**DOE Final Report for
DE-FG02-02ER15327**

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

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Introduction

The following is a final report for DE-FG02-02ER15327, titled “Nanobiogeochemistry of Microbe/Mineral Interactions: A Force Microscopy and Bioinformatics Approach”. This research was conducted by the PI for a period of approximately one year while he was a professor at the University of Maryland. This report includes a summary of research activities and a list of abstracts and articles that were published while the PI was at the University of Maryland.

This award is a collaborative research effort between Dr. Steven K. Lower at the University of Maryland 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 will submit a separate report summarizing his work.

Brief summary of proposed work

In the original proposal (section 5, General Approach and Hypotheses, and Section 6, Research Plan), the planned research program for this three-year 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. Dr. Lower has been involved in tasks 2 and 3 but, as these are under the supervision of Dr. Hochella, these tasks will not be discussed herein.

Work completed by the PI at the University of Maryland

Task 1: Biological force microscopy study of membrane proteins and lipopolysaccharide interactions with mineral surfaces

For this task we have 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 illustrates intermolecular forces detected when *S. oneidensis* approached the (010) surface of goethite in anaerobic solution. 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 1A). Apparently the force fields around the mineral and bacterium did not create spheres of influence greater than a few nanometers in

diameter. The theoretical Debye length for these solutions was 3-10 nm, which is consistent with the observed separation over which forces were detected upon approach of a cell towards the mineral. The forces measured upon approach changed significantly after *S. oneidensis* were allowed to make contact with the goethite surface for several minutes (Figure 1B). No longer were attractive forces detected at the cell-mineral interface (at least upon approach). At first glance, this may seem counterintuitive as the surfaces of the mineral and bacterium are expected to have opposite charges at circumneutral pH (overall pI of *S. oneidensis* ~2-4 and pzc of goethite 7-9). However, as will be discussed below, this may be due to the selective localization of proteins at the *S. oneidensis*-goethite interface. Amine groups on proteins could lead to a localized point of positive charge thereby leading to a non-attractive force between the cell and mineral, which is expected to have a positive charge.

The above discussion focuses on forces at the *S. oneidensis*-goethite interface as a bacterium *approaches* the (010) surface of goethite. Now we will turn our attention to forces detected as a bacterium is pulled away from the mineral surface. Measurements upon retraction reveal strong attractive forces between the cell and goethite surface that extend outwards to over 300 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. These adhesive bonds formed very rapidly as the time required to collect each approach-retraction cycle was less than ~1 second.

A notable feature of these retraction data, particularly for those experiments in which *S. oneidensis* were allowed a longer period of time in contact with goethite (Figure 2B and 2C), are saw-tooth like features. Research by others has lead to the hypothesis that *S. oneidensis* produces proteins to shuttle electrons from the cell to ferric iron in oxyhydroxides, like goethite, under anaerobic conditions. 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 model, can be used to

determine the force (F) necessary to stretch a polypeptide chain in a solvent 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 (i.e., length of the stiff segment of the chain, which is 0.4 nm for the distance between two C_α atoms in a polypeptide), and L is the contour length (i.e., length of the completely stretched polypeptide chain, which can be estimated by knowing a protein's molecular weight and assuming 110 Da per amino acid residue). With this model one can plot a theoretical relationship between force and distance (or extension length, x), and compare it to the experimentally derived extension data embodied in the retraction profiles (e.g., see Figure 2B and 2C). Figure 2 shows that the measured data seem to exhibit a signature for a 150 kDa protein (Figure 2B) as well a smaller 126 kDa protein (Figures 2c). Interestingly, other researchers (e.g., C. Myers) have detected a 150 kDa protein in the outer membrane of *S. oneidensis*, which is a putative iron reductase. Further, the hypothetical proteome for *S. oneidensis* contains several high molecular weight proteins that are putative outer member proteins. For example, a search of the genome of *S. oneidensis* (<http://www.tigr.org/tdb/mdb/mdbcomplete.html>) for putative cell envelope proteins reveals the following proteins, arranged by molecular weight (in kDa): 125.6, 134.9, 145.6, 145.9, 148.2.

In previous studies we have shown that *S. oneidensis* does not display these types of protein signatures for either diaspore (ALOOH) under aerobic or anaerobic conditions, nor for goethite under aerobic conditions. Taken together, this suggests that *S. oneidensis* produces specific proteins to mediate contact with the (010) surface of goethite under anaerobic conditions (i.e., when electron transfer is expected to occur between the *S. oneidensis* and ferric iron). In a

complementary study, Dr. Brian Lower (Virginia Tech) has used two-dimensional gel electrophoresis to study protein expression patterns for *S. oneidensis* grown with oxygen versus Fe(III) as the terminal electron acceptor. His results confirm that *S. oneidensis* produces high molecular weight proteins (e.g., proteins of ~126 and 150 kDa), but only under anaerobic conditions with Fe(III) as the terminal electron acceptor. These results will be presented in a Report prepared by Dr. Hochella.

Conclusions

This project was very successful during the ~1 year of research conducted at the University of Maryland. The research resulted in 3 abstracts and one manuscript. The collaboration between the University of Maryland and Virginia Tech has proven to yield complementary data. Our collective efforts have shown that *S. oneidensis* seems to recognize the surface of specific iron oxyhydroxides (e.g., goethite) such that it produces mineral-specific proteins.

Publications

Abstracts

- 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.

Articles

- Kendall, T.A. and Lower, S.K. (2004) Intermolecular forces at the interface of biological and mineralogical particles. *Advances in Agronomy*. 82: 1-54.