus Gen-Nov-Sp-Nov, a Motile, Magnetic Coccus. *Antonie Van Leeuwenhoek J. Microbiol.* 54, 483-496.

Mohamed KJ, Rey D, Rubio B, Dekkers MJ, Roberts AP, Vilas F (2011) Onshore–offshore gradient in reductive early diagenesis in coastal marine sediments of the Ria de Vigo, Northwest Iberian Peninsula. *Cont. Shelf Res.* 31, 433–447.

Monteil CL, Vallenet D, Menguy N, Benzerara K, Barbe V, Fouteau S, et al. (2019) Ectosymbiotic bacteria at the origin of magnetoreception in a marine protist. *Nat Microbiol* 4, 1088-1095.

Monteil CL, Perrière G, Menguy N, Ginet N, Alonso B, Waisbord N, Cruvellier S, Pignol D, Lefèvre CT (2018) Genomic study of a novel magnetotactic Alphaproteobacteria uncovers the multiple ancestry of magnetotaxis. *Environ. Microbiol.* 20, 4415–4430.

Monteil CL, Menguy N, Prévéral S, Warren A, Pignol D, Lefèvre CT (2018) Accumulation and dissolution of magnetite crystals in a magnetically responsive ciliate. *Appl. Environ. Microbiol.* 84, e02865-17.

1141 Montgomery P, Hailwood EA, Gale AS, Burnett JA (1998) The magnetostratigraphy of
1142 Coniacian late Campanian chalk sequences in southern England. *Earth Planet. Sci. Lett.*
1143 156, 209-224.
1144 Morillo V, Abreu F, Araujo AC, de Almeida LGP, Prast AE, Farina M, de Vasconcelos ANTR,
1145 Bazylinski DA, Lins U (2014) Isolation, cultivation and genomic analysis of magnetosome
1146 biomineralization genes of a new genus of South-seeking magnetotactic cocci within the
1147 Alphaproteobacteria. *Front. Microbiol.* 5, 72.
1148 Moskowitz BM, Bazylinski DA, Egli R, Frankel RB, Edwards KJ (2008) Magnetic properties of
1149 marine magnetotactic bacteria in a seasonally stratified coastal pond (Salt Pond, MA, USA).
1150 *Geophys. J. Int.* 174, 75-92.
1151 Muxworthy AR, Williams W (2008) Critical Superparamagnetic/Single-Domain Grain Sizes in
1152 Interacting Magnetite Particles: Implications for Magnetosome Crystals. *J. R. Soc. Interface*
1153 6, 1207–12.
1154 Neilands JB (1995) Siderophores: structure and function of microbial iron transport
1155 compounds. *J. Biol. Chem.* 270, 26723-26726.
1156 Paoletti LC, Blakemore RP (1986) Hydroxamate production by Aquaspirillum magnetotacticum.
1157 *J. Bacteriol.* 167, 73-76.
1158 Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, Hugenholtz P
1159 (2018) A standardized bacterial taxonomy based on genome phylogeny substantially
1160 revises the tree of life. *Nat. Biotechnol*. 36, 996-1004.
1161 Petermann H, Bleil U (1993) Detection of live magnetotactic bacteria in south-atlantic deep-
1162 sea sediments. *Earth Planet. Sci. Lett.* 117, 223-228.
1163 Pósfai M, Lefèvre CT, Trubitsyn D, Bazylinski DA, Frankel RB (2013) Phylogenetic significance
1164 of composition and crystal morphology of magnetosome minerals. *Front. Microbiol.* 4, 344.

1165 Pósfai M, Buseck PR, Bazylinski DA, Frankel RB (1998a) Iron sulfides from magnetotactic
1166 bacteria: Structure, composition, and phase transitions. *Am. Mineral.* 83, 1469-1481.
1167 Pósfai M, Buseck PR, Bazylinski DA, Frankel RB (1998b) Reaction sequence of iron sulfide
1168 minerals in bacteria and their use as biomarkers. *Science* 280, 880-883.
1169 Qian X-X, Liu J, Menguy N, Li J, Alberto F, Teng Z, Xiao T, Zhang W, Wu L-F (2019) Identification
1170 of novel species of marine magnetotactic bacteria affiliated with Nitrospirae phylum.
1171 *Environ. Microbiol. Rep.* 11, 330-337.
1172 Rivas-Lamelo S, Benzerara K, Lefèvre CT, Jézéquel D, Menguy N, Viollier E, Guyot F, Férard C,
1173 Poinsot M, Skouri-Panet F, Trcera N, Miot J, Duprat E (2017) Magnetotactic bacteria as a
1174 new model for P sequestration in the ferruginous Lake Pavin. *Geochem. Perspect. Lett.* 5,
1175 35-41.
1176 Roberts AP (2015) Magnetic mineral diagenesis. *Earth-Sci. Rev.* 151, 1-47.
1177 Roberts AP, Florindo F, Villa G, Chang L, Jovane L, Bohaty SM, Larrasoaña JC, Heslop D, Fitz
1178 Gerald JD (2011) Magnetotactic bacterial abundance in pelagic marine environments is
1179 limited by organic carbon flux and availability of dissolved iron. *Earth Planet. Sci. Lett.* 310,
1180 441–452.
1181 Roberts AP, Florindo F, Chang L, Heslop D, Jovane L, Larrasoaña JC (2013) Magnetic Properties
1182 of Pelagic Marine Carbonates. *Earth-Sci. Rev*. 127, 111-139.
1183 Roberts AP, Hu P, Harrison RJ, Heslop D, Muxworthy AR, Oda H, Sato T, Tauxe L, Zhao X (2019)
1184 Domain State Diagnosis in Rock Magnetism: Evaluation of Potential Alternatives to the Day
1185 Diagram ». *J. Geophys. Res.: Solid Earth* 6, 5286-5314.
1186 Rong C, Zhang C, Zhang Y, Qi L, Yang J, Guan G, Li Y, Li J (2012) FeoB2 functions in magnetosome
1187 formation and oxidative stress protection in *Magnetospirillum gryphiswaldense* strain
1188 MSR-1. *J. Bacteriol.* 194, 3972-3976.

