

Botanical Iron Biominerals: Electron Diffraction and Microscopy Identification

M. Gajdardziska-Josifovska*, M. A. Schofield*, D. P. Robertson*, R. G. McClean†, W. F. Kean†, and C. V. Sommer‡

* Department of Physics, † Department of Geosciences, ‡ Department of Biological Sciences, University of Wisconsin-Milwaukee, P.O.Box 413, Milwaukee, Wisconsin 53201

Characterization of soil profiles often shows enhanced magnetism in the topsoil, attributed to higher concentrations of ultra-fine grained ferrimagnetic iron oxides. An inorganic [1] and a bacterial [2] route have been suggested for the formation of nanocrystalline magnetite in soil, and the soil magnetite controversy is considered unresolved. The discovery of magnetite nanocrystals in the famous Martian meteor ALH 84001 [3], and their assignment as possible signs of ancient bacterial life on Mars, has fueled renewed interest in the fundamental question how to distinguish nanocrystalline biominerals from their equivalent nanocrystalline minerals. The distinguishing parameters appear to be the nanocrystal morphology, size distribution, and self-assembly. Based on the first two properties, all terrestrial and extraterrestrial biogenic magnetite is currently attributed to magnetic bacteria and their magnetofossils. However, both the Martian magnetite and the majority of soil magnetite nanocrystals do not display the tell-tale linear self-assembly of bacterial magnetite. Soil by definition supports plant life and it is known that plants, like microbes and animals, use and store iron in their cells. Magnetite nanocrystals have recently been found in animals [4] and humans [5], and we reported the discovery of botanical magnetite [6,7]. Here we summarize our recent reported findings and plan to present new results from our ongoing research.

The search for magnetite nanocrystals in complex organisms is akin to the proverbial needle in a haystack problem. While there are established biochemical methods for the extraction of the iron biominerals, these techniques destroy the nanocrystal self-assembly. Fig. 1 shows botanical iron oxide nanocrystals extracted from normal grass from a rural Wisconsin site, and deposited randomly on a carbon film. Electron microscopy (TEM, HREM), diffraction (SAD) and spectroscopy (EDX) studies were performed in an Hitachi H-9000 NAR, operated at 100 keV and 300keV. Digital SAD patterns (Fig. 1b), after numerical unwrapping (Fig. 1c) show similarities with magnetite and differences with the postulated ferrihydrite structure for phytoferritin. The botanical magnetite identification is confirmed by developing a modified biochemical extraction procedure that preserves its ordered self-assembly in micron-sized, three-dimensional agglomerates (Fig. 2a,b). The majority of magnetite nanocrystals display cubo-octahedral shapes (Fig. 1a, and Fig. 2a), with a minority of hexagonal prism morphologies (not shown here). Each morphological group has a narrow size distribution typical of intracellular boundary organized biomineralization processes. In addition to crystalline magnetite (Fe_3O_4), we identify $\epsilon\text{-Fe}_2\text{O}_3$, and haematite ($\alpha\text{-Fe}_2\text{O}_3$) nanocrystals with typical sizes in the 1 – 50 nm range and agglomerate sizes up to 4 μm . The three dimensional agglomerates are built with different biomineral nanocrystals in three distinct modes of biological self-assembly: 1) ordered magnetite (Fig. 2 a,b); 2) semi-ordered mixture of magnetite and $\epsilon\text{-Fe}_2\text{O}_3$ (Fig. 2 c,d); and 3) random haematite (Fig. 2 e,f). These self-assemblies correspond to prior TEM reports of crystalline, paracrystalline and amorphous phytoferritin arrangements in sectioned cell samples. Based on these findings we propose that botanical magnetite is an important contributor to the terrestrial topsoil magnetism.

- [1] B. A. Maher and R. M. Taylor, *Nature* **336** (1988) 368.
 [2] W. E. Fassbinder, et al., *Nature* **343** (1990) 161.
 [3] D. S. McKay, et al., *Science* **273** (1996) 924.
 [4] J. M. Cowley, et al. *J. Struct. Biology* **131** (2000) 210.
 [5] C. Quintana et al., *Cellular and Molecular Biology* **46** (2000) 807.
 [6] M. Gajdardziska-Josifovska, et al., *European Journal of Mineralogy* **13** (2001) 863-870.
 [7] R.G. McClean, et al. *European Journal of Mineralogy* **13** (2001) 1235-1242
 [8] The electron microscopy studies were supported in part by the grant NSF/DMR-9553148.

