X-ray microprobe investigations of mineral-metal-microbe interfaces

K.M. KEMNER[1], S.D. KELLY, [1] M.I. BOYANOV[1], B. LAI[1], S. GLASAUER[2], S. LANGLEY[3], C.F. KULPA, JR.[4], T.J. BEVERIDGE[1] AND K.H. NEALSON[5]

[1]Argonne National Laboratory, Argonne, Illinois. [kemner@anl.gov, skelly@anl.gov, mboyanov@anl.gov, blai@aps.anl.gov]
[2]University of Guelph, Guelph, Ontario. [sglasauer@uoguelph.ca, tjb@uoguelph.ca]
[3]University of Ottawa, Ottawa, Ontario. [Lang053@uottawa.ca]
[4]University of Notre Dame, Notre Dame, Indiana. [Kulpa1@nd.edu]
[5]University Southern California, Los Angeles, California. [knealson@usc.edu]

Bacteria have a key role in determining a contaminant's speciation and thus its mobility in the environment. The metabolism and surface properties of bacteria can change greatly when the cells are planktonic (free floating) versus in a biofilm (surface adhered), and the microenvironment in and near actively metabolizing cells can differ significantly from the bulk environment. To understand the microscopic physical, geological, chemical, and biological interfaces that determine a contaminant's macroscopic fate, the spatial distribution and chemical speciation of contaminants and elements that are key to biological processes must be characterized at micron and submicron lengths. We have used x-ray fluorescence microscopy (100-nm spatial resolution) and microspectroscopy to investigate the spatial distribution of 3*d* elements in both planktonic and surface-adhered *Pseudomonas fluorescens* cells, as well as the chemical speciation and distribution of Cr introduced to the cells as Cr(VI). We used similar approaches to investigate the distribution and chemical speciation of iron in internal iron-rich precipitates in *Shewanella oneidensis* CN32. The objectives were to (1) determine the spatial distribution, concentration, and chemical speciation of metals at, in, and near bacteria and bacteria-geosurface interfaces; (2) identify the metabolic processes occurring within the microbes; and (3) identify the interactions among metals, mineral surfaces, and bacteria near these interfaces. Results will be presented.

Fishing at the nanoscale

Z. SUO[1], F.T. ARCE[1], R. AVCI[1], B. SPANGLER[2], M.H. SCHWEITZER[3], K. THIELTGES[2], J. WITTMEYER[3] AND R. BOYD[1]

[1]ICAL, MSU Physics, EPS 264, Bozeman, MT 59717 USA (avci@physics.montana.edu)
[2]MSU Chemistry, Bozeman, MT 59717 USA
[3]NC State, Raleigh, NC 27695

We give a broad description of AFM in the study of biological systems at nanoscale and focus specifically on the chemistry of tethering biomolecules to AFM tips. The motivation behind this work is that, once fully developed, this approach has great potential for detecting and localizing receptor-ligand interactions in the natural environment of the biomolecules found in the Martian meteorites. The interaction of an unmodified AFM tip with biomolecules has been used extensively in pull-off experiments with biomolecules such as collagen and titin to probe their mechanical and physical properties. We have used such an approach to determine the integrity and functionality of collagen molecules dating back to the Cretaceous period. However, the interaction of the unmodified tip with the biomolecules is a detriment in the study of specific receptor-ligand interactions. Hence, we used oligo(ethylene-glycol) and poly(ethylene-glycol) to prevent the nonspecific binding of proteins to the tip surface by adopting a *flowers-in-the-meadow* approach. This approach employs a mixed monolayer consisting of *short* "*meadow*" tethers interspersed with *longer*, antibody-terminated "*flower*" tethers. We examine several methods for passivating the AFM tip surface with novel *bidentic* tethers, which provide a stronger connection to the surface because the removal of each tether molecule from the surface requires *simultaneously* breaking the two bonds. Furthermore, we find that the *p*H value of the buffer used for these studies has considerable influence on the nonspecific binding between the antibody attached to the tip surface and the Cytochrome *c* protein chosen for these studies. By manipulating the tip modification and the *p*H of the buffer we are able to minimize the contribution of nonspecific binding to the AFM force curves and isolate the specific antibody-antigen interaction events.