1189 Rong C, Huang Y, Zhang W, Jiang W, Li Y, Li J (2008) Ferrous iron transport protein B gene
1190 (*feoB1*) plays an accessory role in magnetosome formation in *Magnetospirillum*
1191 *gryphiswaldense* strain MSR-1. *Res. Microbiol.* 159, 530-536.
1192 Raschdorf O, Bonn F, Zeytuni N, Zarivach R, Becher D, Schüler D (2018) A quantitative
1193 assessment of the membrane-integral sub-proteome of a bacterial magnetic organelle. *J.*
1194 *Proteomics.* 172, 89-99.
1195 Ridgwell A, Zeebe RE (2005) The role of the global carbonate cycle in the regulation and
1196 evolution of the Earth system. *Earth Planet. Sci. Lett.* 234, 299-315.
1197 Rosing MT (1999) ^{13}C-Depleted carbon microparticles in >3700-Ma sea-floor sedimentary
1198 rocks from west greenland. *Science* 283, 674–676.
1199 Sakaguchi T, Arakaki A, Matsunaga T (2002) *Desulfovibrio magneticus* sp nov., a novel sulfate-
1200 reducing bacterium that produces intracellular single-domain-sized magnetite particles. *Int.*
1201 *J. Syst. Evol. Microbiol.* 52, 215-221.
1202 Savian JF, Jovane L, Giorgioni M, Iacoviello F, Rodelli D, Roberts AP, Chang L, Florindo F,
1203 Sprovieri M. (2016) Environmental magnetic implications of magnetofossil occurrence
1204 during the Middle Eocene Climatic Optimum (MECO) in pelagic sediments from the
1205 equatorial Indian Ocean. *Palaeogeog. Palaeoclimatol. Palaeoecol.* 441, 212–222.
1206 Schleifer KH, Schüler D, Spring S, Weizenegger M, Amann R, Ludwig W, Kohler M (1991) The
1207 genus Magnetospirillum gen. nov. description of *Magnetospirillum gryphiswaldense* sp.
1208 nov. and transfer of *Aquaspirillum magnetotacticum* to *Magnetospirillum*
1209 *magnetotacticum* comb. nov. *Syst. Appl. Microbiol.* 14, 379-385.
1210 Schüler D, Baeuerlein E (1998) Dynamics of iron uptake and Fe_3O_4 biomineralization during
1211 aerobic and microaerobic growth of Magnetospirillum gryphiswaldense. *J. Bacteriol.* 180,
1212 159-162.

1213 Schüler D, Baeuerlein E (1996) Iron-limited growth and kinetics of iron uptake in
1214 *Magnetospirillum gryphiswaldense. Arc. Microbiol.* 166, 301-307.
1215 Schultheiss D, Handrick R, Jendrossek D, Hanzlik M, Schüler D (2005) The presumptive
1216 magnetosome protein Mms16 is a poly(3-hydroxybutyrate) granule-bound protein (phasin)
1217 in Magnetospirillum gryphiswaldense. *J. Bacteriol.* 187, 2416-2425.
1218 Schulz-Vogt HN, Pollehne F, Jürgens K, Arz HW, Beier S, Bahlo R, Dellwig O, Henkel JV,
1219 Herlemann DPR, Krüger S, Leipe T, Schott T (2019) Effect of large magnetotactic bacteria
1220 with polyphosphate inclusions on the phosphate profile of the suboxic zone in the Black
1221 Sea. *ISME J.* 13, 1198-1208.
1222 Schumann D, Raub TD, Guerquin-Kern J-L, Wu T-D, Rouiller I, Smirnov AV, Sears SK, Lücken U,
1223 Tikoo SM, Hesse R, Vali H (2008) Gigantism in unique biogenic magnetite at the Paleocene-
1224 Eocene Thermal Maximum. *Proc. Natl. Acad. Sci.* 105, 17648-17653.
1225 Shapiro OH, Hatzenpichler R, Buckley DH, Zinder SH, Orphan VJ (2011) Multicellular photo-
1226 magnetotactic bacteria. *Environ. Microbiol. Rep*. 3, 233-238.
1227 Simmons SL, Bazylinski DA, Edwards KJ (2006) South-seeking magnetotactic bacteria in the
1228 Northern Hemisphere. *Science* 311, 371-374.
1229 Simmons SL, Sievert SM, Frankel RB, Bazylinski DA, Edwards KJ (2004) Spatiotemporal
1230 distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond.
1231 *Appl. Environ. Microbiol.* 70, 6230-6239.
1232 Siponen MI, Legrand P, Widdrat M, Jones SR, Zhang W-J, Chang MCY, Faivre D, Arnoux P,
1233 Pignol D (2013) Structural insight into magnetochrome-mediated magnetite
1234 biomineralization. *Nature* 502, 681-684.
1235 Siponen MI, Adryanczyk G, Ginet N, Arnoux P, Pignol D (2012) Magnetochrome: a c-type
1236 cytochrome domain specific to magnetotactic bacteria. *Biochem. Soc. Trans.* 40, 1319-1323.

