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BIOspheres of Mars: Ancient and Recent Studies

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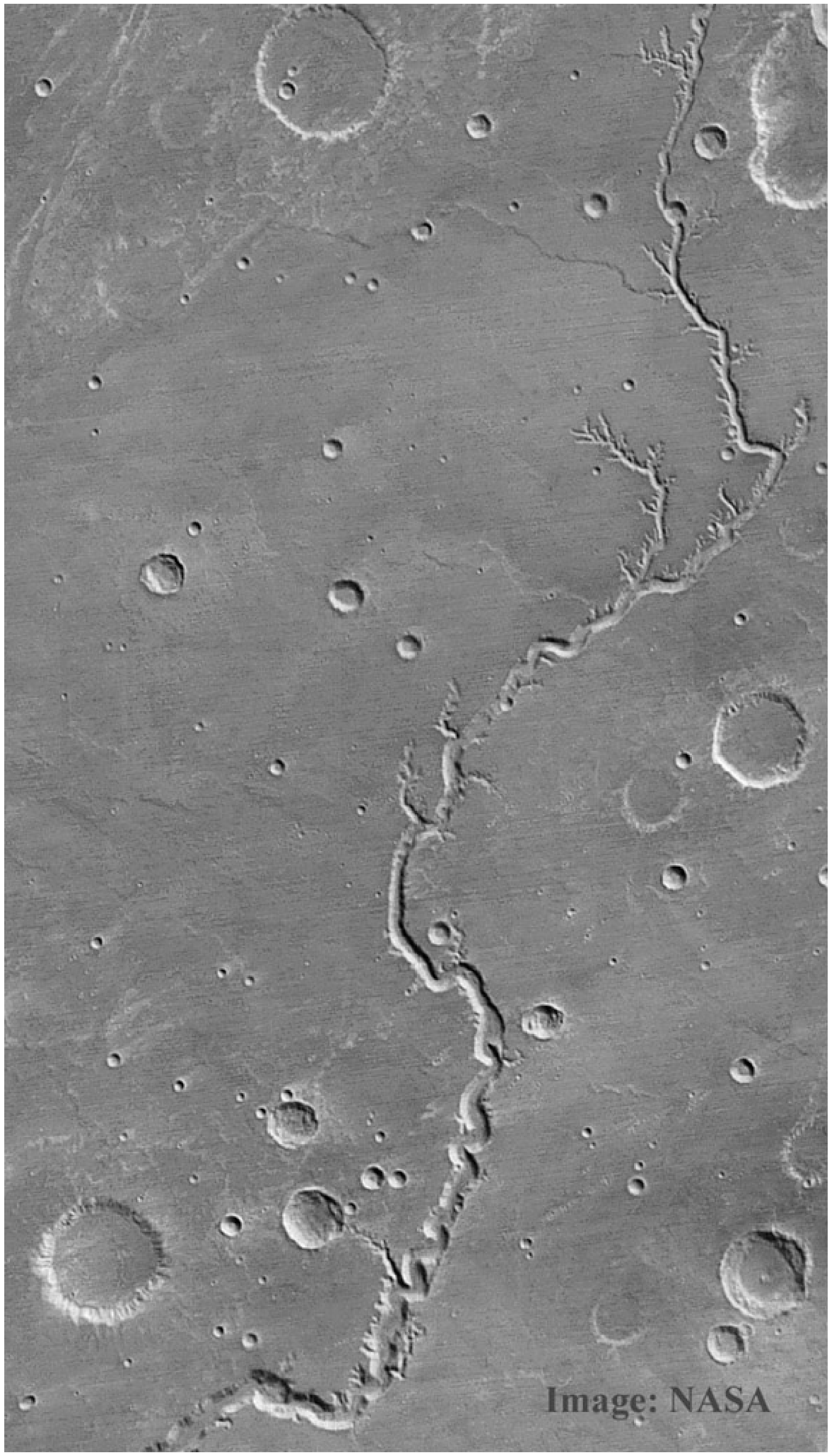
Bio M A R S



A proposal in response to Cooperative Agreement Notice 02-OSS-02
to become a member of the NASA Astrobiology Institute

- BIOMARS -

BIOsphere of **M**ars: **A**ncient and **R**ecent **S**tudies

Submitted by:

The Regents of the University of California
The Center for Integrative Planetary Science*
University of California Berkeley
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* Unit will administer award

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American Type Culture Collection
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VOLUME I

EXECUTIVE SUMMARY

Overview: Mars is an exciting, and comparatively accessible target for astrobiological studies aimed at detection of current or past extraterrestrial life. We will analyze the evolution of the Martian hydrosphere and surface topography to understand the history of water distribution and investigate atmospheric processes that may have contributed to a UV shield. Our goal is to identify the types of sites on Mars that experienced long-term fluid flow and may be, or have been, conducive to life. We will characterize biomes that develop in analogous Earth environments, conduct experiments to determine limitations for life in these habitats, and identify features that constitute indicators of life. We propose robot-based sampling and *in situ* analyses of terrestrial sites so as to develop methods for dealing with the challenges of remote geomicrobiological investigations. Our work will provide constraints for selection of optimal sites for future Mars exploration and methods for sample analysis, and ultimately will be relevant to the question 'did life evolve elsewhere in the universe'.

Habitat constraints from Mars and modeling: Early microbial life probably originated on Earth in environments characterized by redox disequilibria. Habitats may have developed on Mars in redox gradients between reduced basaltic rocks and oxidized fluids and/or gases. Element cycling driven by fluid flow through such redox gradients could underpin (or could have underpinned) a substantial biosphere. We will analyze Mars planetary evolution to develop models for the timing and scale of hydrosphere development and subsurface water circulation. We will couple these hydrosphere models to geomorphological models based on terrestrial field site analyses and experimental geomorphological studies to allow detailed interpretation of Mars surface features. This will permit analysis of the history, form, and timing of fluid flow events that shaped the planetary surface and determination of the factors that control them. In parallel, we will explore atmospheric processes that could have contributed to a UV shield and conduct spectroscopic studies to constrain Mars surface mineralogy.

Habitat constraints from Earth: Studies of chemoautotrophically-based ecosystems will focus on terrestrial aqueous environments in basaltic rocks similar to those at the Martian surface. Hydrology, geomorphology, spectroscopy, and geomicrobiology research will begin at sites where groundwater discharge in basaltic andesites or basalts is generating channels with features similar to those on Mars. These springs appear to offer the best chance of sustained water flow and protection from UV radiation. Initially, our studies will be conducted at cold and warm springs associated with basaltic rocks in dry, cold desert environments in Oregon and Idaho. We will refine our choice of study sites as our understanding of Mars' surface improves.

Abundant, redox-active species such as iron and sulfur represent potential energy sources for possible Martian life at springs in basaltic rocks. Recent microbiological studies, geochemical calculations, and experiments indicate that the kinetics of both Fe-silicate and Fe-sulfide mineral dissolution reactions are fast enough to sustain significant biological populations. We will characterize currently poorly understood microbial habitats in the near subsurface in terms of their population structure, aqueous geochemistry, mineralogy, and isotopic signatures in order to determine the form of the record life might leave in similar Martian systems.

Laboratory constraints for habitat development and biosignatures: Results of *in situ* analyses of terrestrial ecosystems will be paralleled by laboratory-based studies that will explore the ranges of temperature, concentration, and pH consistent with life in the these habitats. Biochemical analyses will explore the factors that set these limits. We will analyze the structure, elemental and isotopic composition, microstructure, morphology, and distribution of minerals generated by, or impacted by, life in basaltic-rock hosted systems so as to develop and test potential new biosignatures. Parallel inorganic experiments will be conducted in order to resolve non-biological features and to examine changes in mineralogical biosignatures with time. As yet unstudied isotopic characteristics of Martian meteorites will be determined in order to provide baselines for isotopic biosignatures. Similarly, work on the isotopic evolution of the atmosphere will establish the magnitude and form of non-biological isotope fractionations. Application of state-of-the-art methods for analysis of Martian, and Mars-like rocks will yield procedures that will be useful for future analysis of samples returned to Earth or encountered during remote analysis on the Martian surface. Our results will contribute to selection of sites on the Martian surface with the highest potential for future detailed in situ investigations.

Our team: Our goal is to create a highly interactive, focused NAI team to address a well-defined set of problems. The necessary interactions will be facilitated by close geographic proximity of most team members. Five of the 10 scientific team members are at UC Berkeley, one is at the SETI Institute, and one in Palo Alto. The three other CoIs have essential expertise for study of difficult to cultivate Fe-oxidizing neutrophiles and in situ measurements. All three non-Bay area CoIs have collaborated with the PI on a NASA-funded seed project preliminary to this proposal. Communication between all CoIs will be promoted through work on common sites and processes, shared goals, and virtual (internet-based conferencing) and traditional group meetings.

Our group includes members with strong, integrated field and laboratory-based research programs and experience with study of a diversity of natural environments. Several team members are expert in the development and deployment of state-of-the-art analytical methods (e.g., isotopic analyses, microsensor measurements) to interdisciplinary problems. Our group also includes a robotics engineer and scientists familiar with ancient and recent Mars planetary history. The NAI support will be essential to facilitate the new interactions between hydrologists, geomorphologists, geomicrobiologists, chemists and engineers that are needed to meet the project goals.

Education and pubic outreach: The topics of life on Mars, life in extreme environments, and extraterrestrial exploration easily capture public attention. Our group will use the broad appeal of these subjects to create educational materials designed to foster interest in science, especially geology, chemistry, and biology. Educators from the University of California Berkeley's Lawrence Hall of Science (LHS) will work closely with the BIOMARS team to develop, field-test, and implement materials that incorporate key project concepts and emphasize the interdisciplinary nature of space exploration. LHS is a public science center that is world-renowned for development of high quality middle and high school science curriculum materials based on current research and understanding of how students think and learn. Educational materials will be disseminated through the use of the LHS infrastructure, and its well-established national and international network of educators.

Summary of Personnel

Principal Investigator:

Dr. Jillian Banfield
University of California, Berkeley

Co-Investigators:

Dr. Janice Bishop,
SETI Institute

Dr. Kristie Boering
University of California, Berkeley

Dr. Donald DePaolo
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Dr. William Dietrich
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Dr. David Emerson
American Type Culture Collection

Dr. George Luther
University of Delaware

Dr. Michael Manga
University of California, Berkeley

Dr. Eric Roden
University of Alabama

Dr. Mark Yim
Palo Alto Research Center

Education and Public Outreach

Kevin Cuff
Lawrence Hall of Science

Dr. Herbert Thier

RESEARCH, TRAINING, MANAGEMENT

1) INTRODUCTION

Is there, was there, life on Mars?

Life may take many forms. However, all life forms require a bioavailable source of energy. The range of energy types utilized by modern life on Earth is vast. However, the range of abundant energy sources available to early or primitive current life on Mars is probably more restricted. Disequilibria between reduced components of Martian rocks or hydrothermal fluids (e.g., H_2, Fe^{2+} and S^-) and oxidized constituents of the soil (Foley et al. 2001; Bell et al. 2000), dust, and atmosphere (e.g., Fe^{3+}, O_2) provide specific targets.

Observations of Earth systems reveal that energy utilization causes changes in elemental and isotopic composition, and in the structure, morphology, and distribution of minerals. These changes constitute biosignatures that can potentially be interpreted to answer the question “is there, was there, life on Mars?”. These biosignatures may be interpretable even if the form of life differs from that familiar to us on Earth.

Schieber (2001) noted that subsurface depositional environments are likely to provide more frequent and diverse opportunities for preservation of biosignatures than the shallow-water carbonate structures (e.g. stromatolites) that have been the mainstay of life detection in the early Earth rock record (Knoll, 1989; Schopf and Klein, 1992; Schopf, 1993). Subsurface habitats characterized by redox disequilibria and protection from UV radiation may be important in the search for evidence for microbial life on Mars. Such sites are probably also the only ones with long term access to water.

We consider it probable that if life forms evolved on Mars, biochemical functions would require at least some water. Recent studies indicate that water occurs in the modern Martian subsurface and that discharge of some type of fluid has occurred, at least episodically, at the surface of Mars. We contend that it is possible to develop a detailed understanding of the Martian hydrosphere and history of fluid discharge through integration of hydrological and geomorphological modeling, experimental studies, and field data for analog systems. Likely sites for development of ancient and recent Martian biomes (defined here as a chemically and biologically distinctive habitat) may be identified by targeting environments that provide energy, water, and protection from UV radiation. Based on study of patterns and locations of fluid discharge on Mars, we will select a tractable number of appropriate Earth analog sites for detailed geomicrobiological and chemical analyses. Our goal is to determine how to detect evidence for past or current life on Mars through analysis of these analog systems. Our work will involve studies of microbial communities and their biosignatures at these field sites and in the laboratory. We will adapt existing robotic devices to permit sampling and in situ analyses of otherwise inaccessible exteme environments. This will allow us to develop appropriate protocols for remote geomicrobiological investigations. The proposed research will enable design of missions to achieve maximum probability of detecting evidence for Martian ecosystems, either through in situ measurements on Mars or in samples returned from Mars.

Setting the scene: Biome constraints from past studies of Mars

A key element of the proposed work is determination of how the Martian surface and near-surface conditions now, and over geologic time, might have provided opportunities for the emergence and proliferation of life. We begin this analysis by using the results of prior studies to constraint our approach and to lay the foundation for proposed investigations.

The rocks comprising the surface of Mars are low Mg-, low S-andesites and basalts (Bandfield et al., 2000; McSween et al., 1999). Based on data from the SNC meteorites, Martian rocks are reduced and contain primary ferrous iron (probably the most abundant, redox-active constituent of the Martian surface) and some reduced sulfur (*e.g.,* McSween, 1985). However, data collected by Viking and Pathfinder indicate that the Martian dust and soil are highly oxidized, iron rich, and that sulfate is a major component (Bell et al. 2000; Foley et al. 2001). These first order observations highlight important disequilibria between the oxidized Mars atmosphere and the reduced subsurface (Table 1). Such disequilibria could form the basis for a diversity of energy-generating systems utilized by Mars life forms. Terrestrial analogs for possible Mars biomes based on disequilibria between iron (and sulfur) compounds are targeted here.

Table I: Pathfinder data (wt %) for soils and rocks from Foley et al. (2001). Please consult the original reference for details.

SOILS	Na2O	MgO	Al2O3	SiO2	P2O5	SO3	Cl	K2O	CaO	TiO	Cr2O3	MnO	Fe2O3
A-2	4.1	9.7	9.8	40.0	0.8	5.9	0.7	0.5	5.9	0.8	0.3	0.5	21.0
A-4	4.2	9.0	9.9	40.1	1.0	6.8	0.8	0.5	5.5	1.2	0.4	0.4	20.2

ROCKS	Na2O	MgO	Al2O3	SiO2	P2O5	SO3	Cl	K2O	CaO	TiO	Cr2O3	MnO	FeO
A-3	4.7	2.4	12.1	53.2	0.6	2.0	0.5	1.1	5.6	0.7	0.1	0.3	16.6
A-7	6.1	5.9	10.6	46.4	0.5	4.4	0.8	0.7	6.4	0.8	0.1	0.4	16.9

In addition to possible chemical energy sources, it is important to consider the physical constraints (radiation, fluid distribution, temperature) that may have limited (spatially and temporally) the development of life on Mars. For example, protection from UV radiation may be an important consideration. The extent to which atmospheric processes early in Mars history could have limited radiation at the planet's surface is unclear (see below). However, UV protection afforded by rocks and the subsurface may have been important in localizing life over much of Mars' history.

A key question is the persistence of liquid water on the Martian surface over time. Given the current P-T conditions, liquid water is not stable at the surface of Mars today. There is evidence for abundant fluids early in Martian history. The flat northern hemisphere (Smith et al., 1998; Smith et al., 1999) has been argued to represent evidence for a past standing body of water (Baker et al., 1992; Head et al., 1999). The development of the ancient volcano-tectonic provinces such as Tharsis is thought to play a critical role in hydrothermal circulation (e.g., Wilson and Head, 2000) and global groundwater flow patterns (e.g., Phillips et al., 2001). There is some evidence of recent surface volcanism (Garvin et al., 2000; Hartmann et al., 1999), but these features are not associated with many possible recent water-related features. Relict geothermal systems indicate abundant hydrothermal activity early in Mars history (Farmer, 1996), and thus have been targets in the search for life on Mars (Farmer and Des Marais, 1999; Walter and Des Marais, 1993). There are also indications of possible sedimentary systems. For example, thermal emission spectrometer studies revealed a large, layered deposit of coarse-grained hematite at Sinus Meridiani (Christensen et al., 2000) that may have been deposited in water.

Surface temperatures are especially important components of analysis of potential Mars habitats and the form and distribution of water over Mars history. The cooling of the planetary interior with time and the variation of solar insolation during obliquity cycles (or tilt of the spin axis) are important contributors to Mars' subsurface and surface thermal evolution, respectively. Although a warm, wet early Mars has been proposed, it is possible that the Martian surface was not much above freezing for extended periods (Squyres and Kasting, 1994). Warm near-surface temperatures in relatively recent times may be related to obliquity cycles (Ward, 1974) that periodically increase and decrease the amount of solar insolation at the Martian surface. The obliquity of Mars is currently $\sim 25^{o}$. When obliquities exceed $\sim 45^{o}$ the amount of insolation is such that even at current levels of internal heat loss the polar caps would melt (Fanale, 1992). Thus, Mars could periodically develop liquid water close to the surface.

The long-term loss of heat generated during Mars accretion, core formation, and the decay of radiogenic elements in the interior dictates the surface heat flow (Schubert et al., 1992; Solomon and Head, 1990). The presence of old (> 4 Ga) magnetic anomalies (e.g, Acuna et al., 1999; Connerney et al., 1999) suggests that a core dynamo was active early in Martian history. The lack of younger magnetic anomalies may reflect a rapid cooling of the core, or a change in style of mantle convection from the mobile to stagnant lid regime (e.g., Nimmo and Stevenson, 2000). Ancient surface heat flow estimates from MGS gravity and topography indicate that the northern lowlands (Smith et al., 1998) was a locus of high early heat loss (Zuber et al., 2000). This spatially non-uniform surface heat flow could be consistent with low-mode convection (Zhong and Zuber, 2000) or plate tectonics (Sleep, 1994*)*. A convective model has been proposed to explain the early focused high heat flow in the northern lowlands indicated by gravity and topography data (Zuber et al., 2000). A later-stage (<4 Ga) reactivation of the Martian dynamo also has been advocated (Schubert, 2000), which implies significant cooling of the interior, but spread over a broader period of Martian history. Constraints implied by current models for the core dynamo generation cannot easily explain possible recent evidence for liquid water at the Martian surface (Malin and Edgett, 2000) but may provide useful insight into plausible ancient hydrological processes.

Surface temperatures depend in part on the properties of the Martian atmosphere. Degassing of the interior during cooling leads to the release of volatiles that likely resulted in a thicker early atmosphere and liberated water on the surface, thus producing conditions potentially favorable for the development of life. Mechanisms of atmospheric loss (Owen, 1992) and the fate of what appears to have been abundant surface water (Carr, 1996) are not completely understood.

The surface topography of Mars provides abundant evidence for fluid flow, but quantitative interpretation of the nature of the events that occurred is not yet possible. Martian geomorphological features such as sinuous channels that have eroded back into volcanic strata appear to represent 'sapping channels' that, on Earth, are known to form as the result of long term, localized groundwater discharge. If such a process yielded the channels on Mars (cover image, courtesy of NASA), the steep channel terminations should provide relatively protected habitats with sustained fluid flow. Thus, the origins of these channels represent important targets for geomicrobiological studies. We propose to explore processes that generate channels with features similar to the Martian channels and develop an understanding of the size, frequency, and timing of fluid discharge events. This will require evaluation of factors that control fluid flow in the Martain subsurface and that localize regions of surface discharge. Thus, the first major goal of our work is to answer the question:

“Where are the sites most likely to yield evidence for past or current life on Mars?”

Biome development on Mars: constraints from Earth

Although we cannot assume that, if life evolved on Mars, it took a form analogous to life on Earth, we can learn a great deal about how to search for life from studies of Earth biogeochemical systems. Even if the design principles for Martian life differ significantly from those we are familiar with, it is reasonable to assume that their metabolism leaves an imprint on the environment. In particular, many energy metabolisms that rely upon catalysis of otherwise sluggish or kinetically prohibited reactions generate products that differ from those in a metabolism-free system. For example, the presence of abundant, nanometer-sized sulfide minerals may be a biosignature because sulfate reduction is extremely slow at temperatures below 150 °C (see review in Druschel et al. 2002) and temperatures > 150 °C will lead to rapid sulfide mineral grain growth (Huang et al. 2002).

In the work proposed, we will focus on basalt and basaltic andesite-hosted oligotrophic environments that we believe are the best possible terrestrial analogs for life-sustaining environments on Mars. The sites chosen for initial work are groundwater springs in basaltic andesites and a subsurface, volcanic-hosted system where acidophilic (acid-loving) chemoautotrophic microorganisms (CO_2-fixing organisms that utilize inorganic energy sources) are sustained primarily by pyrite dissolution. Through analysis of these communities we will answer the question:

"where do microbial populations occur in basaltic and basaltic andesite terrains and what factors limit their distribution?”

We will combine spatially- and temporally-resolved information about microbial community structure, metabolic characteristics with details of mineral structure and mineral surface topography, elemental composition, and isotopic composition in order to define rigorous biosignatures for each environment. Through collaborations between microbiologists, mineralogists/ mineral physicists, and geochemists, we will answer the question:

"how can we establish the existence, or preexistence, of microbial communities through analysis of the physical and chemical characteristics of rocks and minerals?"

TEAM EXPERTISE, EXISTING LINKS, AND PROJECT MANAGEMENT

The following sections describe a proposed series of investigations, each of which will draw upon the expertise of a significant fraction of the researchers that will comprise our NAI team. Before describing these studies, we briefly introduce our investigators. More details are provided in the vitae listed in the later part of this proposal. Each of the individuals has a strong, independent research program that is directly relevant to the proposed research and some are collaborating with other members of the NAI group.

(1) Dr. Jill Banfield, University of California Berkeley

Dr. Banfield is a geomicrobiologist with a background in mineralogy, and interests in geochemistry of surface processes, microbial ecology, and microbial evolution. A major current activity in her research group is genomics-based analysis of extremely acidophilic archaea and bacteria and development of community genomics methods (Tyson et al. 2002, 2003a,b; Banfield et al. 2003; Hugenholtz et al. 2002a,b). The genomic data will be used to create gene expression arrays to monitor microbial activity in acidic biogeochemical systems. The other major focus in her group is study of the size-dependent structure, properties, and reactivity of nanoparticle products of chemical weathering and microbial metabolism. Dr. Banfield has prior experience working at both the Abert Lake and Richmond Mine field sites.

NAI focus: Geomicrobiology of Fe in acidic systems studied via a combination of mineralogical, geochemical, and molecular biological approaches (SSU rDNA analyses, genomics-enabled, microarray-based analyses. Iron-oxide/hydroxide biomineral formation and aging at neutral pH. Response of mineral surfaces to biologically-produced compounds.

Dr. Banfield has current collaborations with Drs. Emerson, Roden, and Luther (supported by a seed grant from NASA) and Dr. Dietrich.

(2) Dr. Janice Bishop, SETI Institute

Dr. Bishop is a planetary spectroscopist with expertise in mineral physics and chemical alteration on Mars. She has many years of experience measuring the spectroscopic properties of aqueous minerals (Bishop and Murad, 1996; Bishop et al., 1994, 1999, 2002b,c; Murad and Bishop, 2000). She also has experience characterizing and collecting geologic samples in the field for lab studies (Bishop et al., 1998; Bishop and Murad, 2002; Bishop et al., 2002a). Much of Dr. Bishop's work is applied to understanding the geochemical environment on Mars through characterization of the minerals observed on the surface. She is a Co-I on the CRISM hyperspectral (0.4 - 4.0 μm) instrument on the 2005 Mars Reconnaissance Orbiter. She has many years of experience measuring the spectroscopic properties of fine-grained Mars analogue materials and comparing them with spectra of Mars (Bishop et al., 1993, 1995, 1998, 2002d).

NAI focus: The spectral properties of weathered rocks in terrestrial analog sites to provide reference data for analysis of mineral alteration zones associated with sites on Mars targeted for geomicrobiological investigations. Mars surface chemistry and mineralogy.

(3) Dr. Kristie A. Boering, University of California, Berkeley

Dr. Boering is a chemist and earth scientist with expertise in experimental and observational atmospheric chemistry and its coupling to climate on earth and other planets on time scales from months to billions of years. Her research involves laboratory experiments on novel photochemical isotope effects and the generation and properties of aerosols, observations of radiatively and chemically important trace gas species from the ground to the middle stratosphere on earth, and numerical modeling to integrate from the molecular to the global scale.

NAI focus: Experimental simulations of atmospheric chemistry in Mars-like atmospheres over the course of Martian history in order to constrain climate and habitability uncertainties, including the stability of liquid water on the Martian surface and whether or not a UV shield in the form of an aerosol layer could have formed under various atmospheric composition scenarios. Experimental simulations of potential "abiotic" carbon isotope signatures generated in the Martian atmosphere which might otherwise be interpreted as a biomarker. Providing an atmospheric perspective to fellow NAI BioMARS members working on surface and subsurface processes and predicting potential habitats for life.

(4) Dr. Donald J. DePaolo, University of California, Berkeley

Dr. DePaolo is a geochemist with expertise in the application of isotopic techniques to problems in geochemistry and geophysics. He has worked on Ca isotope fractionation in food chains, Sr isotope geochemistry of the oceans, and is currently involved on studies of Fe isotope fractionation in the weathering cycle.

NAI Focus: Measurement of the isotopic characteristics of Martian meteorites to provide baselines for biosignature studies. Isotopic characterization of minerals formed in biogeochemical processes.

(5) Dr. William Dietrich, University of California, Berkeley

Dr. Dietrich is a geomorphologist interested in channel formation, soil production, hydrologic processes, and the geomorphic transport laws responsible for landscape evolution. Recently he has collaborated in studies directed at understanding the processes controlling the recent gullies on Mars.

NAI focus: Channel formation and evolution processes on Mars and terrestrial analogs in order to estimate duration and magnitude of water-driven channel processes.

