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Evolution of a Habitable Planet

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THE EVOLUTION OF A HABITABLE PLANET

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EXECUTIVE SUMMARY

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Achievements

The Penn State Astrobiology Research Center (PSARC), created five years ago as part of the NASA Astrobiology Institute, was composed of 16 (Co)-PIs and their research teams from The Pennsylvania State University (13), The University of Pittsburgh (2), and SUNY Stony Brook (1). The investigators represent a wide range of disciplines: geochemistry (7), paleontology (1), atmospheric chemistry (2), geomicrobiology (2), evolutionary genomics (2), and biochemistry and microbiology (2). The proximity of all members has enabled close interaction and a variety of collaborative research, teaching, and public outreach programs. PSARC has supported all or part of the research/education/PO activities carried out by 142 persons (16 (Co-)PIs, 22 research associates and postdoctoral fellows, five research assistants, two technicians, 49 graduate students, 37 undergraduate students, and five staff in administration/IT/EPO). This proposal requests funds from NASA to continue our research activities and to promote education and outreach programs in Astrobiology during the next five years.

Our primary research goal over the past five years (under the theme *Coevolution of the Earth and Life)* has been to increase understanding of the connections between the rise of major life forms during the early history of Earth (between ~3.8 and 0.5 billion years ago) and the evolution of the environment (especially atmospheric O_2, CO_2, and CH_4). This goal has involved investigations of: (1) pre-biotic atmospheric chemistry; (2) hydrothermal and photochemical reactions involving organic compounds and sulfide minerals; (3) evolutionary genomics; (4) biogeochemistry of methanogens and psychrophiles; (5) microbe-mineral interactions; (6) carbon isotope characteristics of organisms in laboratory and natural ecosystems; and (7) biogeochemical investigations of Precambrian soils and sedimentary rocks. Important accomplishments from our research include, but are not restricted to: (i) recognition of the importance of photochemical reactions in controlling atmospheric and oceanic chemistry prior to ~2.3 billion years ago (Ga); (ii) development of a theory for a methane-rich atmosphere and sulfate-poor oceans in the Precambrian; (iii) introduction of several new hypotheses for the rise of atmospheric O_2 and glaciation on early Earth; (iv) discovery of the oldest (2.6 Ga) remnant of microbial mats on land; (v) recognition from genomics of early (~1.2 Ga) diversification of plants, animals, and fungi; (vi) novel insights into microbial phylogenetics and into the biogeochemistry of methanogens and psychrophiles; (vii) increased understanding of microbe-mineral interactions; and (viii) recognition of the important roles played by anaerobic organisms, often living in consortia, in the evolution of the environment and life on Earth.

New Directions in Science and Research

For the next five years, our research to be conducted by 17 (Co)-PIs will build on these achievements while turning our focus toward critical issues concerning *planetary habitability*. Some of the important new frontiers include:

1. The impact of early Earth microorganisms: methanogens, sulfate-reducers, cyanobacteria, S-reducers, fermenters, sulfide-oxidizers, and eukaryotes. When did they arise and how did environmental changes (e.g., the rise of O_2) influence their evolution? How did these organisms influence the environment (atmospheric contents of CH_4, CO_2, and O_2; ocean chemistry; climate) of early Earth? How did they influence the global cycles of C, S, nutrients (N and P) and various redox-sensitive metals (e.g., Fe and Mo) and the evolution of other organisms? What was the impact of these organisms on CH_4 and kerogen recycling during different stages of our planet's evolution, and how effective was such recycling?

2. The roles of photochemical reactions, especially of sulfur species in the atmosphere S and of S and Fe species in waters. How did various photochemical reactions influence Earth's early biosphere? What specific reactions and conditions led to the creation of mass independent fractionation (MIF) of S isotopes before ~2.0 Ga? How much sulfate in the early oceans was created by atmospheric photochemical reaction of volcanic SO_2, and how much by photochemical reactions involving pyrite in shallow water?

3. Detection of biosignatures on other planets. What are the likely biogenic gases on other planets, and how will they be found? What other astronomical methods are useful in the search for habitable planets?

Our approaches to answer these questions are multi dimensional. For the same questions, we seek clues from: (i) biogeochemical investigations on Precambrian rocks and modern ecosystems, (ii) laboratory (bio)chemical experiments in simulated environments, (iii) analytical investigations of genomic sequences, and (iv) numerical modeling. These approaches are summarized as follows:

Section I. Geochemical Record of the Early Earth's Biosphere. Research will focus on "fresh" drill core samples of sedimentary rocks (3.5 – 2.7 Ga in age) from the Pilbara district in Australia. Drilling will be completed under the Archean Biosphere Drilling Project, a new initiative by the NAI. By applying a variety of geochemical methods (e.g., molecular biomarkers, stable and radiogenic isotopes) on these and other drill core samples from various Precambrian districts, we will determine whether the organic matter preserved in Archean sedimentary rocks is biogenic or abiotic, and if biogenic, we aim to identify the organisms responsible. We will also determine temporal and spatial variations in the chemistry of these ancient sedimentary rocks, including MIF of S isotopes; C, N, and Fe isotopes; and the abundance ratios of bioessential metals.

Section II. Photochemical Reactions of Sulfur and Iron in the Early Earth. To understand the mechanism(s) for the creation of MIF of S isotopes and to aid in the reconstruction of the Precambrian S cycle using the isotope record in rocks, we will conduct a series of photochemical reactions of SO_2 by utilizing a reflectron time-of-flight mass spectrometer (RETOF-MS) and a femtosecond laser system coupled with the pump-robe technique. We will also conduct photochemical experiments on Fe-bearing compounds to aid in understanding the formational mechanisms of banded iron formations and seawater sulfate. Modeling of atmospheric reactions and isotopic analyses of the experimental products will also aid in interpretation of the isotope record.

Section III. Genomic Record of the Earth's Early Biosphere. We will continue analyses of protein and DNA sequences of key organisms, especially geologically significant microbial lineages, to better understand their relationships, times of origin, and history of lateral gene transfers. We will also strive to improve methods of analysis and integration of genomic data with the fossil record, biomarkers, and other aspects of biosphere history.

Section IV. Laboratory Microbial Simulations: Astrobiological Signatures. In order to relate the biogeochemical signatures in Precambrian rocks to specific organisms and environments and to understand the response of organisms to their environment, we will investigate the biological, biochemical, isotopic, and genetic response of pure cultures and consortia of important microbes (e.g., methanogens, sulfate-reducers, and cyanobacteria) under simulated Precambrian environments. Special focus will be placed on the chemical and isotopic signatures of inorganic substrates, biogenic gases, and microbial lipids, and on the responses of anaerobes to oxygen and aerobes to methane and sulfide.

Section V. Modern Analogues of Precambrian Microbial Ecosystems. We will conduct a comprehensive multidisciplinary investigation of Fayetteville Green Lake, New York, a modern analogue of the Proterozoic marine biosphere. This provides a unique opportunity to link the physical and chemical properties of a water body to biological, biogeochemical, and genetic characteristics of both the planktonic and benthic ecosystems. Additionally, the biochemistry and genetics of microbial communities in polar ice will help us understand how organisms survived through the Snowball Earth during the Precambrian and how to detect life on the Europa. Investigations of extreme soil environments will help constrain the Precambrian terrestrial environment.

Section VI. Planetary Habitability and Life Detection. We seek to (i) investigate the chances of finding habitable planets around different types of stars; (ii) aid in the search for life on habitable planets of our Solar System and of other stars; and (iii) integrate studies of Earth's atmospheric evolution with astronomical observations. This will be approached by a combination of theoretical, observational, and laboratory-based research.

Section (VI) is an entirely new component of PSARC involving two astrophysicists and an atmospheric scientist to extend the knowledge acquired from studies on the Earth system to the search for life on other planets, and also to develop more complete educational and public outreach programs in Astrobiology. The goals of our proposed research are closely related to Astrobiology Roadmap Goals 1, 2, 4, 5, and 7.