1237 Sparks NHC, Mann S, Bazylinski DA, Lovley DR, Jannasch HW, Frankel RB (1990) Structure and
1238 Morphology of Magnetite Anaerobically-Produced by a Marine Magnetotactic Bacterium
1239 and a Dissimilatory Iron-Reducing Bacterium. *Earth Planet. Sci. Lett.* 98, 14–22.
1240 Spring S, Amann R, Ludwig W, Schleifer KH, Vangemerden H, Petersen N. (1993) Dominating
1241 role of an unusual magnetotactic bacterium in the microaerobic zone of a fresh-water
1242 sediment. *Appl. Environ. Microbiol.* 59, 2397-2403.
1243 Staniland S, Williams W, Telling N, Van Der Laan G, Harrison A, Ward B (2008) Controlled
1244 Cobalt Doping of Magnetosomes in Vivo. *Nat. Nanotech*. 3, 158-162.
1245 Suzuki T, Okamura Y, Calugay RJ, Takeyama H, Matsunaga T (2006) Global gene expression
1246 analysis of iron-inducible genes in *Magnetospirillum magneticum* AMB-1. *J. Bacteriol.* 188,
1247 2275-2279.
1248 Tanaka M, Brown R, Hondow N, Arakaki A, Matsunaga T, Staniland S (2012) Highest Levels of
1249 Cu, Mn and Co Doped into Nanomagnetic Magnetosomes through Optimized
1250 Biomineralisation. *J. Mat. Chem*. 24, 11919.
1251 Thomas-Keprta KL, Bazylinski DA, Kirschvink JL, Clemett SJ, McKay DS, Wentworth SJ, Vali H,
1252 Gibson EK, Romanek CS (2000) Elongated Prismatic Magnetite Crystals in ALH84001
1253 Carbonate Globules. *Geochim. Cosmochim. Acta* 64, 4049–81.
1254 Treiman AH (2003) Submicron magnetite grains and carbon compounds in Martian meteorite
1255 ALH84001: inorganic, abiotic formation by shock and thermal metamorphism. *Astrobiology*
1256 3, 369–392.
1257 Tronc E, Belleville P, Jolivet J-P, Livage J (1992) Transformationof ferric hydroxide into spinel
1258 by Fe^{II} adsorption. *Langmuir.* 8, 313-319.
1259 Uebe R, Ahrens F, Stang J, Jäger K, Böttger LH, Schmidt C, Matzanke BF, Schüler D (2019)
1260 Bacterioferritin of *Magnetospirillum gryphiswaldense* is a heterotetraeicosameric complex

composed of functionally distinct subunits but is not involved in magnetite biomineralization. *mBio* 10, e02795-18.

Uebe R, Schüler D (2016) Magnetosome biogenesis in magnetotactic bacteria. *Nat. Rev. Microbiol.* 14, 621-637.

Vali H, Förster O, Amarantidis G, Petersen N (1987) Magnetotactic Bacteria and Their Magnetofossils in Sediments. *Earth Planet. Science Let*t. 86, 389-400.

Vali H, Weiss B, Li Y-L, Sears SK, Kim SS, Kirschvink JL, Zhang CL (2004) Formation of Tabular Single-Domain Magnetite Induced by *Geobacter Metallireducens* GS-15. *Proc. Natl. Acad. Sci.* 101, 16121-16126.

Wang Y, Casaburi G, Lin W, Li Y, Wang F, Pan Y (2019) Genomic evidence of the illumination response mechanism and evolutionary history of magnetotactic bacteria within the Rhodospirillaceae family. *BMC Genomics* 20, 407.

Wang Q, Wang X, Zhang W, Li X, Zhou Y, Li D, Wang Y, Tian J, Jiang W, Zhang Z, Peng Y, Wang L, Li Y, I J (2017) Physiological characteristics of *Magnetospirillum gryphiswaldense* MSR-1 that control cell growth under high-iron and low-oxygen conditions. *Sci. Rep.* 7, 2800.

Wang S, Chen Y (2017) Origin of magnetotaxis: Vertical inheritance or horizontal transfer? *Proc. Natl. Acad. Sci.* 114, E5016–E5018.

Weiss BP, Sam Kim S, Kirschvink JL, Kopp RE, Sankaran M, Kobayashi A, Komeili A (2004) Ferromagnetic resonance and low-temperature magnetic tests for biogenic magnetite. *Earth Planet. Sci. Lett.* 224, 73-89.

Werckmann J, Cypriano J, Lefèvre CT, Dembelé K, Ersen O, Bazylinski DA, Lins U, Farina M (2017) Localized iron accumulation precedes nucleation and growth of magnetite crystals in magnetotactic bacteria. *Sci. Rep.* 7, 8291.