Optical detection of organic molecules in extreme environments

P.G. CONRAD[1], A.L. LANE[1], R. BHARTIA[1], AND W.F. HUG[2]

[1]Jet Propulsion Laboratory, Cal. Inst. of Technology, 4800 Oak Grove Dr., Pasadena, CA 91109, (conrad@jpl.nasa.gov, Arthur.L.Lane@jpl.nasa.gov, Rohit.Bhartia@jpl.nasa.gov)
[2]Photon Systems, Inc. 1512 Industrial Park St., Covina, CA 91722 (W.Hug@photonsystems.com)

In situ detection and identification of organic molecules in extreme environments on Earth can be almost as challenging as it is on another planet, depending upon the nature of the "extreme" classification. For example, deep sea deployments are particularly challenging because of high pressures, cold, and the physical properties of water (eg., absorption of infrared light, etc.). We have successfully used an optical instrument that measures laser-induced native fluorescence (LINF) and resonance-enhanced Raman scattering in several extreme environments, including deep sea hydrothermal vents, arctic and antarctic deserts, and hot deserts. The context of these measurements has been to provide targeting of rock samples for more rigorous analysis of chemical biosignature content. Our overall goal is development of a rapid survey instrument for deployment *in situ* on another planet, for detection and characterization of organic molecules.

Here we summarize the results of the LINF and Raman scattering measurements in the polar deserts (Svalbard and Antarctic Dry Valleys), Death Valley, CA (USA) and underwater at Pacific Hydrothermal Vent sites, presenting our state of the art with respect to molecular specificity and limits of detection. We also present the effects of environmental factors such as temperature, pressure, humidity, ambient light and lithology on the detection of organic molecules in these extreme environments.

Interaction of amino acids and peptides with minerals to produce biosignatures observable by laser desorption fourier transform mass spectrometry

J.R. SCOTT[1], B. YAN[2] AND D.L. STONER[3]

[1]Chemistry Department, Idaho National Laboratory, Idaho Falls, ID 83415-2208 (scotjr@inel.gov)
[2] Department of Chemistry, University of Idaho, Idaho Falls, ID 83402 USA (yanbz@if.uidaho.edu)
[3]Department of Chemistry, University of Idaho, Idaho Falls, ID 83402 USA (stondl@if.uidaho.edu)

Unequivocal identification of life within the fossil record and understanding present day biogeochemical processes depends on our ability to accurately characterize mineralogical or chemical signatures that have arisen from the interaction of microorganisms with geological matrices. Laser desorption mass spectrometry (LDMS) has been proposed as a method to search for biosignatures on mineral surfaces for identifying signs of past or present terrestrial or extraterrestial life. Matrix-assisted laser desorption/ionization (MALDI), coupled with mass spectrometry, has been highly successful for analyses of biomolecules. MALDI uses a matrix, usually an aromatic carboxylic acid, to interact with the laser light to desorb from the surface and carry the biomolecule analyte into the gas phase. In addition, the matrix assists in ionizing the analyte directly or indirectly. The analysis of mineral surfaces by MALDI requires overspraying the surface with a matrix. In some cases it may not always be practical or prudent (e.g, space exploration) to utilize a matrix and solvent technique (i.e., MALDI) to search for signs of life. Without the use of a matrix, it will be necessary to rely on the mineralogy associated with the biomolecule for desorption and ionization processes.

To achieve our long term goals of examining microbial biosignatures that are present in heterogenous minerals, we are examining the efficacy of LDMS. The interactions of a range of biomaterials and minerals are being examined in our laboratory in order to assess and optimize the conditions for LDMS observation of biosignatures. We have observed that different minerals have varying desorption efficiencies depending on laser wavelength. Furthermore, the types of cations formed during the desorption event vary depending on the mineral type, which is important because ionization of the bioanalytes occurs through cation attachment. In this study we compare the production of biosignatures from sodium and potassium rich surfaces with minerals dominated by iron.

DGT, microsensor and molecular genetic characterization of biogeochemical processes in an extreme arctic environment

LIANE G. BENNING[1], ROB G. J. MORTIMER[1] AND ANDREW STEELE[2]

[1]Earth and Biosphere Institute, School of Earth and Environment, University of Leeds, Leeds. LS2 9JT, UK (liane@earth.leeds.ac.uk)

[2]Geophysical Laboratory, Carnegie Institution of Washington, Washington DC, 20015, USA

Here we present data from field-testing of modern geo-biological techniques in the extreme environments of the northernmost arctic warm springs (Jotun springs, 79.5°N, Bockfjord, Svalbard, Norway). These springs are characterized by ~ 2mm to 4 cm thick layered bacterial communities and mats, which result from extreme redox, biological and inorganic gradients.