Education and Public Outreach Programs

Public Outreach: A one-week Astrobiology workshop for high school teachers (~25 each year), conducted for the past four years, will continue. Also, in collaboration with WPSX, we have produced two 15-minute TV segments on Astrobiology for PBS' "What's in the News" program. These programs were viewed by more than five million K-12 students, and we plan to produce two more segments in the next five years. The College of Earth and Mineral Sciences at PSU plans to open a new museum by Fall 2003, where ~ 1400 sq. feet of space will be devoted to Astrobiology exhibits. We will also conduct a "Traveling Astrobiology Museum" program in which astrobiology exhibits will tour public schools (K-12 grades) in Pennsylvania and neighboring states.

Undergraduate Minor in Astrobiology: An inter-college undergraduate program in Astrobiology was established effective Fall 2000 semester at PSU. Approximately ten students from the Departments of Geosciences, Astronomy, Microbiology, Biology, and Mathematics have already received this B.S. minor. Student enrollment will grow substantially in the future.

Dual-Title Ph.D. Degree Program in Astrobiology: The proposal to create a dual-titled Ph.D. degree program in Astrobiology at PSU is currently under Faculty Senate review. Approximately ten graduate students from Geosciences, Biology, Chemistry, and Astronomy and Astrophysics departments are expected to enroll in the dual-title program effective Fall 2003 semester.

University Commitment: PSU's strong commitment to support NAI/PSARC programs is demonstrated by substantial support, which includes the creation of *two additional tenure-track faculty positions in Astrobiology*, pending funding of this grant proposal.

The Evolution of a Habitable Planet

INTEGRATED RESEARCH AND MANAGEMENT PLAN

Introduction and Overview

The research proposed here will be carried out during the next five years by the PI (Ohmoto) and 16 Co-PIs of the Penn State Astrobiology Research Center (PSARC) and their research staff and students. The 17 (Co-)PIs represent a wide range of expertise in Astrobiology: Atmospheric Chemistry (J. Kasting), Experimental Chemistry and Physics (A.W. Castleman), Geochemistry (M. Arthur, S. Brantley, R. Capo, L. Kump, H. Ohmoto, M. Schoonen, and B. Stewart), Paleontology (M. Patzkowsky), Microbial Biogeochemistry (K. Freeman and C. House), Evolutionary Genomics (B. Hedges), Biochemistry and Microbiology (J. Brenchley and J. Ferry), and Astronomy and Astrophysics (S. Sigurdsson and A. Wolszczan); Castleman, Sigurdsson, and Wolszczan have replaced the retiring R. Minard (Organic Chemistry) and M. Nei (Evolutionary Genomics).

Much of the early history of Earth's surface environment involves changes in the redox state of the atmosphere, oceans, and sediments. The chemical milieu that existed during the origin and early evolution of life has been viewed as strongly reducing (Miller & Urey, 1974) or oxidizing (Towe, 1994). This issue is inextricably intertwined with the Faint Young Sun Paradox, which has been addressed by invoking an atmosphere rich in greenhouse gases, either methane and ammonia (Sagan & Mullen, 1972; Sagan & Chyba, 1997; Pavlov et al., 2000a, 2001) or carbon dioxide (Kasting 1987, 1993). The establishment of an oxygen-rich atmosphere and ocean allowed for the evolution of energetic new life forms and perhaps extinction of some O_2-intolerant organisms. For these reasons, *the major research focus of PSARC has been directed toward our understanding of the connections between the evolution of the environment (especially atmospheric pO_2, pCO_2, and pCH_4) and organisms (both anaerobes and aerobes) and ecosystems during the period of ~3.8 Ga to 0.5 Ga.*

We have pursued the above goal using a variety of approaches, including: (a) modeling of photochemical reactions in the pre-biotic atmosphere; (b) laboratory experiments on hydrothermal and photochemical reactions involving organic compounds and sulfide minerals; (c) evolutionary genomics; (d) analyses of the biogeochemistry and genomics of methanogens and psychrophiles; (e) experimental microbe-mineral interactions; (f) carbon-isotope characteristics of individual organisms and consortia in laboratory and modern natural ecosystems; (g) search for biogeochemical signatures (molecular biomarkers; kerogen C isotopes; pyrite S isotopes); and (h) search for geochemical signatures for the redox states of the atmosphere and oceans in sedimentary rocks and soils of Precambrian ages. Results of these investigations have been published in 159 papers (including those submitted and in review but excluding abstracts of papers presented at national and international meetings) in refereed journals and books, including 23 published papers in *Science*, *Nature*, and *Proceedings of National Academy of Science* (see List of Publications, Volume II). Important accomplishments include, but are not restricted to: (i) recognition of the importance of photochemical reactions in controlling the atmospheric and oceanic chemistry prior to ~2.3 Ga (Pavlov et al., 2001a, b; Pavlov & Kasting, 2002); (ii) development of a theory of a methane-rich atmosphere and sulfate-poor oceans in the Precambrian (Pavlov et al., 2001a, b); (iii) formulation of several new hypotheses for the causes of rise of atmospheric O_2 and glaciation on the early Earth (Kump et al., 2001; Pavlov et al., 2000a; Pavlov & Kasting, 2002; Lasaga & Ohmoto, 2002); (iv) discovery of the oldest (2.6 Ga) remnant of microbial mats (probably of cyanobacteria) on land (Watanabe et al., 2000); (v) recognition from genomics of early (~1.2 Ga) diversification of plants, animals, and fungi (Wang et al., 1999; Heckman et al., 2001); (vi) novel insights into microbial phylogenetics (Hedges et al., 2001; House & Fitz-Gibbon, 2002; Sheridan et al., in press) and into biogeochemistry of methanogens and psychrophiles (Ferry, 1999; Sheridan & Brenchley, 2000); (vii) increased understanding of microbe-mineral interactions (Brantley et al., 2001); and (viii) recognition of the important roles played by ecosystems of anaerobic organisms (e.g., methanogens and sulfate-reducers) in the evolution of environment and life on Earth (Kasting et al., 2001; Orphan et al., 2001, 2002).

Although we have made considerable progress toward achieving the initial goal of our research, many important questions still remain unresolved. For example, the sequence of oxygenation is unclear: an oxidized surface ocean may have been sandwiched between anoxic deep waters and atmosphere for some time before complete oxygenation (Kasting, 1993), initial oxidation may have been modest (Canfield, 1998), or the spread of O_2 may have been abrupt, and complete (Ohmoto, 1996). The most elusive element has been an explanation for this change in redox state. Was it simply the advent and proliferation of oxygenic photosynthesis (Hedges et al., 2001), or might a gradual rise in the oxygen fugacity of volcanic gases finally have ceased to serve as an effective sink for cyanobacterial O_2 (Kasting et al., 1993; Kump et al., 2001; Catling et al., 2001; Holland, 2002)? In either case, atmospheric oxygenation undoubtedly had climatic consequences, perhaps creating an interval of time during which the greenhouse effect was insufficient to prevent global glaciation (Pavlov et al., 2000**).** Biomarkers of cyanobacteria and eukaryotes have been found in ~2.7 Ga black shales from Western Australia by Brocks et al. (1999) and by our group (Eigenbrode et al., 2001). However, questions remain as to whether or not the pre-2.7 Ga sedimentary rocks contain biomarkers of any anaerobic and/or aerobic organisms. A recent controversy over the origin of organic matter in the 3.46 Ga Apex chert from Western Australia, whether it represents fossil material (Schopf, 1993) or a remnant of abiogenic hydrocarbons in a submarine hydrothermal vent (Brasier et al., 2002), illustrates how little we know about the origins, distribution, and evolution of organisms on the early Earth.

