

## **Nanoscale Processes in the Environment: Nanobiogeochemistry of Microbe/Mineral Interactions**

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This project involves the application of nanoscience to the fields of fundamental and applied environmental geochemistry and biogeochemistry [1-4]. There is also an educational outreach component to this study which involves the development of nanoscience curriculum components for high schools in Virginia, and potentially nationally. Abstracted descriptions of the research and educational project accomplishments to date can be found below. Methods and tools used in our work include atomic force microscopy (used primarily as a force sensing system, but also for imaging), confocal laser scanning microscopy, transmission and field emission scanning electron microscopy, *ab initio* and semi-empirical molecular modelling, DLVO modelling, mineral synthesis methods, porous media flow columns, and internet site development. The research team for this project consists of four professors, one research professor, one post-doc, and three PhD candidates, all at Virginia Tech. Fields of expertise covered by the group include mineralogy, geochemistry, biogeochemistry, environmental microbiology, cognitive teaching and learning, and educational outreach.

***Probing ligand-mineral interactions at the nanoscale:*** We have developed a chemical force microscopy (CFM) technique that probes the forces of interaction in aqueous solution between complex organic molecules and mineral surfaces in both distance and force nanospace. Using this method, previously unknown interactions between microbially produced siderophores and iron oxide mineral surfaces have been revealed [5].

Iron is a required nutrient for all organisms including bacteria, fungi and plants. The insoluble nature of iron in oxidizing, circumneutral aqueous environments, however, limits concentrations to levels well below the  $10^{-7}$ - $10^{-8}$  M required for bacterial growth. A response to this limitation is the extracellular release of low molecular weight biomolecules known as siderophores. With formation constants ( $K_f$ ) on the order of  $10^{20}$ - $10^{50}$ , the siderophore-Fe(III) complex is highly stable and thermodynamically favorable under environmental conditions. Indeed, much is known about siderophore interaction with soluble sources of iron; however, the largest source of iron in soils are solid forms, specifically, oxide minerals. Siderophores are known to release ferric iron from minerals, but the nature of the interaction of siderophores with the surface and the associated dissolution mechanism has been unknown. The CFM techniques used in this study have allowed us to directly measure the forces of interaction between siderophores and mineral surfaces for the first time [5]. These measurements give important clues about the specific nature of these interactions.

A protein coupling technique was employed to covalently attach the siderophore azotobactin to a hydrazide terminated AFM tip. The activated tip was probed against two minerals: goethite ( $\alpha$ -FeOOH) and its isostructural Al-equivalent diaspore ( $\alpha$ -AlOOH). Upon contact with each mineral surface, the adhesion force between azotobactin and the iron containing goethite was two to three times the value observed for the isostructural Al-equivalent diaspore. The relative force affinity for the iron containing mineral (versus aluminum) correlates with the difference between the aqueous complex formation constants estimated for azotobactin and Fe(III) (aq) ( $K_f=10^{28}$ ) and Al(III) (aq) ( $K_f\sim 10^{16}$ ). Further, the adhesion force between azotobactin and goethite significantly decreases (4 nN to 2 nN) when small amounts of soluble iron ( $0.1 \mu\text{M FeCl}_3\cdot 6\text{H}_2\text{O}$ ) are added to the system at pH 3.5 suggesting a significant specific interaction between azotobactin's chelating groups and the mineral surface. Specifically, plateau features in the force data generated upon tip retraction from

the surface indicate these chelating groups are associated with azotobactin's C-terminal hydroxamate moiety.

