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Correlative *in situ* Analysis of Magnetosome Magnetite Biomineralization

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Magnetotactic bacteria are widely used as a model system for the study of molecular mechanisms of magnetite magnetosome biomineralization. These microorganisms form magnetite nanocrystals with consistent species-specific morphologies, nearly perfect mineral crystal structures and well-defined magnetic properties. Bacterial magnetite biomineralization is a complex process involving a number of simultaneously occurring different steps, and understanding magnetosome magnetite biomineralization is of fundamental interest to devising the strategies for bioinspired synthesis of magnetic materials at the nanoscale [1]. Exploring the new analytical approaches aimed at specimen characterization *in situ* opens up new ways for the study of dynamics of process at the single-cell level and the nanometer-scale resolution. As many metabolic processes, including iron uptake by the bacteria, occur concurrently with the magnetite crystal formation and growth within the microorganism, development of powerful correlative optical and electron microscopy techniques suitable for accessing the cellular function and ultrastructure with necessary resolution, are crucial in understanding both types of processes.

Current studies point to a number of steps involved in magnetosome growth, including cellular uptake of soluble iron, its complexation with membrane proteins, and nucleation and growth of a mature magnetosome. However, the molecular mechanism of biomineralization in magnetotactic bacteria and the magnetosome nucleation and growth process remains unclear. Magnetosome magnetite biomineralization in *Magnetospirillum magneticum* strain AMB-1 was probed *in situ* by using a continuous flow fluid cell scanning transmission electron microscopy (S/TEM) holder platform in combination with the conventional electron microscopy imaging and analytical spectroscopy approaches. The bacteria were studied using a high angle annular dark field (HAADF) detector, where upon the biomineralization of iron, the high atomic number of magnetosomes chains in the bacteria provided high contrast allowing imaging of the microorganisms. STEM visualization *in situ* was followed by correlative fluorescence imaging to probe the integrity of bacterial cell wall membrane and confirm the bacteria's viability, as schematically shown in Figure 1. A fluorescent dye mixture was flowed through the fluid cell. As a result, the microorganisms with intact cell wall membrane were stained with a green fluorescent protein and considered viable. Bacterial cells with damaged cell wall membrane were stained red with propidium iodide and considered dead [2]. We discuss the effects of the electron beam and confinement in the fluid cell and outline the steps to mitigate electron beam-induced damage to the bacterial cells.

While the crystal structure of magnetosome magnetite is well-established, far less is known about the chemical environment of the biomineral at the early stages of magnetosome formation. Current effort is focused on probing the magnetosome magnetite biomineralization both *in vivo* and in *vitro* by using the combination of correlative STEM-FM imaging, electron diffraction, and analytical spectroscopy. To this end, we employed the combination of electron diffraction and EELS analyses to probe the crystallinity

and chemical bonding in the nascent magnetosome particles. Newly formed magnetosome particles are mostly amorphous, whereas the fully grown magnetosomes are unambiguously indexed to magnetite. We have identified the narrow "transition range" corresponding to the onset of crystal lattice formation and utilized the peak fitting analysis of EEL spectra to monitor the evolution of chemical bonding in magnetosomes and correlated these findings with the emergence of crystalline lattice [3].

In-vivo imaging of viable bacteria is a first step in directly observing biomineralization of magnetosomes in live magnetotactic bacteria. Our approach can be expanded to the *in vivo* characterization of a wide range of inorganic structures biomineralized by various microorganisms, and as such it is expected to have a direct impact on the understanding of biological processes. Assuming the radiation damage to the specimen is mitigated, additional characterization will include X-ray fluorescence study of magnetotactic bacteria in the fluid cell for monitoring the cellular dynamics *in situ*. These studies will complement the *in situ* HAADF imaging effort, as shown in Figure 2 [4].

References:

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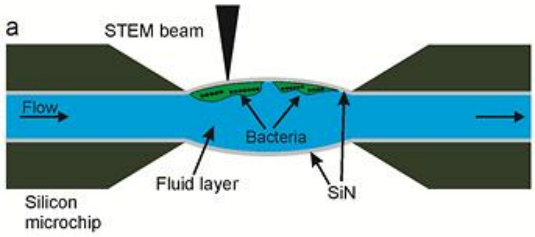

Figure 1. Schematics of HAADF-STEM imaging of magnetotactic bacteria in the Fluid Cell.

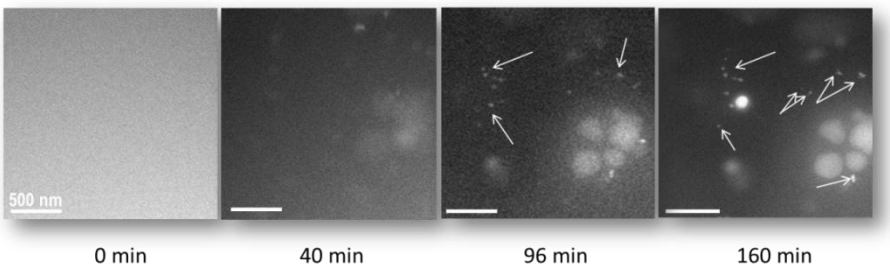

Figure 2. *In situ* magnetosome magnetite biomineralization by AMB-1 grown under low-iron conditions is observed after delivering iron-containing growth medium to the fluid cell. The continuous change in contrast is related to accumulation of iron preceding the magnetosome formation. Arrows point to formed magnetosomes.

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