Examining the Polymerization of Recombinant MamK onto the Membrane of Magnetosomes Using the High-Speed Atomic Force Microscope

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Magnetotactic bacteria (MTB), such as *Magnetospirillum magneticum* AMB-1, biomineralize nanometer-sized magnets within their cells called magnetosomes. These organelles consist of a crystal of magnetite that is approximately 50 nm in size and surrounded by a membrane, which form a chain that can be dozens of magnetosomes in length. The chain of magnetosomes is held together with an actin-like protein called MamK [1]. These proteins form short filaments that bind to many magnetosomes, which keep them in a stable, rigid line as well as anchor the entire chain to both ends of the cell. Little is known about how MamK polymerizes, other than it forms filaments which can be visualized in the transmission electron microscope (TEM). In the TEM, the filaments form bundles, however individual filaments can be discerned that are approximately 6 nm wide [2]. Our purpose is to examine MamK *in vivo* under physiological conditions to determine its dynamics and how it polymerizes. To perform this experiment we used the high-speed atomic force microscope to image isolated magnetosomes to which we added monomeric MamK. The expected result is that we will be able to visualize MamK filaments polymerizing along the chains of magnetosomes.

High-speed atomic force microscopy (HS-AFM) is a microscopy technique that has several advantages over other microscopy techniques; primarily that it can analyze cells in physiological conditions (i.e. buffer solutions), it has single nanometer resolution, can scan the sample several times each second, and it uses piconewton forces when scanning the sample. To prepare our sample, we isolated magnetosomes from 10 L of AMB-1 cells from early stationary growth phase and diluted the magnetosomes to 125 mg/mL in 10 mM TRIS buffer. Approximately 3 µL of this was deposited onto 0.01% poly-L-lysine coated mica and imaged in the HS-AFM. We first imaged the sample to confirm that the magnetosomes bound to the mica and were still intact. We then added recombinant monomeric MamK that was purified from E. coli and diluted to approximately 0.02 µM in PBS, pH 7. MamK was added to the magnetosomes bound to the mica and 10 mM ATP was added to begin polymerization of MamK.

The magnetosomes appeared intact given that they formed chains, had a membrane, and were the correct size. However, when the solution of monomeric MamK with 10 mM ATP was added to the magnetosomes, no apparent MamK filaments were visible. However, the magnetosome chains increased in diameter and became more diffuse; but there were no obvious filaments appearing along the magnetosomes. Previously we confirmed that we could visualize filaments of MamK in the HS-AFM, however, when we mixed MamK with magnetosomes, we do not see any polymerization. It is difficult to isolate the exact reason why we did not see MamK filaments when we added monomeric MamK to the magnetosomes, therefore our future work will involve determining how to get MamK to polymerize when added to magnetosomes.

References:
[1] Taoka, A., Asada, R., Wu, L.-F., Fukumori, Y., *J. Bac.* **189** (2007), p. 8737.

[2] Komeili, A., Zhuo, L., Newman, D. K., Jensen, G. J., *Science* **311** (2006), p. 242.

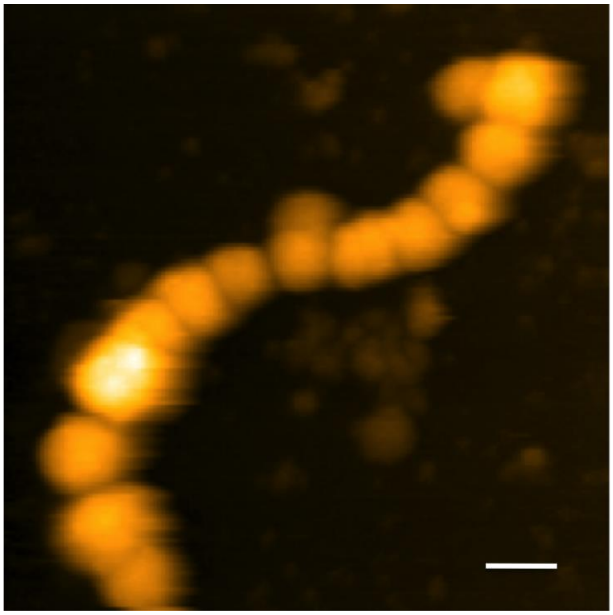

Figure 1. A chain of magnetosomes that was isolated from *M. magneticum* AMB-1, deposited on poly-L-lysine coated mica and imaged in the high-speed atomic force microscope. Scale bar equals 5 nm.

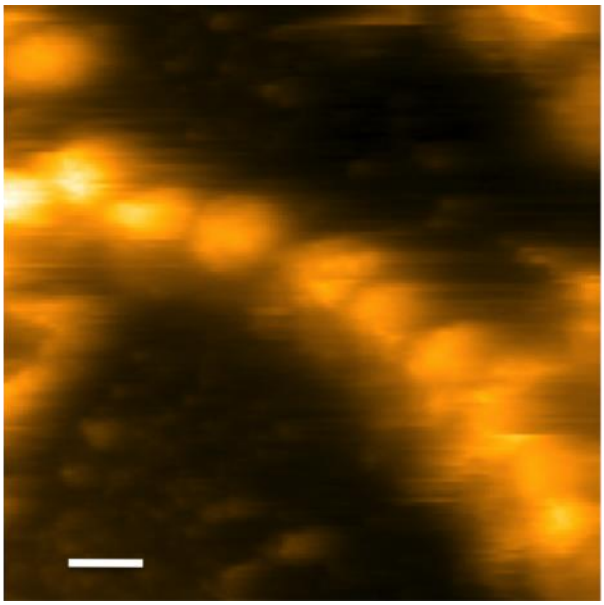

Figure 2. A different chain of magnetosomes that was isolated from *M. magneticum* AMB-1 and deposited on mica but with monomeric MamK with 10 mM ATP added to the magnetosomes. The chain of magnetosomes became wider and more diffuse which we believe is due to the MamK, however no filaments were observed. Scale bar equals 5 nm.