Dr. Dietrich currently collaborates with Dr. Banfield as part of the NSF sponsored National Center for Earth-surface Dynamics

(6) Dr. David Emerson, American Type Culture Collection

Dr. Emerson is a microbiologist with specific expertise in culturing and isolation of novel organisms that oxidize iron in near neutral pH solutions. His research interests focus around Fe-based lithotrophy in low temperature environments (e.g., soils) and in hydrothermal vent systems.

NAI focus: Analysis of bacterial communities associated with aqueous and solid Fe(II) phase oxidation through enrichment culturing and culture-independent molecular (SSU rRNA-based) detection.

Dr. Emerson currently collaborates with Drs. Roden, Luther, and Banfield, and the NAI group at the Carnegie Institute, Washington DC.

(7) Dr. George Luther – University of Delaware

Dr. Luther is an inorganic chemist with expertise in geochemistry. His research includes the application of microsensor measurements of aqueous solutions (including those from extreme environments) to understanding biogeochemical systems.

NAI focus: Fe biotic and abiotic redox reactions, including mechanisms of electron transfer. Characterization of the patterns of dissolved Fe speciation in redox-stratified Fe cycling environments via voltammetric techniques applied at macro (cm-m scale) and micro (µm-mm scale) spatial scales. Electrode technology will be coupled with robotic systems through interaction with Dr. Yim. His group will make real time chemical speciation analyses of Fe and S in microbial culture experiments and work with other team members to demonstrate what chemical species microbes are using for growth.

Dr. Luther has current collaborations with Profs. Banfield, Emerson, Roden and other NAI teams.

(8) Dr. Michael Manga, University of California Berkeley

Dr. Manga's work in hydrogeology has focused on using springs to study subsurface hydrologic and geologic processes in volcanic arcs. New ongoing work is addressing the problem of how hydrologic systems interact with earthquakes, in particular how permeability and fluid pressure changes in response to earthquakes (a combination of field studies, theoretical work, and numerical studies) and how earthquakes respond to changes in fluid pressure (based on data analysis). Manga's research group is also involved in a numerical and experimental study of the global evolution of the Martian mantle, focusing presently on the thermochemical dynamics of early Mars.

NAI focus: Study of hydrosphere evolution on Mars through field, lab, and theoretical studies.

(9) Dr. Eric Roden – University of Alabama

Dr. Roden is an environmental microbiologist with interests in biogeochemical cycling in sedimentary environments, with a specific focus on the redox cycling of iron and the physiological ecology of Fe-reducing and Fe-oxidizing bacteria.

NAI focus: Biogeochemical studies of the structure and function of microbial Fe cycling communities using traditional and microscale chemical profiling techniques, process-level studies of microbial metabolism and mineral transformation, and molecular biological detection of functional bacterial groups.

Dr. Roden currently collaborates with Drs. Luther and Emerson on studies of microscale Fe redox cycling in layered, redox-stratified microbial communities and with Dr. Banfield on growth of Fe-oxidizing bacteria on silicate mineral substrates.

(10) Dr. Mark Yim, Palo Alto Research Center

Mark Yim heads a team at the Palo Alto Research Center called the Smart Electro-Mechanical Systems Area. Researchers in this group have degrees in a variety of fields including Computer Science, Mechanical Engineering, Electrical Engineering Product Design and Fine Art. The group focuses on the coupling of computation with electro-mechanical systems to exploit the rapid increases in computational ability to enable radically new capabilities in electro-mechanical systems.

The team has built modular, reconfigurable robot systems that have demonstrated a variety of firsts including, self-reconfiguring for locomotion with two topologically different gaits (rolling loop to snake-like), snake-like concertina gaits through unstructured holes (exploring gopher holes), a robot that can climb: stairs, fences,
poles, over loose rubble, through 4" pipes etc. While in general these systems promise to be versatile (through the ability to chance configurations), robust (through redundancy) and low cost (through batch fabrication from many repeated modules), two near term application of these systems include search and rescue and planetary exploration.

NAI focus: Adaption of modular, reconfigurable robots for geomicrobiological investigations.

(11) Mr. Kevin Cuff, Lawrence Hall of Science, University of California, Berkeley

Kevin Cuff is currently the director of the Student Radon Research Project, an NSF sponsored instructional materials development at the University of California’s Lawrence Hall of Science (LHS). Mr. Cuff is a geologist by training, with graduate experience in gas emission research on active volcanoes, who has been developing inquiry-based instructional materials and programs at LHS for the past 13 years. He is the author of several extremely popular books that contain earth science-related inquiry-based lessons for secondary school students, and has recently developed a project-based curriculum module that engages students in authentic earth science research activities.

NAI focus: Mr. Cuff will contribute to the education and public outreach effort.

(12) Dr. Herbert Thier

Herbert D. Thier is currently an Academic Administrator Emeritus at the Lawrence Hall of Science, University of California, Berkeley. He is Founding Director of the Science Education for Public Understanding Program [SEPUP] and a number of other grants at the University. Since 1963 he has been leading Instructional Materials Development and Teacher Enhancement projects in science at the Lawrence Hall of Science. He received (with M. Linn), the JRST *Research in Science Teaching Award*, of the National Association for Research in Science Teaching in1975. Thier received the Distinguished Service to Science Education Award, of the National Science Teachers Association in 1994 and the Distinguished Service to Science Education Award, of the Connecticut Science Supervisors Association in1996.

NAI focus: Dr. Thier will be responsible for the education and public outreach component. He will coordinate interactions between the EPO team and the scientific investigators.

Project Management

As PI, Banfield will coordinate all activities within the NAI. She will be assisted in this effort by Susan Sullivan, a program assistant (temporary hire) known to all team members through her contributions to preparation of this proposal. Banfield will oversee the geomicrobiological studies (Thrusts 2 and 3) and coordinate the field-based reasearch with Dietrich, the lead investigator for the Mars history and physical constraints group (Thrust 1, see below).

Roden, Emerson, Luther, Banfield will be responsible for sampling and geochemical characterization of the specific environments targeted. Yim will interact with Luther and Banfield to supplement the field effort via robot-enabled sampling of otherwise inaccessible regions. The Roden, Emerson, and Banfield laboratories will be responsible for most of the microbiological investigations, in collaboration with Luther, who will provide fine-scale geochemical measurements. Emerson and Roden contribute expertise in microbial physiology and isolation of new organisms, Roden provides microbial system modeling skills, Roden, Emerson, and Banfield routinely use molecular biological methods, and the Banfield group contributes expertise in mineralogy and genomics. Complementing these programs are Luther, Boering, and DePaolo, who bring to the NAI expertise in solution, gas, solid characterization and isotopic analyses. Bishop contributes knowledge of Mars exploration and remote- and laboratory-based mineral spectroscopic analysis, Banfield, who has expertise in mineralogical characterization via electron microscopic and other methods, and Dietrich and Manga, who provide expertise in field, laboratory, and modeling-based studies of hydrological and geomorphological processes.

All CoIs will contribute to a 'BIOMARS' project web site (already established to share field site information and photographs for proposal development). Sharing of this expertise through exchange visits, shared field work, and regular meetings, will greatly strengthen investigations. Streaming video conferencing capabilities have been established in the EPS department at UC Berkeley; this technology will be used for regular internet-based meetings.

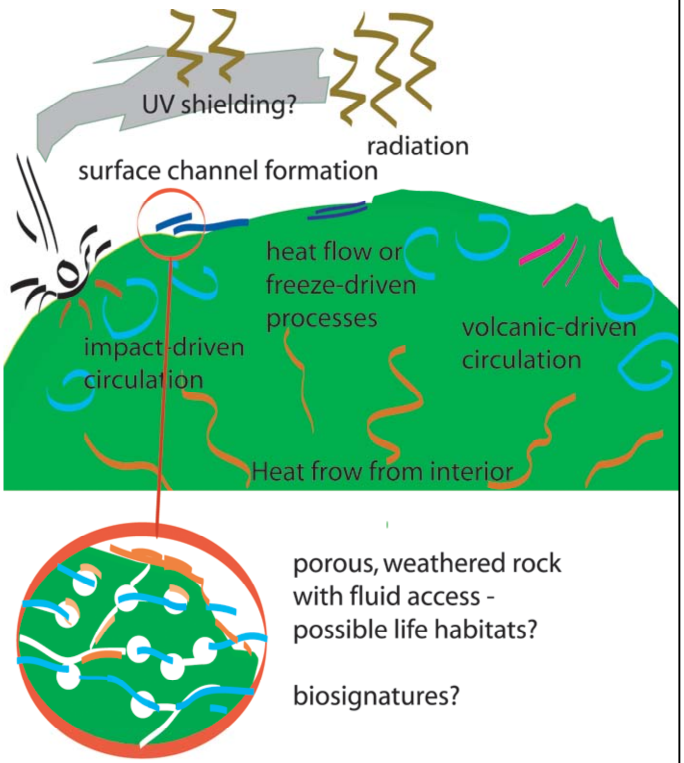
Relationship between the NAI and CIPS, and specific UC Berkeley Commitments

UC Berkeley has launched a major effort to pursue research and education in planetary science, planetary environments, and astrobiology. Two years ago, UCB created an organized research unit called the Center for Integrative Planetary Sciences (CIPS), which is directed by Dr. Geoff Marcy. CIPS has hired several new faculty (the PI and one CoI for this proposal) and instituted several educational initiatives to support a new planetary/astrobiology program. A new undergraduate curriculum has been established in planetary science under the auspices of two departments, "Astronomy" and "Earth and Planetary Science". These new undergraduate programs integrate courses in chemistry, geophysics, astronomy, biology, and atmospheric science into a coherent curriculum. The CIPS faculty will provide complementary leadership in astrobiology research and teaching, thus greatly strengthening the visibility and impact of the proposed NAI.

As described in the attached letter from Dr. Marcy, CIPS will commit a significant fraction of its resources to the NAI, should it be funded. Specifically, they propose to **provide $50,000 per year** to establish a competitively awarded **"UC Berkeley Postdoctoral Fellowship in Astrobiology and Planetary Science"** (see letter from Dr. Marcy).

As part of the proposed activities for the NAI, Banfield and colleages will develop a new undergraduate general science course that will cover planet formation, origin of life, organism-environment interactions and the search for life in the universe. This course will be coordinated via the CIPS infrastructure. **Administrative support** for course management and coordination between the NAI and CIPS (separate from the NAI administrative support requested in the budget) and **computer support** will be provided by CIPS (see letter from Dr. Marcy). For additional details, please see the section on institutional commitment.

PROPOSED WORK AT A GLANCE



THRUST 1:

ATMOSPHERIC PROCESSES
PI: Boering

MINERALOGY
PIs: Bishop, Banfield

SURFACE PROCESSES
PIs: Dietrich, Manga, Bishop

HYDROLOGIC PROCESES
PIs: Manga, Dietrich

THRUST 2:

GEOMICROBIOLOGY
PIs: Emerson, Roden, Banfield, DePaolo, Luther

THRUST 4:

ROBOTIC GEOMICROBIOLOGY
PIs: Luther, Yim, Banfield

Section 3.1 **THRUST 1**: Locations most likely to be optimal for life on Mars

Section 3.1.1 Hydrology – form, timing, volume of fluid flow near the Martian surface
Section 3.1.2 Mars surface processes: sites of sustained fluid flow on Mars?
Section 3.1.3 Atmospheric processes: the form and effectiveness of a UV shield
Section 3.1.4 Mineralogy and physical properties of solids in Martian biomes

Section 3.2 **THRUST 2**: Life associated with Mars rocks – habitats and biosignatures

Section 3.2.1 Microbial populations in chemoautotrophically-based ecosystems
Section 3.2.2 Physiology, possible role of biomineralization in energy generation
Section 3.2.3 Mineral Biosignatures associated with microbial Fe cycling
Section 3.2.4 Isotopic biosignatures
Section 3.2.5 Mineral surfaces as biological indicators
Section 3.2.6 Exploring life's extremes in an acidic Fe-S system

Section 3.3 **THRUST 3**: Novel in situ measurements, robotic geomicrobiology

Section 3.3.1 Development of sensors for in situ biogeochemical measurements
Section 3.3.2 Robotic-based geomicrobiology

3) PROPOSED WORK

Thrusts 1 and 2 (see diagram on previous page) will tackle identification of the most hospitable sites for life on Mars, analysis of the types of communities that may colonize them, and how these communities may be detected. Thrust 3 complements Thrust 2 by adding new geochemical and sampling approaches. Four approaches will be used in this work: Mars image analysis, study of terrestrial analog field studies, laboratory simulations and experiments, and numerical modeling.

The common element for the work in the three thrust areas is the set of field sites to be studied. These will provide constraints for hydrologic, geomorphologic, and geomicrobiological models and serve as sources of microorganisms for experiments and sites for biosignature analysis.

Field sites

Initially, mineralogical, geochemical, geomicrobiological, hydrologic, and geomorphological field work will be conducted at three field sites in the western US. Alternative sites may be selected as work evolves over the five-year grant period.

The first site locations are in the Miocene andesitic and basaltic volcanics and tuffs that occur near Abert Lake, Oregon. Abert Lake is a closed-basin saline, alkaline lake that occurs in graben. The lake is bounded by the spectacular ~ 2000' high basaltic andesite and basaltic Abert Rim (Fig. 1). The rim is capped by lavas considered to be approximate equivalents of the Columbia River Basalts of northern Oregon. The lake is a

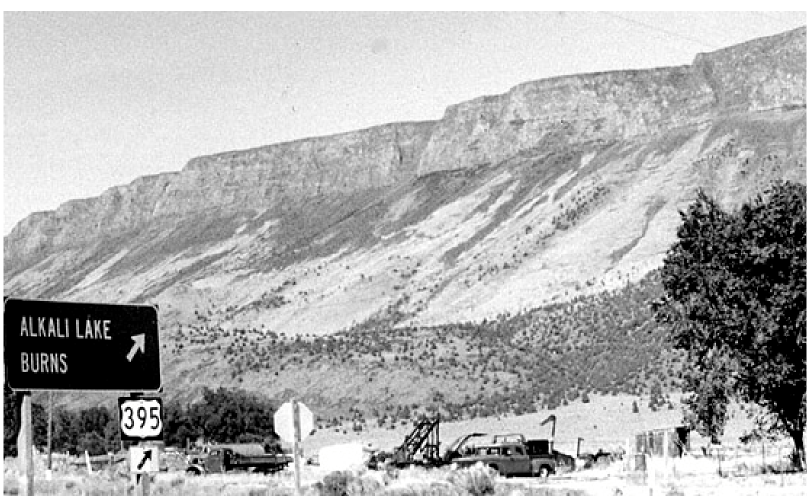


Figure 1: Photograph looking north-east toward the Abert Rim scarp. The site is relatively dry and cold, with relatively light vegetaion cover. The numerous springs associated with basaltic andesite rocks are targest Mars biome analogs.

remnant of the Pleistocence pluvial Lake Chewaucan (Phillips and Van Denburgh, 1971). The hydrology, geochemistry, history of the lake were documented by Phillips and Van Denburgh (1971), Van Denburgh (1975), and Dieke Jones (1980). The mineralogy of the volcanics, weathering products, and nature of diagenetic reactions in the lake sediments were reported by Banfield et al. (1991a,b). The area receives 12-14" rain per year, with large daily, monthly, and annual ranges in temperature (Allison 1982). The surface water runoff is limited (thus groundwater discharge features should be evident) and vegetation cover is relatively light. The geology of the region was described by Walker (1963) and

Jones and Weir (1983) and the geodynamic evolution is reviewed by Humphreys et al. (2000).

An important reason for selection of the Abert region for study is that the erosional features associated with the escarpment (Fig.2–panel B) resemble features interpreted as fluid-formed channels on Mars (Fig. 2–panel A). In addition we have identified several sites of possible seepage-driven erosion (possible analogs for the large, ancient seepage channels on Mars). Furthermore, the Abert Lake region, and surrounding areas, contains numerous active springs with a wide range of discharge temperature. In some cases, the spring waters smell of hydrogen sulfide and show clear evidence of mineral precipitation and biological activity. Recent hydrologic studies designed to explore the geothermal potential of the area have provided evidence for late Quaternary hot spring activity (e.g., Jellinek et al., 1996). Thus, the area has the potential to yield sites for studies of current geomicrobiological processes as well as places for analysis of biosignatures of extinct systems.

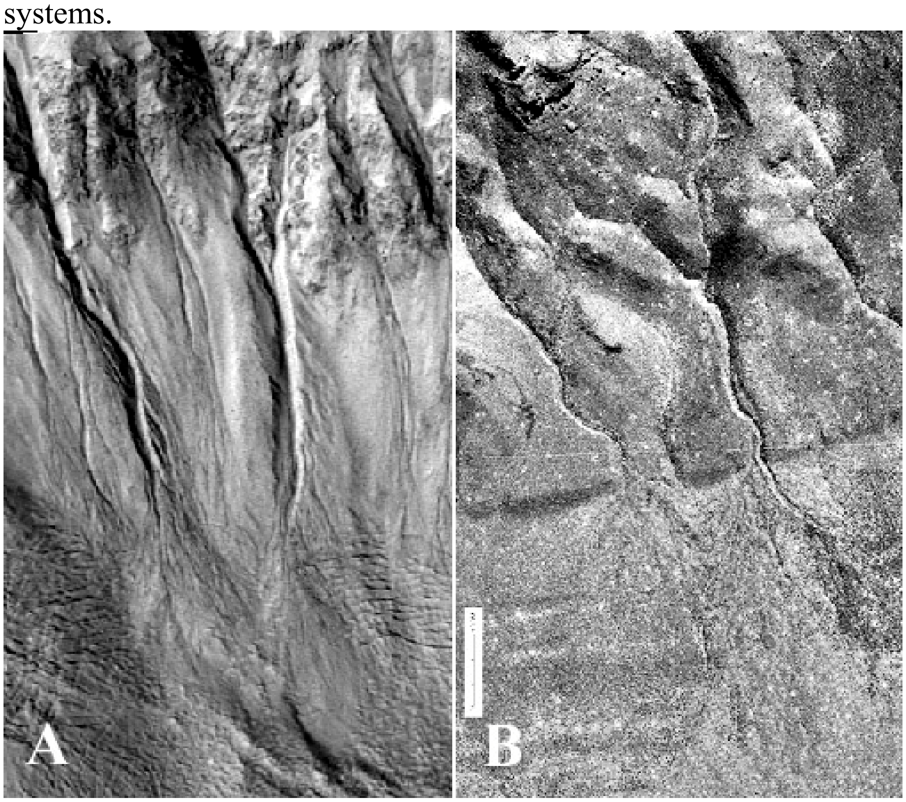


Figure 2

Aerial photos:

(A) Channels on Mars (see below) and (B) channels associated with the Abert Rim. Many potential analog sites were identified along the rim, and to the west of Abert Lake.

The second site for our work is an area adjacent to the Snake River, Idaho. The Snake River Plain extends for ~500km across southern Idaho. Compressional stress associated with mountain-building processes in the Mesozoic (>65 Ma) may have contributed to downwarping of the region. Subsequent crustal extension that created the Basin and Range province caused widespread normal faulting, so the Snake River Plain is also a large graben structure. During the last 15 Myr, numerous basalt flows have partially flooded the Snake River Plain (Malde and Powers, 1962; Armstrong et al., 1975). These basalts are, in general, more permeable than the underlying basement rocks, and so they provide conduits for water that drains into the Plain from the surrounding uplands or that falls as precipitation on the Plain itself. Some flows are more conductive than others, and where these units intersect the present-day canyon (>100 m deep) of the

Snake, springs are abundant (Meinzer, 1927). Estimates of the combined discharge of the springs in the Twin Falls and Bliss area are >150 cubic meters/sec (Stearns, 1936). The water temperature is ~16 ^{o}C, which indicates that the groundwater flow is discharging geothermal heat. Indeed, Brott et al. (1981) suggest that spring discharge removes most of the geothermal heat flow from the Snake River plain.

The most important feature of the SnakeRiver area is that discharge of large volumes of groundwater possibly caused formation of channels that resemble the sinuous channels on Mars (see cover photo: credit NASA/JPL/MSSS]. The Snake River was inundated by the Bonneville floods, and there are scabland channels along the perimeter of the canyon. The sites we have selected, however, were not created by the Bonneville releases, as they are located in a wide and deep section of the canyon where the floods did not significantly overtop the Snake Canyon walls (according to data in O'Connor, 1993;and confirmed via personal communication, 2003). The access, scale, bedrock composition and high outflow discharge rate of these features may them ideal for analog studies. Like the Abert area, the rocks are basaltic and the vegetation not extensive.

The third site for our work is a subsurface metal sulfide deposit that is undergoing active weathering to generate large volumes of acid solution. The volcanic rock-hosted deposit is pyrite (FeS_2)-dominated (>95%). At this time, we do not know that large pyrite accumulations occur, or occurred, on Mars. However, metal sulfide deposits may have developed in association with relict hydrothermal systems (and been subsequently removed by weathering). In fact, the red-orange Martian dust has compositional features not unlike neutralized acid rock drainage (high concentrations of ferric iron and sulfate; also see Figure 9, below), and there have been some suggestions that the soils are somewhat acidic.

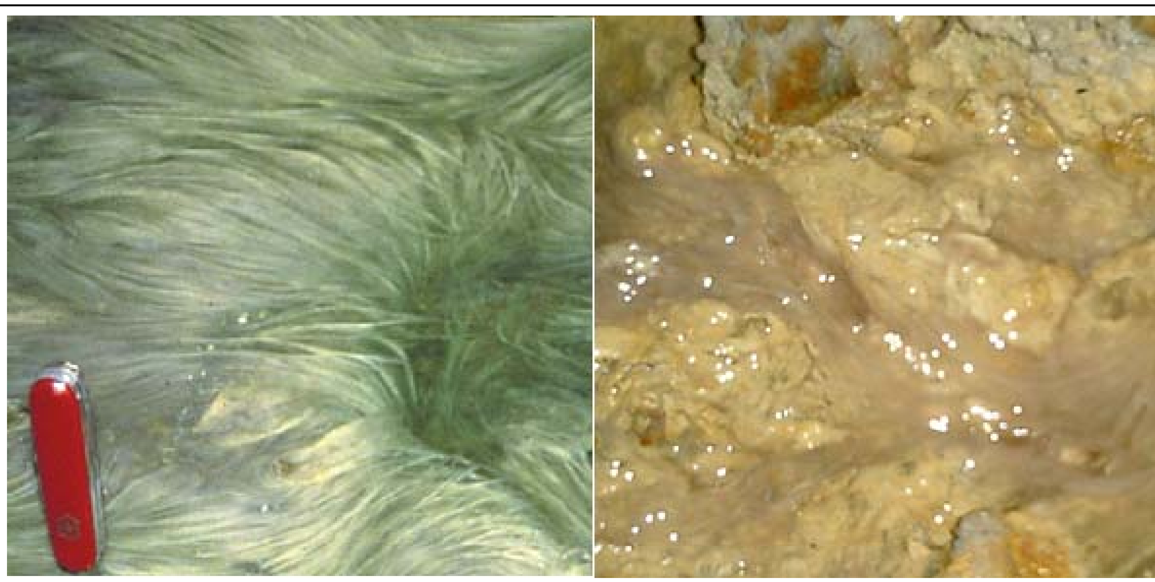


Figure 3:Biofilms in pH 0.7 solution in the Richmond Mine. At this site, our goals are to determine the limits for life in chemoautotrophic Fe-S systems, and what sets them.

Our third field site, located in the Richmond Mine at Iron Mt., CA, is of special interest because it hosts a very active chemoautotrophically-based subsurface biosphere (see Fig.

3) that is sustained by iron and sulfur oxidation. Temperatures within the deposit generally exceed 40 °C due to exothermic oxidative dissolution of pyrite. Pyrite dissolution process yields very acidic (pH 0.5–1), metal-rich (near molar concentrations of $FeSO_4$), toxic metal-rich (few to tens of milimolar Zn and Cu) solutions that pose a variety of challenges to the extremophile microbial communities that colonize the system.

THRUST 1: WHAT ARE THE OPTIMAL LOCATIONS FOR LIFE ON MARS?

An overriding goal of our work is to integrate information on geodynamics and heat flow, heat-driven crustal fluid flow, fluid-driven surface topographical evolution, fluid- and atmosphere-driven mineral weathering, and geological, biological, and radiation-driven atmospheric chemical process that dictate fluid chemistry and radiation exposure at the planetary surface so as to provide the best possible constraints for understanding the potential for biosphere development over the history of Mars. This work will be carried out by the "planetary" subgroup team (Manga, Dietrich, Bishop, Boering) and will be coordinated by Dietrich ("planetary group" lead investigator").

3.1 Mars hydrology: where was the water, why, how much, and when?

3.1.1 Mars hydrogeological investigations

Subsurface water dynamics play a key role in establishing and maintaining habitable environments. We thus propose to develop and extend a set of models in order to provide constraints on the location, discharge rate and temperature of groundwater that emerges at the surface, and hence evaluate the settings (in space and time) favorable for microbial life. Our focus will be on understanding the hydrogeologic environment in which seeps and small springs occur because they can be a source of near-surface moisture for extended periods of time. Seeps and springs are also features for which there are terrestrial analogs in which we can both study the microbial communities and test hydrologic models. A good understanding of sites with modest groundwater discharge, however, also requires an understanding of the properties and evolution of the entire hydrologic system on Mars.

The behavior of water in the subsurface cannot be studied in isolation from other regions and processes on Mars. The flow of water in the subsurface depends on the hydraulic head gradients driving flow, vertical and horizontal temperature gradients within the groundwater system, and hydraulic properties (e.g., permeability, porosity, compressibility) of the host rocks. Hydraulic head gradients will change in response to the geodynamic and exogenic processes that affect surface deformation. Temperature gradients change in time as the planet cools and vary in space due to magmatic activity. The permeability of groundwater systems depends in part on the chemistry of groundwater, which in turn depends on the composition and pressure of the atmosphere. Consequently, the geodynamic history of a planet, the style and rate volcanic and magmatic activity, and the evolution of the atmosphere and hydrologic cycle, will all influence dynamics of groundwater systems.

Subsurface hydrogeologic processes can be conveniently separated into local, regional, and global scales. At the local scale, there are three model problems of interest (see Figure 4).