Major Questions

Our research during the next five years will address the remaining key issues and explore new frontiers in “the evolution of a habitable planet.” The important new questions for our future research are summarized in the following three groups:

(1) **Questions related to the impact of important microorganisms on the early Earth, methanogens, sulfate-reducers, cyanobacteria, S-reducers, fermenters, sulfide-oxidizers, and eukaryotes**: When did they evolve? How did they influence the environment (the atmospheric contents of CH_4, CO_2, and O_2; ocean chemistry; climate) of the early Earth? How did they influence the cycles of nutrients (P, N) and bioessential elements (Fe, Mo, Zn, Cu, etc)? How did they influence the evolution of other organisms? How did the changes in environment (e.g., rise of O_2) influence their evolution? How was CH_4 recycled during different stages of our planet’s evolution, by sulfate-dependent methanotrophy, aerobic methanotrophy, or through abiotic mechanisms (e.g., high-temperature reactions with ferric-bearing rocks)? During the

Phanerozoic era, the long-term global carbon cycle is characterized by the burial of atmospheric CO_2 as organic C (kerogen) and carbonate in sedimentary rocks and by the complete recycling of both kerogen and carbonate in sedimentary rocks to CO_2 (and HCO_3^-) during weathering. In the absence of atmospheric O_2 in the Archean, how did the global C cycle operate? What were the recycling mechanisms for kerogen? The global sulfur cycle in the Phanerozoic era is characterized by sulfate-rich oceans, an abundant formation of biogenic pyrite by sulfate-reducers in the oceans, and the complete conversion of pyrite S to SO_4^{2-} during weathering of rocks under an O_2-rich atmosphere. The S cycle is closely linked to the geochemical cycles of O, C, Fe, and many other redox-sensitive elements, and the evolution of a variety of organisms. How did the global S cycle operate in the Precambrian? Were Archean oceans sulfate-poor? Why was there relatively little variability in the carbon isotopic composition of the ocean during the Mesoproterozoic (1600-1000 Ma)? Was its deep ocean sulfidic, and if so, what effect did this have on the biogeochemical cycling of essential nutrients including trace metals? What were the fluxes of extraterrestrial materials (meteorites and cosmic dust) and their effects on the early biosphere?

(2) **Questions concerning the roles of photochemical reactions, especially of sulfur species in the atmosphere and of sulfur and iron species in waters**: What are the specific reactions and conditions that led to the creation of mass independent fractionation (MIF) of sulfur isotopes in rocks older than ~2.0 Ga (Fig. 1)? Is the MIF of S isotopes the "smoking gun" for the rise of atmospheric O_2? Why are there serious discrepancies between the MIF record in rocks and the MIF characteristics found by the previous photochemical experiments (Farquhar et al., 2000)? How much sulfate in the early oceans was created by atmospheric photochemical reactions of volcanic SO_2, and how much by photochemical reactions involving pyrite (FeS_2) in shallow water? How did various photochemical reactions influence the early Earth's biosphere?

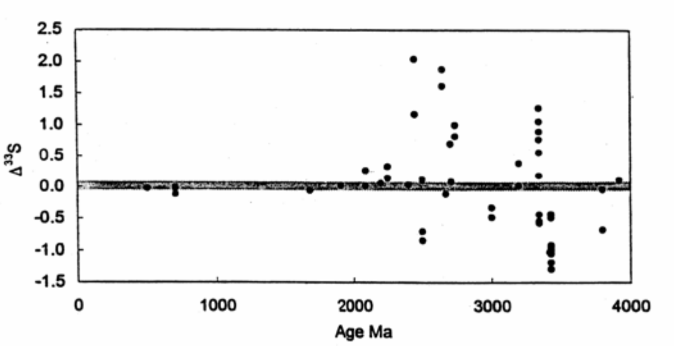

Fig. I. MIF factors of S isotopes (Δ□□S values) in sedimentary rocks vs. their age (Farquhar et al., 2000). The magnitudes of deviation in the $^{34}S/^{32}S$ and $^{33}S/^{32}S$ ratios of a sample (*i*) from the corresponding ratios in Canyon Diablo troilite are expressed by δ□□S_i and δ□□S_i values, respectively. The magnitude of fractionation among the isotopes of the same element during normal (bio)chemical reactions is roughly proportional to the difference in their masses, leading to the relationship of: δ□□S_i = 0.514δ□□S_i. The deviation from this relationship, Δ□□S = δ□□S_i - 0.514δ□□S_i., is defined as the MIF factor. When Δ□□S > |±0.1|‰, the sulfur is "mass independently fractionated".

(3) **Questions concerning biosignatures on other planets**: What are the likely biogenic gases on other planets, and can we observe them spectroscopically? What other astronomical methods might be useful in the search for habitable planets?

These questions are related to many of the goals of the NASA Astrobiology Roadmap, especially to Goal 1 "Understand the nature and distribution of habitable environments in the Universe", Goal 2 "Explore for past or present habitable environments, prebiotic chemistry and signs of life elsewhere in our Solar System", Goal 4 "Understand how past life on Earth interacted with its changing planetary and Solar System environment", Goal 5 "Understand the evolutionary mechanisms and environmental limits of life", and Goal 7 "Determine how to recognize signatures of life on other worlds and on early Earth".

Research and Management Plan

Our approaches to answer the above questions are multi-dimensional. For the same questions, we seek clues from: (i) biogeochemical investigations on Precambrian rocks and modern ecosystems, (ii) laboratory (bio)chemical experiments in simulated environments, (iii) analytical investigations of genomic sequences, and (iv) numerical modeling. In this proposal, we have grouped our approaches into six, closely linked "Sections". Each Section of this proposal has a Section Leader and a group of Co-PIs (Subsection Coordinators) who are responsible for coordinating research projects focused on specific scientific problems. The PI (Ohmoto) will coordinate efforts to integrate the activities of all the Sections. While it is necessary to subdivide the research this way, we consider all of the sections to be intimately related. Our approaches are summarized as follows:

Section I. Geochemical Record of the Earth's Early Biosphere (Ohmoto-Leader, Arthur, Capo, Freeman, House, Kasting, Kump, and Stewart). Research will focus on "fresh" drill core samples of sedimentary rocks (3.5 – 2.7 Ga in age) from the Pilbara district in Australia. Drilling will be completed under the Archean Biosphere Drilling Project, a new initiative by the NAI. By applying a variety of geochemical methods (e.g., molecular biomarkers, stable isotopes) to these and other drill core samples from various Precambrian districts, we will determine whether the organic matter preserved in Archean sedimentary rocks was biogenic or abiogenic and, if biogenic, we will identify the original organisms. We will also attempt to identify the existence of any systematics in the temporal and spatial variations in sedimentary rocks, specifically the MIF of S isotopes; C, N, and Fe isotopes; and the abundance ratios of bioessential metals.

Section II. Photochemical Reactions of Sulfur and Iron in the Early Earth (Kasting-Leader, Arthur, Castleman, and Schoonen). To understand the mechanism(s) for the creation of MIF of S isotopes and to aid in the reconstruction of the Precambrian S cycle using the S isotope record in rocks, we will conduct a series of photochemical reactions of SO_2 by utilizing an innovative experimental technique developed by Castleman: a reflectron time-of-flight mass spectrometer (RETOF-MS) and a femtosecond laser system coupled with the pump-robe technique. We will also conduct photochemical experiments on Fe-bearing compounds to aid in understanding the formational mechanisms of banded iron-formations and seawater sulfate. Modeling of atmospheric reactions and isotopic analyses of the experimental products will help focus interpretations of the S isotope record in rocks.

Section III. Genomic Record of Earth's Early Biosphere (Hedges-Leader, House, and Patzkowsky). We will continue analyses of protein and DNA sequences of key organisms, especially geologically significant microbial lineages, to better understand their relationships, times of origin, and history of lateral gene transfers. We will also strive to improve methods of analysis and integration of genomic data with the fossil record, biomarkers, and other aspects of biosphere history.

Section IV. Laboratory Microbial Simulations: Astrobiological Signatures (House-Leader, Arthur, Brantley, Ferry, Freeman, and Ohmoto). In order to relate the biogeochemical signatures in Precambrian rocks to specific organisms and environments and to understand the response of organisms to their environment, we will investigate the biological, biochemical, isotopic, and genetic response of pure cultures and consortia of important microbes (e.g., methanogens, sulfate-reducers, and cyanobacteria) under simulated Precambrian environments. Special focus will be placed on the chemical and isotopic signatures of inorganic substrates, biogenic gases, and microbial lipids, and on the responses of anaerobes to oxygen and aerobes to methane and sulfide.