1284 Williams TJ, Lefèvre CT, Zhao W, Beveridge TJ, Bazylinski DA (2012) Magnetospira thiophila
1285 gen. nov., sp. nov., a marine magnetotactic bacterium that represents a novel lineage
1286 within the Rhodospirillaceae (Alphaproteobacteria). *Int. J. Syst. Evol. Microbiol. 62*: 2443-
1287 2450.
1288 Witt A, Fabian K, Bleil U (2005) Three-Dimensional Micromagnetic Calculations for Naturally
1289 Shaped Magnetite: Octahedra and Magnetosomes. *Earth Planet. Sci. Lett.* 233, 311–24.
1290 Doi: 10.1016/j.epsl.2005.01.043.
1291 Yamazaki T, Kawahata H (1998) Organic carbon flux controls the morphology of
1292 magnetofossils in marine sediments. *Geology* 26, 1064-1066.
1293 Zhu K, Pan H, Li J, Yu-Zhang K, Zhang S-D, Zhang W-Y, Zhou K, Yue H, Pan Y, Xiao T, Wu LF
1294 (2010) Isolation and characterization of a marine magnetotactic spirillum axenic culture
1295 QH-2 from an intertidal zone of the China Sea*. Res. Microbiol. 161*, 276-283.
1296

Figure legends

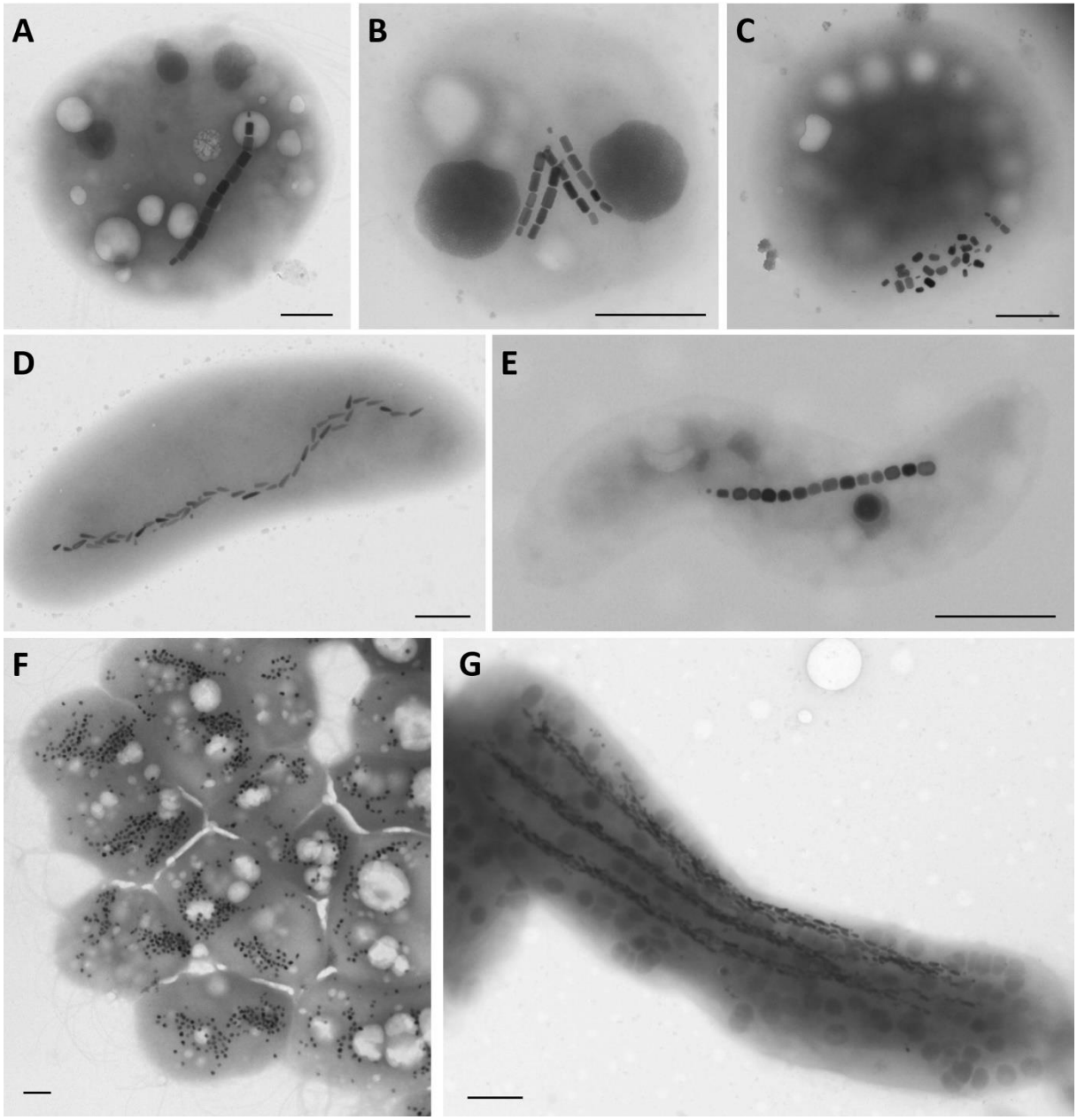


Fig. 1 Transmission electron microscope images of magnetotactic cocci (A-C), curved rod (D) spirillum (E), disaggregated multicellular magnetotactic prokaryote (MMP) (F) and *Ca*. Magnetobacterium bavaricum-like (G) isolated from various freshwater (B, C, E and G) and marine (A, D and F) environments. Magnetotactic cells in (A-E and G) produce elongated prismatic (A-C and E) or bullet-shaped (D and G) magnetite magnetosomes while the MMP (F) produce octahedral greigite magnetosomes. Scale bars represent 0.5 µm.

