DOE Final Report for
DE-FG02-04ER15590

Nanobiogeochemistry of Microbe/Mineral Interactions: A Force Microscopy and Bioinformatics Approach

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Introduction to final report

The following is a brief final report for DE-FG02-04ER15590, titled "Nanobiogeochemistry of Microbe/Mineral Interactions: A Force Microscopy and Bioinformatics Approach". This report includes a summary of research activities and a list of abstracts and articles that have been generated as a result of the funding.

This award is a collaborative research effort between Dr. Steven K. Lower at the Ohio State University and Dr. Michael F. Hochella, Jr. at Virginia Tech. This report contains only those aspects of the research that are being supervised by Dr. Lower. Where appropriate, this report will discuss the linkage between these two related but distinct research efforts. Dr. Hochella submitted a separate final report.

Brief summary of the original proposed work

In the original proposal (section 5, General Approach and Hypotheses, and Section 6, Research Plan), the planned research program for this project was divided into three major tasks. These included research involving (1) biological force microscopy to study intermolecular forces between whole bacterial cells and minerals, (2) chemical force microscopy to study interactions between discrete biomolecules and mineral surfaces, and (3) molecular modeling of interactions between functional groups on biological and mineralogical surfaces. Dr. Lower was in charge of task 1, biological force microscopy, and he also participated in task 2, chemical force microscopy. Below is a brief discussion of the work conducted by Dr. Lower and his group.

Brief summary of work completed under Task 1

Task 1: Biological force microscopy

This section provides a brief overview of research related to Task 1. More extensive discussions of this research can be found in three peer-reviewed publications, which were published as a direct result of this work. These publications are:

Lower, S. K. (2005) Directed natural forces of affinity between a bacterium and mineral. *American Journal of Science.* 305: 752-765.

Lower, B. H., Hochella, M. F., Jr., Lower, S. K. (2005) Putative Mineral-Specific Proteins Synthesized by a Metal Reducing Bacterium. *American Journal of Science*. 305: 687-710.

Lower, B. H., Yongsunthon, R. F. P. Vellano, III, Lower, S. K. (2005) Simultaneous force and fluorescence measurements of a proteins that forms a bond between a living bacterium and a solid surface. *Journal of Bacteriology*. 187: 2127-2137.

This task focused on interactions between iron oxyhydroxides and the dissimilatory metal reducing bacterium *Shewanella oneidensis*. *S. oneidensis* were grown to mid-exponential phase and attached to the end of a force-sensing cantilever creating a so-called biologically-active-force-probe. This probe was used in a force microscope to quantitatively measure pico- to nano-Newton forces as living cells of *S. oneidensis* approached, made contact with, and were subsequently retracted from the (010) surface of goethite (FeOOH). In a typical experiment,

approach and retraction forces were measured after varying the contact time between *S. oneidensis* and goethite in aerobic or anaerobic solutions supplemented with lactate as an energy source.

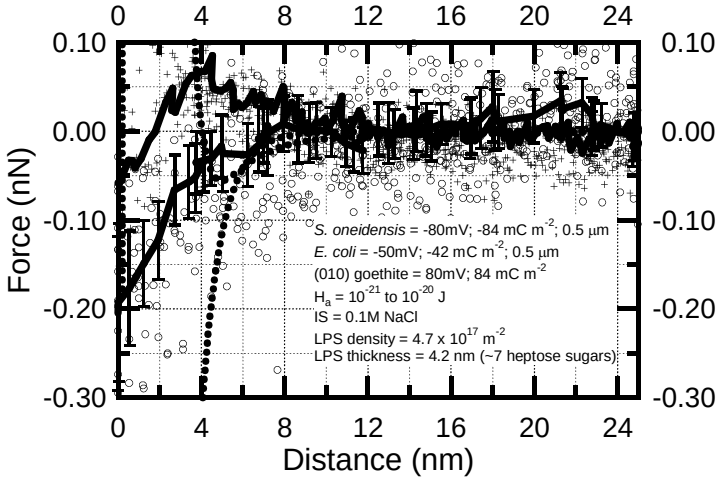

Figure 1. Force-distance measurements for *S. oneidensis* approaching the (010) surface of goethite (open circles) or *E. coli* approaching the (010) surface of goethite (cross symbols). The solid black lines correspond to the average trace bounded by the 95% confidence interval. The dotted lines correspond to model predictions for intermolecular forces between goethite and the two bacteria. See text for details.