High-resolution DGT (diffuse gradient thin film gels), and high-resolution microsensor (pH, O_2 and H_2S @ mm scale) measurements helped elucidate the depth behaviour of metals and redox active species across the interfaces between the 3 main redox zones. In addition, field-based molecular genetic characterization of the bacterial communities and laboratory based microscopic characterization of the inorganic and biogenic components in these layers when combined with the high-resolution DGT and redox measurements helped reconstruct a detailed biogeochemical profile.

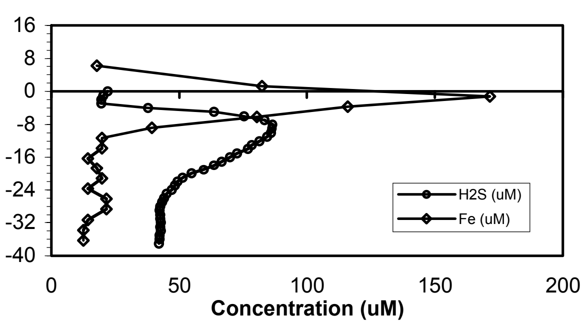

Figure 1. Depth profile in the Jotun3 spring showing the Fe and H_2S behaviour accross the O_2/H_2S interface (at 0 mm)

Viruses from extreme environments

KENNETH STEDMAN[1]

Department of Biology and Center for Life in Extreme Environments, Portland State University. (kstedman@pdx.edu)

Viruses are ubiquitous and vastly outnumber microbes and other organisms in all environments studied to date (Brussow and Hendrix, 2002). Viruses from extreme environments have only recently been discovered and are poorly characterized relative to animal viruses and viruses in marine environments. Characterized viruses of extremely thermophilic Archaea have unique morphology unlike any other viruses. Additionally, their genome sequences do not resemble any other known sequences (Prangishvili et al., 2001). Study of these viruses has led to insights into virus origins, evolution and antiquity (Rice et al., 2004), the stability and structure of viruses as well as the development of genetic tools for the study of thermophilic Archaea (Jonuscheit et al,. 2003). These viruses offer unique opportunities to study the role of viruses in hydrothermal environments and their potential for preservation. The current state of knowledge of viruses from extreme enviroments will be discussed.

References

Brussow H. and Hendrix R.W., (2002) *Cell* **108** 13-16.

Jonuscheit M., Martusewitsch, E., Stedman, K. M. and Schleper C., (2003) *Molecular Microbiol.***48** 1241-1252.

Prangishvili D., Stedman, K.M., and Zillig, W. (2001) *Trends in Microbiology* **9** 39-43

Rice G. Tang, L., Stedman, K., et al. (2004) *Proc. Natl. Acad. Sci. USA* **101** 7716-7720.

A mechanism for preservation of ~3.5 billion-year-old microbial alteration textures in pillow basalts from the Barberton Greenstone Belt

N.R. BANERJEE[1,2], H. FURNES[1], T. CHACKO[2], K. MUEHLENBACHS[2], H. STAUDIGEL[3], AND M. DE WIT[4]

[1]Department of Earth Science, University of Bergen, Allegt.41, Bergen 5007, Norway (banerjee@ualberta.ca)
[2]Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, AB T6G 2E3, Canada
[3]Scripps Institution of Oceanography, University of California, La Jolla, CA 92093-0225, USA
[4]CIGCES, Geological Sciences, University of Cape Town, Rondebosch 7701, South Africa