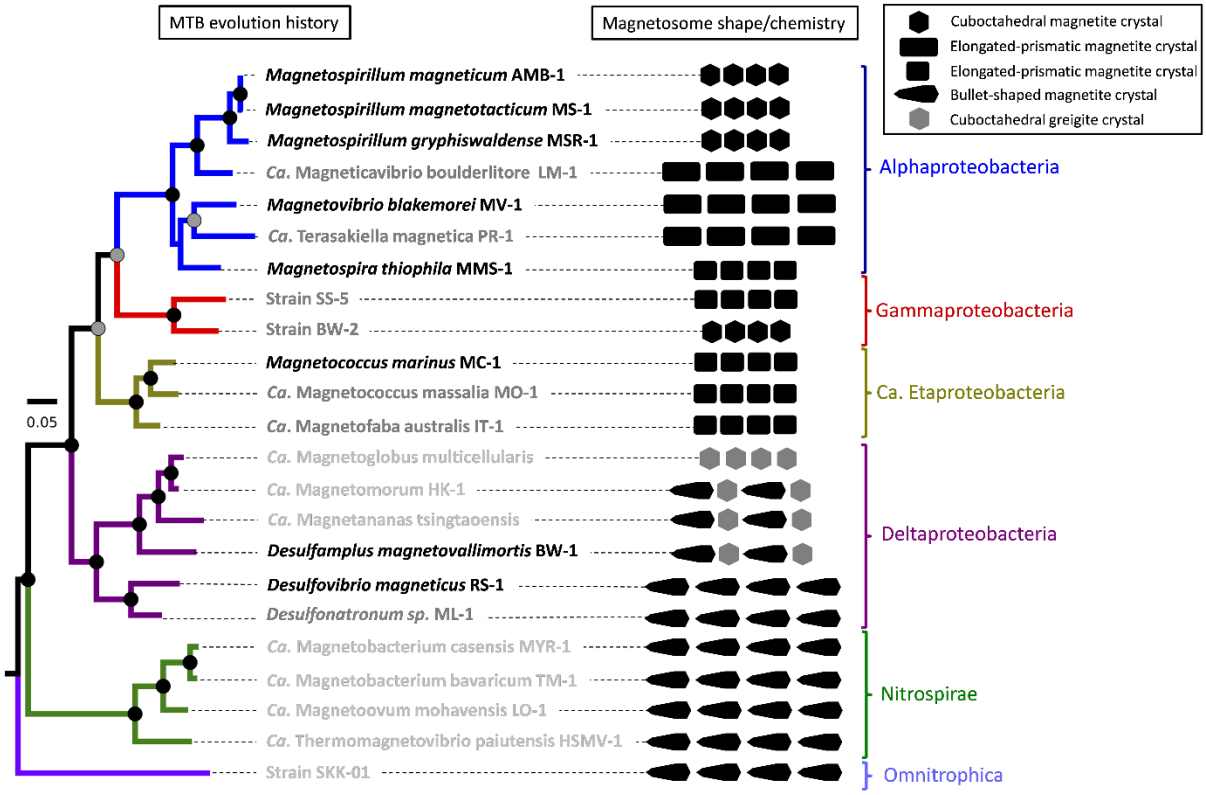


Fig. 2 Correlation between MTB evolution and magnetosome shape and composition. The maximum likelihood tree was built with 16S rRNA gene sequences of selected MTB for which magnetosomes were already characterized. The tree was rooted with the Omnitrophica sp. strain SKK-01. Cultured and taxonomically characterized MTB are written in dark, cultured but not yet taxonomically characterized MTB are written in dark gray while uncultured MTB are written in light gray.