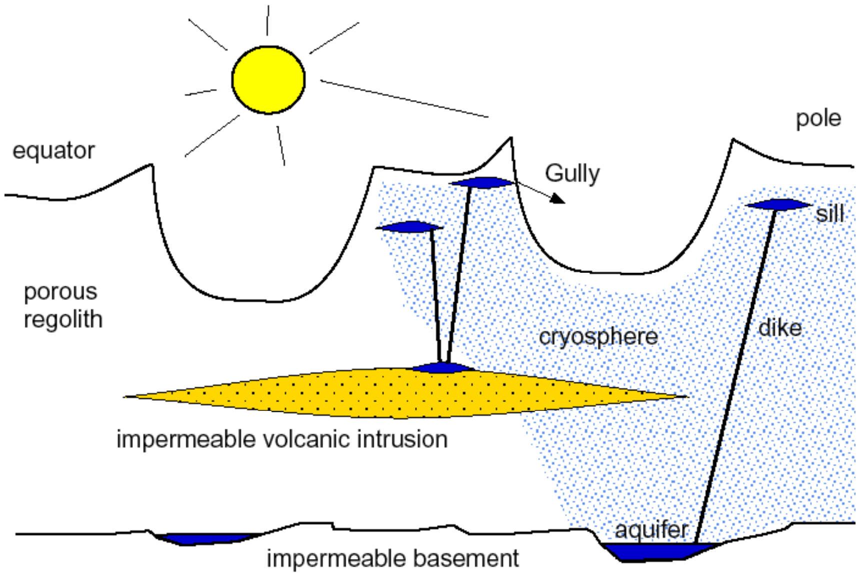


Figure 4:

Illustration (redrafted from Gaidos, 2001) of "local" processes that influence the location and magnitude of groundwater discharge.

First, do intrusions provide a source of heat that may allow expulsion or drainage of groundwater (e.g., Squyres et al. 1987; Gulick, 1998; McKenzie and Nimmo, 1999; Mellon and Phillips, 2001; Harrison and Grimm, 2002) and potentially provide the heat and redox gradients required for microbial life? Second, does the formation of impact craters produce sufficiently large hydraulic gradients and heating of the subsurface to permit surface discharge (Tanaka et al., 1998)? It has been argued that individual impacts not only affect the groundwater system, but were large enough to inject water into the atmosphere, induce rain, and hence cause runoff and erosion (Segura et al., 2002). Third, as the Martian heat flow decreases and the cryosphere thickens, how does fluid pressure evolve and what are the implications for groundwater discharge (Gaidos, 2001)? In all three problems, we would like to predict groundwater temperature, flow rates, and the time scales over which the hydrologic system evolves. Dr. Manga and students have studied spring-systems in a variety of settings on Earth, including thermal springs along active faults, and basalt-hosted cold and thermal springs in the Oregon and California Cascades. These studies have focused on using measurements made at springs to characterize subsurface hydrological properties (Manga, 1996, 1999), quantify surface water-groundwater interactions (Manga, 1997), and study subsurface geologic processes (Manga, 1998; James et al., 1999, 2000; Saar and Manga, 2003). Models developed for these (and ongoing) studies of springs can be extended to Martian settings noting that a key difference is the presence of a cryosphere, which acts as a dynamic confining unit (Carr, 1979).

At the regional scale, the formation of features such as Tharsis may provide controls on the location and orientation of valley networks (e.g., Phillips et al., 2001) through changes in the crustal stress, flow geometry, and hydraulic gradients (e.g., Wilson and Head, 2002). It is at the global scale (see Figure 5) that we can best develop insight into the evolution of the hydrological cycle and water balance (Clifford and

Parker, 2001). Thus, both regional and global scale hydrogeologic systems provide the framework within which we must understand and model local hydrologic processes.

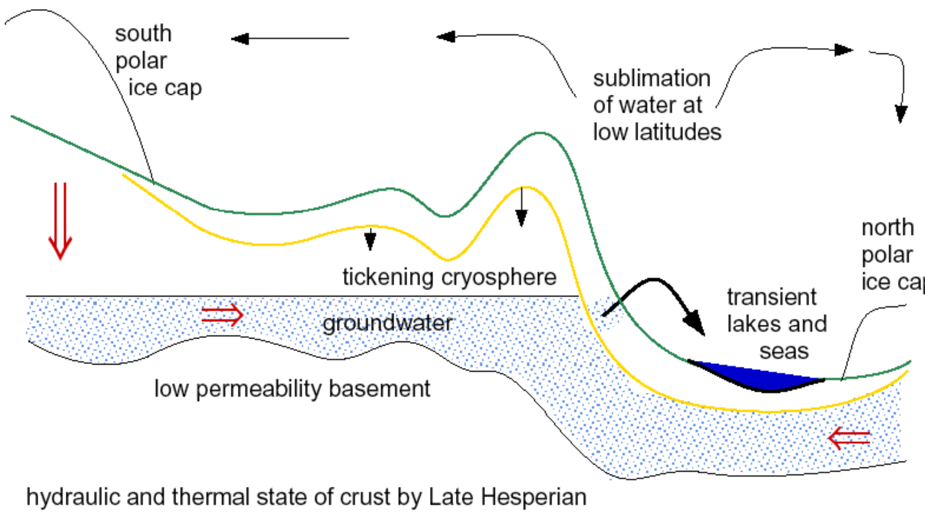


Figure 5: Illustration (redrafted from Clifford and Parker, 2001) of one model for the global hydrologic system during the Late Hesperian

The development of regional and global hydrogeologic models requires understanding the evolution of heat flow (which affects the thickness of the cryosphere) and stresses in the lithosphere. Our research group at Berkeley (Professors Mark Richards and Michael Manga, postdoc Mark Jellinek, and graduate students Helge Gonnermann, Mark Wenzel and Dave Stegman) is also involved in developing numerical and laboratory models of the global and regional (Tharsis) geodynamic evolution of Mars. The results of this work, coupled with those from previous studies, provide the needed constraints on heat flow (e.g., Schubert and Spohn, 1990; Reese et al., 1998; Zuber et al., 2000), stresses (Tanaka et al., 1991), and rate of volcanism (Hauck and Phillips, 2002). The latter is a source of water, CO_2 (which influences climate and chemical weathering), and other volatiles.

The models to be developed by the Manga group as part of the NAI collaboration will extend previous models used to study Martian hydrogeological processes (e.g., Carr, 1979; Forsyth and Blackwelder, 1998; Goldspiel and Squyres, 2000; Clifford and Parker, 2001; Gaidos, 2001; Anderson et al., 2002) in several respects.

- First, we propose to include poroelastic dynamics in order to describe fluid-solid coupling. For example, changes in hydrogeologic properties such as storage and permeability will arise from the volume change of water as it freezes and melts. That is, not only does fluid pressure change due to freezing (Goldspiel and Squyres, 2000), but as shown by Gaidos (2001) properties of the porous material itself will change. The large discharges represented in the outburst floods imply large changes in pore pressure, which in turn will influence subsurface properties, and should be important in understanding the development of chaotic terrain (e.g., Cabrol et al., 1997). The theory of *linear* poroelasticity is straightforward to include in hydrogeologic models (e.g., Wang, 2000). *Nonlinear* processes (such as consolidation and liquefaction) are still best characterized empirically; nevertheless, while they cannot be included as rigorously in models, their effects can be parameterized (e.g., Manga et al., 2003).

- We will model the evolution of porosity and permeability through weathering reactions following ideas and models developed for terrestrial analogs (e.g., Bolton et al., 1999; Fontaine et al., 2001; Lowell and Yao, 2002 for some recent studies that illustrate a range of approaches). Weathering reactions will be influenced by the partial pressure of CO_2 (e.g., Elwood and Madden, 2002), providing a connection between the evolution of the atmosphere and subsurface properties.
- We will develop models to relate the geodynamic evolution of Mars to its hydrogeologic properties following ideas developed by Rojstaczer (2002). In more detail, the amount of water and its rate of flow within the subsurface are controlled by the porosity and permeability of the subsurface. The standard procedure for Martian models is to use values similar to those on Earth, though scaled to account for the lower Martian gravity (e.g., Clifford and Parker, 2001). However, permeability and porosity on Earth are dynamic properties that reflect a balance between the rate at which the subsurface must cycle water, the hydraulic gradients caused by topography that drive this flow, the rate at which porosity and permeability are reduced by chemical and physical processes, and the regional stresses that can create new porosity and permeability. Typical upper-crustal terrestrial permeabilities of 10^{-14} m^2 (Ingebritsen and Manning, 1999) have been calculated by Rojstaczer (2002) through such a balance. The permeability in the lower crust of the Earth is constrained by the rate at which fluids are expelled by metamorphic reactions (Manning and Ingebritsen, 1999), which on Mars are limited by cooling rate and would be much lower than on Earth. Given the very different hydrologic cycle and geodynamic evolution (heat flow and stresses) of the Earth and Mars, there is no *a priori* reason to assume that Martian permeability and porosity distributions need resemble the Earth's.

As made clear in the review by Clifford and Parker (2001), care must be taken to account for the transfer of water between the atmosphere, the cryosphere, the subsurface (both saturated and unsaturated zone), and bodies of surface water.

Our proposed attempt to develop better models for the evolution of hydrogeological dynamics on Mars may appear poorly constrained. Nevertheless, there are several observations that provide bounds (in space and time) on acceptable models, and we anticipate that ongoing and future studies (including those described in this proposal) will add further constraints. Most important are estimates of the time (e.g., Burr et al., 2002), location and elevation (Carr, 2002), geologic setting (Gilmore and Phillips, 2002), and magnitude of groundwater discharge (Gullick, 2001) in groundwater seepage channels. In fact, because of the importance of understanding properties of surface discharge (rates, temperature, time scales) the surficial component of this proposal focuses on understanding the mechanics of channel formation in groundwater-dominated systems. The large outflow channels, however, also provide important constraints on groundwater systems (e.g., Carr, 1996). Additional constraints are provided by impact crater morphology (Carr et al., 1977; Stewart et al., 2001), evidence (or lack of) of hydrologic activity in craters (Cabrol et al., 2001; Russel and Head, 2002), density of the upper crust (Nimmo, 2002), the presence and age of hydrothermal alteration (Newsom et al., 2001;

Treiman et al., 2002), and the strength of faults which provides insight into fluid pressures (Barnett and Nimmo, 2002),

One of the better ways to constrain our models will be to apply them to more easily studied analog settings on Earth, including the proposed field sites in eastern Oregon and Idaho. In our past work, see Figure 6, we have shown that we can successfully model spring systems and obtain new insight intro subsurface hydrogeologic processes.

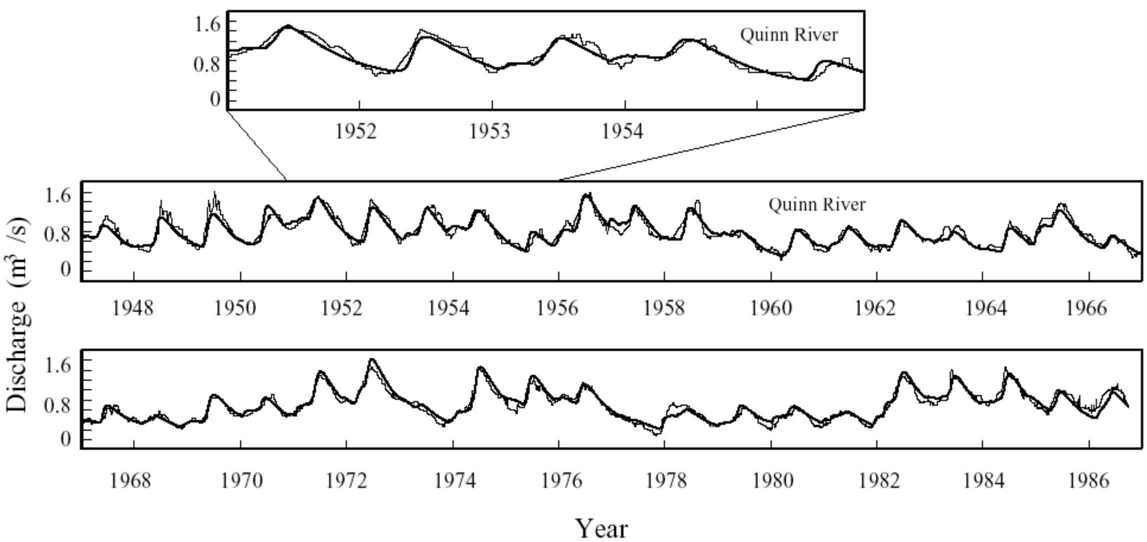


Figure 6: Predicted (bold curve) and measured (thin curve) discharge at Quinn River Spring in Oregon. The prediction is based on a calibrated groundwater flow model and allows us to determine subsurface hydrogeologic properties such as permeability, aquifer thickness, heat flow (from Manga, 2001).

Terrestrial studies are essential because they allow us to test some aspects of the models that we will apply to Mars, such as the evolution of porosity, permeability, flow rates, and water temperatures (e.g., the study springs in permafrost of Andersen et al., 2002). While we have a limited range of atmospheric conditions on Earth, we can find spring systems in areas with wide ranges of groundwater recharge rates, discharge temperatures, and ages of the host aquifers. Co-I Manga has worked extensively on the spring and groundwater systems in the Oregon Cascades and eastern Oregon where the host rocks are dominated by basaltic rocks, as they are on Mars. As a starting point for our work, we will investigate fluid flow associated with basaltic and basaltic andesite spring systems near Abert Lake, in south central Oregon and high discharge springs adjacent to the Snake River, Oregon. However, as we learn more about the hydrology and surfical processes at these sites, we may redirect our work to other locations. In Oregon, we have access to spring systems in which recharge rates vary from < 1 cm/year to > 1 m/year, discharge temperatures range from about 1^{o}C to boiling. The spatial scale of the groundwater systems ranges from 100s of meters to 100 km. Another location in which models can be tested is Hawaii, where saline water (Martian groundwater may have a high salinity) interacts with basaltic rocks. As in Oregon, we can study groundwater discharge at springs that range from small to large, and cold to hot. Springs provide insight into the averaged or integrated behavior of groundwater systems. Our models will either predict or require knowledge of the spatial distribution of porosity, permeability, temperature and flow rate; data made available from the Hawaii Scientific Drilling Project can be compared with models.

3.1.2 Mars surface processes: where are sites of sustained fluid flow on Mars?

One goal for our team is to evaluate the geomorphological characteristics of channels on the Martian surface to provide information about the history of fluid discharge.

The valley networks of Mars provide compelling evidence that at times in Mars' history surface water carved deep canyons and formed well-developed branching drainages (e.g. Cabrol and Grin, 2001). The origin of the surface water, the mechanisms of channel formation, and the estimates of magnitude, duration and frequency of surface water flow are debated, however (e.g. Craddock and Howard, in press; Carr and Malin, 2000; Grant and Parker, 2002, Segura et al., 2002). The small gullies recently made visible by the Mars Orbiter Camera (MOC) (Malin and Edgett, 2000a) have been interpreted as possible evidence of relatively recent water releases from the near surface, although alternative models have been proposed (e.g. Musselwhite et al., 2001). Layered deposits visible on crater rims suggest that standing bodies of water may have existed on Mars in ancient times (e.g., Malin and Edgett, 2000b; Grant and Parker, 2002). These and other observations constrain the atmospheric and hydrologic modeling proposed here as part of the effort to reconstruct the conditions under which life may have originated and persevered on Mars. One goal of the surface processes component of the proposed research is to extract more quantitative information about rates of discharge and time scales of flow in ancient and recent channel systems in order to provide more precise constraints on atmospheric and hydrologic models.

This proposal focuses on life in hydrothermal spring environments because springs provide a sustained flow of liquid water, heat, a supply of nutrients, and shielding from radiation. We therefore propose to focus on surficial features on Mars that appear to have been eroded by flows originating in the subsurface: valley networks, some of which are thought to have been formed by sustained or repeated surface flow from groundwater systems in the ancient past; and the recent gullies, which may have been formed by episodic releases of water from the shallow subsurface. Understanding the characteristics of the flows that formed these features will place important constraints on the possible origin and sustained presence of life on Mars.

Several debates have emerged regarding these two types of channel features. With regard to the valley networks, three primary issues can be identified (Carr and Malin, 2000): 1) the source of the water (groundwater outflow versus overland flow due to precipitation), 2) the mechanism of channel growth (headward advance of sapping fronts versus simultaneous incision along the entire channel), and 3) the relative duration and magnitude of the surface flow events (short-lived, episodic releases versus sustained surface runoff). The strong evidence of repeated release and reworking of the surface, at least in the Noachian (e.g. Grant and Parker, 2002; Craddock and Howard, in press), raises the challenging issue of how the groundwater system is recharged. With regard to the recent gullies, several formation mechanisms have been proposed, although most evidence suggests that water played a role (e.g. Stewart and Nimmo, 2002). The gulles are cut into scree slopes, which may themselves be an indication of widespread, water-related activity. Scree slope inclinations on Mars are consistently lower than the angle of repose, prompting Perron et al. (submitted) to propose that freeze-thaw activity may be reshaping the slopes. Hence, near-surface hydrologic activity may be more sustained and more extensive than previously considered.

The Dietrich group, with input from the Manga group, will conduct a detailed study of each of the two types of channel features visible on the Martian surface. Each of these two foci will include quantification of geographic and geometric properties of the Martian features using Mars Global Surveyor (MGS) data, and experimental simulations and terrestrial field analog studies to identify quantitative, mechanistic relationships between channel form, flow characteristics, and substrate (bedrock) properties.

The most interesting valley networks on Mars from an astrobiological standpoint are the kilometer-wide, amphitheater-headed channels that have been interpreted as groundwater sapping features (e.g., Baker et al., 1990). These channels, such as Nirgal Valles (Fig. 7A) do not appear to be active at present, but may have been an ideal place for the development of life early in Martian history, as they appear to have been formed by sustained or repeated flow of water from a subsurface source over a long period of time. Headward advance of a "sapping" channel occurs when enhanced weathering and erosion at a seepage site leads to undermining and collapse of the headwall and sidewalls around the seepage site. Features of the Martian channels that suggest groundwater sapping include amphitheater-shaped headwalls, hanging tributary valleys, long main valleys with short tributaries, little change in cross-sectional dimensions with downstream distance, irregular tributary junction angles, and low drainage density (Laity and Malin, 1985; Howard, 1988; Higgins, 1984). In some cases these channels are discontinuous, suggesting subsurface collapse (Carr and Malin, 2000). There is debate, however, as to whether the channels were created by progressive headward advance of seepage fronts. Carr and Malin (2000) suggest that incision by overland flow released from a stationary spring head could have produced the observed morphology. These authors note that the most recent channels visible in the canyons are much smaller than the canyon dimensions, and that the canyons are sinuous in a way thought to be inconsistent with groundwater sapping processes.

Using MOC images and MOLA topographic data, we will quantify the detailed morphology and geographic distribution of these features in order to extract more information about the magnitude of flows responsible for their formation (see Grant and Parker, 2002, for an example). We will then conduct laboratory experiments and field studies of analogous terrestrial features to answer three key questions: (1) how much water is needed to cut the canyons on Mars over such great lengths (hundreds of kilometers); (2) was this incision vertical (by surface flows) or lateral (by groundwater sapping); and (3) over what time scale did the erosion occur? As suggested in earlier analyses by Howard (1988, and modeled by Goldspiel and Squyres, 2000), the volumes of groundwater flow can be estimated through an erosion theory. In both studies, however, it has been assumed that the eroding material is cohesionless. This simplifies the problem, but is probably inappropriate for Mars. Here we propose to develop a mechanistic understanding of erosion mechanisms and water flow needed to cause channel head advance by groundwater sapping of cohesive materials. We will conduct extensive laboratory experiments, building on the pioneering work of Howard and McLane (1988) and Kochel et al. (1985), to first document the differences in morphology and erosion mechanics of seepage erosion versus vertical channel incision from a spring source. Then we will build a sufficiently large-scale tank to enable us to measure groundwater head gradients, flow rates and erosion of a seepage head formed in weakly cohesive material. We have found through extensive experiments on bedrock channel incision that we can use a measure of tensile strength to scale rock resistance appropriately (Sklar and Dietrich, 2001).

The Box Canyon, Idaho study area (Fig. 7B) is an ideal analog site to study groundwater sapping. The channel is morphologically similar to those on Mars in many respects, including its sinuous channel path. In the vicinity are numerous other actively forming sapping channels with springs at their heads (Stearns, 1936). We will obtain detailed topographic data (using LIDAR) and combine this with measured outflow to model the groundwater flow field to the seepage heads. Since steady erosion of bedrock walls cannot be observed over short time scales, we will document the long-term rate of seepage head advance using cosmogenic radionuclide exposure-age dating of exposed surfaces and quantify the pattern of fracture development and block failure at the seepage face. The wide range of channel head advances in various canyons in the area will then provide multiple solutions to constrain the development of a groundwater-driven erosion theory. These channels are located along a reach of the Snake River Canyon where the Bonneville floods did not significantly overtop the canyon walls, but backwater sediments should have been deposited in them and we will look for such deposits to add in dating rates of erosion. Our study of groundwater sapping channels will seek quantitative, mechanistic relationships between channel form; flow characteristics such as discharge, head gradient, and longevity; and substrate properties such as erodibility and conductivity.

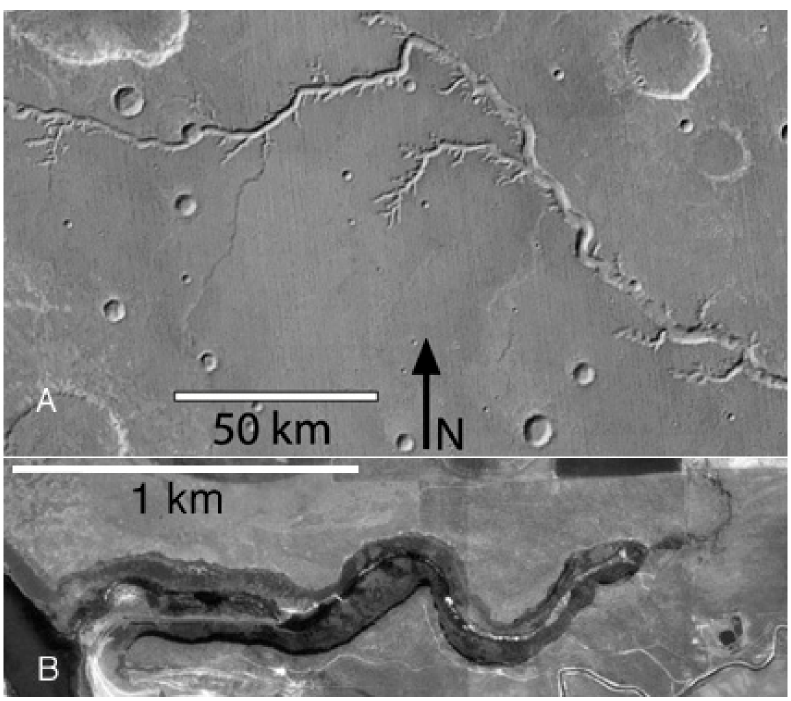


Figure 7

A: Channels of Nirgal Valles (Mars) that are interpreted to have formed by sustained groundwater discharge

B: Channel of Box Canyon, Snake River, Idaho

Our study of sapping channels (which may have fostered the development of life deep in the Martian past) will be complemented by an ongoing study of channel features that formed in the recent past (10s of Myr) and may still be active. Kilometer-long channels formed on debris slopes, dubbed "gullies" by Malin and Edgett (2000a), are evidence of recent geomorphic activity and are the most probable location for the sustained presence of life on Mars. They appear to have been formed by short-lived mass flows containing a pore fluid, most likely water (Stewart and Nimmo, 2002). These flows appear to have originated at depths of ~100m, which suggests that there may be near-surface (but shielded) reservoirs of liquid water fed by groundwater and/or atmospheric deposition. Their origin at distinct rock layers or alcoves (Gilmore and Phillips, 2002) also implies groundwater release. The observation that some gullies have experienced multiple flow events (Malin and Edgett, 2001) suggests that there may be recharge of the fluid reservoirs, a requirement for the sustained presence of life.

The goal of our ongoing study of gully formation is to develop a mechanistic theory for channel incision by mass flows on scree slopes. We have already constructed a database of MOC and MOLA data for a 30º x 30º region on Mars that will allow us to measure the topographic characteristics and channel geometries of gullies. We have also begun preliminary experimental work to link model channel geometry to mass flow properties, including fluid content. By combining this understanding with estimates of the solid volume evacuated by a gully, we intend to infer the volume of fluid released from a near-surface reservoir. We propose to extend our analysis of MOC images and MOLA data to include a global survey of gullies. Using the procedure described above, we would estimate the time-averaged discharge of groundwater wherever gullies are present. This would provide important constraints on models of current hydrologic conditions in the Martian subsurface.

We also propose to conduct fieldwork at the BioMARS analog site at Abert Lake, Oregon, to test the relationships that come out of our laboratory experiments. In the Abert Lake site there are small channel networks that appear similar in form and scale to the recent Mars gullies. We will conduct field studies to determine the origin of these channels, distinguishing between mass flow channel incision and fluvial incision (see Stock and Dietrich, in press), and quantifying the amount of water needed to cause the erosion.

3.1.3a Atmospheric processes: the form and effectiveness of a UV shield

Atmospheric chemistry has profound implications for the climate and habitability of Mars throughout its history. The presence and stability of greenhouse gases and aerosols, for example, will regulate climate or force climate change. Photochemical processes in the atmosphere may also produce UV radiation shields (e.g., ozone on Earth), which would influence both radiative transfer (and therefore climate) and the stability of organic compounds in the lower atmosphere and at the surface. Thus, analysis of the Martian atmosphere is vital to understanding of the opportunities and challenges for early life on Mars, as well as the importance of habitat features that provide radiation protection.

Critical links between atmospheric chemistry, climate, habitability, and indicators of habitation in terrestrial-like atmospheres are currently being studied using models of early Earth (e.g., prebiotic and prephotosynthetic), current Earth, and other planetary atmospheres [e.g., Zahnle, 1986; Kasting, 1992; Pavlov et al., 2000]. A large number of parameters needed for these models, however, are untested experimentally. The co-I for this proposal with expertise in atmospheric science, Dr. Boering, will investigate factors influencing atmospheric composition and chemistry relevant to Martian atmospheres at various times in its history through laboratory experiments. The experiments will provide new insights into the interdependence of photochemical lifetimes of greenhouse gases, the potential for the formation of photochemical hazes and whether these hazes may have warmed or cooled the atmosphere and surface and could have served as a UV shield.