Numerous studies of volcanic basaltic glass from the oceanic crust have demonstrated the importance of endolithic microbes in the alteration process. Microbial dissolution features are ubiquitous within the upper few hundred meters of modern oceanic crust, having been discovered in basalts of all ages, wherever fresh glass is preserved. Recent work in ophiolites and greenstone belts has extended the evidence for microbial alteration of oceanic basalts as far back as the Archean in ~3.5 Ga pillow lavas from the Barberton Greenstone Belt (BGB) in South Africa [1]. The BGB pillow lavas are metamorphosed to greenschist facies but are exceptionally well-preserved and undeformed in places. The formerly glassy rims of the BGB pillow lavas are easily recognized in outcrop and contain micron-sized, microbially generated, tubular structures mineralized by titanite. These structures are interpreted to have initially formed during microbial etching of the originally glassy pillow rims. Here we present new data from pillow lavas in recent oceanic crust and Phanerozoic ophiolites that elucidates an early and effective mechanism for the preservation of delicate microbial etching textures. We demonstrate that titanium is enriched in areas of microbial dissolution of basaltic glass. Early seafloor hydrothermal circulation of calcium- and silica-bearing fluids soon after formation of the microbially etched tubes causes the residual titanium to be sequestered in titanite. The early precipitation of sub-micron titanite grains within the biologically etched tubes serves as an agent for preservation that may persist for geologically extended periods of time in the absence of later penetrative deformation.

References

[1] Furnes et al., (2004) *Science* **304**, 578.

Dinosaur soft tissues

MARY HIGBY SCHWEITZER[1], JENNIFER L WITTMEYER[2], JOHN R HORNER[3] AND RECEP AVCI[4]

[1]North Carolina State University and North Carolina Museum of Science, and Museum of the Rockies, Montana State University, schweitzer@ncsu.edu
[2]North Carolina State University wittmeyer@ncsu.edu
[3]Museum of the Rockies, Montana State University jhorner@montana.edu
[4]Dept. of Physics, Montana State University avci@physics.montana.edu

In the summer field season of 2003, the Museum of the Rockies recovered elements of the oldest recorded Tyrannosaurus rex at the base of the Hell Creek Formation in Eastern Montana. Demineralization of bone tissues from the femur of this animal revealed the presence of soft, transparent and pliable soft tissues. In addition to the soft vessels, three populations of microstructures with cell-like morphology and internal structure were identified. These microstructures exhibited at least two different modes of preservation. Preliminary analyses indicate that original molecular components may be preserved as well. We examine the modes and degree of preservation, and discuss possible biogeochemical and environmental interactions that may have contributed to preservation.

Direct detection and discovery of gene resources from the environment

YUTAKA KAWARABAYASI

AIST, higashi 1-1, tsukuba, Ibaraki 305-8566, Japan (kawarabayasi.yutaka@aist.go.jp)

Although many cultivable microorganisms, including thermophiles and other extremophiles, were isolated, it is generally said that over 99 % of total microbes are now still uncultivable. These uncultivable microbes are expected to influence to the surrounding environment, e. g. effect to gas concentration or metal selection. Thus, it can be thought that identification and monitoring of such uncultivable microbes alive in the environment are necessary for recognition of their influences.

For detection of uncultivable microorganisms, the shotgun libraries were constructed from the environmental DNA prepared from Southern Eastern Pacific Rise (S-EPR), Suiyo seamount and Beppu hot spring in Japan. End sequencing of the randomly selected clones in each library indicated that there is no sequence in the public database identical to the end sequences, revealing that all sequences isolated from the environment were novel and unique. As results of determination of entire nucleotide sequence and detection of protein-coding regions, the clones with eukaryotic features, containing the intron-like sequences or tetraplet repetitive sequences, were dominantly detected in the S-EPR library. Analyses of entire sequences of clones from Beppu hot spring and Suiyo seamount indicated that the microorganisms were dominant in both sampling places. The aminoacyl-tRNA synthetase genes isolated indicated the evidence that novel archaea and bacteria are present in both the thermal places. To investigate thermostability of each gene products, the ORFs isolated on the clones from Suiyo seamount were used for construction of expression vector in *E. coli*. 50 % of gene products were recovered as soluble proteins, then the maximum temperatures stable after treatment at different temperature were analyzed. The results indicated that the proteins encoded on the same DNA fragment exhibited similar features of thermostability.

To identify the novel genes from the environment, shotgun library with 4 kb insert fragment was constructed using DNA prepared from Kirishima hot spring. Each DNA molecule prepared was stored in the independent wells in 96-well plates. It was revealed that the DNA molecules mixed in each line, column and plate were useful for searching novel genes from the environment, by checking of identification of genes homologous to the well-characterized ones.