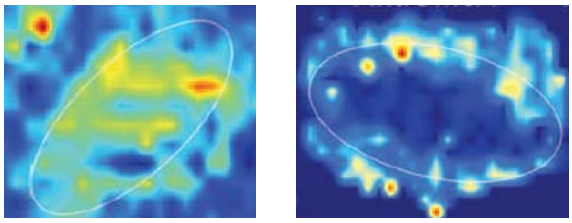
Tips for Nanomapping

Atomic force microscopy technique maps proteins on Shewanella *surface*

An international team used surface science and imaging capabilities at the Department of Energy's EMSL to determine the location, with nanoscale resolution, of MtrC and OmcA – two *Shewanella oneidensis* MR-1 surface proteins that can affect environmental quality. Bacteria such as *Shewanella* exchange electrons with minerals, yielding effects such as changes in the migration of environmental contaminants and water purity. MtrC and OmcA are cytochromes, or surface-bound iron-containing proteins that facilitate this exchange between *Shewanella* and iron. Carried out, in part, as a contribution to EMSL's Biogeochemistry Grand Challenge, the team's imaging studies offer a deeper understanding of the role MtrC and OmcA play in electron exchange and may lead to enhanced bioremediation methods.



Ig-RFM showed that MtrC is distributed across the Shewanella *surface (left). OmcA is localized to the cell-hematite interface (right). Both MtrC and OmcA are in the EPS (white lines indicate the bacterium boundary).*

Comprised of participants from The Ohio State University; Pacific Northwest National Laboratory; Corning Incorporated, Johannes Kepler University of Linz, Austria; Ecole Polytechnique Fédérale de Lausanne, Switzerland; and Umeå University, Sweden, the research team used EMSL's oxygen plasma-assisted molecular beam epitaxy capability to grow hematite (Fe_2O_3) thin films. *Shewanella* cells were allowed to attach to the thin films; the iron in the hematite serves as an electron acceptor. EMSL's dynamic force-scanning probe microscope for single-molecule force spectroscopy as well as antibody-recognition force microscopy (Ig-RFM) were then used to map the locations of MtrC and OmcA on the live *Shewanella* surface. A relatively new technique, Ig-RFM uses a nanometer-scale, flexible antibody-coated tip that is moved across a sample surface. When the antibody, in this case anti-MtrC or anti-OmcA, comes in contact with its binding partner, a measurable force is required to separate the two. Force measurements indicate that MtrC is distributed rather uniformly on the bacterial surface, and OmcA is localized at the cell-mineral interface. Both cytochromes locate to the extracellular polymeric substance, which is made up of the secretions that help bacteria bind to surfaces.

Scientific Impact: Electron exchange is a basic function carried out by bacteria such as *Shewanella*. Understanding the mechanism of this exchange is of fundamental importance and may lead to advanced bioremediation strategies. In addition, this work supports EMSL's goal to predict biological functions from molecular and chemical data.

Societal Impact: Understanding microbe-mineral electron exchange is important to developing enhanced bioremediation methods for contaminated environments, such as the DOE's Hanford Site in Richland, Washington.

Reference: Lower BH, R Yongsunthon, L Shi, L Wildling, HJ Gruber, NS Wigginton, CL Reardon, GE Pinchuk, TC Droubay, JF Boily, and SK Lower. 2009. "Antibody Recognition Force Microscopy Shows that Outer Membrane Cytochromes OmcA and MtrC Are Expressed on the Exterior Surface of *Shewanella oneidensis* MR-1." *Applied and Environmental Microbiology* 75(9)2931-2935. DOI:10.1128/AEM.02108-08. This research was featured on the journal's May 1, 2009 cover.

Acknowledgement: This work was supported by the DOE-OBES Geosciences Research Program, EMSL's Biogeochemistry Grand Challenge, the DOE-OBER Genomics-Genomes to Life Program, and the National Science Foundation.