Section V. Modern Analogues of Precambrian Microbial Ecosystems (Kump-Leader, Arthur, Brenchley, Capo, Freeman, House, and Stewart). A modern analogue of the Proterozoic marine biosphere is Fayetteville Green Lake, New York. We will conduct a comprehensive, multidisciplinary investigation of the ecosystems in this lake to understand the connections between: (a) the changes in the physical and chemical characteristics of the water body, and (b) the changes in biology, biochemistry, and genetic characteristics of both the planktonic and benthic ecosystems. We will also carry out investigations on the biochemistry and genetics of microbial communities in polar ice in order to increase our understanding of how organisms survived through the Snowball Earth during the Precambrian and to predict the nature of organisms that might exist on Europa. We will investigate the biogeochemical processes of the formation of modern soils in order to aid in deciphering the record in Precambrian paleosols.

Section VI. Planetary Habitability and Life Detection (Sigurdsson-Leader, Capo, Freeman, Kasting, Stewart, and Wolszczan). In this section, we seek to integrate studies of Earth's atmospheric evolution with astronomical observations. The research objectives here are: (i) to investigate the chances of finding habitable planets around different types of stars; and (ii) to aid in the search for life on habitable planets of our Solar System and of other stars. The objectives will be approached by a combination of theoretical, observational, and laboratory-based research.

Many of the problems addressed in our investigations are controversial: the timing of the establishment of an oxygen-rich atmosphere, the interpretation of variations in the isotopic compositions of Precambrian rocks, and the fidelity of the Archean geochemical and fossil record. Diverse views are held on all of these and other issues, and are expressed in this proposal. Indeed, our group is not of one mind on all these issues, and that is reflected in what follows. We consider this a strength of our group: it provokes lively internal debates and hones our arguments and sparks further investigations. We all agree that in the end the ideas most strongly supported by observations, modeling, and experiments will prevail.

I. GEOCHEMICAL RECORD OF THE EARTH’S EARLY BIOSPHERE

Ohmoto (Leader), Kump, Arthur, Freeman, House, Kasting, Capo, and Stewart

This section presents an integrated study of the redox and biological evolution of the Earth preserved in the Precambrian rock record. A major focus of our field-related biogeochemical investigations will be high quality core samples (3.5-2.7 Ga in age) recovered from the Pilbara district in Western Australia under the Archean Biosphere Drilling Project (ABDP), a major new initiative of the NASA Astrobiology Institute (Section 1.1). We will use a variety of geochemical approaches to unravel the nature of marine and terrestrial biospheres on the early Earth, using ABDP and other samples (Sections 1.2.1-1.2.5). The results of these studies will be integrated with laboratory and theoretical investigations of the evolution of the Earth’s early atmosphere, oceans, and biosphere (Sections 1.3, 1.4, and 2).

1.1. THE ARCHEAN BIOSPHERE DRILLING PROJECT (ABDP)

1.1.1. Overview

The most serious problem for paleontologists and geochemists attempting to understand the evolution of organisms and their biogeochemical environments from the ancient rock record has been the difficulty in obtaining important sequences of “fresh” rocks, i.e., rocks that have not been severely altered by post-depositional processes. This problem has been highlighted during discussions among researchers of the Mission to Early Earth (MtEE) Focus Group of NAI. Precambrian rocks, especially those of Archean age (>2.5 Ga), have generally been subjected to high-grade metamorphism ($T > 300$ °C and $P > 2$ kb), often hydrothermal alteration, and a history of recent subaerial weathering (sometimes >10 million years) that has destroyed or altered the original biosignatures and mineralogical characteristics. Road cuts and tunnels can provide relatively “fresh” exposures, but they only intersect the most interesting geologic formations by chance. Exploration drilling typically focuses on areas exhibiting strong hydrothermal alteration, making these samples less desirable for geochemical studies.

The Archean Biosphere Drilling Project (ABDP) is an international drilling project to be undertaken by the Astrobiology Drilling Program (ADP), a new initiative of NAI. The main objectives of the ABDP are to recover “fresh” continuous sections of several key geologic formations (mostly sedimentary rocks 3.5-2.7 Ga in age) from the Pilbara district, Western Australia and use them to conduct systematic paleontological and biogeochemical investigations of the early Earth. These investigations will address (a) the nature of the biosphere, including the types and distribution of organisms in the oceans, lakes, and on land; (b) paleoclimate; (c) the chemistry of the atmosphere and oceans; (d) cosmic fluxes on the early Earth; and (e) geochemical fluxes of O, C, S, Fe, N, and other bioessential elements in the atmosphere-ocean-land mass-mantle system.

After several years of field surveys, the ABDP group has selected seven drilling sites where the target formations have been least affected by metamorphism, hydrothermal processes, and/or subaerial weathering subsequent to their formation. Our strategy is to drill several relatively shallow holes below the current and ancient groundwater tables rather than one or two long holes, in part for financial reasons: drilling five 300 m-deep cores is less expensive than drilling one 1000 m-deep core. More importantly, due to post-Archean tectonic movements, no area exists in the Pilbara district where one or two deep drill holes will intersect all the geologic formations of interest.

The planning and execution of the drilling, core logging, sample distribution, and research will be coordinated by the ADP, including Bruce Runnegar (Director of NAI), representatives of the MtEE Focus Group, and representatives of the following four research groups: Hiroshi Ohmoto (PSARC, representing NAI), Munetomo Nedachi (Kagoshima University and other Japanese universities; PSARC Associate Member), Arthur Hickman (Geological Survey of Western Australia (GSWA)), and Mark Barley (University of Western Australia (UWA); PSARC Associate). With major funding from the Japanese Ministry of Education to M. Nedachi's group and from the NAI to PSARC, drilling will commence June 2003 and the final drilling will be completed by October 2003. Under the coordination of Ohmoto, Nedachi, and the ADP Advisory Committee, more than 50 researchers will be involved in various investigations of the ABDP drill cores, including: ~40 from the Japanese group; ~5 from the UWA, ~5 from the GSWA, and ~15 from PSARC (Ohmoto, Arthur, Freeman, Kump, House, Capo, and Stewart and their Research Associates and graduate students).

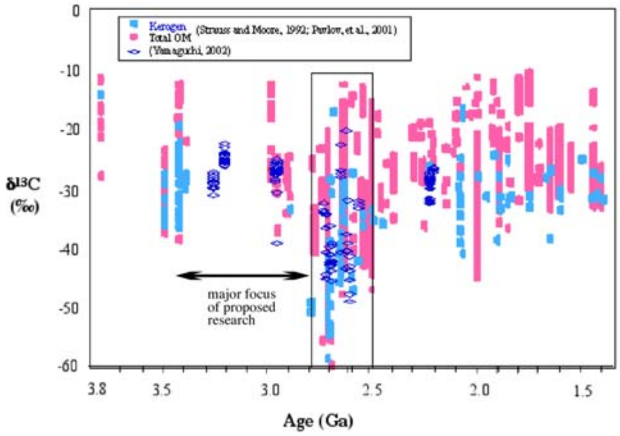

Fig 1.1 Carbon isotope record of sedimentary organic matter through Archean and early Proterozoic ages.

1.1.2. ABDP Drilling Targets

The ABDP will primarily focus on a variety of sedimentary rocks and submarine volcanic rocks in the Pilbara district of Western Australia that are older than 2.7 Ga (e.g., Buick et al., 1995, 2002). This district was selected primarily because (1) biogeochemical data (e.g., biomarkers and carbon isotope ratios) on rocks older than 2.7 Ga are very scarce (Fig. 1.1), and (2) Hickman and his GSWA colleagues have found that some areas of the Pilbara district were only subjected to very low-grade metamorphism (zeolites facies), and that many sedimentary units rich in organic carbon (black shales and black cherts) exist in these areas. Therefore, there is a high probability of discovering microfossils and a variety of molecular biomarkers of Archean age. The seven drilling sites (DDH 1 to 7) are located in a ca. 300 km x 200 km area (Fig. 1.2).