3.1.2b Photochemical formation rates and optical properties of haze particles in simulated Martian atmospheres

For the early Earth, and, by analogy, early Mars, photochemical models suggest that photolysis of CH_4, perhaps produced biotically by methanogens, in an atmosphere of CO_2, N_2, and water vapor could produce a photochemical haze [Pavlov et al., 2000, 2001a]. Depending on the particles' chemical composition and size distribution, the haze could act to significantly warm the atmosphere and surface [Sagan and Chyba, 1997] or cool it [McKay et al., 1991; Pavlov et al., 2001a]. It could also provide an early UV-shield, which would increase the stability of organic molecules at the surface and the photochemical lifetimes of other UV-labile greenhouse gases, such as NH_3 [Sagan and Chyba, 1997]. Current photochemical models are sensitive to the atmospheric CH_4/CO_2 ratio and predict that CH_4 begins to polymerize when this ratio exceeds unity [Pavlov et al., 2000; Zahnle 1986]. These models, however, include a number of approximations, some of them quite arbitrary, for the rates of particle formation, and no laboratory data exist to test their predictions.

While experimental work has been done in the laboratory using shock discharges to simulate the chemistry of hydrocarbon hazes thought to occur on Titan's N_2 atmosphere [e.g., Ehrenfreund, et al, 1995; Coll et al., 1999], no experiments have been done investigating the photochemical polymerization of CH_4 in an atmosphere containing CO_2, N_2, H_2O, the likely composition of the early Martian atmosphere. We propose to measure particle formation rates and optical properties in model gas mixtures exposed to UV radiation using a new technique (Adamkovics and Boering, submitted) developed and already operational in our laboratory (Figure 8).

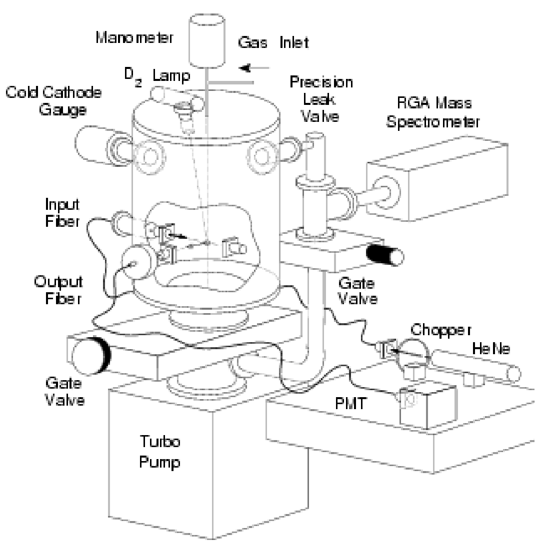


Figure 8: Experimental setup for measuring photochemical formation rates and optical properties of organic aerosols by UV irradiation of gaseous precursors simulating various possible compositions of Martian atmospheres. Complex gas-phase species formed are measured with an online mass spectrometer while aerosol formation and optical properties are measured by light scattering as a function of detection angle. Isotopic analyses of gas-phase species and particulates can also be made on collected samples offline, as can scanning electron microscopy analyses for the morphology and size distribution of the particulates.

Gas phase species will be measured in real time with an online mass spectrometer, while particle formation will be simultaneously monitored by optical scattering. By measuring the angular dependence of particle scattering, the particle size distribution and complex index of refraction will be determined "*in situ*" (i.e., while still suspended in the gas phase as opposed to measurement after deposition to a thin film). The *in situ* optical properties will be compared with collection of the particulates followed by offline field emission scanning electron microscopy (FESEM) to examine particle size and morphology. We will complement these *in situ* and SEM studies with offline chemical and isotopic analyses of the particulate material by collection of the aerosol and subsequent analysis using Gas Chromatograph-Mass Spectrometry (GC/MS) and Gas Chromatograph-Isotope Ratio Mass Spectrometry (GC/IRMS).

This new suite of laboratory measurements will significantly enhance our understanding of the mechanism(s) and kinetics of haze formation in a Martian-like atmosphere and will guide a more accurate representation of these processes in models, which, in turn, will allow more reliable predictions of Martian climate and the UV flux to the Martian surface to be made.

3.1.4 Mineralogy and physical properties of solids in Martian biomes

The analysis of the nature of fluid flow associated with channels in the Martian surface relies upon understanding of the properties of the materials through which the fluids are flowing. Similarly, geomicrobiological analyses depend upon an understanding of the mineral chemistry and reactivity, as these factors can control the options for metabolic energy generation and the physical characteristics of habitats.

Knowledge of the nature of Mars surface materials relies upon compositional data collected from a few sites on Mars and spectral information obtained by orbiting instrumentation. Interpretation of the minerals present depends upon the availability of appropriate reference spectra. Thus, spectra from Mars analog sites will be important in the iterative process of determining the nature of potential habitats associated with Mars surface channels.

The co-I with expertise in remote characterization of the mineralogy of planetary surface materials, Dr. Bishop, will provide constraints on the mineralogy and physical properties of materials in channels on the Martian surface. The spectral measurements will be performed using a FieldSpec Pro FR purchased for this project from Analytical Spectral Devices (ASD) that covers the range 0.35 to 2.5 µm and has a spectral resolution of 2-3 nm.

During year one spectral measurements will be made using the ASD visible/NIR instrument at SETI/NASA-ARC of mineral standards in the lab with a solar simulated NIST traceable irradiance source, and outdoors using this lamp and solar radiation in separate tests. Using the lamp and a fixed sample/standard distance will produce spectra that are readily reproducible, while using direct solar radiation outdoors will introduce variation in the source that will lead to atmospheric lines and increased noise. Measuring samples outdoors with the lamp can help constrain these differences. As some field measurements at variable distances will be desired, it will be necessary to gain an understanding of how the field conditions affect/mask the spectral properties of the rocks/minerals. These tests will ensure that the users are familiar and experienced with the instrument and the spectral character of the minerals of interest in order to maximize field time and facilitate selection of rocks to be collected for the study.

Spectra of minerals, soils and rocks will need to be measured in a dehydrated environment in order to obtain spectra that can be readily compared with spectra on the surface of Mars. A number of clay minerals, iron oxides/oxyhydroxides, some carbonates, some sulfates, and several Mars analog soils have already been measured under dehydrated conditions (e.g. Bishop and Pieters, 1995; Bishop and Murad, 1996, 2002; Bishop et al., 1995, 1996, 1998a,b, 1999, 2001, 2002a,b,c,d,e) and are available in Co-I Bishop's library for this project. Co-I Bishop and her student/lab technician will convert these data from the lab spectral resolution to the spectral resolution of spectrometers measuring data on the surface and in orbit around Mars. During years one to two Co-I Bishop will work with PI Banfield and the team to decide which mineral/soil spectra in Co-I Bishop's library are relevant to the project. Mineral standards will include a variety of clay minerals, opal and silica polymorphs, carbonates, sulfates, and iron oxides/oxyhydroxides.

During years two and four Co-I Bishop and her student/lab technician will visit field sites with the co-Is in order to take spectral measurements of rocks and terrain, help document the field sites, and collect samples for detailed lab studies. The spectrometer will be taken to the Aber Lake and the Snake River sites. The Snake Rivers site is of particular interest because we expect big outcrops of alteration minerals (iron oxides, clays, sulfates, carbonates, etc) due to the high groundwater discharge. For the acid Richmond Mine, rocks will be brought back to the lab for spectroscopic characterization.

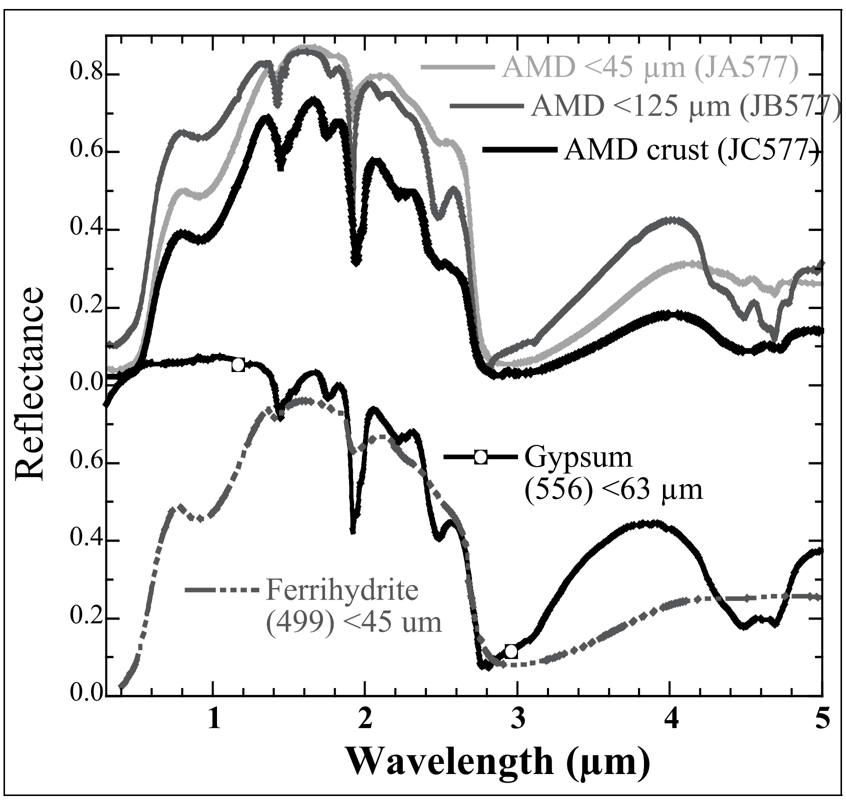


Figure 9: Visible/NIR reflectance spectra of neutralized acid mine drainage (AMD) material from iron mountain measured under dehydrated conditions. Similar reflectance spectra are shown of two main components of this sample, ferrihydrite and gypsum, for comparison. These spectra cover the spectral range of Omega (Mars Express) and represent a dry Mars-like sample state.

During years two to five portions of the rocks collected in the field will be crushed and dry sieved into size separates for detailed analysis. Because spectral properties of minerals change with the grain size, it is important to measure both coarse and fine size fractions. The size fractions will include coarse material containing rock chips and fragments 125-250 µm in size, material less than 125 µm, and material less than 45 µm. If the rocks contain altered rinds or are otherwise only partially weathered, then the alteration products are normally most abundant in the <45 µm fraction. The mineralogy of these samples will be determined by X-ray diffraction and transmission electron microscopy. The Bishop group will identify minerals at the field sites for which spectra are not currently available, obtain reference minerals, and their spectra. In all samples, mineral particle size will be determined confirmed scanning electron microscopy and, in the case of ultra-small particles, by peak broadening analysis of X-ray diffraction spectra. Compositional data will be obtained via X-ray fluorescence and mass spectroscopic measurements. Reflectance spectra of the size separates of these samples will be measured at the NASA-supported RELAB facility at Brown University.

Bishop and her group will read these data into ENVI and process them for several sets of Mars spectral data. Emphasis will be placed on convolving the data to the Omega spectrometer (0.4 - 5.0 µm wavelength range, 2003 Mars Express mission) and CRISM spectrometer (0.4 - 4.0 µm wavelength range, 2005 Mars Reconnaissance Orbiter mission) because these instruments will be measuring data where a number of aqueous minerals important to our project can be detected (e.g. through bands due to OH, water, carbonate and sulfate). As shown in Figure 9 spectra of the AMD material contains several bands due to gypsum and ferrihydrite that could be detected in the Omega spectra of Mars. CRISM will be measuring data both in a low spectral resolution global survey mode as well as a high spectral resolution mode for selected spots on the surface. Co-I Bishop is a CRISM team member and will be working with both the Omega and Crism datasets; she will therefore be informed about changes in the specific spectral channels measured by both instruments. Spectral analyses will be performed in order to determine limitations on abundance for the minerals of interest to our project for the various instruments and measurement modes. During years three to five Co-I Bishop and her group will perform similar work on the spectral data of samples measured in this project.

3.2 THRUST 2: LIFE ON MARS: HABITATS AND BIOSIGNATURES

Introduction

The overriding questions to be addressed in the geomicrobiological studies relate to the nature of microbial populations that can colonize relatively hostile, rock-dominated habitats somewhat analogous to those expected on Mars, the factors that limit colonization, and the form of the record of the existence of these communities. Work in this area will be the focus of the "geomicro" subgroup (Emerson, Roden, Luther, Banfield) and will be coordinated by Banfield ("geomicro team" lead investigator).

Figure 10 illustrates the general approach we take to field and experimental geomicrobiolgoical studies. Because of the extensive information already available on microbial populations and biogeochemical processes occurring at the Richmond Mine (Edwards et al. (1999; Bond et al., 2000b; Bond et al., 2000a; Edwards et al., 2000; Baker et al., 2003, Druschel, Baker et al. in review; Baker et al., submitted), the focus of work in this acid habitat will be on microbial adaption and the processes and factors that limit microbial colonization. In contrast, work at the Abert and Snake River sites will focus on poorly understood connections between silicate mineral weathering, iron cycling, microbial community structure, and biosignature formation.

Solids are likely to yield the best durable biosignatures in the Martian samples. Solid products of metabolism are most likely to be preserved in the circum-netural pH, basalti rock-dominated systems. Particular attention will be paid to relationships between mineralization, cell distribution, and cell preservation in the solid materials will be characterized. Emphasis will be placed on the structure, chemistry, and morphology of solids formed by inorganic and biological processes.

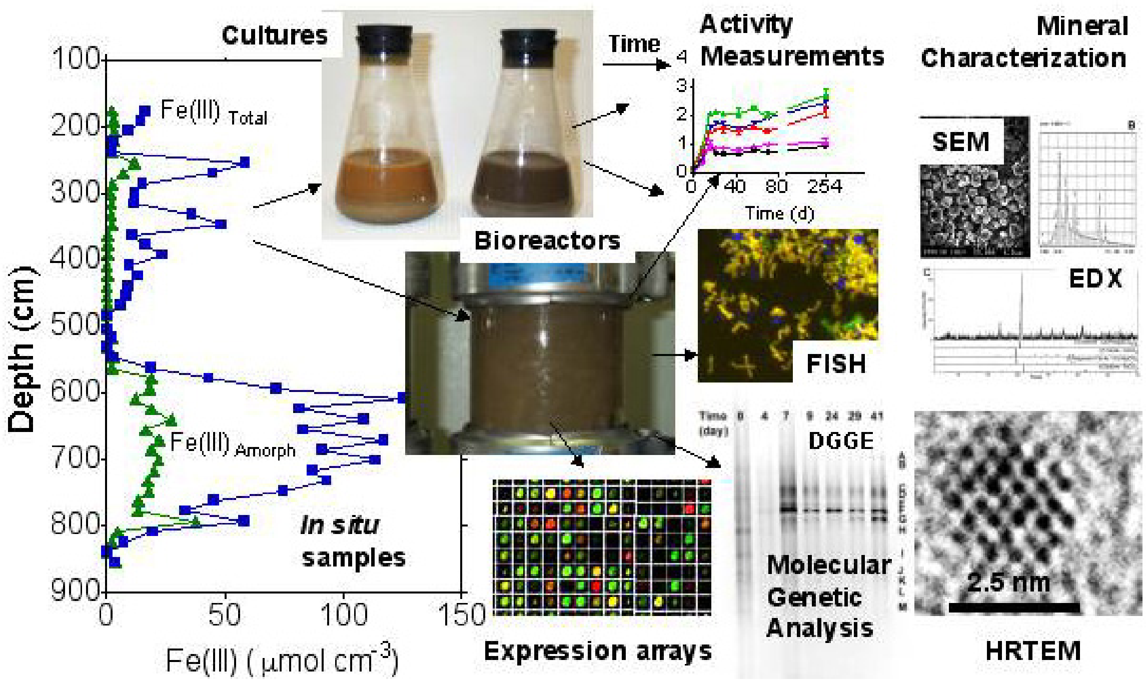


Figure 10: Schematic diagram illustrating our approach: Methods include scanning (SEM) and transmission electron microscopy (HTEEM), energy-dispersive (compositional) analysis (EDX) and fluorescence in situ hybridization (FISH).

Recent considerations of potential sources of energy for subsurface life on Mars have focused on the availability of both energy (H_2, CO) and oxidant (O_2) in the Martian atmosphere (Weiss et al., 2000). However, the existence of lithotrophic microbial communities on Earth that are driven by energy from geologic processes (e.g. H_2 generation coupled to tectonic activity or mineral weathering; Stevens and McKinley, 1995; Chapelle et al., 2002) suggest the possibility that life on Mars could be supported by subsurface as well as atmospheric sources of energy. Reduced and oxidized Fe mineral phases represent prime candidates for redox-active compounds capable of supporting subsurface life on Mars and other extraterrestrial environments (Jakosky and Shock, 1998; Santelli et al. 2001, Irwin and Schulze-Makuch, 2001; Schulze-Makuch, 2002).

When solutions flow through rocks, minerals dissolve. In basaltic rocks, weathering of abundant ferromagnesian silicates (e.g., olivine, pyroxene, amphibole) and sulfides (e.g., pyrite) begins with oxidation of ferrous iron bearing minerals. These reactions consume oxidants (e.g., nitrate or dissolved oxygen. Subsequent interaction between these oxidant-depleted fluids and minerals yields ferrous iron rich solutions that can migrate through the subsurface. Even in weathered basaltic rocks from Abert Rim that are exposed to Earth's atmosphere, evidence of significant iron mobility (and subsequent redeposition) has been documented (Banfield et al. 1991). Redox gradients form in subsurface regions where reduced, iron-bearing fluids mix with more oxidized fluids, or where the fluids have increased access to the atmosphere.

Ezymatic oxidation of iron by microorganisms at near neutral pH is often precluded by extremely rapid inorganic oxidation kinetics. However, reaction kinetics are slow in redox gradients where the oxygen concentration is low, and in very acid solutions (see below for discussion of acidic solutions). The two target environments for microbial iron oxidation in near-neutral pH systems are (i) in proximity to surfaces of Fe-rich minerals that are undergoing the early stages of dissolution in groundwater solutions and (ii) in gradient zones where reduced and oxidized fluids mix and are cycled.

The existence of "iron bacteria" able to oxidize ferrous iron in circum-neutral pH solutions has been known for over a century. However, comparatively little effort has been directed towards their investigation. In the last few years, however, there have been a number of important new discoveries in this area. For example, although the ability of iron oxidizing bacteria to conserve energy from iron oxidation remained uncertain for many decades, it is now well established that at least a subset of species (including members of the well known *Gallionella* genus) are able to fix CO_2 using energy derived from iron oxidation (i.e., they are autotrophs, thus can underpin a subsurface biosphere). Additionally, a greater diversity and more widespread distribution of iron oxidizing organisms in near-surface environments has been demonstrated, both microaerobically (Emerson and Moyer, 1997; Emerson, 2000; Sobolev and Roden, 2001) and anaerobically through coupling of Fe-oxidation to nitrate-reduction (Straub, et al 2001; Hauck, et al 2001). To date, only a handful of key papers have appeared because the number of researchers working on iron-oxidizing microorganisms has been small and cultivation and characterization of these organisms is difficult. Profs. Emerson and Roden, leaders in this effort, bring key expertise in this area to the NAI team. Given its potential importance on Mars (and on Earth), the biological and geochemical importance of chemoautotrophic iron-oxidizing prokaryotes in basalt-dominated systems is an important focus of this proposal. This metabolic group may be especially relevant to early life on Mars if the ability to utilize ferrous iron as an electron donor was an early evolutionary development, as some analyses suggest.

Simultaneous with the recognition of the wider range of habitats for iron-oxidizing neutrophiles (microorganisms living in near-neutral pH solutions) has been acceptance of the importance of deep subsurface microbial communities. Much of the analysis on deep subsurface communities has focused on hydrogen and methane-based metabolisms (e.g., Stevens and McKinley, 1995, Chapelle et al., 2002). We will not attempt to duplicate this effort. Rather, we will focus on nearer-surface systems where iron oxidation is coupled to oxygen or nitrate reduction.

Microoganisms may be able to utilize ferrous iron released by dissolution of iron-bearing minerals. The redox potential of ferrous iron phases (including structural ferrous iron in primary or secondary minerals) can be substantially lower than that for dissolved ferrous iron. Thus, ferrous iron bearing minerals may provide a unique basis for energy metabolism. Springs with visible, orange, iron oxide accumulations are not uncommon. These may be the excellent analogs for Martian biomes, such as those that may have been associated with the Sinus Meridiani deposit. We speculate that a very significant range of iron oxidizing organisms will be discovered in appropriate rock-dominated microaerophilic environments (e.g., at the interface between solutions and ferrous iron-rich minerals).

Ferric iron mineral products represent a potential electron acceptor for anaerobic metabolism of organic carbon or hydrogen. Together, iron-oxidizing and iron-reducing life forms may regulate a coupled iron and carbon cycle.

We propose a detailed investigation of the biogeochemistry and microbial community structure of subsurface and near-surface environments at the Abert Lake and Snake River study sites, which preliminary analyses suggest may provide plausible analogs to potential Fe-based life systems in shallow subsurface environments on Mars (for example, where water generated by geothermal heating of subsurface ice deposits; Carr, 1996) may permit development of microbial communities based on Fe(III) oxide reduction, Fe(II) oxidation, or coupled Fe reduction and oxidation (Figure 11). The motive for these studies stems from our assertion that careful documentation of the biogeochemical characteristics and microbiological composition of terrestrial Fe-based life systems is an appropriate first step toward development of the knowledge base and analytical techniques that will eventually be required to detect signatures of existing or past life on Mars and other extraterrestrial bodies.

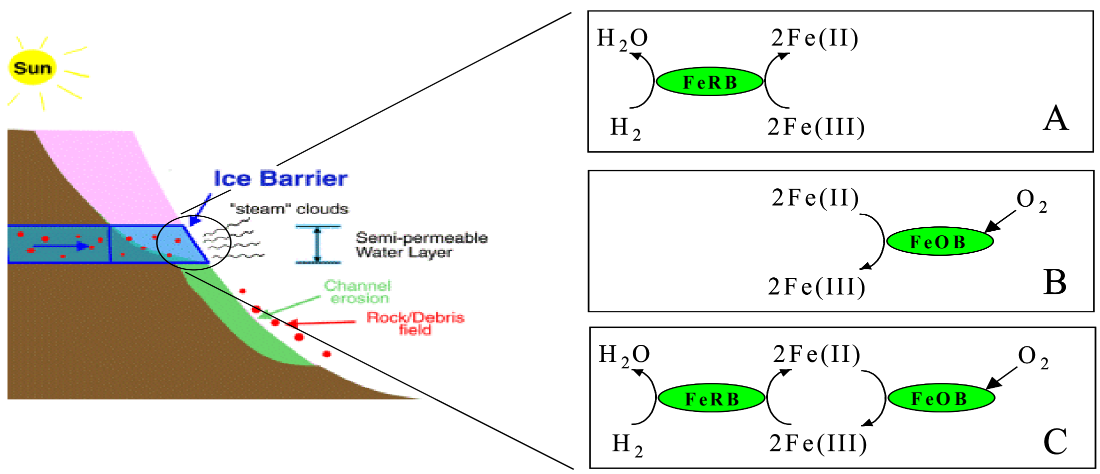


Figure 11. Potential setting for subsurface Fe-based microbial life on Mars driven by (A) Fe(III) oxide reduction (geothermal H_2 source), (B) Fe(II) (e.g. andesite) oxidation, or (C) coupled Fe(III) reduction and Fe(II) oxidation. FeRB = Fe(III)-reducing bacteria; FeOB = Fe(II)-oxidizing bacteria.

We hypothesize that basaltic rocks will host significant populations of organisms that are sustained by Fe cycling, especially by iron oxidation. We will characterize the structure and activity of microbial populations to test this idea. Mineralogical biosignatures at the field sites will proceed along three interrelated lines (1) detailed studies of in situ geochemical distributions correlated with information about microbial community structure; (2) process-level studies of mineral transformations coupled to Fe metabolism by pure or mixed cultures retrieved from field samples; and (3) controlled studies of microbial Fe redox metabolism in bioreactors seeded with organisms from the field site.

3.2.1 Microbial populations in chemoautotrophically-based ecosystems at field sites

In order to determine the size of microbial populations sustained by iron cycling in near-surface environments, soils, weathered rocks, solutions, and associated microorganisms will be collected from sites of groundwater discharge at the Abert Lake and Snake River spring sites. Similarly, organisms to be used in the study of the factors that limit growth of microbial populations sustained by metal sulfide mineral dissolution will be collected from the Richmond Mine site. Robotic sample collection from otherwise inaccessible regions of the Richmond Mine is described in Section 3.3.2

The environmental chemistry will be determined at the time of sample collection of samples from all three sites. This work will benefit from real time microanalysis (hundreds to sub 100 μm-scale) of O_2, pH, Eh, T, ferrous iron and sulfur compound concentrations. Chemical analyses will include determination of oxygen and pH using standard electrochemical microsensors, soluble Fe(II) and Fe(III) gradients using voltammetric microsensors, at microscale ($\leq$ 100 μm) resolution. Novel aspects of the in situ geochemical measurements are discussed in section 3.3.1. At the Abert and Snake River sites, analyses will utilize well-established 'peeper' technology, where multiple membrane-enclosed diffusion chambers separate microbes and pore water and allow measurement of important pore water chemistry in the absence of microbes. Solid phases will be identified by direct electron microscopic and x-ray diffraction characterization. In some cases, refrigerated microtome frozen subcores will be microtome sectioned. Where appropriate, selective chemical extraction-based analysis of the solid-phase materials will be conducted.

How large a subsurface microbial population might be sustained by dissolution of ferrous iron-rich silicates comprising basalts and basaltic andesites on Earth, thus possibly associated with weathering of basaltic rocks near the Martian subsurface? What is the size of populations sustained by coupled iron oxidation and reduction or other processes occurring in the basaltic rocks? What sorts of microorganisms occur, and what factors control their distribution?

In order to answer these questions, microbial populations at the Abert and Snake River sites will be evaluated via a combination of culture-based methods (Emerson and Roden). Firstly, the microbial populations sizes will be determined by direct total cell counts and quantification of extracted lipids, as well as by Most Probable Number (MPN) assays for aerobic and anaerobic Fe-oxidizers and for Fe-reducers, S-reducers and heterotrophs. In each case the base medium will be based on the chemistry of the field site to the best of our ability to determine what that is. Attempts for both enrichments and MPNs will also be done using solid mineral phases (Emerson laboratory). In all cases, a subset of the highest dilution MPN tubes that show growth will be used for continued enrichment and isolation of the physiologically most relevant microbes (see Section 3.2.2).