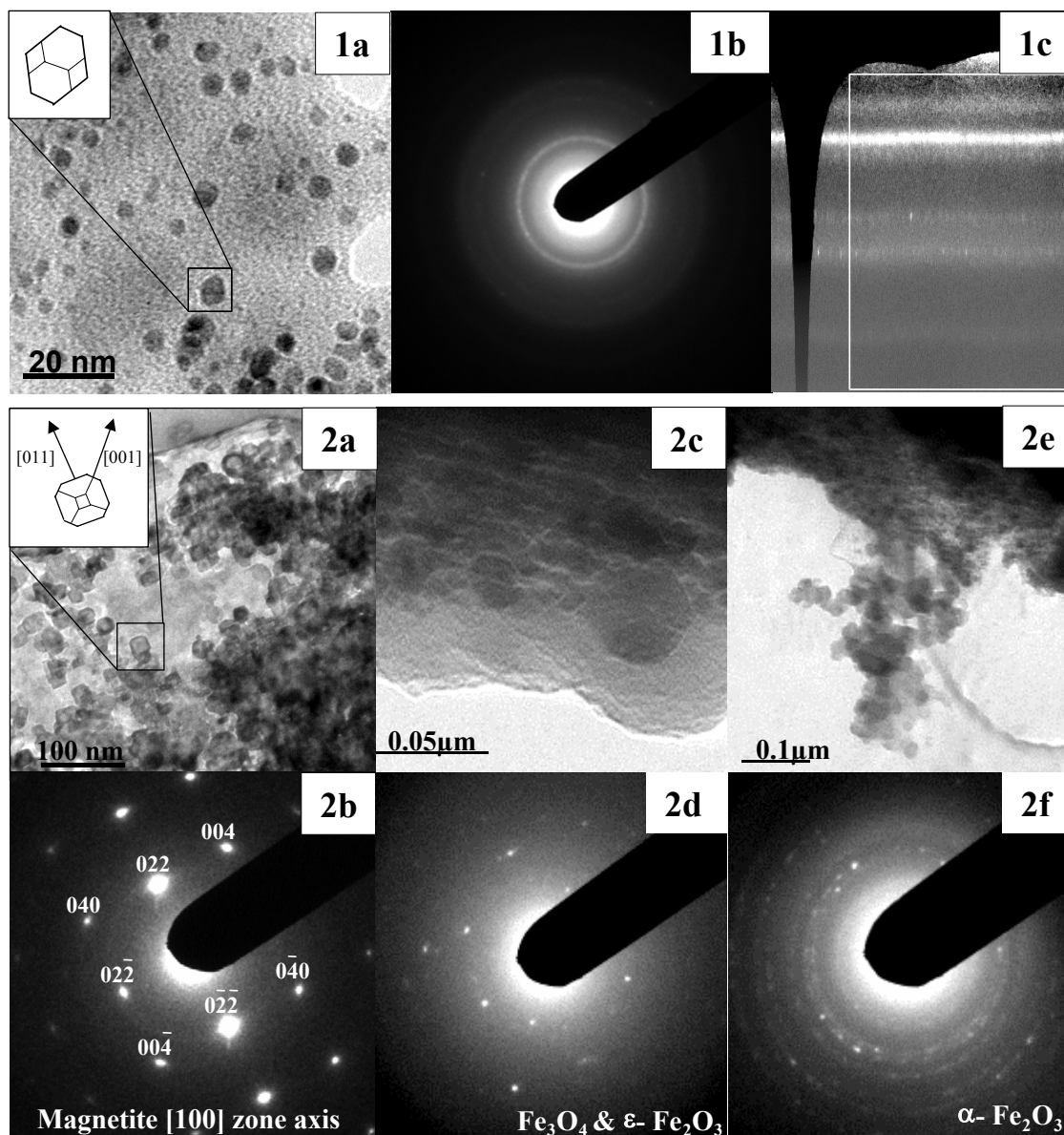


FIG. 1. a) Bright field TEM image of extracted phytoferritin nanocrystals from grass. b) Digital SAD ring pattern from nanocrystals in area (a) is numerically unwrapped in (c) to allow averaging.

FIG. 2 Representative TEM images and SAD patterns of phytoferritin agglomerates from disrupted grass cells reveal three distinct modes of biological self-assembly as function of nanocrystal structure: magnetite nanocrystals with ordered self-assembly (a,b), mixture of ϵ - Fe_2O_3 and magnetite nanocrystals with semi-ordered orientation (c,d), and haematite nanocrystals with random orientations (e,f).