Figures 1 illustrates intermolecular forces detected when *S. oneidensis* approached the (010) surface of goethite. As *S. oneidensis* approached to within 10 nm of the goethite surface, attractive forces (negative sign) caused the bacterium to make contact with the mineral to a maximum force of -0.2 nN (Figure 1). As a control experiment, forces were also measured between goethite and another Gram negative bacterium (see Fig. 1). The observed forces for *E. coli* were significantly different than those observed for *S. oneidensis*. In particular, a repulsive force was detected when *E. coli* and goethite were separated by 4 to 8 nm.

The observed forces between *S. oneidensis* and goethite are well described by summing the theoretical van der Waals and electrostatic interactions (see dotted line in Fig. 1). There is, however, a notable discrepancy between the observed forces and model results at separations <6 nm (see Fig. 1). The measured forces are significantly less than predicted at this length scale. This is because the actual force gradient exceeds the spring constant of the cantilever (0.07-0.11 nN nm^{-1}), causing the cantilever to jump to contact.

The above discussion focuses on forces at the *S. oneidensis*-goethite interface as a bacterium *approaches* the (010) surface of goethite. However, something very different is observed when the bacterium is pulled away from the mineral surface. Measurements upon retraction reveal strong attractive forces between *S. oneidensis* and goethite that extend outwards to 500 nm (Figure 2). We believe this is due to the formation of adhesive bonds between the mineral and biopolymers on the cell surface. The length dimension of various macromolecules are such that forces within 10-50 nm are consistent with lipopolysaccharides; whereas longer range, attractive forces are more likely due to outer membrane proteins.

A notable feature of these retraction data are saw-tooth like features (see Fig 2). To determine whether the saw-tooth features in the retraction profiles were actually protein signatures, we used a theoretical model designed to predict the way in which proteins should unfold. This model, called the worm-like chain (WLC) model, can be used to determine the force (F) necessary to extend a polypeptide chain to a length of (x). It is given by the following

equation: $F(x) = (kT/b)\ [0.25\ (1 - x/L)^{-2} - 0.25 + x/L]$, where, k is the Boltzmann's constant, T is the temperature, b is the persistence length (0.4 nm), and L is the contour length. Figure 2 shows that the measured data seem to exhibit a signature for polypeptides composed of 725 and 1365 amino acids. Assuming 110Da per amino acid, these contour lengths corresponds to proteins that are ~80 and 150 kD, respectively.

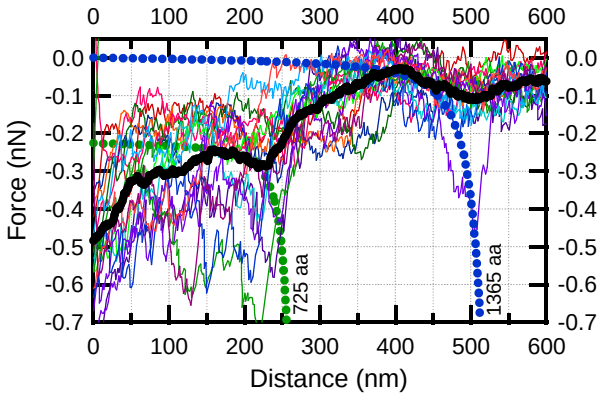

Figure 2. AFM retraction measurements between S. oneidensis and goethite. Solid black line represents the average force-distance profile. The dotted lines correspond to the theoretical unfolding trajectories of putative outer membrane proteins.

In a complementary study, Drs. Brian Lower and Mike Hocehlla used two-dimensional gel electrophoresis to study protein expression patterns for *S. oneidensis* grown with oxygen versus Fe(III) as the terminal electron acceptor. Their results confirm that *S. oneidensis* produces high molecular weight proteins (e.g., proteins of ~80 and 150 kDa), but only under anaerobic conditions with Fe(III) as the terminal electron acceptor. These results were presented in Dr. Hochella's final report.

Brief summary of work completed under Task 2

Task 2: Chemical force microscopy

This section provides a brief overview of research related to Task 2. This research is presented in more detail in the following two manuscripts.

Lower, B.H., Liang Shi, Ruchi Yongsunthon, Timothy C. Droubay, David E. McCready, and Lower, S.K. (2006) Biomechnical properties of outer membrane cytochromes MtrC and OmcA from *Shewanella oneidensis*. *Biophysical Journal*. In preparation.

Lower, B.H., Liang Shi, Ruchi Yongsunthon, Timothy C. Droubay, David E. McCready, and Lower, S.K. (2006) Dynamic force spectroscopy of specific bonds that form between an iron oxide surface and outer membrane cytochromes MtrC and OmcA from *Shewanella oneidensis* MR-1. *Journal of Bacteriology*. In review.