The microbial populations in basaltic rock-dominated habitats will be documented via molecular phylogenetic analysis (primarily SSU rRNA gene sequencing work by Emerson, Roden, and Banfield laboratories). Using the sequence data, we will design fluorescence in situ hybridization (FISH) probes to quantify population abundances at the species and higher levels. This work will follow methods reported by Edwards et al. (2000) and Bond et al. (2000), and Bond and Banfield (2001). Real time PCR (qPCR) quantitation of specific populations of the Fe-oxidizing microbial community will be carried out in the Emerson laboratory using Taq-man primer-probe combinations. The probes may be the same used as for FISH analysis of neutrophilic iron-oxidizing bacteria (FeOB). In addition, we will also use terminal restriction fragment length polymorphism (tRFLP) as a tool to assees overall community diversity at the field sites. These latter techniques will done using established procedures for qPCR (Suzuki, et al 2000) and tRFLP (Liu, et al, 1997) Data from different sites and conditions will be compared in order to understand the factors limiting microbial populations and how population structural changes relate to geochemical changes.

Biomineral products in basalt and basaltic andesite habitats will be characterized in detail using optical and electron microscopic methods, X-ray diffraction, electron microprobe-based compositional analyses, and isotopic analyses (isotopic work is described in section 3.2.4).

3.2.2 Physiology, and possible role of biomineralization in energy generation

To complement the field-based studies of bacterial communities and associated biosignatures, the physiology of isolates will be studied in the laboratory. Particular attention will be given to identification of neutrophilic Fe-oxidizers and establishing the ecological roles (e.g., in carbon and nitrogen fixation) of different phylotypes. Culture-based studies to identify iron-oxidizing organisms will be critical if we are to determine their abundance and impacts on their geochemical environments.

Neutrophilic Fe-oxidizers from the field sites will be cultivated using bioreactors and other growth methods (see above) useful for carrying out basic physiology and ecological studies. At present our research group has 9 strains of lithotrophic, neutrophilic FeOB in pure culture isolated from a wide range of environments that can be comparative studies (see Table 2 in the Results of Previous Support section, below). In addition, we will isolate new FeOB and iron-reducing bacteria (FeRB) from the field sites and from the bioreactors. The isolates will be extensively characterized, including physiology studies aimed at determining optimal growth conditions and substrates. The forms of C- and N-metabolism will also be determined using standard isotope methods, ^{14}C-uptake and $^{12}C/^{13}C$ ratios for C and ^{15}N-uptake for N. The phylogeny of the isolates will be determined uisng 16S rDNA analyses, and their genotypes will be evaluated used –rep-PCR (De Bruijn 1993, Versalovic et al. 1998), and their phenotypes using MALDI-TOF-MS. These latter results will be compared with existing data for other species from other sites. This work will be conducted primarily in the Emerson laboratory. The cultivation of iron-cycling microorganisms from relevant environments is essential for experiments to investigate the impact of active cells on mineral surface evolution and for experiments designed to measure isotope fractionations. In essence, these pure culture studies will 'ground-truth' our in-situ studies.

Pure cultures will be analyzed with microelectrodes to determine, in detail, the physico-chemical parameters for growth. These cultures will be the used in bioreactor studies designed to explore microbial communities and biosignature development.

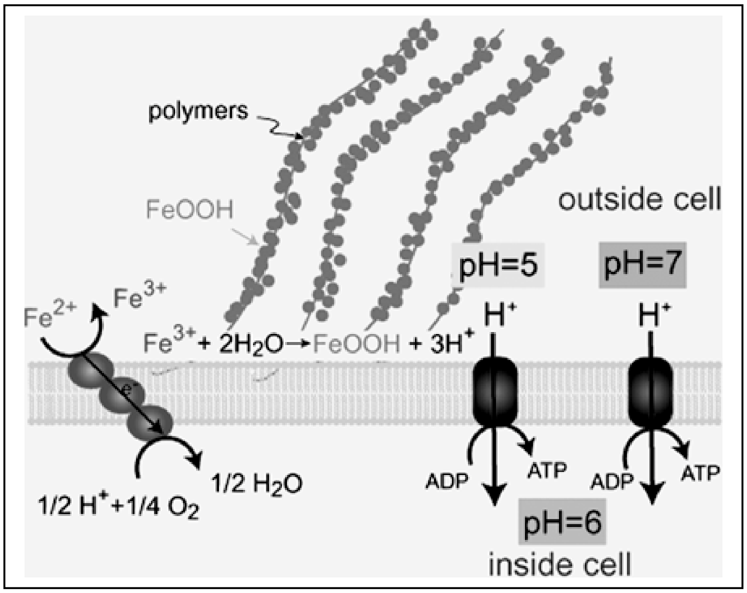


Figure 12:

Diagram indicating possible connections between proton generation, pH gradients, proton motive force, and energy generation. Increased energy generation is possible if proton supply, rather than Fe^{2+} supply, is rate limiting. Diagram: Chan and Banfield.

A hypothesis developed by the Emerson and Banfield groups posits a special and unusual connection between mineral precipitation and energy generation by iron-oxidizing neutrophilic microorganisms. Metabolisms based on iron oxidation in neutral and alkaline environments colonized by iron-oxidizing bacteria are thermodynamically possible, but the energy yield is only moderate. Protons must be pumped against a pH gradient (a challenge not faced by acidophilic iron-oxidizing prokaryotes). As noted by Emerson (2000), mineral precipitation liberates protons. If mineral precipitation occurs in proximity cells, the proton electrochemical gradient is changed, leading to an increase in the proton motive force (pmf). We postulate that the reason for extensive (and expensive!) production of extracellular polymer strands is to localize iron oxyhydroxide precipitation adjacent to the regions of the cell surface where ATP synthase complexes are concentrated. This could result in a higher energy yield if the rate of energy generation by the cell is limited by proton supply, not by supply of ferrous iron. This idea is illustrated in Figure 12.

As shown, rate of utilization of electrons from Fe^{2+} is directly linked to the rate of supply of protons (it is unknown whether proton pumping accompanies flow of electrons from Fe^{2+} to O_2; it appears that they are not in acidophiles). Either the supply of Fe^{2+} or protons could limit the rate at which energy can be generated. If the latter, energy generation can be increased if proton transport into the cell is accelerated by increase in the pmf due to proton release following iron oxyhydroxide precipitation. We will test this hypothesis by measuring microscopic pH environments in proximity to active cells, by altering the concentrations of ferrous iron and pH to establish growth limitation, and by determining if there is a correlation between sites of polymer secretion and ATP production. If requested genome sequence for an isolate (PV-1, a mesophilic iron-oxidizing bacterium from a hydrothermal vent site (Emerson & Moyer, 2002) becomes available (DOE Joint Genome Institute microbial sequencing proposal, resubmission encouraged), the additional prediction of a correlation between the location of iron oxidizing enzymes (e.g., cytochromes, multi-Cu-oxidases) and polymers structures will be tested. If our hypothesis is correct, the implication is that intimate association of polymers and minerals should be widespread and important. This work will be conducted via collaboration between the Emerson and Banfield groups.

3.2.3 Mineral Biosignatures associated with microbial Fe-oxidation and Fe cycling

We will extend the field-based studies of iron- and sulfur-cycling microbial communities by studying the structure, dynamics and products of these systems in the laboratory. Process-level studies will be conducted with pure and/or mixed cultures obtained from the field sites. CoI Roden, working closely with Co-Is Emerson and Luther, will lead this aspect of the proposed work. The goals will be to provide information on the rates, pathways, and end-products of specific enzymatic processes, e.g. microbial reduction of Fe(III) oxides, oxidation of soluble and solid-phase Fe(II) compounds, and sulfate-reduction. In addition, bioreactors (Figure 13) will be used to investigate the ability of microorganisms obtained from the field sites to thrive and generate biogeochemical and mineralogical signatures of life under conditions that approximate those which may be present in shallow subsurface environments on Mars. The reactors (1-10 dm in length) will be packed with selected Fe-bearing mineral phases (e.g. Fe-rich basaltic glass, Fe(II) silicate minerals, in mixtures containing some cases FeS_2 (to simulate basaltic rocks), and Fe(III) oxides in a quartz matrix. Reactors will be inoculated with a consortium of organisms from field sites. The reactors will be designed to allow for control of temperature, pressure, water content, and gas composition. The water content (a crucial factor for life in the Martian subsurface) and rate of fluid flow through the reactors will be regulated by altering the degree of pore space saturation through control of hydrostatic pressure at the outflow end of the reactor (e.g. Seyfried and Rao, 1987). We anticipate that macroscale (tens of cm to mm) and microscale (mm to micron scale) **chemical gradients** associated with bacterial processes will develop within the bioreactors.

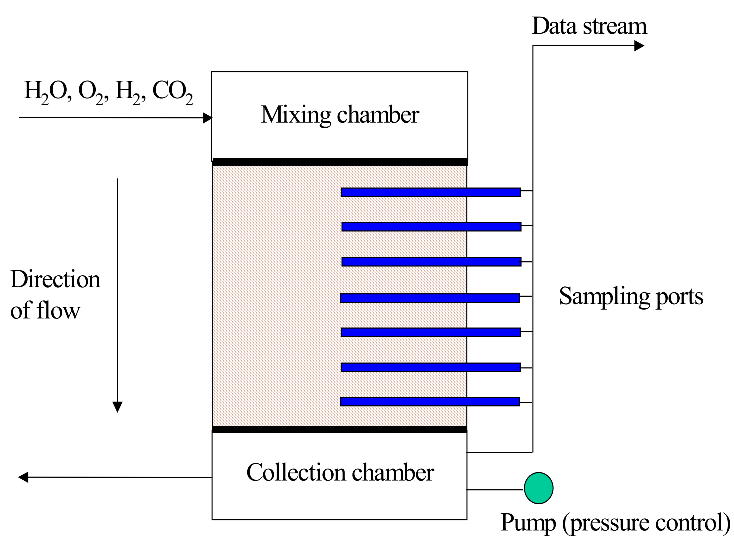


Figure 13 Diagram of bioreactors to be used for studies of Fe-based microbial metabolism under conditions approximating the Martian subsurface environments.

Methods described in section 3.3.1 will be critical for gradient characterization measurements, to be provided by Co-I Luther. Chemical heterogeneities should be paralleled by **variations in the microbial communities**, to be documented by Co-Is Roden and Emerson, and Fe-bearing mineral phases formed, to be documented by PI Banfield. The evolution of redox gradients, development of microbial communities, and alteration of mineralogical properties will be followed over time in order to gain insight into the potential biogeochemical structure and mineral biosignatures of Fe-based subsurface life. Several of the necessary techniques have already been applied to studies of Fe(II) oxidation and coupled Fe(III) reduction/Fe(II) oxidation by pure cultures of Fe(II) oxidizing and reducing microorganisms (Sobolev and Roden, 2002; Roden et al., 2003).

Distributions of redox-active and related chemical species that either consumed (e.g. O_2, H_2, CO_2, Fe(III), S(-II)) or produced (e.g. Fe(II), SO_4^{2-}, H^+) as a result of Fe-driven microbial metabolism will be compared to those predicted on the basis of non-reactive fluid and/or gas transport plus abiotic chemical reactions (using standard **numerical reactive-transport modeling** techniques; Steefel and MacQuarrie, 1996; Boudreau, 1997) in order to provide independent verification of the role of microbial activity in controlling redox speciation in the reactors. Co-I Roden has recently employed such techniques for analysis of biogeochemical Fe cycling in freshwater wetland sediments (Roden, 2003) and experimental microbial Fe cycling microcosms (Roden and Sobolev, 2001).

Microbial communities that develop in the bioreactors will be sampled in a limited way during operation, and more extensively upon termination, as described above for the community analysis of the field sites. **Bioreactor microbial population analysis** methods will include FISH and real time PCR quantitation of specific groups, MPN quantitation and enrichment, as well as either tRFLP or 16S rDNA clones libraries to assess overall community diversity within an individual bioreactor and for comparison to the field sites.

An important characteristic of neutrophilic microbial iron oxidation, especially when it occurs at sites of fluid mixing (e.g., groundwater entering a swamp; intersection of subsurface fluid flow paths, etc.), is the generation of polymers that become loaded with iron oxyhydroxide minerals. In modern systems, the activity of iron-oxidizing bacteria is evidenced by distinctive, highly mineralized cells and cell products. In modern samples, these are highly recognizable, especially when they are produced by *Leptothrix* and *Gallionella* spp. (Figure 14). In the last few years, micropaleontological studies have revealed compelling evidence that these same structures are preserved for millions of years as evidenced by analysis of ancient hydrothermal sites and 'red beds' where it was evident that Fe oxidation and cycling were occuring. Filamentous Fe-oxides and Fe-silicates analogous to extant Fe-oxidizing bacteria such as *Gallionella* spp. and *Leptotrhix ochracea* can be found in the fossil record at ancient hydrothermal vent sites, as well as other sites where the physico-chemical conditions appear conducive for Fe-oxide precipitation (Juniper and Fouquet, 1988; Juniper and Sarrazin, 1995; Little, et al 1999; Preat, et al 2000). A recent review of a wide variety of specimens suggested these types of biosignatures could be quite common (Hofmann and Farmer, 2000) The most dramatic of these are microfossils associated with the Pilbara Craton in Australia that are dated to an age of 3.2 bya, and that are associated with pyritic deposits (Rasmussen, 2000).

Preliminary work in aquifer and ocean systems reveals that the **morphology** of polymer-mineral aggregates varies significantly with habitat, and that many of the resulting biomineral accumulations have features that are highly diagnostic of their biological origin. Consequently, we will examine the detailed nature of mineralized polymer products of Fe-oxidizing prokaryotes. Mineral-polymer mixtures from the field site, cultures, bioreactors, and in situ studies (see below) will be characterized by optical, scanning electron, and transmission electron microscopy. These methods provide information about the size, shape, and structure of minerals and the way they are associated with polymers. Comparison of mineralized biopolymers from different species will enable us to determine the conditions under which these potentially very important biosignatures form. In parallel, we will synthesize iron oxyhydroxides via a variety of inorganic methods and characterize these with the same techniques used to study the natural biomineral materials. We will also conduct polymer mineralization experiments, following the approach of Nesterova et al. 2002. It may be to distinguish biomineral signatures that strongly indicate the preexistence of polymers such as large prebiotic molecules or cell degradation products because preliminary evidence indicates that iron oxyhydroxides can preserve evidence of these (e.g., micron-scale mineralized polymer filaments are reported by Chan et al. in review and shown in Figure 14 as curvy filaments).

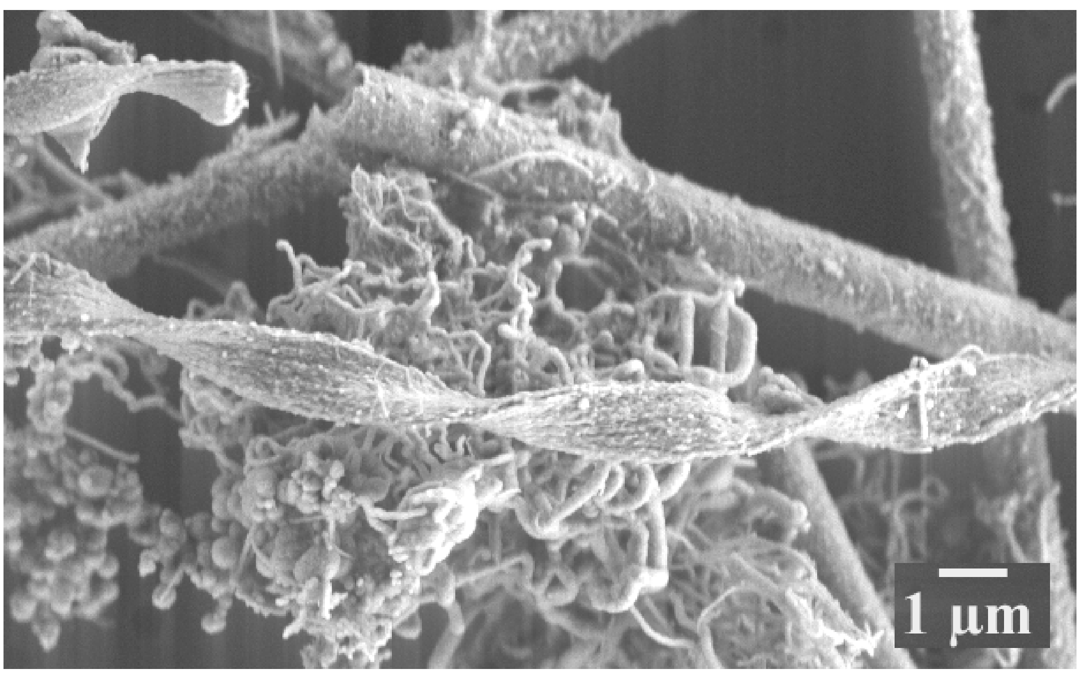


Figure 14: Scanning electron microscope image of distinctive mineralized biopolymers: sheaths, stalks, and fibrils

Some Fe-oxidizing microorganisms generate Fe-oxide products but apparently do not generate energy from this reaction (*L. discophora* and *S natans*). In other cases (ES-1 and PV-1), iron oxidation is associated with energy transduction (Emerson & Moyer, 1997 & 2002; Neubauer, et al 2002). HRTEM analysis of the polymers-mineral materials will be conducted to determine if this difference influences the type of minerals formed. This work will include physiology experiments, where cells are grown under different oxygen concentrations, Fe concentrations, temperature, and pH, as well as shaking/physical regimens, all aimed at understanding how these conditions may effect Fe-oxide mineralization by these two groups.

Microorganisms generate very distinctive mineral products. For example, nanocrystalline magnetite can be produced directly by magnetotactic bacteria (Blakemore, 1975) or indirectly through production of Fe(II) by dissimilatory Fe(III)-reducing bacteria that is later incorporated into magnetite (Lovley et al., 1987) and sulfate-reducing bacteria can generate extremely small metal sulfide particles. In principle, Fe-reducing organisms could have been responsible for generation of the carbonate-embedded magnetite nanocrystals in the Martian meteorite Allan Hills 84001 (McKay et al., 1996). Although recent detailed electron microscopic evidence suggests that the magnetite nanocrystals in the Allan Hills 84001 meteorite are not of biological origin (Barber and Scott, 2002), this by no means rules out the possibility that biosignatures of Fe-based life on Mars and in other extraterrestrial environments may eventually be identified. Rather, these recent analyses provide important information for constraining when potential mineralogical biosignatures such as magnetite do and do not provide valid evidence of biological activity. Given the vast amount of prior work on magnetite as a biosignature, will examine the basaltic rock-dominated habitats and bioreactors studied for evidence of magnetite produced biologically, focusing primarily upon its distribution and mineralogical context.

The relationship between organic compounds, especially exopolymers, and Fe biomineralization is of considerable interest. Organic molecules could be stabilizing aqueous Fe (III) complexes (see section 3.3.1). Large exopolymers are probably critical in biomineralization and biosignature preservation. Consequently, an important aspect of the culture work will be to determine the **chemistry of polymers** (especially sheaths, stalks, and fibrils) formed by neutrophilic and acidophilic Fe-oxidizing bacteria. Preliminary studies indicate that it is common for all the neutrophilic Fe-oxidizers isolated in Dr. Emerson's laboratory to form a variety of exopolymers (when Fe oxides are removed with hydroxylamine, large amounts of organic polymer remains). It is possible that some exopolymers form a matrix or scaffold upon which Fe-oxidation occurs, whereas others are passive substrates on which Fe-oxyhydroxide nanoparticles accumulate. In addition to the role polymers could play in localized pmf generation, as hypothesized above, they may also provide a means by which the cells can prevent themselves from becoming encased in an impermeable crust of Fe-oxides. Based on studies of sheath structures formed by heterotrophic Fe and Mn-oxidizing bacteria, these exopolymers may be complex acidic polysaccharides (Emerson and Ghiorse 1993).

We will determine the nature of these biopolymers because we hypothesize that their specific nature will influence the composition, structure, defect structure (crystallinity), and particle size of the associated Fe-oxide and Fe-oxyhydroxide minerals. This work, to be carried out by the Emerson group, will involve pure cultures already on hand (Table 2) and newly isolated species, as they become available. The principle composition of sugars/polysaccharides, proteins, and lipid content will be determined.

Assuming that it is principally polysacchride, then an analysis of the major carbohydrate moieties, e.g. amino sugars, uronic acids, neutral sugars, etc. will be performed. This latter work will be accomplished most readily by sending samples to the Center for Complex Carbohydrate Research at the University of Georgia, which provides a reasonable fee-based service for these types of analysis.

Relationships may exist between the nature and rate of production of the polymer matrix and the environmental conditions. This will be examined by determining the ways in which environmental conditions regulate exopolymer production in cultures. The experiments will utilize conditions similar to those described above for effects of environmental conditions polymer-biomineral formation and will be performed in parallel with those experiments in Dr. Emerson's laboratory.

In the proposed work, our team will examine the detailed compositional characteristics of polymer-mineral assemblages associated with iron-oxidizing microorganisms that colonize basalt-spring systems. We will determine the ways in which the iron oxyhydroxides differ in **element and trace element composition and morphology** from inorganic products formed due to rapid inorganic iron precipitation. Thus, we will investigate whether a biological origin can be established in the absence of preserved cell materials. This work will involve continuation of the collaborations among Emerson, Roden, Banfield.

An important question that must be dealt with when developing biosignatures for analysis of ancient samples is **'how well do they age?'**. As noted above, the biomineral products formed by iron oxidation have characteristics that are highly diagnostic of the presence of microorganisms (e.g., Fig. 14, Chan et al., in prep; Banfield et al. 2000) and polymers (Chan et al. in prep). Although some of the most obvious features survive in some geologic settings (see above), it is unclear to what extent the distinctive morphological, microstructural, and microchemical characteristics of these complex biomaterials will survive over long time periods. We anticipate that subtle features of ancient iron oxide deposits that are crucial to biosignature analysis may be lost. For example, the distinctive particle size, submicron-scale impurity content, and crystal orientation of nanoparticles may be eliminated by crystal growth and phase transformation reactions. It is important to know which of the interpretable characteristics (e.g., submicron-scale mineralized tubes formed by *Lepthothrix* sheaths and ribbon-like structures formed by *Gallionella*) may be retained. Because little is known about the extent to which the morphology and microstructure of biomineral products are preserved over geologic time, we will experimentally investigate aging of biomineral materials.

We hypothesize that the presence of polymers, and their specific composition and structure, may be important determinants of the evolution of textural characteristics of polymer-mineral composites during diagenesis. Consequently, Dr. Banfield's laboratory will study thermally-driven changes in cell-(and cell product-) mineral assemblages in order to understand biosignature preservation in ancient samples (e.g., size, microstructure, morphology, composition). The fate of microbial and simulated polymer-mineral assemblages will be evaluated in conducting coarsening experiments at between 40 and 150°C under hydrothermal conditions, in air, and under vacuum. This work will involve biomineral materials generated in the Roden and Emerson laboratories, inorganically precipitated mineral controls, and synthetic polymer-mineral aggregates. Samples will be characterized by powder X-ray diffraction, BET, and high-resolution transmission electron microscopy (HRTEM). The kinetics of growth and transformation reactions will be monitored using peak area analysis and peak broadening analysis of X-ray diffraction data (see methods of Penn and Banfield, 1998).

Isotopic fractionation commonly accompanies biomineral formation. **Isotopic composition** of minerals formed as the result of microbial iron and sulfur cycling represents an important component of the NAI team's proposed research (below).

3.2.4 Isotopic biosignatures

i) Biological and inorganic isotope fractionations recorded in rocks

A vital aspect of this work is to establish baselines for non-biological isotope fractionation effects. This will be accomplished through the work of Dr. Boering, dealing with atmospheric isotope fractionations that may be recorded in minerals, and via measurements on Martian meteorites and materials from terrestrial analog systems via Dr. DePaolo.

Dr. DePaolo's work will focus on analysis of Fe and Ca (but also meaure S, H, O, as appropriate) isotopes, which are fractionated mainly by biological processes. His group will determine whether the magnitude and sense of the fractionations are discriminatory, thus diagnostic of biological processes.

As noted above, Fe is a redox sensitive element and may be critical to microbial metabolism on Mars. Recent work suggests that biologically produced Fe- bearing minerals may have distinctive isotopic compositions. Microbially-mediated iron reduction (Beard et al., 1999), organic chelation (Brantley et al., 2001), and anoxygenic photosynthesis (Croal et al., 2002; Johnson et al., 2002) fractionate iron isotopes ratios by 1.3 to 1.6‰, concentrating the light isotope preferentially in the dissolved state.

However, recent studies of natural and experimental systems have also demonstrated that abiotic processes can fractionate iron isotopes. Bullen et al. (2001) investigated the abiotic precipitation of ferrihydrite, a ubiquitous process in the natural environment, and showed that the ferrihydrite precipitate was enriched in the heavy isotope by about 1 to 2‰ relative to dissolved Fe^{+2}(aq) (Bullen et al., 2001). More recent results (Skulan et al., 2002) suggest that equilibrium precipitation of goethite alone does not fractionate iron significantly. Skulan et al.'s (2002) results exclude the Fe^{2+}-Fe^{3+} oxidation step, while Bullen et al.'s (2001) study includes this step. In addition, Skulan et al. (2002) suggest that kinetic isotope effects during relatively rapid precipitation of goethite can create a solid that is 1.3‰ lighter than the Fe^{3+}(aq) in solution.

The interpretation of the abiotic experiments is complicated by issues of aqueous speciation, which may also fractionate iron isotopes. Spectroscopic studies (Schauble et al., 2001) indicate that iron isotope fractionations between coexisting species in solution may be significant (δ^{56}Fe range of 7.8‰ at 25°C between various chloride and hydroxide iron species). Experimental evidence (Johnson et al., 2002) indicates a +2.8‰ fractionation between Fe^{3+} and Fe^{2+} in solution.