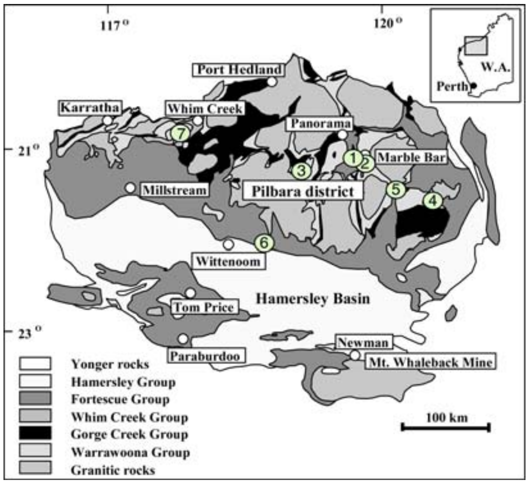

Fig 1.2 Sites of ABDP.

The Marble Bar chert (DDH 1) is one of the oldest chert formations (3.47 Ga). Black shales in DDH 2-4 are some of the oldest marine black shales (3.46-2.9 Ga), >100 m in thickness, and rich in organic carbon (>2 wt%) and pyrite (>0.2 wt%). DDH 5 and 6 offer some of the oldest (2.76-2.7 Ga) lacustrine shales, >100m in thickness. A ~20 m thick sericite-rich zone in the Mt. Roe Basalt (DDH-7; 2.7 Ga) has been cited by Rye and Holland (2000) as the best example of a paleosol formed under an anoxic atmosphere, while Nedachi et al. (2002a, b) describe biomarkers of eukaryotes, cyanobacteria, and kerogens in this sequence and suggest that it could instead represent tuff layers deposited in a shallow marine environment that were altered by CH_4-rich hydrothermal fluids (T≈150°C).

1.1.3. Drill cores from other Precambrian Districts

During the past five years, the PSARC has collected a large number of drill cores of a variety of sedimentary and igneous rocks (~3.4 to ~1.8 Ga in age) from the Kaapvaal Craton in South Africa, the Hamersley district in Western Australia, the Abitibi, Wawa, and Wagigoon districts of Ontario, Canada, and the Outokump district in Finland. Because drilling of these was for mineral exploration, many of the samples were affected by hydrothermal fluids. Nevertheless, these drill cores have already yielded significant mineralogical and biogeochemical data (e.g., the contents of C, H, N, S, P, and major and trace elements, and $\delta^{13}C$ values of kerogen; Watanabe et al., 1997; Kakegawa et al., 1998; Yamaguchi, 2002; Watanabe, 2002). We will continue investigating these core samples as supplements to the ABDP cores.

1.2. RESEARCH AREAS: THE EARTH'S EARLY BIOSPHERE

1.2.1. Origins of Organic Matter in Archean Sedimentary Rocks

Major Questions: The recent controversy regarding the 3.46 Ga Apex chert, i.e., whether the organic matter (OM) is remnant of cyanobacteria-like organisms, and formed on a shallow continental shelf (Schopf, 1968; Schopf, 1993), or synthesized by the Fisher-Tropf reaction in a deep submarine hydrothermal vent (Brasier et al., 2002), illustrates how little we know about the genesis and environment of formation of OM in Archean rocks. Therefore, the first major question to be addressed in our investigations is:

(1). What were the origins and environments of OM in Archean cherts? Specifically, is the OM biogenic or abiotic? If biogenic, what type(s) of organisms produced it? What were the mechanism and environment for the formation of chert? (This last question will be addressed in Section 1.2.4). The Marble Bar chert (DDH 1) is an attractive drilling target because it is older than the Apex chert (3.47 vs. 3.46 Ga), but it has a very similar geologic environment (>10 m thick chert beds occur in a thick (> 2 km) succession of pillow basalt).

Our preliminary investigation on outcrop samples has already revealed an abundance of organic matter in some chert layers (Fig. 1.3). The kerogen associated with pillow lavas is also an important research target because it may represent a remnant of microbial mats that developed on the seafloor during quiescent periods of submarine hydrothermal activity, while those in the chert/jasper units may represent microbial communities that developed during intensive periods of submarine hydrothermal activity.

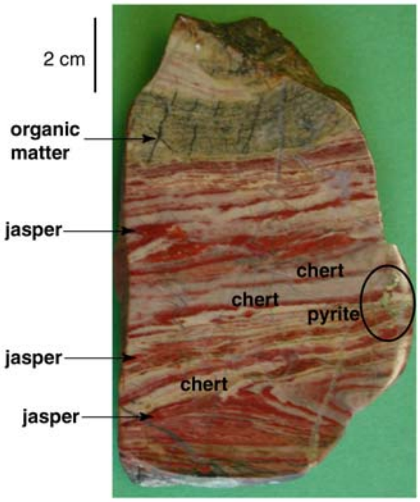

Fig 1.3. Marble Bar Chert/Jasper. Red color is due to an abundance of fine-grained hematite crystals.

Organic matter in shales from the ABDP target area is not a product of abiological hydrocarbon synthesis, because these thick formations (>100 m) extend over >100 km^2 of areas with uniformly high (>0.2 wt%) organic C contents. The organic matter in these shales is most certainly composed of various mixtures of the remnants of organisms that lived in the surface zone (planktonic organisms) of the oceans (or lakes), in a deeper water column, and on the sediment/water interface (benthic organisms), as well as possibly some "old" (detrital) kerogen. Considering the possible importance of methane in the Archean atmosphere, we add the following important questions for our investigations:

(2) Who were the most important primary producers (autotrophs) in the Archean? Were they methanogens that utilized the CO_2 and H_2 in the Archean atmosphere for organic synthesis (reaction 1-1), anoxygenic photoautotrophs, such as green sulfur bacteria (reaction 1-2), or oxygenic photoautotrophs, such as cyanobacteria (reaction 1-3)?

$$CO_2 + 2H_2 \Rightarrow CH_2O + H_2O \qquad (1\text{-}1)$$

$$CO_2 + 2H_2S \Rightarrow CH_2O + 2S^0 + H_2O \qquad (1\text{-}2)$$

$$CO_2 + H_2O \Rightarrow CH_2O + O_2 \qquad (1\text{-}3)$$

(3) What was the principal mechanism for the generation of CH_4 by methanogens? Was it by directly utilizing the CO_2 and H_2 in the atmosphere (reaction 1-4) or by the fermentation (reaction 1-5) of the OM that was generated by reaction (1-2) or (1-3)?

$$CO_2 + 4H_2 \Rightarrow CH_4 + 2H_2O \qquad (1\text{-}4)$$

$$2CH_2O \Rightarrow CO_2 + CH_4 \qquad (1\text{-}5)$$

(4) Who were the principal methane-oxidizing microbes? Were they methanotrophs that utilized dissolved O_2 (reaction 1-6) or anaerobic methanotrophs (Archaea and/or Bacteria) that utilized dissolved SO_4^{2-} (reaction 1-7)?

$$CH_4 + 2O_2 \Rightarrow CO_2 + 2H_2O \qquad (1\text{-}6)$$

$$CH_4 + SO_4^{2-} + 2H^+ \Rightarrow CO_2 + H_2S + 2H_2O \qquad (1\text{-}7)$$

Recycling of CH_4 is an important issue, because without the recycling, the conversion of crustal carbon to reduced forms, CH_4, "oil slicks" (Lasaga et al., 1971), and kerogen, might have been a substantial part of the carbon cycle influencing the habitability of particular microbial niches.