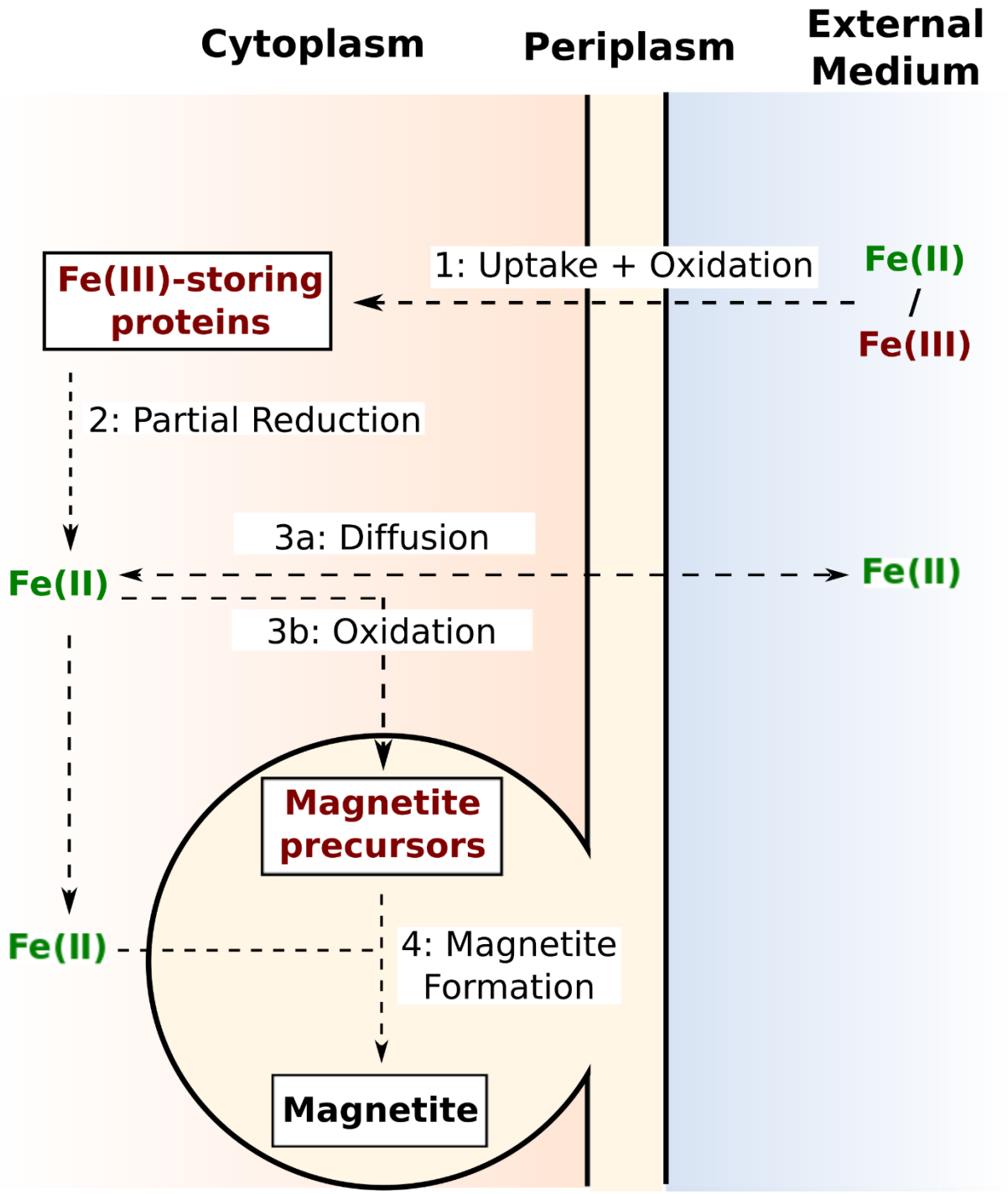
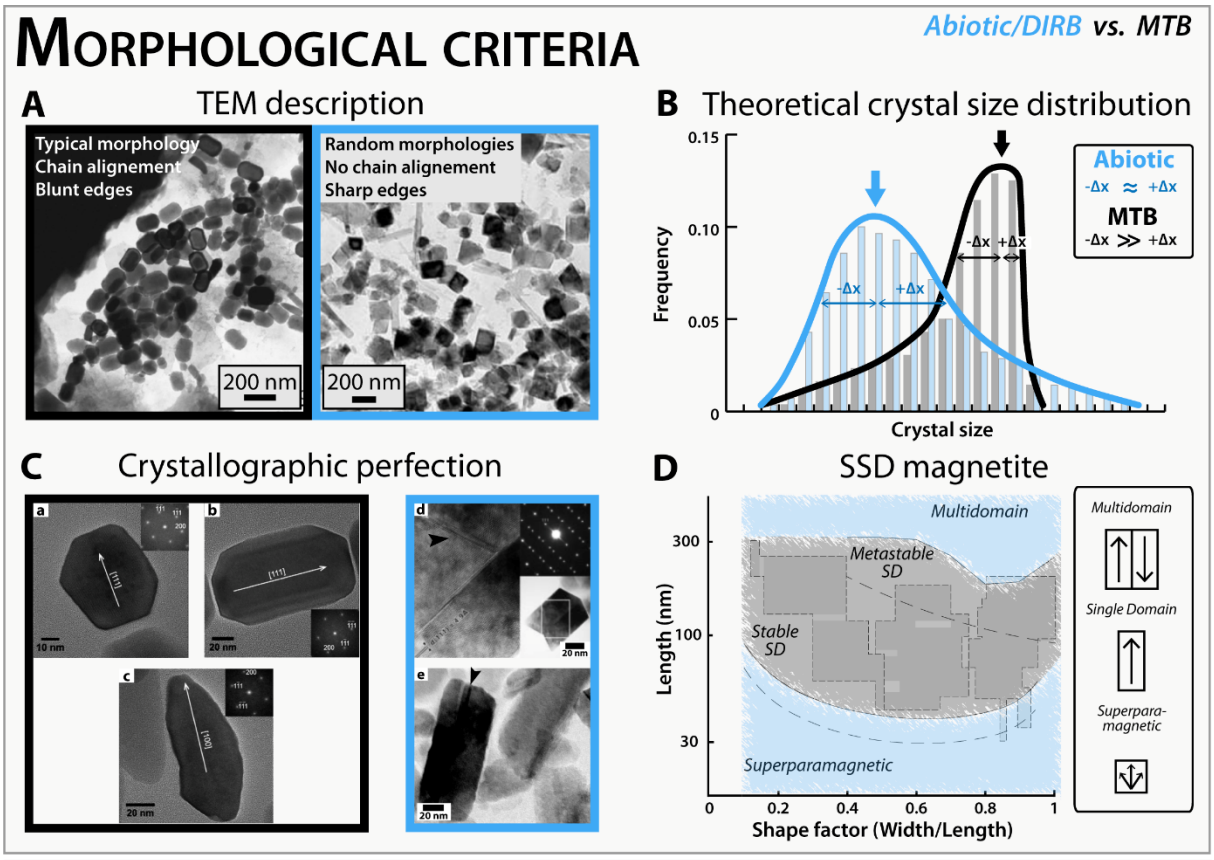
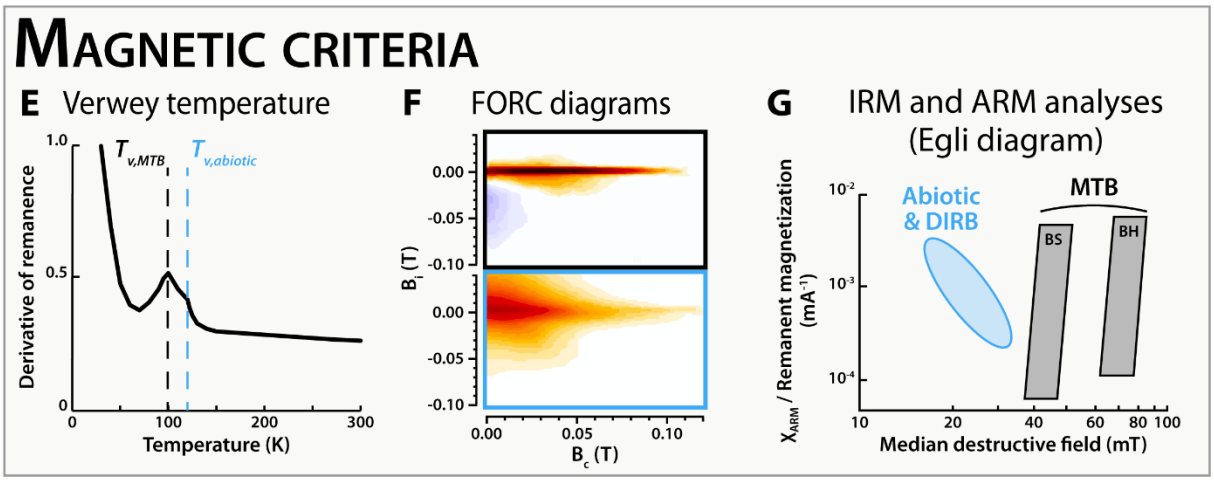