Hence, there is a significant likelihood that the Fe isotopic composition of minerals could be indicative of a biological origin. This cannot at present be proven. The best way to approach this problem would be by studying natural terrestrial systems that might be analogous to those on Mars, and establishing just how large Fe isotopic effects are in these systems.

What we know about terrestrial rocks so far is that igneous rocks do not vary significantly in their δ^{56}Fe values, with a total range between 0.1 and 0.6‰ (Beard and Johnson, 1999; Beard et al., 1999; Johnson and Beard, 1999; Johnson et al., 2003). However, some natural samples do have significant variations; these include chemical (± biological) precipitates such as marine Fe-Mn nodules and crusts (Beard and Johnson, 1999; Beard et al., 1999; ZHU et al., 2000), and soils, stream waters, and precipitates from streams (Bullen et al., 2001; Fantle and DePaolo, 2002). It is possible that all of these observed fractionations are ultimately due to microbial processes, but this cannot be proven yet because the appropriate systematic studies have not been done.

Although not necessarily essential to life on Mars, Ca may be included in biominerals. On the Earth, Ca isotopes are apparently significantly fractionated in nature almost solely by biological processes (Skulan et al., 1997). Typical mineral matter for terrestrial organisms has δ^{44}Ca values that are about 1.5‰ lighter than the Ca source (Skulan and DePaolo, 1999). Some marine organisms (certain foraminifers) can apparently produce larger fractionations, up to about 3‰ (Nagler et al., 2000). Calcium bearing minerals could therefore provide a potentially conclusive test for the existence of life on Mars. If there was never any life, there should be very little Ca isotope fractionation – the existence of life would essentially be required if significant Ca isotopic variability were observed.

The work plan for this approach will be integrated with other work on terrestrial analogue sites. Fe and Ca bearing minerals associated with biological activity (both in situ and in experimental systems) will be measured for isotopic composition to determine the natural range of values that can be expected. Co-I Roden has already participated in NASA-supported studies of Fe isotopic fractionation coupled to dissimilatory Fe(III) oxide reduction (Johnson et al., 2002). These samples will be compared to precipitates in systems where biological activity is not a factor, and analyzed in the context of experimental results from our experiments and those in the literature. In addition, we will characterize the Fe and Ca isotopic composition of carbonate and other minerals in Martian meteorites. The meteorite data may be highly interesting whether or not significant fractionations are observed. They will also be useful as baseline data for future sample returns.

ii) Isotopic fractionation in the Martian atmosphere: constraints for interpreting inorganic fractionations recorded in rocks

Recent studies on Martian meteorites have shown that isotopic signatures in oxygen and sulfur that could only have been produced by photochemical processes in the atmosphere have been preserved in the rock record [Farquahar et al., 1998, 2000a, 2000b; Bao et al., 2000]. It is possible that atmospheric photochemistry producing hydrocarbon hazes (discussed above in section 3.1.3) could also result in a carbon isotopic signature that is preserved in the Martian rock record. If the particles produced from the precursor CH_4 are isotopically light, it is also possible that a carbon signature from atmospheric processes could be misinterpreted as a biologic signature. Indeed, this atmospheric scenario has been proposed as an alternative explanation for the presence of isotopically light kerogens (degraded organic carbon) found in the geologic record 2.8 Gy ago on Earth [Pavlov et al., 2001b]. For example, the current interpretation for Earth's rocks is that the very light kerogens are created by an additional fractionation of the carbon isotopes due to the rise of methanotrophic organisms that consumed the methane produced by methanogens when O_2 levels rose high enough in Earth's atmosphere to support methanotrophs [Hayes, 1994]. Yet, this interpretation would have to be investigated further if the carbon isotopic composition of a photochemical haze produced from the photolysis of CH_4 in an N_2, CO_2, and H_2O (i.e., early Earth-like) atmosphere is isotopically light. Using the experimental technique outlined above in section 3.1.2b, we can test the hypothesis of Pavlov et al [2001b] that photochemistry alone could impart an isotopic signature in carbon in the martian rock record that could potentially be mistaken for a signature of metabolism. Particles formed in our reaction chamber with a simulated martian atmosphere will be collected and analyzed offline for the bulk and compound-specific $\delta^{13}C$ values. These new measurements will provide a baseline for the contribution of abiotic atmospheric processes to the isotopic composition of carbon in martian rocks and meteorites.

3.2.5 Mineral surfaces as biological indicators

Although its clear that heterotrophic microorganisms can profoundly affect both the rates of silicate mineral weathering by producing acids and complexing ligands (Barker et al. 1997), little attention has been focused on the possibly distinctive effects of microbial activity on surface topographic evolution.

Microbial activity in proximity to mineral surfaces may lead to changes in surface structure and composition (see Banfield et al. 2001 for review). For example, recently Welch et al. showed that surface etching of apatite (the primary mineral source of phosphorus to the biosphere) is dramatically different in the presence of organic ligands compared to inorganic ions (Welch et al. 2002). Similarly, Teng and Dove (1997) showed that surface step geometries on calcite are highly dependent on surface-binding ligands. We will investigate the ways in which microorganisms impact their surroundings primarily though studies of chemoautotrophic prokaryotes growing in proximity to metal sulfide surfaces and microbial communities living in the porous surfaces of phosphate and silicate minerals in basaltic andestite rocks from the Abert Lake region. We will compare surfaces dissolving in the absence of cells and after exposure to microbial communities in laboratory experiments (section 3.2.3). Surface characterization will be by field-emission scanning electron microscopy and via surface compositional analysis (e.g., x-ray photoelectron spectroscopy). Particular attention will be paid to phosphate mineral surfaces, as these have been shown to focus microbial activity in weathered rock (Taunton et al. 2000). An example result may be the finding that insoluble elemental sulfur accumulates on dissolving metal sulfide surfaces but is absent on metal sulfide surfaces in biological experiments containing S-oxidizing prokaryotes.

Our investigations of mineral surface features potentially diagnostic of microbial activity also will involve situ studies of biosignature formation at the field sites. It may be difficult to convincingly establish that features of field-collected samples can uniquely be interpreted as biosignatures because the initial state of these materials is unknown.

Consequently, in addition to laboratory experiments, we will conduct **in situ studies** to investigate biosignature formation at the Abert and Snake River sites. In these studies, samples of well-characterized minerals will be emplaced at the field sites for periods ranging from a few months to up to 4 years. Samples will be retrieved and characterized in terms of microbial surface colonization, morphology change, and compositional change. In the first experiments, carefully prepared, precharacterized (dimensions, surface features, weight) slices of Fe-rich volcanic glasses, olivine, and pyroxene will be placed into microaerophilic sites in the field. Analogous slices of metal sulfide minerals (e.g., marcasite, pyrite, arsenopyrite) will be placed at another site (these experiments are similar to those reported previously by the Banfield group in the Richmond Mine, see Edwards et al.1998). It is anticipated that the minerals will dissolve at different (but predictable, and measureable) rates, releasing ferrous iron (and sulfide ions) into the environment. Cell numbers will be evaluated using methods noted above; FISH analyses and qPCR methods will be used to evaluate population structure. Mineralogical methods (see above) will be used to document changes in surface structure and to identify mineral precipitates. Isotopic features (e.g., Fe, H, and O) will be determined, as described below. Addition of a fresh source of energy (e.g., the reactive sulfide mineral in ground water) may locally (and conveniently) stimulate metabolic activity and mineral dissolution and precipitation phenomena of interest.

In one experiment, the Banfield group will emplace a kilogram of fine-grained, sterilized pyrite into a confined region at the Abert site. This creates the potential for considerable local acidification. This experiment is designed to explore how rapidly microbial colonization of distinctive habitats occurs. Are there a few cells of acidophiles in basaltic rocks (e.g., 'everything is everywhere'?) available to colonize the acid habitat, or are microbes rapidly dispersed from remote acidic sites? This experiment will run for five years.

3.2.6 Exploring life's extremes in an acidic Fe-S system

Our third field site is a very acidic system associated with a volcanic-hosted metal-sulfide deposit. In combination with work on neutrophiles described above, research at this site will extend out scope to cover much of the pH range for biological iron oxidation (from ~ possibly < 0 to 9 (environments with pH ~ -3.5 were described in currently inaccessible regions of the Richmond Mine by Nordstrom et al. 2000).

The Richmond Mine sustains a remarkably productive subsurface biosphere (Fig. 3), and provides an analog for possible localized microbial habitats associated with volcanic-hosted iron sulfide accumulations on Mars. Acid solutions form when oxidative dissolution converts pyrite to aqueous ferrous iron and sulfuric acid. Microbes play a key role in environmental regulation because they catalyze the slow (inorganic) reoxidation of ferrous iron, producing soluble Fe(III) which is the primary sulfide oxidant at low pH. Microbial catalysis of iron oxidation can dramatically increase rates of metal sulfide oxidation, allowing microbial communities to regulate the pH at values optimal for their survival.

The extremely acidophilic microbial communities that populate this site have been extensively studied by the Banfield group. Prior research has revealed the structure of microbial communities (at the level of species, a defined by SSU rDNA analysis) and explored correlations between species makeup and geochemical conditions. The work to date has been limited to pH > 0.5 habitats and has not evaluated the limits of life for these organisms or those in other mine habitats, or the metabolic processes that are important in setting these limits.

The Richmond Mine acid mine drainage system at Iron Mt., CA, offers a unique opportunity to explore the answers to these questions because (i) it contains regions more extreme than virtually anywhere else (estimated to have temperatures > 65 °C in combination with extreme acid) and (ii) it is one of the first systems for which extensive community genomic data are available (~ 20 mb at the time of submission of this proposal, ~140 million mb anticipated). These data are the product of separate DOE and NSF-funded genomic sequencing and analysis efforts to evaluate lateral gene transfer (DOE) and ecological response to normal system perturbations (NSF).

In the proposed work, samples will be robotically collected from the most extreme (and currently unstudied) environments within the Richmond Mine system. This has recently become possible because extensive tunnel reconstruction (part of the ongoing remediation effort) has provided access to entry points to old stopes (caverns created by mining) and tunnels. We have EPA and mine owner approval to deploy robots into regions that cannot safely be accessed by human investigators. Preliminary to this proposal, we tested a prototype robot in the environment (see section 3.3.2). This test provided important information about modifications needed in order to sample in the extreme environments of the tunnel system. The proposed robot modification work is described in section 3.3.2. One of the two objectives for the robotic sampling is to obtain samples of organisms adapted to the most extreme conditions possible for characterization and to be used as innoculum for experiments to investigate extreme adaption. The other objective is to develop geomicrobiological-robotics protocols for remote sampling.

The robotics project will represent a collaboration between the Yim (Robotics), Luther, and Banfield groups. The Luther group's special contribution will be in in situ analysis of sulfur intermediates and measurement of soluble Fe(II) and Fe(III). A particular goal of this project is to implement these electrodes on the robot so that relevant data can be collected at the sampling location.

Organisms will be isolated in the Banfield laboratory. Isolation procedures for a subset of organisms have been used successfully by this group (however, this has not been a priority). The physiology of existing uncharacterized and new isolates will be analyzed.

In the proposed laboratory experiments to explore extreme environmental adaption (relative to the normal habitat for these organisms), we will use both batch cultures and specially designed bioreactors that replicate the natural environment. Use of bioreactors will probably be critical to permit co-cultivation of microbial populations. A diagram of the proposed bioreactor is shown in Figure 15. Important features are darkness (to simulate the subsurface), a long reactive path length with adjustable gradient (flow rate), and controlled temperature, pH, humidity, and gas mixture. The system will include computer-controlled sensors to permit continuous monitoring of most physical and chemical parameters (ionic strength monitoring, temperature, pH) and chemical and biological 'scrubbing' prior to recirculation. The system is designed to be compact and modular, making many duplicate systems practical. Microbial populations size and makeup will be monitored by FISH, direct cell counts, and (if necessary) quantificaiton of total lipids. Sampling will target planktonic, biofilm, and sediment-attached cells.

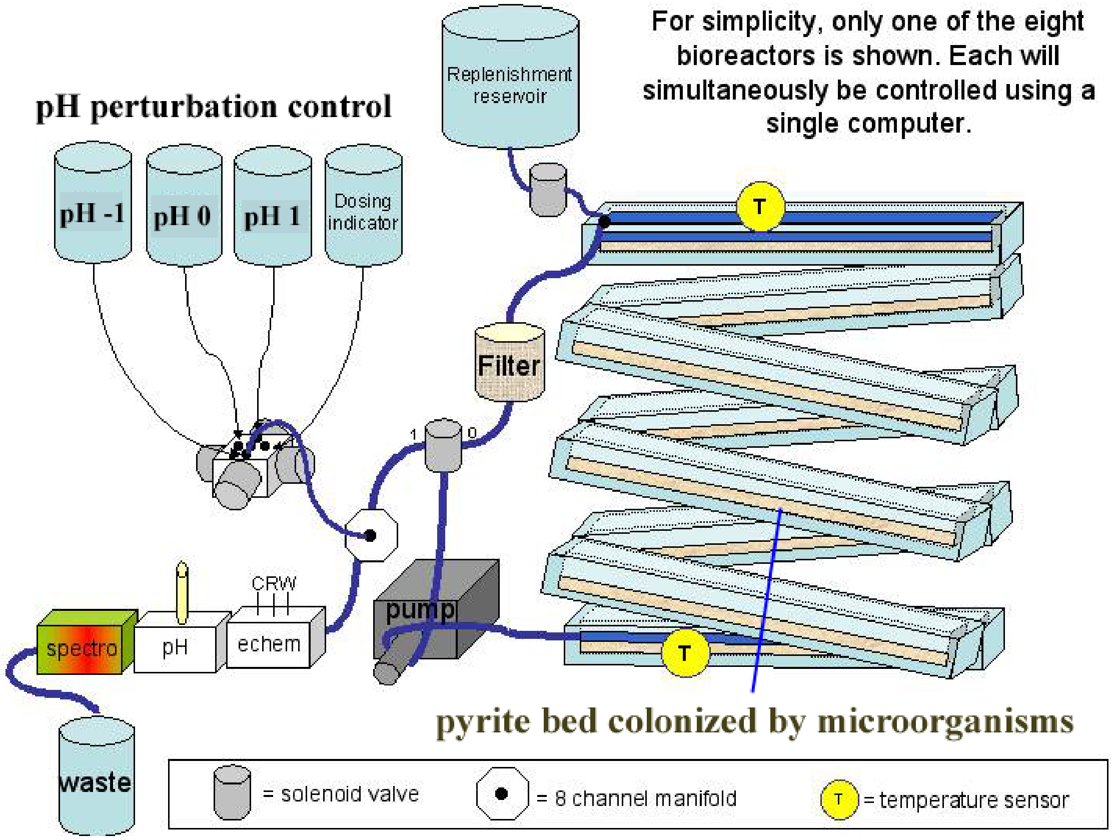


Figure 15: Proposed variable gradient bioreactors. The bioreactor will be enclosed (dark), temperature, fluid flow, humidity (gas mixture) and pH be controlled.

The Banfield group will evaluate the response, at the level of gene expression, of microorganisms colonizing pH 0.5-1.0 habitats to environmental challenges. Our objective will be to determine how organisms respond, and the sequence of events that marks failure to adapt (i.e., what determines the limits of adaption). Extremes to be investigated include cold, low water abundance, high ionic strength, and extremely low pH. At this time, we know that some microorganisms can grow at pH values as low as ~ 0 and temperatures in the range of 30-50 °C. Our goal will be to determine the limits of pH and temperature tolerance.

Information about gene expression (pathway- and organism-specific activity) of members of the populations over time (and in response to changed environmental conditions) will be obtained by using gene expression arrays (e.g., Wu et al. 2001). These are currently being designed for above mentioned parallel projects in the Banfield laboratory. The arrays are possible because genomic data for a pH 0.7 community will be available. Current estimates of the array size for this project is up to ~10,000 proteins, this corresponding to all genes for 6 species (species defined at the 16S level) and evidence for relatively little genome variability within species (Tyson et al. 2003).

Although the extreme acid study sites have high chemoautotrophic microbial activity, it may be difficult to detect most categories of biosignatures in them. Typically, the entire sulfide mineral assemblage is dissolved and very little secondary mineral precipitation occurs (unless some pH neutralization occurs). Furthermore, the long-term durability of organic products (e.g., lipids) at low pH is limited. Potentially promising biosignatures for this system are primarily in the form of etching patterns on sulfide mineral surfaces. These are potentially difficult to interpret (Edwards et al. 2000b). However, at least some (as yet unidentified) microorganisms do etch themselves into surfaces, creating extremely deep etch pits distinct from those formed inorganically. Attention will be given to characterizing these microbe-mineral interfaces (primarily via differential interference contrast and optical microscopy, SEM and FISH with existing or new probes). However, the primary questions to be pursued in this proposed NAI effort relate to microbial adaption and tolerance under relevant environmental extremes.

3.3 THRUST 3: NOVEL IN SITU MEASUREMENTS AND ROBOT-BASED GEOMICROBIOLOGY

Introduction

A key aspect of the proposed work is integration of Mars remote sensing, field-, and laboratory-based studies. **In situ measurements** and **sampling** will be important aspects of this work.

- In both the field and laboratory studies, **high-resolution geochemical measurements** of ion concentrations, redox state, fluxes, and temperature at the macro (cm-m scale) and micro (µm-mm scale) spatial scales will provide crucial information about metabolic options, active microbial metabolic processes, constraints for microbial cultivation-based studies, and biosignature analyses.

- As the ultimate question (about life on Mars) will probably be answered largely by remote investigation (e.g., involving robots), we feel that it is important to develop the **methods and approaches needed for robotic-based studies** in parallel with development of strategies for the scientific investigations.

It is <u>not</u> our goal to stimulate a major robot development program under this NAI. Rather, our intention is to work closely with scientists who are actively involved in robot development in order to explore the challenges and opportunities. If the NAI is awarded, other interations with NASA robotics scientists (e.g., Dr. Silvano Colombano, NASA Ames, who has been to the Richmond Mine site with the Banfield group) will be sought (to be funded by other avenues) in order to broaden this effort.

3.3.1 Development of sensors for in situ biogeochemical measurements– simultaneous detection of multiple chemical species that are biologically relevant

Until Dr. Luther's group developed a solid-state gold-amalgam (Au/Hg) voltammetric microelectrode for sediment work (Brendel and Luther, 1995), it was not possible to measure non-gaseous biologically important species in freshwater and seawater solutions with microelectrodes for two reasons. First, the membrane-covered electrodes are not permeable to ions (other than the proton) and second, the potentiostats for the application of potential were relegated to only one fixed potential where more than one species may be electroactive. These two features did not allow for the determination of the important redox metals Fe(II) and Mn(II). Using the solid-state gold/amalgam (Au/Hg) microelectrode, Dr. Luther's group is able to measure dissolved O_2, H_2S, I^-, Fe(II), Mn(II), polysulfides and FeS_{aq} (Luther et al, 1998; Rozan et al, 2000; Theberge and Luther, 1997) because each redox species, if present, produces a current that can be detected in one potential scan [this is analogous to varying wavelength and monitoring absorbance in UV-VIS spectroscopy]. A key advance from our present work is the finding that FeS_{aq} was present in Contrary Creek, VA waters rather than Fe^{2+} alone (see results of previous work). This means that microbes are using a different source of reduced Fe than expected.

Also germane to this proposal, the electrode measures soluble Fe(III)species (see Huettel et al, 1998; Taillefert et al, 2000, 2002), which are metastable to precipitation only in the presence of organic ligands and are molecular clusters of unknown composition. These clusters are transportable, used by microbes (Dollhopf et al, 2000) and are intermediates in the formation of iron-oxide nanocrystals, as found by Banfield's group (e.g., Banfield et al. 2000).

Dr. Luther's group uses a portable electrochemical system designed with an instrumental company (Analytical Instrument Systems, Inc.) to monitor *in situ* redox species from environments ranging from microbial mats to sediments (Luther et al, 1999; 2001) to hydrothermal vents (Luther et al, 2001, 2002; Nuzzio et al, 2002). The calibration / validation of the electrodes and initial salt marsh results were described in Brendel and Luther (1995). Several papers, as noted above, have been published describing field research with the solid-state microelectrode and an *in situ* intercomparison of the O_2 data from the solid state electrode with the traditional Clark O_2 microelectrodes. The comparison yielded excellent agreement (Luther et al, 1999). Also, other trace metals (Cu, Pb, Cd, Ni, Zn) can be measured (Sundby et al, 1999). Most importantly, the electrodes have shown that chemical speciation drives hydrothermal vent ecology (Luther et al, 2001) and that molecular biology corresponds with *in situ* redox measurements (Glazer et al, 2002). Both Fe and S speciation can be measured and these redox species are a significant chemical source for microbes in areas ranging from ground waters, seeps, acid mine drainage sites (AMD), etc**. Thus, the electrode can be used to prospect for specific life forms.** The Luther group proposes this use in this proposal, and to couple this technology with the robotics equipment of Dr. Yim to analyze chemistry in extreme environments.

In addition to *in situ* measurements in the field, Au/Hg electrodes allow the possibility of studying primary and secondary diagenetic and microbial reactions in lab studies in real time. Recently, Dr. Luther's group (Dollhopf et al, 2000) has used the microelectrodes to follow Fe(III) and Mn(IV) reduction in cultures of *Shewanella puterfaciens*, strain MR-4. These solid-state electrodes are robust and will be used in culture experiments with other team members. With Dr. Roden's group (Roden et al, 2002; see results of previous support), we have already demonstrated the electrode's ability to investigate Fe dynamics in a gel experiment, which contained Fe oxidizing microbes above Fe reducing microbes. Thus, we propose to use our electrodes in the proposed bioreactor system for real time measurement of redox chemical species (see section 3.2.3). The electrode system will permit us to obtain more accurate kinetic data on microbial processes and assess which redox chemical species are used by microbes. Traditional sampling and analytical methods can create artifacts and lead to incorrect interpretation of chemical and biological processes (Luther et al, 2001, 2002).

3.3.2 Robotic-based geomicrobiology: sampling inaccessible regions of the Richmond Mine

The challenges of sample collection and site characterization in some underground regions of the Richmond Mine have some parallels to sample retrieval and analysis on Mars: the samples occur in places where it is currently impossible for people to go; the habitats are extreme, simultaneous imaging, environmental characterization, and sample selection are required. Robots have the potential to solve these problems.

However there are significant differences as well. Subsurface locomotion requires mobility that is different than the Martian terrain explored in the past. Not only are there mobility constraints in climbing over obstacles (e.g., rocks), but size constraints requiring small profiles and as 'sediment' (pyrite accumulations in the tunnels) conditions that maybe submerged by acid solutions. In the context of the Richmond Mine system, the environment has regions that have extreme pH and temperature. As noted above, work to date has been concentrated on the 'moderate and moderately extreme' sites (pH 0.5 – 2) but more acidic environments occur in pools in caverns in other parts of the mine system. At this time, we have no idea of the types of microorganisms that will be encountered at temperatures above 50 °C or pH values of close to, and below 0. Our first goal will be firstly to determine if such organisms exist.

Since the requirements for specific in situ geomicrobiology and biogeochemical experiments in these areas involve mission specifications that are significantly different than those used in current robotic exploratory missions, these experiments will also lead to determining and validating requirements for possible future robotic mars missions.

We have conducted a preliminary test of a modular robot, known as a 'PolyBot', in the tunnel system and determined the types of modifications necessary to allow it to function in the mine environments. We propose to carry out these modifications, then develop methods to permit sample collection and necessary *in situ* measurements. Robotics work will be carried out by the Yim group, who will work with the Banfield group to develop sampling protocols and test the system.

PolyBot is a modular reconfigurable robot (MRR) that is compact, light, durable, and versatile (Figure 16). It is made up of repeated modules that may be arranged into different configurations depending on the task. The system promises to be extremely versatile (from reconfigurability), robust (from redundancy) and low cost (from economies of scale from repeated units). The Yim group (Yim 1993; Yim et al. 2003) has shown that MRRs possess the basic relevant abilities that make it potentially invaluable in extraterrestrial planetary exploration: it can move over a variety of terrains, including rough gravel, through a gopher hole using a snake-like concertina gait and through a man made obstacle course (made by US marines). NASA Ames recognized the promise of such as system and adopted an early prototype design of PolyBot in their system called “snakebot” to study the usefulness for use in planetary exploration. This system received a large amount of international press.

The snake-like concertina gait demonstration was the first exploration of its kind inside an unstructured natural tunnel by a snake-like robot, and may be particularly useful for more highly constrained areas in the Richmond mine system. Even more apropos, early demonstrations of the system were shown exploring the surfzone (under water tested at a local beach), which included a simple liquid sampling tool on it as initial analysis for the Richmond mine exploration. Because it has been designed as a generic tool, PolyBot can also manipulate complicated and heavy objects, as required for sampling and delivery and operation of other *in situ* test equipment (see section 3.3.1). In addition, PolyBot has demonstrated its versatility over a variety of challenges, including riding a tricycle, climbing fences, stairs, poles, manipulating boxes, and self-reconfiguration; some in tethered and some in untethered forms (Yim et al. 2000, Yim et al. 2002). The ability to reconfigure its modules is potentially very useful in Mars exploration: small robots can rapidly explore some areas and join up in order to traverse difficult areas. Reconfigurable versions may be alternately able to form the shape of a rolling hoop, walking spider or slithering serpent, as needed, allowing them to access a greater diversity of places. The modular form ensures redundancy, a useful strategy to deal with component failure.

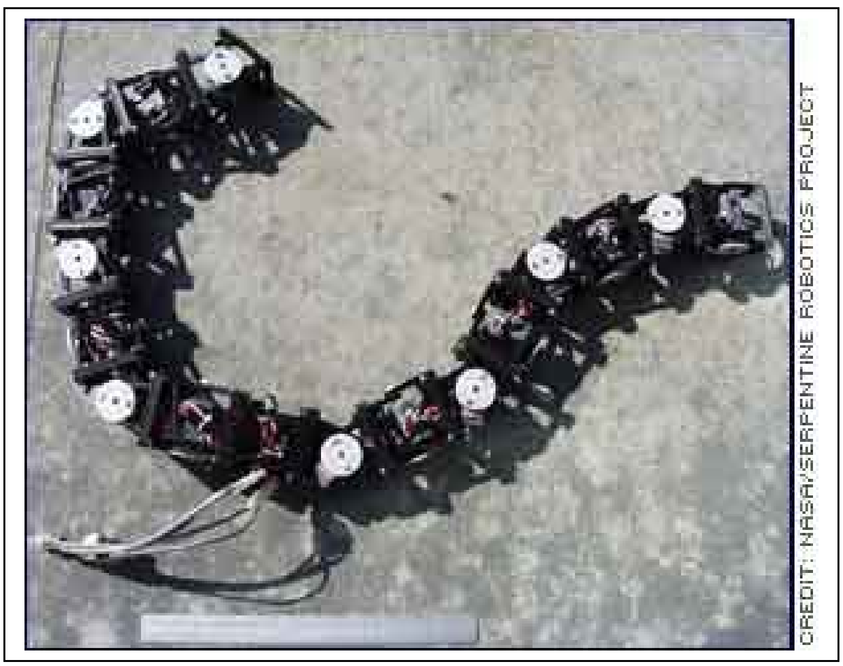


Figure 16: Photograph of a modular, reconfigurable robot similar to that used in a preliminary test in the Richmond Mine. The robot will be modified to improve durability in acid solutions, and interfaced with tools to permit *in situ* chemical measurements and collection of samples from regions where people can not go.