Research conducted under Task 1 suggests that *S. oneidensis* selectively targets at least two outer membrane proteins to its interface with an iron oxide. These proteins presumably assist in the transfer of electrons between the bacterium and Fe(III) in the mineral. To further explore this hypothesis, AFM force measurements were performed on two outer membrane, *c*-type cytochromes purified from *S. oneidensis*. These two proteins – MtrC (SO1778; also known as OmcB) and OmcA (SO1779) – were selected because they have previously been shown to play an important role in the reduction of metals like Fe(III).

Recombinant MtrC and OmcA were expressed in *S. oneidensis*, purified, and then covalently attached to gold substrates via C-terminal Cys residues. AFM was used to measure

forces between each of these two proteins and force probes that were functionalized with a hematite thin film using molecular beam epitaxy. Figure 3 shows approach and retraction force measurements for the MtrC-hematite and OmcA-hematite pairs. These force spectra reveal a unique sawtooth shaped force signature for each cytochome. This force-signature was noted in 76% and 39% of the measurements for MtrC and OmcA, respectively.

The WLC model was used to determine whether the sawtooth force-profile corresponds to the theoretical unfolding trajectory for each protein. The observed and predicted force-extension relationships agree very well (see Fig. 3) indicating that a specific bond forms between both cytochromes and the hematite surface.

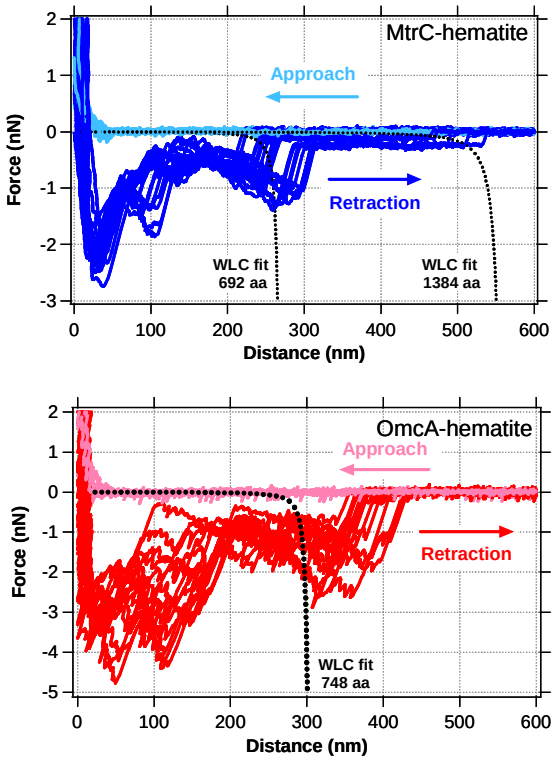

Figure 3. AFM force measurements between MtrC and hematite (top) vs. OmcA and hematite (bottom). Dotted lines labeled 692 and 748 aa correspond to the WLC model force-extension relationship for recombinant MtrC and OmcA, respectively.

Figure 4 provides a comparison of AFM measurements on whole cells of *S. oneidensis* vs. purified cytochromes from *S. oneidensis*. There is a very strong correlation between what is observed for the purified proteins and the whole cells. The sawtooth force-signature observed around 250-300 nm is present in both the whole cell as well as the cytochrome data. The magnitude of force for the cytochrome signature is "stronger" than the whole cell data (compare Figs. 2 and 3). This may be a "real" difference related to some type of shielding event that occurs when a cytochrome is present within the outer membrane of a whole cell. Or, this difference could simply be due to the fact that the cantilevers used in these different studies were calibrated using different methods, which are know to yield slightly different values for spring constants.

In either case, the strong correlation supports our original hypothesis that MtrC and/or OmcA form a directed, specific bond with the surface of iron oxides. It is very difficult to say with certainty that the whole cell sawtooth observed near 300 nm is due solely to one or the other cytochrome. As shown in Fig. 3, both cytochromes are able to form a specific bond with hematite but each protein has unique binding attributes. On the one hand, OmcA binds to hematite with a stronger force than MtrC. On the other hand, MtrC forms a bond with hematite more frequently and the nature of this bond is much more resilient than OmcA (data not shown). It is therefore more likely that either cytochrome could be the origin of the "whole cell" sawtooth near 300 nm as each cytochrome provides a unique function in binding and/or reduction of Fe(III) in minerals.

There is another striking similarity between the whole cell and protein data at a length scale of ~500 nm (see Fig. 4). At this scale, the data for *S. oneidensis* MR-1 exhibit a notable sawtooth binding event. While not a classic sawtooth, the data for MtrC reveal a longer range attractive force with a bond rupture distance of ~500 nm. The predicted contour length of MtrC is ~277 nm (see Fig. 3). Therefore, the attractive force detected at length scales greater than 300 nm cannot be attributed to a single molecule of MtrC. Perhaps, this longer range binding feature originates from the unraveling of a dimer of MtrC. Additional AFM studies with mutagenically altered forms of MtrC or OmcA in combination with modeling or computer simulations will be needed to confirm/refine this hypothesis.