Fig. 3 Schematic model of iron biomineralization in MTB. Reduced or oxidized species are incorporated into bacteria and stored as Fe(III) species. Partial reduction of this pool leads to production of Fe(II) for trafficking to magnetosomes. Magnetite and its potential precursors are precipitated from oxidation of Fe(II) which could happen either in the cytoplasm or in the magnetosome membrane.


MORPHOLOGICAL CRITERIA
Abiotic/DIRB vs. MTB
A TEM description
Typical morphology
Chain alignement
Blunt edges
200 nm
Random morphologies
No chain alignement
Sharp edges
200 nm
B Theoretical crystal size distribution
Frequency
Crystal size
Abiotic
-Δx ≈ +Δx
MTB
-Δx ≫ +Δx
C Crystallographic perfection
D SSD magnetite
Multidomain
Metastable SD
Stable SD
Superparamagnetic
Length (nm)
Shape factor (Width/Length)
Single Domain
Superpara-magnetic


MAGNETIC CRITERIA
E Verwey temperature
Derivative of remanence
Temperature (K)
F FORC diagrams
G IRM and ARM analyses (Egli diagram)
Abiotic & DIRB
MTB
BS
BH
Median destructive field (mT)

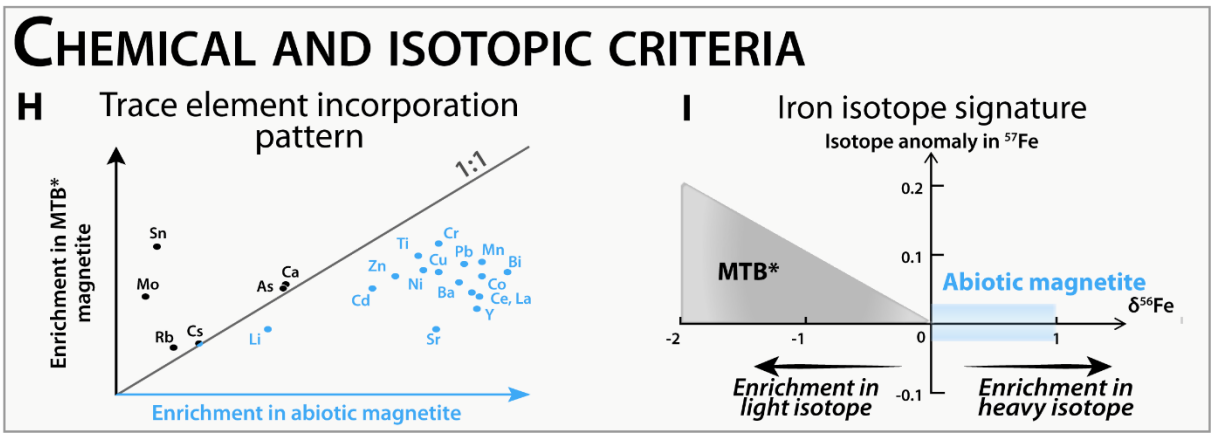

CHEMICAL AND ISOTOPIC CRITERIA
H Trace element incorporation pattern
Enrichment in MTB* magnetite
Enrichment in abiotic magnetite
1:1
I Iron isotope signature
Isotope anomaly in 57Fe
MTB*
Abiotic magnetite
δ56Fe
Enrichment in light isotope
Enrichment in heavy isotope