(5) What was the burial flux of organic matter considering primary production (e.g., photoautotrophy and chemautotrophy), and carbon remineralization through fermenters, methanogens, and sulfate reducers? What portion of this flux represents carbon produced from oxygenic photoautotrophy (reaction 1-3)? This information is critical in estimating global O_2 production fluxes (e.g., Holland, 1984; Lasaga & Ohmoto, 2002).

(6) Recycling of "old" kerogen to CO_2 by aerobic reactions (biological and abiological) is an essential element of the Phanerozoic carbon and oxygen cycles. This recycling has produced an essentially constant ratio (~1/4) of organic C to carbonate C and nearly constant $\delta^{13}C$ values (~-25‰ and ~0‰ for organic C and carbonate C, respectively) in Phanerozoic sedimentary rocks (e.g., Holland, 1984; Lasaga & Ohmoto, 2002). If kerogen is not expected to decompose during weathering under an anoxic atmosphere (Ohmoto, 2003), then what were the recycling mechanisms of "old" kerogen in the Archean? Did the ratio of detrital kerogen to the organic matter in (5) change with geologic history, and can it be related to the evolutionary history of atmospheric oxygen?

Kerogen with the most negative $\delta^{13}C$ values in Precambrian sedimentary rocks (~-60‰) was extracted from a few samples of the 2.7 Ga Tumbiana Formation in the Hamersley district, Western Australia (Strauss & Moore, 1992). These very negative $\delta^{13}C$ values suggest the existence of a microbial community in a 2.7 Ga lake that consisted of: (a) methanogens that produced isotopically very light CH_4, (b) microbes that oxidized the CH_4 to isotopically very light CO_2, and (c) microbes that used the isotopically light CO_2 for organic synthesis. However, interpretations vary among researchers. For example, Hayes (1994) suggests that methanotrophs utilizing atmospheric CO_2 and O_2 provided the principal mechanism for methane recycling (reaction 1-6), and that ~2.7 Ga ago cyanobacteria and methanotrophs (aerobes) evolved and the oceans became stratified with an oxygenated surface zone and an underlying anoxic water body. Hinrichs et al. (2002) suggest that sulfate-reducing microbes (SRM) provided the principal mechanism for methane recycling (reaction 1-7) and that these microbes evolved around 2.7 Ga. Pavlov et al. (2001) suggest a linkage between the appearance of cyanobacteria and the formation of an organic haze-rich atmosphere at ~2.7 Ga. All of these interpretations have been based on a very limited set of data from weathered outcrops. Our multidisciplinary investigations, described below (1.2.1.1-1.2.1.3), will be carried out systematically on a much larger number of samples.

1.2.1.1. Geochemical analyses of kerogen-bearing rocks (Ohmoto)

Although we expect to find biomarkers in many of the drill core samples, nearly 100% of organic matter (OM) in Archean rocks occurs as kerogen. The previous studies carried out by Ohmoto's group on Precambrian shales (mostly 2.7-2.0 Ga in age) from South Africa, Canada, and Australia (e.g., Watanabe et al., 1997; Yamaguchi, 2002; Watanabe, 2002) have demonstrated the origins of kerogen and its depositional environment can be constrained from systematic investigations, including: (a) microscopic observations using optical and electron microscopes to determine the modes of occurrence (layers vs. disseminated), grain size, morphology, and textures of kerogen in rocks; (b) analyses of the abundance ratios of C, H, N, S, P, and major elements (especially Al, Ti, Si, and Fe) in bulk rocks and extracted kerogen; and (c) analyses of $\delta^{13}C$ and $\delta^{15}N$ values of kerogen and whole rocks. We will adopt the same approach to the studies on the drill core samples.

1.2.1.2. Biomolecular analyses (Freeman and House)

Biomarker compounds associated with cellular membranes preserved in Archean sedimentary rocks provide evidence for a wide diversity of microbial life on the early Earth. For example, published reports (Brocks et al., 1999; Summons et al., 1999) document abundant 2-methylhopanes in the Late Archean, suggesting the presences of cyanobacteria cells at that time. At PSU, Freeman and House's groups have been working to expand the investigation of Late Archean biomarkers with a goal of evaluating isotopic and molecular signatures in the context of geographic locations, stratigraphic position, and sample lithology. A key objective of this project is to evaluate authenticity of the biomarkers, and to identify possible contamination. Initial work has focused on kerogen and lipids preserved in core and outcrop samples of Late Archean (2.8-2.5 Ga) sedimentary rocks from across the Hamersley Province in Western Australia (Fig. 1.4).

Molecular analyses include both extracted compounds and those released by pyrolysis of the insoluble organic matrix (kerogen). We have employed a sequential strategy, whereby samples are treated to solvent extraction, mineral digestion (with HCl and HF), an additional solvent extraction, and pyrolysis in a H_2 atmosphere. Molecular structures provide supporting evidence for the authenticity of the extracted compounds. The ancient nature of the extracted biomarkers is supported by similarities in aromatic compounds, thermally mature molecular isomer data for the extracts, and the lack of any indicators for contamination or young age-specific biomarker patterns in any fraction. This work is continuing with additional molecular and isotopic analyses. Molecular structures extracted from Late Archean samples provide evidence for all three domains of life: the Archaea (acyclic isoprenoids), the Bacteria (hopanes) and the Eukaryotes (diverse sterane structures). We have tentatively identified trace quantities of compounds associated with green sulfur bacteria (aryl isoprenoids); we have also found 3-methyl hopanes in low quantities, which signify inputs from either aerobic methane oxidizing bacteria or acetogenic organisms. We have also identified series of dimethyl and diethyl alkanes, which are associated with inputs from sulfide-oxidizing bacteria. In Figure 1.4, we highlight branches on a genome-based tree (Wolf et al., 2002; House et al, submitted) that reflect the broad taxonomic diversity of organisms recorded by Late Archean biomarkers.

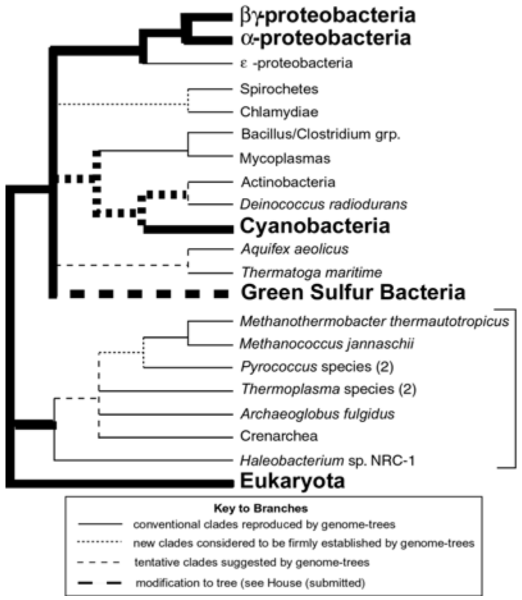

Figure 1.4. Genome-base phylogenetic tree modified from Wolf et al. (2002) and House, etal. (submitted). Highlighted lines indicate representation among late Archean biomarkers (Eigenbrode et al., 2003).

We will continue this line of inquiry by extending it to other samples of similar age and by employing older samples recovered by ABDP. We will initially conduct elemental and isotopic analyses of kerogen in order to evaluate thermal maturity and the overall association of $\delta^{13}C$ signatures with lithology and age. We will then follow a strategy that parallels our methods noted above, using a sequential process to isolate free compounds and compounds associated with the kerogen matrix released through mineral digestion and stepped H_2 pyrolysis. Work will target the Tumbiana Formation (2.7 Ga) and the Mosquito Creek Formation (2.9 Ga) in order to compare results with our current study (which inlcudes Tumbiana samples). We will also extend our findings further back in time with samples from the Sulfur Spring Formation (3.2 Ga), although we note that recovery of biomarker compounds become increasingly less probable in older units.