***Nanoscience studies of the transport of microparticles (including bacteria) in porous media:***

Sticking efficiency ( $\eta$ ) is the probability of attachment of a particle to a collector and is determined by the forces of attraction and repulsion between these surfaces at the nanoscale. In this study the sticking efficiency of carboxylated polystyrene microspheres was derived from force versus distance data collected using atomic force microscopy (AFM). The small, spherical, negatively charged microspheres are a suitable force measurement substitute for a single bacterial cell. The microsphere-silica glass system provides an experimental foundation for future experiments involving bacterial cells and mineral surfaces. Individual microspheres measuring 1  $\mu\text{m}$  in diameter were attached to the ends of silicon nitride cantilevers and brought into contact with a silica glass collector surface in aqueous solution. Contact-mode AFM was used to measure the interaction forces with picoNewton resolution as the surfaces were moved together and subsequently separated at a rate of approximately 3  $\mu\text{m}/\text{sec}$ . Intersurface potential energies were determined by integrating force data with respect to sphere-collector separation distance. Sticking efficiencies are calculated from potential energy using an established method that describes deposition of Brownian particles by convective diffusion. Using this method and our measurements, the sticking efficiency of a carboxylated microsphere adhering to a silica glass surface was measured to be  $2.92 \times 10^{-7}$  equating to adhesion of only 0.00003% of all particles colliding with the collector surface. Although the measured sticking efficiency is low, it is not unreasonable. Each surface has a negative zeta potential at the experimental pH and a relatively strong repulsive barrier to adhesion.

The preliminary results of this study show a promising new method of calculating sticking efficiency using force curve data. Using the AFM technique, sticking efficiencies can be determined directly from measurable forces of interaction between the particle and collector surfaces. This direct method may more accurately describe the transport of microparticles (including bacteria) in porous media by incorporating the nanoscale interactions that control attachment at the nanoscale.

***Manganese oxidation promoted by hematite ( $\alpha\text{-Fe}_2\text{O}_3$ ) particles as a function of particle size:***

Geoscientists have become increasingly aware of the presence of nanocrystalline materials in the solid Earth, hydrosphere, atmosphere, and biosphere. Many of these materials have been observed in laboratory systems to exhibit shifts in electron band energies related to quantum confinement. The phenomenon of properties that change as a function of size is expected in geological environments and must have implications tied to geochemical reactivity; these implications have remained largely unexplored.

We are using a very important geochemical process, the oxidation of manganese, as a test of one possible importance of quantum confinement in the environment and over Earth's history. The mineral hematite acts as a catalyst during the initial stages of the reaction between dissolved Mn(II) and oxygen. Coordinatively undersaturated functional groups at the mineral surface donate electron density to adsorbed Mn(II) ions, causing them to be more susceptible to reaction with dissolved oxygen. We have recently synthesized  $<10$  nm particles of hematite, and soon intend to run a series of kinetic experiments using particles with well-defined sizes in the nanorange. We expect the reactivity (and thus the rate of the oxidation reaction) of different size particles to vary due to quantum size effects altering the electron density distribution between the hematite surfaces and adsorbed manganese. As a result of the complex geometry and chemistry of the hematite surfaces it is difficult to predict the reactivity trends, thus making these experiments all the more important.

However, we may gain valuable predictive insight by applying *ab initio* molecular orbital calculations to this system based on our previous work in related areas [6].

***Development for secondary school science curricula:*** The goal of this curriculum development project is for teachers to collaborate with scientists to bring the nanoscience and technology initiatives out of the labs and into secondary science classrooms. In support of the National Science Education Standards of 1996, our curriculum development project will enable teachers to learn about "cutting-edge" scientific research protocols by working with practicing scientists in the laboratory and collaboratively as a team to develop nanoscale science curricula that can be disseminated and shared with colleagues. To achieve this goal, we conducted the first one-week teacher staff development workshop at Virginia Tech in June 2002 in which project scientists presented scientific content related to nanoscience and its applications. Teachers from regional high schools worked with scientists in their labs using equipment such as the atomic force microscope and field-emission scanning electron microscope to gain practical experience in techniques used to investigate microbe/mineral interactions at the nanoscale. Teachers, researchers, and specialists in national science education standards and curriculum development participated in computer-assisted mathematical modeling and problem-solving to interpret their data, brainstormed about how to incorporate nanotechnology into lesson plans, and planned the four year project in which curricula will be written, tested and revised for broader dissemination in a CD format. As part of the dissemination efforts of the nanoscale curriculum project, a website is being developed to provide a tool for teachers and scientists to communicate with each other and post work-in progress related to curricula development and research. On-line web discussion boards, administering assessment tools, and asynchronous teamwork on papers and presentations are examples of ways in which the project will be facilitated through the website.

## References

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