The Yim group proposes to further harden the robots for the acid environments, adapt them to be able to take and return samples and measurements, tune the mobility for the Richmond Mine, specifically to optimize performance in fine pyrite mud and acid pools, and to test and develop interface and methods for humans to be able to control and semi-teleoperate robots for our tasks (subsurface navigation, sample and return measurement).

The robotics work will begin by testing robots in accessible sites within the mine. The two primary objectives of the first phase of research are to optimize sample collection and in situ analysis methods and to evaluate robot vs. human sampling bias. In the later case, sampling will be conducted in the same area by human and robot and results (biases) examined. Results will be used to fine-tune robotic sampling methods. Once the robot performance is fine tuned, the second phase of sample collection in inaccessible regions will begin. We plan approximately two on site trips per year for this work.

4.0 INITIAL RESULTS: GEOMICROBIOLOGY IN Fe-S SYSTEMS

Approximately 1.5 years ago, a team of investigators (Banfield, PI; Roden, Emerson, Luther, and Sturchio, Co-Is) received two years of seed funding from NASA (~$50,000/ yr/investigator) to conduct preliminary research that represents the foundation for the geomicrobiology part of this proposal (Sturchio, now chair of his Department at the University of Illinois at Chicago, decided not to participate in this full proposal). A summary of collaborative research accomplishments associated with the seed funding is provided below.

i) Can Fe-silicate dissolution sustain Fe-oxidizing bacterial populations?

Experimental work was conducted in the Banfield group, in collaboration with the Emerson and Roden groups, to determine if Fe-oxidizing bacteria can derive metabolic energy by oxidizing Fe(II) released during dissolution of ferrous-iron-rich silicate minerals (e.g., fayalite (Fe_2SiO_4), pyroxene (e.g., $CaFeSi_2O_6$, biotite ~ (K $(Mg,Fe)_3Si_3AlO_{10}(OH)_2$). The initial experiments by the Banfield group used the moderate acidophile *Acidithiobacillus ferrooxidans* (previously known as *T. ferrooxidans)*, which was successfully grown on fayalite (Santelli et al.2001 and Welch et al. 2002). Although this choice of system required use of fairly acidic solutions, it was useful because *A. ferrooxidans* is unable to grow on trace organic components, which are difficult to completely eliminate from experiments. By coupling kinetic analysis of the rates of release of ferrous iron via silicate mineral dissolution as a function of temperature and pH with analysis of the rate of utilization of ferrous iron by bacteria, it was possible to predict that basalt weathering reactions at ocean temperatures should sustain populations of > 10^5 cells/cm^3 (Welch et al., in prep). In research in collaboration involving the Banfield, Emerson, and Roden groups, experiments using isolates of iron-oxidizing bacteria with Fe-bearing silicate minerals as the source of dissolve ferrous iron were undertaken (ongoing work). The goal is to further test the predictions of iron-oxidizing bacteria population sizes sustainable by dissolution of Fe(II)-bearing minerals in basaltic rocks.

ii) Studies of microbial Fe(II) oxidation and Fe redox cycling in circumneutral environments

Research on neutrophilic iron-oxidizing bacteria, and the potential for microbial Fe redox cycling, was conducted at several different field sites. All of these sites have conspicuous flocculated Fe-oxides associated with characteristic structural remains of neutrophilic Fe-oxidizers, principally *Lepthothrix ochracea* and *Gallionella ferruginea*. The Emerson and

Luther groups have studied a surface stream (Contrary Creek) in Virginia which has a pH that ranges from ca. 5.5 to 6.5, and Fe(II) concentrations in the range of 50 to 100 μM. Emerson's group has made monthly measurements of Fe(II), pH, temperature, O_2, and conductivity at several sites in the stream. They also made visual and microscopic observations of the microbial community in the more acidic region of the stream, which indicated that the iron oxides have large numbers (~10^7 to 10^8 mL^{-1}) of tightly associated bacterial cells. Furthermore, they demonstrated proof of concept that tRFLP can be utilized to both document community diversity and track changes in dominant microbial groups in the community.

Roden's group has conducted a preliminary evaluation of the potential interaction between iron-oxidizing bacteria and dissimilatory Fe(III)-reducing bacteria at a groundwater Fe seep located near the campus of The University of Alabama in Tuscaloosa, AL. MPN analyses indicated the presence of 10^3 mL^{-1} and 10^4 mL^{-1} of culturable iron-oxidizing bacteria and dissimilatory Fe(III)-reducing bacteria, respectively, in the seep materials. These findings suggest that both Fe-oxidizing and Fe-reducing bacteria are actively involved in Fe redox cycling in the seep materials. The potential for Fe(III) reduction activity was clearly demonstrated by experiments in which materials were incubated under anaerobic conditions and changes in Fe(II) concentration were monitored over time (Fig. 17, upper panel). Fe(III) oxide reduction in electron-donor amended materials (Fig. 17, lower panel) resulted in the formation of distinctive, black-colored Fe(II)-Fe(III) hydroxide mineral phases. Molecular biological (16S rDNA extraction, PCR amplification, and analysis by construction of clone libraries and/or denaturing gradient gel electrophoresis) and fluorescence in situ hybridization (FISH) studies of the Fe cycling bacterial communities (using 16S rRNA probes available for known iron-reducing bacteria together with newly-developed 16S rRNA probes for circumneutral iron-oxidizing bacteria) in the seep materials are underway, and collaboration with G. Luther on microscale analysis of Fe redox distributions in the seep materials are planned for the near future.

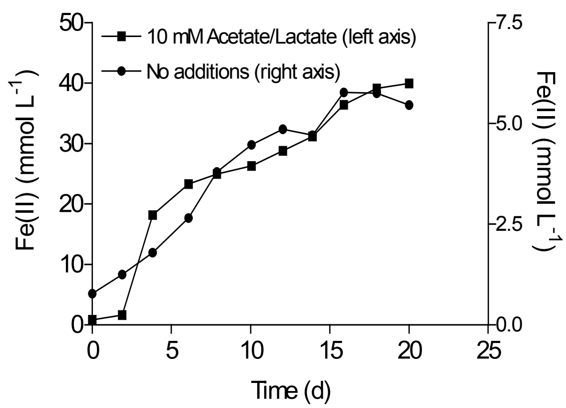


Fig. 17 Fe(III) reduction (upper panel) and reduced Fe mineral production (lower panel) in anaerobically- incubated Fe seep materials with (left) and without (right) electron donor amendment.

The Banfield group has studied biomineralization in the pH 7.2-8.6 carbonate-hosted subsurface tunnels associated with the Tennyson ore deposit in Wisconsin. Approximately 20 cm thick Fe-oxide biomineral accumulations have formed since the mine system was flooded ~ 32 years ago. We have extensively characterized the mineralogy of these materials, and described novel, elongate mineralized cell products (a central few nm wide fiber of akaganeite surrounded by flocculated ferrihydrite (Chan et al. in prep.). We have constructed clone libraries and FISH studies to explore the phylogenetic diversity of the community, made in situ measurements (using 'peepers') of solution chemistry, and developed an understanding of how fluid flow, geochemistry and microbial activity and interconnected in this system to generate highly diagnostic mineral biosignatures (Chan et al. in prep #2). Mixed cultures of iron-oxidizing bacteria have been established for further phylogenetic analysis, and for characterization via collaboration with the Emerson group.

Research involving the Banfield, Sturchio, and Roden groups was carried out at Soda Spring in western Oregon. Chemical data was collected by the Sturchio group for spring waters in which abundant Fe-oxide biomineral accumulations were forming. Results show a gradual decrease in Fe concentration from the vent to Pool 4, whereas all other measured constituents are essentially constant (including Mn). There is a large decrease of all constituents in Pool 6 due to dilution of inflowing stream water. The chemistry of this spring water is consistent with acid attack of intermediate volcanic rock by groundwater that has absorbed magmatic CO_2, with concomitant neutralization of the acidity through hydrolysis of minerals. The high Si (near saturation with amorphous silica) is typical of acid waters that have reacted with glassy volcanics. The low SO_4 and Cl- indicate that the magmatic gases absorbed by the water were already cooled (SO_2 and HCl absorbed at higher temperature). The SO_4^{2-} concentration is similar to that expected from an air-saturated water in which sulfate is generated by consumption of dissolved atmospheric oxygen with sulfide (dissolved or in the form of sulfide minerals). No nitrate (< 0.1 mg/L) was detected by ion chromatography. Stable isotopes (H, O, C) and dissolved gas measurements are currently being conducted. In parallel, the Banfield group has characterized the biomineral products of Fe(II) oxidation and are currently engaged in isolating FeOB from the spring materials. Most biominerals are in the form of ferrihydrite aggregations and some mineralized stalk structures. FISH data on these biomineral samples indicate abundant β-Proteobacteria. Roden's group has demonstrated the presence of cultivatable Fe(III)-reducing bacteria in a majority (5 of 8) spring samples.

iii) Development and application of methods for in situ analysis of microbial Fe cycling communities

An important aspect of both field and laboratory (experimental) based studies of bacterial Fe redox metabolism involves development of field sampling methods that allow *in situ* measurement of important physicochemical parameters across redox boundaries. These data are necessary for understanding of the range of environments suitable for Fe-oxidizing and Fe-reducing microbial communities, as well as for refinement of culture-based strategies that are essential for the metabolic characterization of the relevant organisms. Initial analyses of in situ Fe and O_2 dynamics have been completed using the microanalysis tools developed by Dr. Luther's group.

Field sampling using *in situ* voltammetry was performed at the Contrary Creek site in Virginia. This work has provided a unique data set on the chemical speciation of iron and sulfur that could not have been obtained by other techniques. The data clearly illustrate the importance of *in situ* analysis for identification of the major redox active forms that exist in natural waters. The system was initially thought to be Fe-dominated, based on colorimetric measurements soluble Fe(II) and Fe(III). However, the *in situ* data showed that molecular iron-sulfide clusters, FeS_{aq}, were dominant over dissolved free Fe^{2+}. Figure 18 shows the results of a profile from the Contrary Creek area taken on November 8, 2002. Successive cyclic voltammetry (CV) scans were performed in situ using a glass Au-amalgam microelectrode scanned between a potential of –0.05 and –1.8 vs. Ag/AgCl reference at a scan rate of 2000 mV/sec. Using this method, it has been determined in laboratory experiments with creek water and in other field work that potentials for Fe^{3+} will be at about –0.25 V, O_2 at –0.4 V, H_2S/ HS^- at –0.6 V, $FeS_{(aq)}$ at –1.2 V, H_2O_2 at –1.4, and Fe^{2+} at –1.6 V.

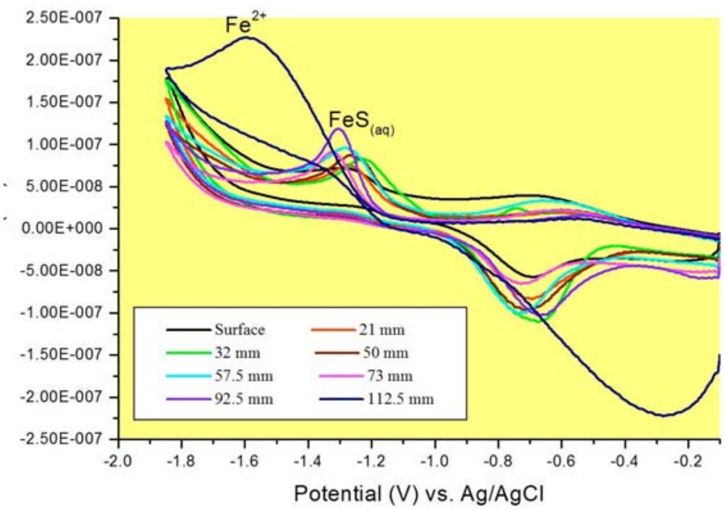


Fig. 18. Cyclic voltammetry scans from the Contrary Creek March area taken on November 8, 2002. Scans were performed in situ at different depths using a glass Au-amalgam microelectrode between a potential of –0.05 and –1.8 vs. Ag/AgCl reference at a scan rate of 2000 mV/sec.

The predominant features of most scans along this profile (and 2 others) taken at lateral positions 5 cm in either direction, showed a forward peak at approximately –1.2 V with the associated return peak at about –0.7 V. Iron [total Fe^{2+}] measured by the ferrozine method for these samples was 171 μM. The in situ CV scans (Fig. 18) show that the majority of the iron (and all the sulfide) is in the form of $FeS_{(aq)}$ clusters. Note that the $FeS(_{aq})$ peaks around –1.2 V (vs. Ag/AgCl) shift up to 100 mV through the profile. This is possibly due to changes in the molecular composition of the cluster (e.g., if it is Fe_4S_2 or Fe_3S_2, etc.) or to complexation of the cluster with organic compounds – we are currently testing these possibilities in the laboratory. Only at the deepest point in the water column did the iron exist as dissolved free Fe^{2+}. Analysis of the O_2 levels show that the stream water was microaerophilic, as there were very low levels of oxygen present (less than 10 μM, but above the Ar-purged standards). These data have altered our thinking of the key redox species that are being used by microbes in this system. We are now testing for sulfur oxidizers in addition to the Fe-oxidizers previously found in this system.

The Luther and Emerson groups have cultivated iron-oxidizing and iron-reducing bacteria the Great Marsh in Delaware. In addition, during a Chesapeake Bay cruise during the last week of July, the Luther and Emerson groups collected in situ voltammetry data and samples for cultivation of Fe(III)-reducing bacteria. The Luther group will carry out culture work on organisms isolated by Emerson and follow Fe chemistry in situ during incubations as documented below with the Roden group.

The Roden and Luther groups have investigated coupled Fe oxidation and reduction in experimental Fe(III) oxide-reducing microcosms in the presence and absence of a newly-isolated neutrophilic Fe(II)-oxidizing bacterium strain TW2 (Sobolev and Roden, 2003). Based on the ability of TW2 to cause unique alterations in patterns of Fe(III) oxide deposition in opposing gradients of Fe(II) and O_2 (Sobolev and Roden, 2001), we hypothesized that this organism might lead to enhanced coupling of Fe redox cycling at the aerobic-anaerobic interface of the microcosms. The existence of such biologically-catalyzed Fe redox cycling in structured (layered) microbial communities has important implications for patterns of biogenic mineral accumulation (Emerson, 2000), as well as Fe isotopic fractionation signatures (Johnson et al., 2002) in bacterial Fe cycling systems – e.g. systems which could conceivably exist on the surface of Mars. Results are described in a manuscript (Roden et al., 2002). In summary, the findings indicate that close juxtapositioning of FeOB and FeRB and rapid microscale Fe redox cycling occurred within the cocultures, such that the majority of the Fe was maintained in the reduced state at the aerobic-anaerobic interface despite the presence of detectable O_2 in the bulk aqueous phase. The lower concentration of dissolved Fe(II) in microcosms (as determined by voltammetric microelectrodes at the University of Delaware) inoculated with TW2 compared to those containing *S. alga* only (Fig. 19) provided direct evidence for enzymatically-enhanced Fe(II) and O_2 scavenging. More detailed studies of the distribution of FeOB and FeRB within Fe cycling aggregates, as well as more rigorous quantification of dissolved and solid-phase Fe(II) and Fe(III) pools

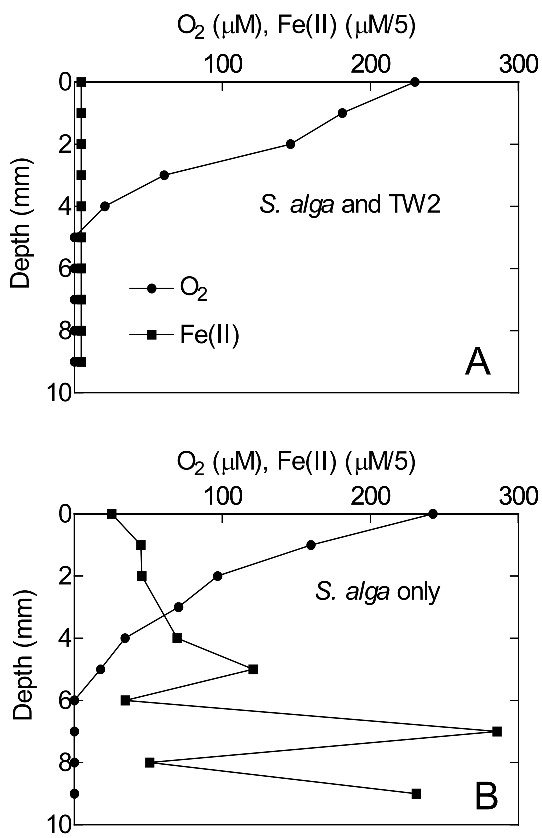


Fig. 19. Voltammetric microelectrode profiles of dissolved O_2 and Fe(II) in Fe cycling microcosms containing both *S. alga* and TW2 (A) or *S. alga* only (B). Data are averages of triplicate profiles. From Roden et al. (2003)

at the redox interface, are planned to verify our conceptual model (Roden et al., 2003) for the behavior of our coculture systems.

In situ voltammetry investigations of the chemistry of mats from salt marshes and a hot spring in Yellowstone showed Fe and O_2 profile data similar to the lab experiments described above.

Strain	Source	Obligate FeOB	Oxide type	Lab
ES-1	Groundwater,	Yes	Particulate	Emerson
ES-2	Groundwater	Yes	Particulate	Emerson
PV-1	Marine hydrothermal vent	Yes	Filamentous	Emerson
JV-1	Marine hydrothermal vent	Yes	Particulate	Emerson
CCJ	Rhizosphere	Yes	Particulate	Emerson
BrT	Rhizosphere	Yes	Particulate	Emerson
LD-1	Rhizosphere	Yes	Particulate	Emerson
Br-1	Rhizosphere	Yes	Particulate	Emerson
TW-2	Wetland	Mixotrophic	Particulate	Roden

Table 2: Strains of neutrophilic Fe-oxidizing bacteria (FeOB) isolated by the Emerson and Roden labs, and available for aspects of the proposed research.

iv) Influence of neutrophilic Fe-oxidation rates.

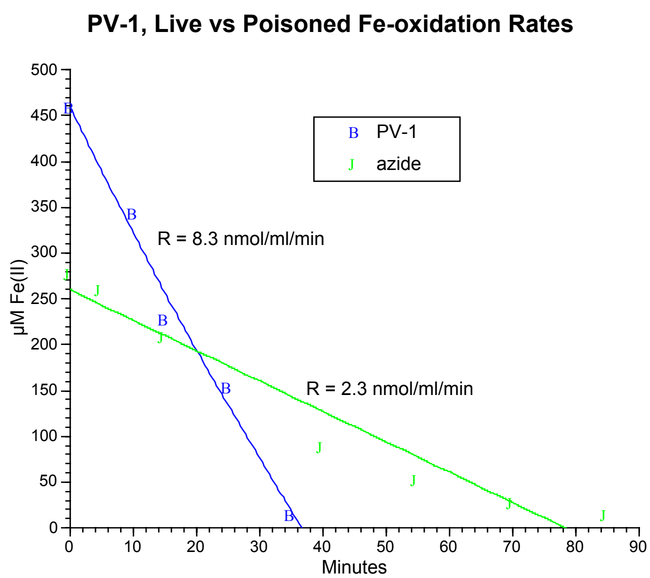


Fig. 20. Graph showing rates of Fe-oxidation for live PV-1 versus PV-1 poisoned with azide. The live rate was determined, then azide was added to the BR and the poisoned rate determined.

A focus of the Emerson lab during the last 18 months has been aimed at understanding the percentage of Fe-oxidation that is directly mediated by microaerophilic FeOB, and how much Fe(II) they need to oxidize to meet their energy requirements. We have used bioreactors that can control pH, temperature, O_2 supply, and Fe(II) supply to study these questions. A simple experiment, illustrated in Fig 20, demonstrates that the marine strain PV-1 may directly mediate nearly 80% of the Fe-oxidation in this system. A more detailed analysis of a freshwater strain, BrT, indicated that it could account for up to 60% of the Fe-oxidation in the system that was directly mediated by the cells and that the growth yield was 0.70 g of CH_2O (a proxy for cell C) per mol of Fe(II) oxidized. Furthermore, there was evidence that while live BrT accelerated rates of biotic oxidation versus abiotic oxidation, the presence of the cells and exopolymers could also suppress the abiotic oxidation of Fe(II) (Neubauer, et al, 2002). The strains of iron-oxidizing bacteria isolated by the Emerson and Roden labs and available for proposed research are listed in Table 2.

v) Connections between geochemistry and metabolism in acid systems

We have investigated the oxidation of tetrathionate ($S_4O_6^{2-}$), which along with thiosulfate ($S_2O_3^{2-}$), is an intermediate in the oxidation of pyrite in the natural environment and acid mine drainage systems. Hydroxyl radical (OH^*) forms on pyrite surfaces and enhances the oxidation kinetics of these intermediate sulfur species. We have also investigated the oxidation rates for tetrathionate in the presence of Fenton's reagent (Fe(II) and H_2O_2 which forms hydroxyl radicals). The reaction leads to sulfate and is too fast to be directly measured using UV-Vis spectrophotometry. Cyclic voltammetry experiments in acidic solutions indicate that the reaction of $S_4O_6^{2-}$ with OH^* goes through an unknown intermediate, tentatively assigned as $S_3O_4^{n-}$, which is stable in an inert atmosphere but not in air. An outer-sphere electron transfer mechanism for the reaction of $S_4O_6^{2-}$ with OH^* to form $S_3O_4^{n-}$ is proposed. Competitive reaction kinetics within the context of the Haber-Weiss mechanism suggests that the rate constant for the oxidation of polythionate with OH^* is diffusion controlled and $\sim 10^8$ M^{-1} sec^{-1}. This work represents a collaboration between the Luther and Banfield groups (Druschel et al., in review).

vi) Fe stable isotopic signatures of biogenic magnetite and Fe(II)-carbonate minerals

Biogenic magnetite and Fe(II)-carbonate minerals were generated in Roden's laboratory using the well-characterized iron-reduciing bacteria *Shewanella putrefaciens* and *Geobacter metallireducens*. The minerals were collected and dried under anaerobic conditions and shipped to the University of Wisconsin for analysis of Fe isotopic (^{54}Fe, ^{56}Fe) composition by multi-collector ICP-MS. These studies provide the first data on the isotopic composition of Fe(II)-bearing minerals generated during dissimilatory bacterial Fe(III) oxide reduction, and are included in a manuscript submitted for publication which describes the current state of knowledge concerning constraints on Fe isotope fractionation during biogeochemical Fe cycling (Johnson et al., 2002). The results indicate that magnetite will have ^{56}Fe/^{54}Fe ratios that are approximately equal to the Fe(II) in solution as the isotopic equilibrium is approached, whereas the ^{56}Fe/^{54}Fe ratios for Fe(II) will be 1-2 ‰ higher than Fe carbonates, where the largest fractionations are measured in Fe carbonates that contain limited Ca substitution. The relative order of δ^{56}Fe values for these biogenic minerals is therefore Ca-bearing Fe carbonate < siderite < magnetite, which is similar to that observed in natural samples such as banded iron formations, as well as that predicted from Fe isotope fractionations calculated from spectroscopic data. The results of these and ongoing collaborative studies open the way for use of Fe stable isotope analyses for assessment of the participation of biological activity in Fe redox cycling in natural sedimentary environments.

5.0 "BIO M A R S" AND THE ASTROBIOLOGY ROADMAP

Mars represents one of the few places where, in the near future, it may be possible to answer the fundamental questions that underpin the study of the origins, evolution, distribution, and future of life in the universe. Our work is primarily designed to tackle the question: '**Does life exist elsewhere in the universe**?" (Astrobiology roadmap (AR), fundamental question 2, goal 2) by targeting the habitat types on Mars with the highest probability of sustaining life and developing biosignatures for detection of past and current life (AR goal 7). The findings (life detection in combination with understanding of early Mars evolution) will impact thinking about the related question 'How does life begin and evolve? (AR fundamental question 1, goal 3). Should it happen that evidence for life is found on Mars, understanding of where it evolved and how life changed in response to changes in the abundance of water and the atmosphere will provide information about the drivers and consequences of dramatic climate change on the millions of years timescale. This understanding can be used to project the outcomes of environmental change on Earth. Thus, the proposed studies will seek information that, in the long term, may address the question: '**What is the future of life on Earth and beyond?**" (AR goal 3).

Although unintentional, Mars exploration may result in **colonization of Mars by Earth-derived microbial life.** The organisms most likely to survive are those that colonize terrestrial Mars-like habitats similar to those studied in our work. Thus, the proposed NAI is directly relevant to the question of the **potential of microbial life to adapt and evolve in environments beyond their planet of origin** (Astrobiology roadmap goal 6). Our work also may have direct relevance in the distant future if there is an attempt to deliberately introduce life to Mars.

6.0 SUMMARY

Three major interconnected research thrusts have been defined. Firstly, we will identify the places on Mars with the highest probability of protection from extreme radiation, bioavailable energy, and long-term access to water. The requirement for UV protection early in Mars history will be deduced from atmosphereic studies. This search will be accomplished by a novel, comprehensive approach in which we will deduce current and past conditions at the Martian surface by coupling studies of core behavior and planetary evolution to models of hydrosphere development and fluid discharge at the surface. Thus, we will generate targets for geomicrobiological studies. Our second research thrust is to determine the limits for life of in Mars-like Earth environments identified in the first research thrust (AR, goal 5), identify the energy options most likely to generate biosignatures, and identify the form of biosignatures expected as the result of utilization of this energy (AR goal 7). Our work will position NASA to optimize the chances of detecting Martian life, if it exists, or once existed.