1.2.1.3. Microfossil isotopic analyses (House)

Geochemical studies have been extremely useful for identifying broad secular trends throughout the Precambrian and for suggesting the involvement of particular microorganisms in the carbon cycle at particular times during the past. Nonetheless, these analyses lack high spatial resolution, and therefore, are missing key information that might be useful in the discovery of biochemical characteristics of the earliest microbial ecosystems. Secondary ion mass spectrometry (SIMS) offers a powerful tool capable of isotopic analyses of 10 – 15 μm spots (Ireland, 1995).

Recently, several papers have shown the usefulness of SIMS for the carbon isotopic analyses of Precambrian graphite (Mojzsis et al., 1996; Ueno et al., 2002), of Precambrian microfossils (House et al., 2000; Kaufman & Xiao, 2001; Ueno et al., 2001), and of modern microbes from environmental samples (Orphan et al., 2001, 2002). In general, there are a number of

Precambrian paleobiological applications for carbon isotopic analyses by SIMS, including linking microfossils to metabolic pathways, constraining estimates of the partial pressure of carbon dioxide in the atmosphere at during the Precambrian, and ascertaining whether or not methane was an important part of an ancient microbial ecosystem. These investigations will be carried out in collaboration with the Center for Astrobiology at UCLA, and in conjunction with the studies in Section (*1.2.1.2)*.

1.2.2. Evolution of the Geochemical Cycle of Sulfur: Mass Independent Fractionation of S Isotopes, Sulfate-Reducing Microbes, and Sulfate in the Precambrian Oceans

Major Questions: In most previous investigations, evidence for sulfate-reducing microbes (SRM) in Archean oceans was sought in the variation of the $^{34}S/^{32}S$ abundance ratio ($\delta^{34}S$ value) of pyrite crystals in Archean sedimentary rocks, because until recently, the only known mechanism for $\delta^{34}S$ variations in surface environments was bacterial sulfate reduction. The SO_4^{2-} content of Archean oceans has also been estimated primarily from the difference in the $\delta^{34}S$ values between pyrite and barite ($BaSO_4$) crystals in Archean sedimentary rocks: $\Delta_{SO4\text{-}py} = \delta^{34}S_{SO4} - \delta^{34}S_{py}$. Such an approach has suggested that SRM evolved by ~3.4 Ga (Ohmoto et al., 1993; Shen et al., 2001). However, depending on the interpretation of the mechanisms controlling the $\Delta_{SO4\text{-}py}$ values, the suggested values for the SO_4^{2-} content of Archean oceans greatly vary from “the same as today” (Ohmoto & Felder 1987), to “less than 1/10 of today” (e.g., Lambert et al., 1978), to “less than 1/100 of today” (Habicht et al., 2002). An important question to investigate is the minimum level of seawater sulfate content required for the anaerobic recycling of methane (Reaction 1-7) to become important.

The discovery by Farquhar et al. (2000) of the MIF of S isotopes in some sedimentary rocks older than ~2.0 Ga (described in Section 1.1) has presented a serious problem in the conventional approach, which has assumed all (or most) pyrite crystals in sedimentary rocks formed by bacterial sulfate reduction, thus relating the $\Delta_{SO4\text{-}py}$ values to seawater SO_4^{2-} content. According to Farquhar et al. (2000), the observed $\delta^{34}S$ fractionation between pyrite and sulfate in Archean sedimentary rocks could have been created largely by atmospheric reactions, rather than by SRM. Some recent researchers (e.g., Pavlov & Kasting, 2002; Runnegar, 2001; Runnegar et al., 2002; Holland, 2002) have even suggested that all pyrite and barite crystals in Archean sedimentary rocks formed from S^0 and SO_4^{2-}, which were produced in an O_3- and O_2-free atmosphere by the UV radiation of volcanic SO_2. Therefore, an important objective of this research is to provide a link between the experimental and theoretical approaches (see Sections 2.1 and 2.2) and the geologic record of MIF of S isotopes, and in so doing, answer the following major questions:

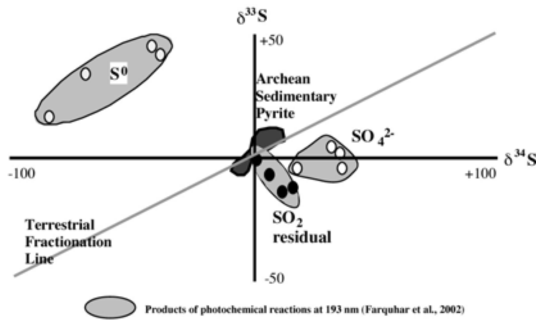

Fig 1.5. MIF of S isotopes, experiment vs. natural data

(1) What are the causes of MIF in geologic samples? Related questions include: (a) What are the reason(s) for a serious discrepancy between the MIF values observed in natural samples and those observed in laboratory experiments (Fig. 1.5)? While most sulfide-bearing geologic samples with $^{33}\Delta$ values outside the 0±0.1 ‰ range are characterized by positive signs for both $\delta^{34}S$ and $\delta^{33}S$ values, S^0 produced by short-wave

UV are characterized by very large and variable negative δ^{34}S and positive δ^{33}S values. (b) What important parameters determine the δ^{34}S, δ^{33}S, and $^{33}\Delta$ values of natural pyrites and sulfates? What mechanism(s) produce at least two well-defined MIF trends in natural samples (see Fig. 2.4 in Section 2.2)? Data obtained from sedimentary and igneous rocks (3.2-2.5 Ga in age) from the Pilbara-Hamersley district (Onoet al., submitted; Runnegar et al., 2002; Ohmoto et al., in prep.) indicate that most of the MIF values in these district fall around two lines: $^{33}\Delta$̣̃□□δ^{34}S + 0.90 or $^{33}\Delta$̣̃□□1‰ (see Fig. 2.4 in Section 2). (c) What are the connections between the MIF of S and volcanism? Recent discoveries of the existence of MIF ($^{33}\Delta$ = +0.6 to –0.4‰) in volcanic ash from the Mt. Pinatubo volcano (Savarino et al., 2002) and the correlation between $^{33}\Delta$ values and Zn contents in the 2.5 Ga McRae shales (Ohmoto, unpublished data) suggest the connection between the MIF and the atmospheric O_2 level could be more complicated than previously suggested.

(2) When did SRM evolve on Earth?

(3) How did the SO_4^{2-} content of normal oceans change through the Archean and Proterozoic eras? The sulfate content of Proterozoic oceans, as well as that of Archean oceans, is of great interest to us, because the disappearance of banded iron formations (BIFs), possible changes in the redox cycles of Fe, Mo, and other elements, and evolution of new organisms in the Proterozoic may have occurred due to the formation of H_2S-rich oceans, which required a high concentration of seawater sulfate (Canfield, 1998; Anbar & Knoll, 2002).

In order to answer the above questions, we will conduct the following investigations (1.2.2.1-1.2.2.2):

1.2.2.1. Multi-geochemical analyses of S-bearing sediments (Ohmoto)

Sulfur isotope analysis alone will not fully answer any of the above questions. Our investigations will be first directed toward identifying and quantifying pyrite crystals (and sulfate minerals) of different origin: (1) those of detrital origin (type-a), (2) those formed during the accumulation of host rocks (i.e., syngenetic and early diagenetic pyrite; type-b), and (3) those formed after the early diagenetic stage (typically by hydrothermal fluids; type-c). "Atmospheric pyrite" (i.e., pyrite formed by utilizing the S^0 produced by photochemical reactions in the atmosphere) and biogenic pyrite belong to type-a. Identification of different types of pyrite (and sulfate) crystals requires detailed petrographic, mineralogical, and geochemical investigations using a variety of microscopes (optical, electron, and X-ray) for the occurrences, morphologies, textures, and chemical compositions of individual pyrite crystals, as well as bulk-rock analyses of the abundance ratios of S, C, and major-, trace- and REE elements. For example, positive correlations can be expected between the contents of pyrite S and organic C if the pyrite crystals were mostly formed by SRM during the accumulation of sediments. This type of multi-geochemical investigation~~s~~ has been routinely carried out by Ohmoto's group (Watanabe et al., 1997; Naraoka & Ohmoto, submitted; Yamaguchi, 2002; Watanabe, 2002).