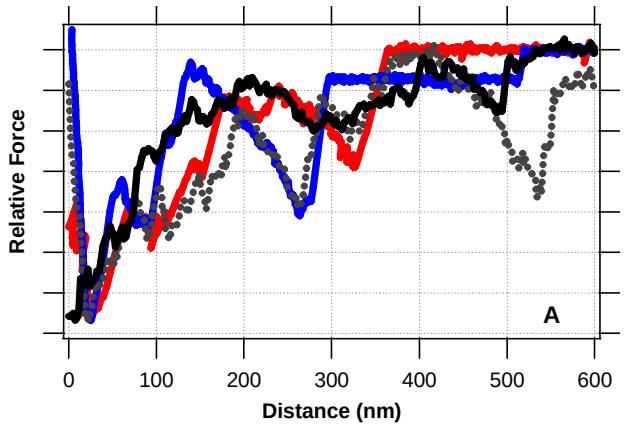

Figure 4. Retraction curves for forces detected between a Fe(III) oxide and *S. oneidensis* MR-1 (black curves), recombinant MtrC (blue curve), or recombinant OmcA (red curve). Curves for the whole cells come from Fig. 2 and Lower et al. (2001). Curves for purified cytochromes come from Fig. 3. The force axis has been "normalized" such that the maximum force in each, individual retraction curve is set to a value of one. This procedure does not alter the distance data, it simply plots force on a relative scale.

Abstracts and publications funded by this DOE award

Abstracts and presentations

Lower, B. H., Hochella, M. F., and Lower S. K. (2005) Proteins produced by iron reducing bacterium to mediate contact with iron oxides. NW Regional Meeting of the American Chemical Society. June 15-18, Fairbanks, AK.

Lower, S. K. (2004 – *Invited Presentation*) "Application of atomic force microscopy to microbial-mineral interactions." *Clay Minerals Society Annual Meeting* in Richland, Washington.

Hochella, M.F., Jr., Lower, S.K. (2003) "Mineral-fluid interfaces and interactions: From molecular to local, regional, and global-scale processes." *Goldschmidt Conference* in Japan.

Lower, S.K. (2003) "Probing reactions at the biotic-abiotic interface." *International Society for Environmental Biogeochemistry* in Japan.

Lower, S.K., Hochella, M.F., Jr., Kendall, T.A., and Lower, B.H. (2003) "Mineral specific biological interactions." DOE meeting at Argonne National Laboratory, Illinois.

Lower, B. H. and Lower, S. K. (2003 – *Invited Presentation)* "Mineral specific proteins synthesized by bacteria." *American Geophysical Union Annual Meeting* in San Francisco, California.

Lower, S. K. (2003 – *Invited Presentation)* "Mineral specific proteins synthesized by bacteria." Interagency Meeting on Nanoscale Science, Engineering, and Technology. National Science Foundation. Arlington, Virginia.

Lower, S. K. (2002 – *Invited Presentation*) "Bacterial adhesion to clay surfaces and implications to environmental transport." *Annual Meeting of the Clay Mineral Society* in Boulder, CO.

Lower, S. K. (2002 – *Invited Presentation*) "Nanoscale interactions between bacteria and minerals." *Annual Conference of the International Association of Geochemistry and Cosmochemistry* in Honolulu, HA.

Peer-reviewed Articles

Lower, B.H., Liang Shi, Ruchi Yongsunthon, Timothy C. Droubay, David E. McCready, and Lower, S.K. (2006) Biomechnical properties of outer membrane cytochromes MtrC and OmcA from *Shewanella oneidensis*. *Biophysical Journal*. In preparation.

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Yongsunthon, R. and Lower, S. K. (2005) Force measurements between a bacterium and another surface in situ. *Advances in Applied Microbiology* 58: 97-124.

Yongsunthon, R. and Lower, S. K. (2005) Force "spectroscopy" of biological molecules on living bacteria cells. *Journal of Electron Spectroscopy and Related Phenomena 150: 228-234.*

Lower, B. H., Yongsunthon, R. F. P. Vellano, III, Lower, S. K. (2005) Simultaneous force and fluorescence measurements of a proteins that forms a bond between a living bacterium and a solid surface. *Journal of Bacteriology*. 187: 2127-2137.

Kendall, T. A. and Lower, S. K. (2004) Forces between minerals and biological surfaces in aqueous solution. *Advances in Agronomy*. 82: 1-54.