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Plan for Strengthening the Astrobiology Community

Institutional Commitment

UC Berkeley has launched a major effort to pursue research and education in planetary science, planetary environments, and astrobiology. Two years ago they created an organized research unit called the Center for Integrative Planetary Sciences (CIPS). CIPS is directed by Prof. Geoff Marcy. CIPS has hired several new faculty (the PI and one of the CoIs for this proposal) and instituted several educational initiatives to support a new planetary/astrobiology program.

A new undergraduate curriculum has been established in planetary science under the auspices of the ``Astronomy" and ``Earth and Planetary Science" Departments. These new undergraduate programs integrate courses in chemistry, geophysics, astronomy, biology, and meteorology into a coherent curriculum. They will form a solid basis for the undergraduate and graduate programs for the NAI.

As part of the proposed activities for the NAI, PI Banfield and colleagues will develop a new course that will cover planet formation, origin of life, organism-environment interactions and the search for life in the universe. This course will be coordinated via the CIPS infrastructure. **Administrative support** for course management and coordination between the NAI and CIPS (separate from the NAI administrative support requested in the budget) and **computer support** will be provided by CIPS (see attached letter from Prof. Marcy).

There are many sources of support available to fund undergraduate research. Our education and outreach efforts will build upon several ongoing programs at UC Berkeley. SUPERB (Summer Undergraduate Program in Engineering Research at Berkeley) offers outstanding underrepresented engineering students the opportunity to gain research experience by participating in research projects with engineering faculty and graduate students. SUPERB is targeted to students of color, first-generation college students, educationally disadvantaged students, or students from historical minority institutions. The Berkeley Edge is a newly established recruitment, retention and advancement program for traditionally underrepresented minority graduate students in science, mathematics and engineering (jointly by the NSF and UCB). The Berkeley Edge goal is to identify, recruit, retain, and assist in advancing talented minority students to the professoriate.

There are several graduate programs available for training of graduate students working in astrobiology. These include opportunities associated with CIPS, Earth and Planetary Science, Environmental Science, Policy and Management, and the Microbiology Program.

As described in the attached letter from Prof. Marcy, CIPS will commit a significant fraction of its resources to the NAI, should it be funded. Specifically, they propose to **provide $50,000 per year** to establish a competitively awarded, advertised **"UC Berkeley Postdoctoral Fellowship in Astrobiology and Planetary Science".**

The Miller Fellowship Program provides funding for Postdoctoral Fellows. Recently, EPS has received approximately one per year. There is currently one Miller Fellow in the Geomicrobiology Program.

Under CIPS, we have already hired three new tenure-track faculty members, namely Drs. Eugene Chiang, Michael Manga, and Jill Banfield. Their research spans areas of planet formation, the chemical composition of planets, and the biogeochemistry of the atmosphere, oceans and mineralogy and surfaces, especially with regard to conditions suitable for biology. We plan additional faculty hires in the next year. CIPS has also hired a new Senior Fellow, Greg Delory, who specializes in the surface morphology and water on Mars.

Support for the proposed research will also be available through the following programs:

1.) The university provides up to two graduate fellowships to prospective graduate students each year. First year graduate students admitted to Earth and Planetary Science or Ecosystem Science are typically covered for at least one semester with fellowships.

2.) The department provides approximately ten Graduate Student Instructor (GSI) positions for graduate students per year.

3.) Department provided $1 million dollars for laboratory facilities and renovations to establish the geomicrobiology program.

4.) The campus has committed $300,000 in matching funds for new TEM and XRD instrumentation (MRI proposal in review).

5.) The department supports the following labs through a commitment of space (1,300 square feet) and staff (one full time staff member): Thin Section Lab, Electron Microprobe Lab, XRD lab and XRF Lab. Equipment in these labs is valued at $2,000,000.

6.) PI Banfield and Co-I Dietrich share an internet-based teleconference system that can be used by the NAI team.

7.) The campus supports the following technical staff: Tim Teague, Analytical Specialist (EMP, XRD, etc); Dave Smith, Machinist.

Banfield Group

Space: Rooms, 274, 452, 453 McCone and 108, 113, 120, 128 Hilgard totaling approximately 3500 square feet of lab space.

UC Paid Staff: $20,000/year in staff support via the AES program.

Equipment Value: $900,000

Dietrich Group

Space: Rooms. 269, 273, 313, 398 McCone totaling approximately 1,000 square feet of lab space.

Equipment Value: $500,000

DePaolo Group

Space: Rooms 5, 465, 467, 469 McCone Hall, totaling approximately 2,000 square feet of lab space.

UC Paid Staff: $21,000 /year in staff support.

Equipment Value: $1.2 million

Manga Group

Space: Rooms 6, 7, and 175 McCone Hall, totaling approximately 2700 square feet of computer and experimental lab space.

Equipment Value: $150,000 value

Boering Group

Space: Rooms DG24-DG26, CG2-CG4 Giauque Lab, Hildebrand Hall, totaling approximately 1,000 square feet of lab space.

UC Paid Staff: $5,000 /year in staff support.

Equipment Value: $800,000

Other Institutions

In addition to the commitment of the University of California, Berkeley, each of the proposing institutions will make available to this effort a variety of facilities and equipment to support NASA's Astrobiology Program. These resources will greatly benefit the implementation of the proposed research effort, the proposed training, education and outreach plan.

The University of Alabama

Dr. Eric Roden occupies an 1100 square foot laboratory in a new energy/environmental sciences building on the University of Alabama campus. Autoclaves for microbiological work are located across the hall. An auxiliary 200 square foot lab is available for reactive transport experiments with sediment cores. Dr. Roden has recently received federal funding for several research projects dealing with microbial Fe transformations in sedimentary environments. The equipment and other infrastructure accumulated through these awards will be fully available for the proposed NAI project. Prof. Roden's laboratory space and associated equipment has a dollar value of at least $0.25 million. Prof. Roden also has direct access to a central Freshwater Analytical Chemistry laboratory, and a Geological Science laboratory, both of which provide a variety of equipment and resources relevant to the NAI project (Volume II, Facilities and Equipment). An Electron Microscopy/Confocal Microscopy Facility housed in the Department of Biological Sciences is also available for research on the project. Each of the above are multi-million dollar facilities supported by both external grants and University funds.

The University of Alabama provides a variety of support mechanisms for research and graduate training in aquatic-environmental sciences which are relevant to the proposed NAI project. The Department of Biological Sciences includes a section in Ecology and Systematics (E&S) which has approximately 15 faculty with research interests in ecology and environmental science. Recently, a University-wide Center for Freshwater Studies (CFS) was initiated at UA. The CFS embraces a broad range of faculty and students with expertise in biological, geochemical, hydrological, and policy/management aspects of freshwater affairs. Several faculty involved in the CFS, together with counterparts at the University of Mexico, were awarded funding for an NSF IGERT project designed to provide interdisciplinary graduate training in ecological, geochemical and hydrological aspects of freshwater aquatic sciences. Prof. Roden, is one of ten UA faculty participants in the IGERT project. The Department of Biological Sciences is also the recipient of a university graduate program enhancement award which includes several graduate research assistantships that are available (on a competitive basis) to support ongoing faculty research lines. The varioussupport structures which these programs provide will be fully available to students and faculty collaborators on the NAI project.

The University of Delaware

Facilities necessary for conducting the proposed research are available at the University of Delaware in Lewes in either Dr. Luther's laboratories or as general equipment. Dr. Luther's laboratory (1500 sq. ft) has a trace metal clean facility (600 sq. ft class 10,000 clean room containing 4 class 100 clean benches) which is equipped with cleaning, storage and analytical facilities. In addition, a 200 square foot class 100 clean laboratory van for use on land and sea is available. UD has an equipment facility, which includes ICp-ES, Alpkem autoanalyzer, Carlo Erbe CNS analyzer.

Seven students have been supported by research assistantships, one more by a teaching assistantship. Also, three postdoctoral students have been supported by research grants and another was supported with his own NSF postdoctoral fellowship. A renovation of $400,000 for trace metal clean facilities has been performed and the estimated total cost of equipment in the laboratory is $600,000. The College of Marine Studies has a fleet of ships available for research; small boats can be rented on an hourly basis and the R/V *Cape Henlopen* is a UNOLS coastal vessel.

American Type Culture Collection

Dr. David Emerson has a research laboratory in a shared ATCC/George Mason University research facility in Manassas, VA. This laboratory has 800 ft^2, and is equipped with a COY anaerobic glove box, multiple incubators, anaerobic gassing station, a PCR machine, a research quality Olympus epiflourescence microscope coupled to a CCD camera, a chemical fume hood, and all the equipment necessary for routine microbiology, including three biological safety hoods. Dr. Emerson also oversees another 800 ft^2 of shared laboratory space for fermentation and process studies. This includes three Applikon fermetners, two 3-liter, and one 5-liter capacity for growing Fe-oxidizers under special conditions, and an HP gas chromatograph for methane and other gas measurements. In addition there is shared instrumentation including digital gel documentation systems, hybridization ovens, high temperature ovens, high speed and ultracentrifuges, spectrophotometers, a scintillation counter, a Bio-Rad Biologic HPLC system, as well as other equipment. For preservation of microorganisms and sensitive biologics, Dr. Emerson has access to a -80^{o}C freezer and a large capacity (4000 vial) liquid nitrogen storage vessel. All freezers and mission-critical equipment are maintained on an alarm system that is monitored 24 hours. His laboratory also has several Macintosh computers (PPC 7500, G# & I Mac), and Pentium-based wintel machines. The total cost of equipment in these facilities is approximately $300,000.

This research complex also houses a state-of-the-art DNA sequencing facility. This includes three ABI 377 flourescent sequencers, two ABI 310 flourescent sequencers, three Robbins 96 well robots, Speed Vacs, and all the front end instrumentation such as preparative centrifuge, agarose gel electrophoresis systems, and acrylamide gel electrophoresis system required for high throughput phylogenetic analysis. He also has access to a network of 36 Silicon Grpahics workstations and a large disk array to support data handling and phylogenetic analysis analysis.

Dr. Emerson also has routine access to the ATCC bacteriology laboratories (he supervises two technicians there, in addition to his research lab.) These labs are well equipped for microbial characterization work; instrumentation includes a MIDI system for fatty acid methyl ester (FAME) analysis, and a riboprinter from Qualicon, as well as a Bio-log system for C-source utilization studies.

SETI Institute

Dr. Bishop's laboratory will be used for sample preparation for spectral analysis. This includes preparation of size separates of samples collected in the field through grinding and sieving. The lab will also be used for weighing, storage and labeling of samples.

Dr. Bishop's office at NASA-ARC is equipped with a computer and software sufficient for the spectral analyses to be performed here, although it is envisioned that these will need to be upgraded over the 5-year duration of the project.

Dr. Bishop also has access to the spectroscopy facilities at the NASA-supported Reflectance Experiment Laboratory (RELAB) at Brown University under the direction of Professor Carlé Pieters. The laboratory is specifically equipped for geologic remote sensing measurements. This includes a bi-directional visible to near-infrared reflectance spectrometer and a biconical Nicolet Nexus FTIR spectrometer. This is a multi-user, NASA-supported facility and a description and user's manual can be found at (http://porter.geo.brown.edu/relab/index.html).

Palo Alto Research Center

Dr. Yim has a lab with a diverse set of prototyping tools including a small 4 axis CNC machine, 100W laser cutter, fused-deposition modeling machine as well as a variety of metal and plastic working tools. These tools can be used to modify already existing modular robotics systems that will be used in this proposal as needed. These include over 200 modules from robot systems that span over 3 generations and multiple iterations of development.

PARC also has a class 100 clean room to perform surface and bulk micromachining on a variety of substrates. The facility contains deposition tools for conductors (e.g., AlCu, MoCr, ITO), semicon-ductors(e.g., poly-Si, a-Si), and insulators (e.g., SiON, SiO2, SiNx).

PARC has many electronics and mechanics prototyping equipment including a 5-axis CNC machine, fused-deposition modeling prototyper, 100W laser cutter, fully equipped and staffed machine room and electronics shop.

A technical information center staffed by trained librarians provides immediate access to technical journals and literature search services. All of these resources will be available to this project.

Parent Science Proposal Title
BIOMARS - BIOsphere of Mars: Ancient and Recent Studies

Education & Public Outreach Proposal Title
Searching for Life on Mars

Summary of Proposed Costs
Year 1: $64, 697; Year 2: $64, 769; Year 3: $64, 710; Year 4: $63, 227; Year 5: $64,477

BIOMARS E/PO Abstract
Key earth, space, and biological science themes encompassed in the BIOMARS Parent Proposal will also serve as the focus for the project's educational and public outreach development efforts. To effectively address these themes from an educational standpoint, BIOMARS' multidisciplinary team of scientists will work closely with educators from the University of California at Berkeley's Lawrence Hall of Science to develop, field-test, and implement a set of materials that employ proven effective teaching and learning strategies. Educational materials that enhance existing middle and high school science curriculum by presenting important, standards-based science and technological education content will be developed. In addition, materials will be produced that increase public awareness and understanding of relevant issues in Astrobiology in particular, and science in general. By integrating aspects of earth science and life science, developed materials will demonstrate the interdisciplinary nature of space exploration. Effective implementation of these materials will be ensured. To achieve BIOMARS educational goals, a partnership has been established between the BIOMARS team of multidisciplinary scientists and science educators from the University of California at Berkeley's Lawrence Hall of Science (LHS). LHS is the university's popular public science center, which is also world-renowned as a leader in the development of high quality, standards-based educational materials based on current research and related understandings of how students think and learn. Project educational materials will be disseminated through the use of the LHS infrastructure, and its well-established national and international network of educators. To ensure that all materials developed effectively address the project's educational goals, all materials will be pilot and field-tested for educational effectiveness prior to their release to the public.

Principal Investigator:
Dr. Jillian Banfield

E/PO Co-Investigators:
Mr. Kevin Cuff (Lead) and Dr. Herbert D. Thier

Goals and Objectives of the E/PO Effort
The key to BIOMARS educational materials becoming truly effective and implemented on a broad scale lies in their ability to become woven into the fabric of contemporary reform-minded education efforts underway throughout the nation and world. As such, our principal goal will be to produce and prepare for dissemination an innovative set of

educational materials that provide strong support to the educational goals of the *National Science Education Standards* (National Academy of Science, 1996), as well as state frameworks and district guidelines. Achieving this goal will help accomplish the related overall project goal of increasing public awareness and understanding of the value and importance of BIOMARS scientific endeavors. To achieve our goals, our main objectives will be to develop materials that:

- Closely correlate BIOMARS-related scientific investigations to inquiry-based learning of important standards-based earth, space, and life science concepts.
- Provide opportunities for students to learn important science content by asking questions, gathering and evaluating scientific evidence, and making decisions based on evidence.
- Encourage students to assess the benefits and tradeoffs associated with their investigations.
- Sustain high levels of enthusiasm and interest, by emphasizing the relationship between students' investigative learning experiences and relevant interests and issues in their own lives.

E/PO Structure and Content

The topics of possible life on Mars, life in extreme environments, and extraterrestrial exploration tend to readily capture public attention. Our group will use the broad appeal of these subjects to engage middle and high school students and general public audiences in activities that increase their understanding of BIOMARS-related content. The education team will work closely with the BIOMARS scientific team to:

1) Generate, test, and implement a project-based learning module for middle and high school science classrooms.
2) Adapt some of these materials for use with the general public.
3) Develop and implement a series of one-day educator training workshops.
4) Develop and implement a series of one-day public events, that include presentations made in a small, public planetarium.
5) Initiate the development of electronic versions of the school and public materials and personal profiles of project scientists.that can be used at home and by schools and public science centers nationwide.

1. BIOMARS Learning Module Development

The module will consist of inquiry-based investigations that provide students with opportunities to evaluate information the way scientists do. They will be project-based in nature, and as such their themes will engage students in grade appropriate, investigative activities whose focus is related to the efforts of the BIOMARS scientific team. Thus, students will work in teams analyzing evidence of past and present fluid distribution and flow on Mars, evidence related to the evolution of Mars' atmosphere and hydrosphere, and evidence of Martian surface feature development throughout its history. They will relate this evidence to the search for and understanding of possible Martian habitats, and use accumulated understandings to make decisions for example on where future sampling on Mars should occur. Overall, the module will be multidisciplinary in nature, and as such it will enable teachers to enhance their existing space science, life science, geology, and technology classroom curricula. By integrating these subjects the module will present concepts that are closely aligned with those that comprise state and national standards documents, and also demonstrate the interdisciplinary nature of space exploration. A BIOMARS Educator's Guide will be produced for users of the module. Educational resources developed will address the following key concepts in earth, life, and space science: What are the requirements for life on earth as we know it? In what extreme environments does life exist on earth? How do organisms survive, and in fact thrive in extreme environments? What do landscapes and rocks tell us about the past? How are conditions on other planets different than earth? How do we detect life on other planets? Is there life on Mars? What would we see on Mars? How did Mars evolve differently from earth over its 4.5 billion year history? These concepts are aligned with the following Grade 6-12 Content Standards addressed in the *National Science Education Standard*s: Science as Inquiry, Earth and Space Science; Life Science, Physical Science; Unifying Concepts and Processes; Science and Technology; and History and Nature of Science.

The following is a tentative list of specific topics from which activities will be developed for inclusion in the module:

a. Investigating Redox Reactions. Students react Ferrous Chloride with Hydrogen Peroxide to investigate the production of free oxygen as a model reaction for what could take place on Mars.
b. Exploring Sulfur-based Reactions. Students initiate chemical reactions involving Ferrous Sulfate to form various precipitates.
c. Investigating Growth in Acid Environments. Students grow various organisms such as yeast and brine shrimp in varying acidic environments.
d. Life on Earth and Beyond. Students explore the issue of the possibility that biomes on Earth may be different than those found elsewhere.
e. Investigating Mars' Surface. Students examine photographs and maps of Mars to develop an understanding of how its visible features may have formed.
f. Exploring subsurface fluid flow. Students investigate fluid flow by injecting fluids of varying viscosities into gel models, and use what they learn to develop ideas about fluid circulation on Mars.
g. **Lessons from the Martian Atmosphere**. Students discuss the relationship of all education project investigations to the students' interests and responsibilities as a citizen. For example investigations regarding the evolution of life in an atmosphere without free oxygen will be related to issues related to protecting the quality of Earth's atmosphere.

2) Adaptation of Materials for Public Use.
Selected activities from the module will be adapted for use by the general public at Science Centers, other public science facilities and for individual and family use through the World Wide Web.

3) Educator Dissemination Workshops.
As a means of popularizing and disseminating the BIOMARS Educator's Guide, a BIOMARS educator workshop will be held at Lawrence Hall of Science each project

year. These workshops will be attended by middle, high school and informal educators from throughout the greater San Francisco Bay Area, who will be recruited from a pool of individuals associated with LHS teacher and informal educator professional development programs. The workshops will include the presentation of background information on the pertinent science and instructional practices associated with the module, as well as direct hands-on experience with its investigations.

4) One-day Public Events . A series of public events will be designed and held, which will be entitled: Mars Astrobiology Days. These events will involve both the project's scientific and educational teams. Presented will be interactive public activities describing the BIOMARS project and its findings, particularly in relation to the lives of the attendees. In addition attendees will have the opportunity to experience some of the activities described in 2 above. Events will take place first at LHS, and possibly later at other Science and Technology Center sites, such as the New York Hall of Science. Presentations will be videotaped for possible classroom use and/or display on a project-related Web site. In addition, programs that include photographs, diagrams, and hands-on activities that highlight research of the BIOMARS scientific team will be presented in the LHS interactive planetarium. Once fully developed and tested, BIOMARS-related planetarium presentations will be inserted into the rotation of standard LHS planetarium program offerings.

5) Scientific Team Profiles. Development of electronic profiles of members of the project's scientific team, including its lead PI, Professor Banfield, will be initiated. These profiles will present a given scientist in an informal, personal manner, by tracing their development from childhood to the present. The profiles are intended to show the public how nascent interests and influences may result in careers within particular fields of study. Development of these profiles will complement an LHS initiative that is in the early stages of development, which is intended to help increase public understanding of science by displaying such profiles in portable kiosks that reside at the Boston Museum of Science, the Minnesota Museum of Science, and LHS.

Capability And Commitment Of The Proposer And The Proposer's Team

Professor Jillian Banfield is the PI for the parent proposal and is fully committed to be a participating member of the E/PO team. Professor Banfield has had significant experience in the development of general science courses and demonstrations designed to capture the interest and to effectively teach science concepts in undergraduate courses for non-science majors (<http://www.geology.wisc.edu/%60jill/306.html eudora="autourl"> http://www.geology.wisc.edu/`jill/306.html). She has designed an on-line course "Science Behind the News" to appeal to high school and undergraduate students and K-12 teachers seeking continuing education opportunities. Development of BIOMARS educational materials will allow her to work with the other science team members and E/PO specialists from LHS to incorporate the science and technological content of her research into the space, earth and biological science curriculum of classroom teachers.

A team of educators from LHS will coordinate BIOMARS E/PO efforts under the leadership of Kevin Cuff and Dr. Herbert D. Thier. Kevin Cuff is a project director within LHS' Center for Curriculum Innovation (CCI), and the former director of LHS geology education group. Over the past decade, Mr. Cuff has directed several CCI endeavors that have resulted in the development of project-based earth and physical science materials that have been published and have achieved widespread circulation. These have included numerous modular units and courses that convey pivotal concepts and help promote the development of essential understandings and inquiry abilities. These materials are also actively used as tools to promote innovation in the preparation of secondary science teachers and graduate preparation in science education. As a ranking member of the CCI staff, Mr. Cuff has intimate knowledge of, and ready access to its teacher professional development networks, which are used to provide ongoing support and education in the use of innovative materials and methods developed at the center. BIOMARS E/PO efforts will greatly complement those of other CCI groups who currently contribute to NASA-funded programs that support education and outreach efforts, including the Kepler Project. Working closely with Mr. Cuff will be Dr. Herbert Thier, the former Academic Administrator and the founding director of the Science Education for Public Understanding Program (SEPUP) at LHS. He has been a leader in the field of inquiry-based instructional materials development for over four decades. In addition to SEPUP, Dr. Thier has played a major role in the development of numerous other renowned programs that together have produced materials used by many millions of students, teachers and other educators nationwide and throughout the world.

Project Management Structure

The management structure for the E/PO component of the proposal will be collegial and cooperative. The strong commitment of the overall Project PI to E/PO activities will help the E/PO team to build strong cooperative relationships with the various project scientists so that their accomplishments can help inform the E/PO effort. All materials developed by the E/PO component of the project will be submitted for scientific review by project scientists. As CoIs, Mr. Cuff and Dr. Thier and will work closely on the overall design and carrying out of the E/PO effort. CoI Cuff will be responsible for day to day efforts of the E/PO project. Furthermore, relationships will be established with CCI projects such as Great Explorations in Math and Science (GEMS), Full Option System Science (FOSS), and SEPUP to ensure that BIOMARS E/PO materials and experiences provide support to widely adopted and used materials developed by these projects.

Contribution to Nationally Recognized and Endorsed Education Reform Efforts

Development of BIOMARS educational materials will result in science learning experiences that stimulate the evolution of a deeper understanding of the nature of science; particularly its reliance on inquiry, creativity, and labor performed by teams of people working toward a common end. In addition, by embedding key inquiry and science concepts in a project-based format, the BIOMARS learning module will provide an innovative set of multidisciplinary investigative experiences that foster changes in the way science is taught and learned. Use of module materials in classrooms and with the public will help develop student and public understanding of science. Furthermore, BIOMARS E/PO educational materials will be congruent with NSES recommendations, which stress the need for new emphasis on inquiry-based learning that focuses on development of deeper understandings of science embedded in the everyday world.

Broader Impact of the Implementation of E/PO Materials

Presentations and materials will be developed that contribute to an improvement in science literacy by promoting increased understandings of the nature of science in general, and that increase public awareness and understanding of BIOMARS scientific endeavors in particular. To ensure that these resources are beneficial to users from a diverse set of socio-economic backgrounds, they will incorporate methodologies such as hands-on inquiry, collaborative learning, multi-sensory observation, and interdisciplinary explorations. Given their emphasis on providing the public with access to scientists engaged in cutting-edge scientific research of great interest to the public at large, components such as the Mars Astrobiology Days, along with their associated planetarium programs will likely become a part of LHS regular public outreach programming. Through the established connections that the LHS-based E/PO team has with museums such as the New York Hall of Science, the Boston Museum of Science, and the Natural History Museum of Los Angeles County, the finished BIOMARS educational materials will be made available to these and other public science centers. The BIOMARS Educator Guide will encourage students to investigate relevant questions and issues from a project or problem based approach that helps them to develop a real and personal interest in an area of concern. The guide will constitute a new educational resource that provides opportunities for students to effectively learn and apply important inquiry skills, while also learning key standards-based earth, biological, and physical science concepts. The interdisciplinary, standards-based nature of the guide will make it easier for the investigations in it to be infused into classroom curricula, which will make the guide more desirable to teachers. Successful development of the guide will therefore lead to the creation of a highly useful, exemplary model of a multidisciplinary, project-based educational resource that can be used to integrate science, mathematics, and technology education studies. This model, along with other project-related findings and educational products will become disseminated throughout the science education community through publication within the LHS Web site and presentations at meetings and conventions. In addition, all educational products will be disseminated through the LHS established partnerships with school districts throughout the United States.

Evaluating the Effectiveness and Impact of the Proposed E/PO Efforts

In addition to piloting and field-testing the BIOMARS educational module and presentations and activities prior to public implementation, we intend to evaluate their infusion into core science programs being used in schools and science centers locally and nationwide. We will do this by tracking not only the degree to which project products are included in the offerings of LHS and its partner science center/museum public programs, but also reports from the field regarding the actual use of the materials in schools. One way that we will accomplish this is by offering potential users materials they can request on the Web. Then we will evaluate the use of the materials placed on the Web by monitoring the number of individuals who register to use them (free of charge), and also by tracking requests for further information and materials as described above. We will also survey teachers who use the materials and attendees at project Public Outreach events to get their impressions of the value of the materials and presentations and how to improve them.