Fig. 4 Morphological, magnetic, chemical and isotopic criteria used to distinguish MTB magnetite (in black) from abiotic/induced magnetite (in blue). **Morphological criteria** (A) TEM images of Miocene magnetofossils from South Atlantic Ocean sediments (on the left; Kopp and Kirschvink, 2008) and abiotic magnetite precipitated from FeSO4 solution (on the right; Arató *et al.*, 2005). (B) Theoretical size distribution histograms showing specific patterns for MTB magnetite and abiotic precipitation of magnetite. (C) Crystal lattices evidenced in (d) abiotically-precipitated twinned nanocrystal of magnetite (Devouard *et al.*, 1998) and (e), DIRB magnetite (*Vali et al.*, 2004); while (a-c) MTB nanocrystals of magnetite from different species exhibits no internal defects (Li *et al.,* 2013). (D) Length versus shape factor (width/length ratio) for magnetite nanocrystals. MTB magnetites mostly fall within the stable single-domain (SSD, grey zone). Superparamagnetic domain (SP) and multidomain (MD) can also contain some bacterial nanocrystals as shown by the grey squared areas obtained by measuring magnetosomes. **Magnetic criteria** (E) Verwey transition of MTB magnetite (above 100°K) and abiotic magnetite (above 120°K) occurs at two different temperatures (Chang *et al.,* 2016). (F) FORC diagrams illustrating the specific pattern expected from MTB magnetite (upper diagram) and an example containing significant amount of detrital magnetite (lower diagram) (from Lascu et al., 2015). The axes represent the grain microcoercivity (BC) and the interaction field illustrating magnetostatic interactions between particles (Bi). (G) Isothermal remanent magnetization (IRM) and anhysteretic remanent magnetization (AMR) measured on samples allow to construct Egli Diagram. This representation separates clusters of MTB magnetite (biogenic soft, BS, with low coercivity, and biogenic hard, BH, with a higher coercivity mostly linked with their elongation) from other forms, but this technique is time-consuming and complex (Roberts *et al.*, 2019). **Chemical and isotopic criteria** (H) Trace element partition coefficients normalized to iron content for MTB and abiotic magnetite. The

grey line indicates similar trace element enrichment in both biotic and abiotic magnetites relative to the growth medium (Amor *et al.*, 2015). MTB* stands for chemical signatures tested on AMB-1 only. (I) Iron isotope composition measured on magnetite produced by the AMB-1 strain (Amor *et al.*, 2018) and abiotic magnetite (Frierdich *et al.*, 2014). Abiotic magnetite is enriched in heavy isotopes with no anomaly on ^{57}Fe while AMB-1 magnetite is enriched in light isotopes and exhibits a ^{57}Fe anomaly. Microscope images were reproduced with permission.

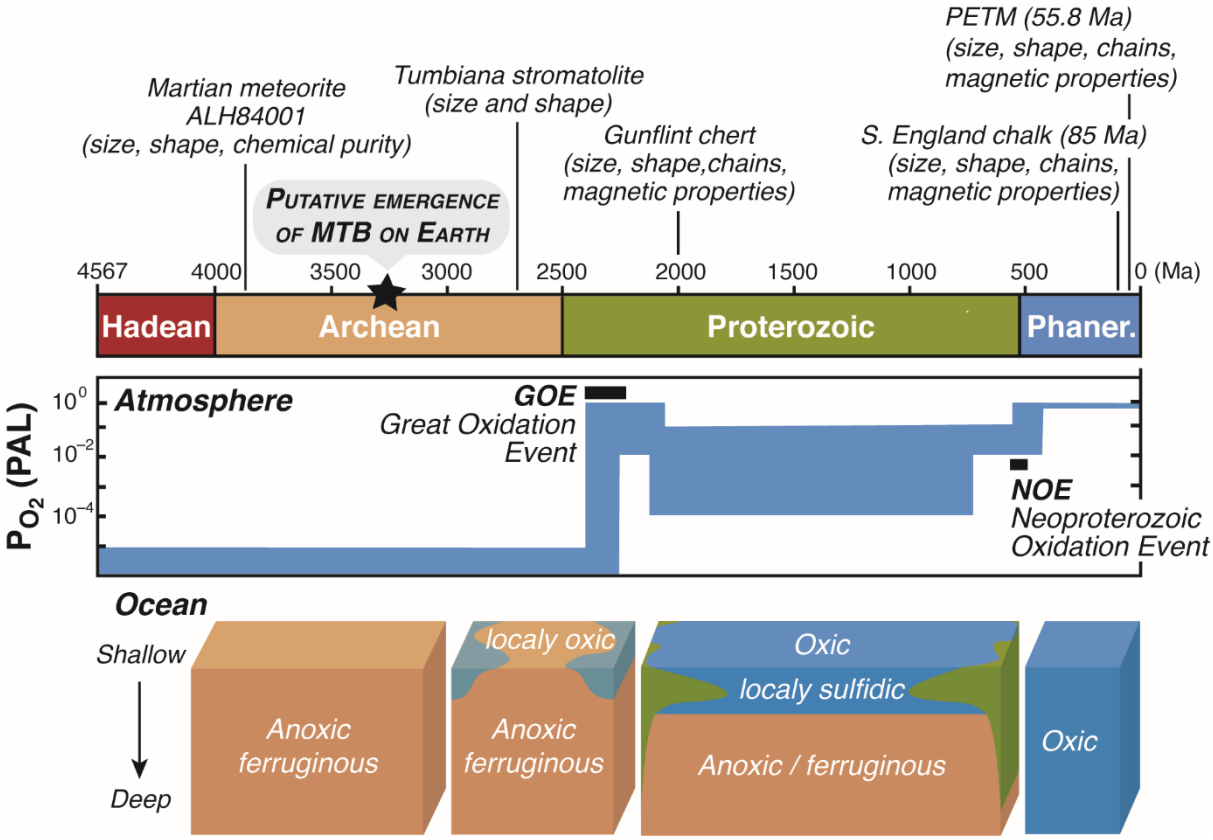


Fig. 5 Main geological eons on Earth's history, evolution of atmospheric oxygen (Kump, 2008 ; Lyons et al., 2014), ocean chemistry (Poulton and Canfield, 2011; Lyons et al., 2014) and occurrences of magnetofossils in the deep time (see main text for details). Oxygen partial pressure expressed in percent of PAL (Present Atmospheric Level).