Following the above types of geochemical investigations, different phases of S-bearing compounds (pyrite, other sulfides, sulfate, and organic S) will be sequentially extracted, converted to Ag_2S, and analyzed for δ^{33}S and δ^{34}S values. We have already analyzed more than 30 samples of sedimentary and igneous rocks from the Pilbara district. Both the δ^{33}S and δ^{34}S values must be determined in order to quantitatively estimate: (a) the fractions of "atmospheric pyrite" and biogenic pyrite in sedimentary rocks, and (b) the true isotopic fractionation factors between the biogenic pyrite and the contemporaneous seawater sulfate. The $^{33}\Delta$ values should fall within 0±0.1‰ if the S atoms in pyrite (and other compounds) have only been involved in normal biological and chemical processes during their entire history; $^{33}\Delta$values outside this range have been attributed to photochemical reactions (Farquhar et al., 2000, 2001). Since the amount of biogenic pyrite in a marine sediment depends on the sulfate content of the seawater, determination of the average contents of biogenic sulfides in Archean shales becomes critically important in constraining the sulfate content of the Archean seawater (Fig. 1.6).

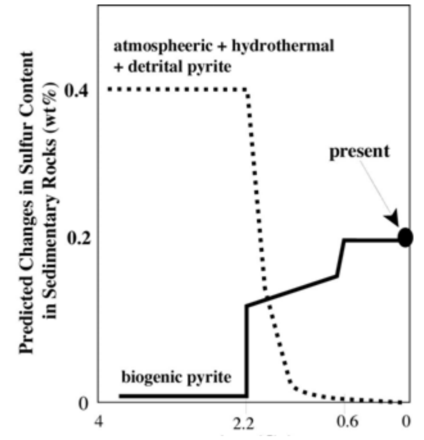

Fig. 1.6. The temporal changes in the contents of biogenic pyrite and of (atmospheric + hydrothermal + detrital) pyrite in sedimentary rocks as expected from the S-cycle models of Canfield et al. (1999, 2000), Habicht et al (2002), Runnegar (2002), and Holland (2002).

The primary focus of our investigations will be the drill core samples from the ABDP. We will investigate sediments that were deposited under a variety of geologic environments, including: (a) lake sediments; (b) carbonates; (c) gray (organic C-poor) shales and sandstones that deposited on an open continental shelf; (d) black (organic C-rich) shales that deposited in marine anoxic basins; and (e) shales that accumulated in deep submarine hydrothermal environments. We will investigate lacustrine sediments because these rocks are expected to contain the highest proportion of “atmospheric pyrite”. We will examine the relationships between the magnitude of MIF and the various geological and geochemical parameters (e.g., stratigraphy, lithology, paleogeography, and volcanic and hydrothermal inputs) in order to understand the causes of MIF of S isotopes and the significance of MIF records in geologic samples.

1.2.2.2. Sulfate analyses of carbonates (Arthur)

Sedimentary anhydrite ($CaSO_4$) is rare in Archean formations, possibly because it was lost during metamorphism and/or by reactions with groundwater. Therefore, previous researchers analyzed barites ($BaSO_4$), which may be the replacements of anhydrite through hydrothermal reactions, to estimate the S isotopic composition of Archean seawater sulfate. However, the SO_4^{2-} in Archean barites may be of hydrothermal origin, not normal seawater. Anhydrite beds are more common in Proterozoic formations, but are still limited in temporal and spatial distributions. In contrast, sedimentary carbonates are more widely distributed; most carbonates contain trace amounts of sulfate, which was probably incorporated into carbonates during precipitation from seawater (or lake water). At PSARC, Arthur’s group has analyzed: (a) the content of trace sulfate in carbonate; (b) its $\delta^{34}S$ and $\delta^{18}O$ values; (c) the $\delta^{34}S$ value of trace pyrite in the carbonate; and (d) the $\delta^{13}C$ and $\delta^{18}O$ values of the host carbonate from ~600 Ma carbonate sequences in Namibia and Arizona, and reconstructed a detailed history of the content and isotopic composition of seawater sulfate during the period of the Snowball Earth (Hurtgen et al., 2002).

We will extend our investigations of sedimentary carbonates to those of the middle and early Proterozoic ages, and also of Archean age, in order to reconstruct the evolutionary history of sulfate in the oceans through geologic time.

1.2.3. The Nutrient Cycles (Arthur, Kump, and Ohmoto)

A combination of techniques, in part described above (Sections 1.2.1.1 and 1.2.2.1), will allow us to evaluate evolution of nutrient cycles in the Archean and Proterozoic oceans. These techniques include studies of the concentration and isotopic composition of nitrogen in preserved organic compounds, and an analysis of organic and inorganic phosphorus concentrations in sedimentary rocks of various lithologies, particularly black shales. A major question is how changes in the ocean-atmosphere redox state influenced cycling of nitrogen and phosphorus. Furthermore, how did evolutionary innovation, for example the origination and prominence of biological nitrogen fixation or the origination and proliferation of denitrifying bacteria, both of which impart strong isotopic signals on organic nitrogen, affect ecosystem productivity? What was the interdependence of environmental evolution, nutrient availability and productivity? For example, what was the balance of nitrogen fixation vs. denitrification following the onset of an oxygenated atmosphere? Another intriguing question is what led to a relatively low and flat carbon isotopic composition of carbonates through most of the Proterozoic, sandwiched between periods of apparently responsive carbon isotope variation in the early Paleoproterozoic and late Neoproterozoic? The solution to these problems may lie in changes in nutrient cycling as a response to redox variations, or, as proposed by Anbar and Knoll (2002), in limitations on nitrogen fixation imposed by certain redox-sensitive trace metals. This work will also test the validity of Canfield's (Bjerrum & Canfield, 2002) hypothesis that the productivity in the Archean oceans was much less than that in the Phanerozoic oceans because a higher proportion of phosphate in the Archean oceans was removed by ferric oxides in BIFs.

There have been several attempts to provide a temporal view of the nitrogen isotopic evolution of Precambrian organic matter. These studies have used either bulk nitrogen isotopic compositions of chert (Beaumont & Robert, 1999) or the isotopic composition of ammonia in low-grade metamorphic rocks (Boyd, 2001; Pinti et al., 2001). We propose to study the isotopic composition of nitrogenous compounds preserved in shales and carbonates. This requires an approach that sequentially examines organic N, absorbed N_2 and mineral-bound N in consideration of the possible effects of diagenesis; the similar approach was proven successful by Yamaguchi (2002) on shales from drill core samples (3.2-2.0 Ga in age) from the Kaapvaal Craton, South Africa. In an attempt to understand what the consequences of oceanic oxygenation might have been for nutrient cycling, we will also utilize a numerical biogeochemical cycle model for oceanic nitrogen and phosphorus that is used to test assumptions regarding the effects of widespread deepwater vs. whole-ocean (atmosphere) anoxia on nitrogen availability to plankton and nitrogen isotopic values for sedimented organic carbon.

In addition to the ABDP core samples, we will study suites of samples of black shale and interbedded lithologies from Labrador (Ramah Group, 1.9 Ga, Hayashi et al., 1997), Ontario Canada (Serpent Fm and Whitewater Group, 1.85 Ga, Fedo et al., 1997), Western Australia (Earaheedy Group, 1.7 Ga, Krapez and Martin, 1999; Bangemall Group, 1.6 Ga, Buick et al., 1995), Arctic Canada (Dismal Lakes Group, 1.3-1.27 Ga, Frank et al., in press) and NW Territories, Canada (Shaler Supergroup, 1.0 Ga, Rainbird et al., 1996), and Neoproterozoic samples from Australia, Great Basin, U.S., and Namibia from our earlier work (Hurtgen et al., 2002; Hurtgen & Arthur, in prep.). We propose further field work in Labrador and NW Territories and additional work in Western Australia in order to better sample units there. Suites of fresh samples are available to us through collaborative studies (T. Frank and T. Lyons: PSARC Associates) for the other sites.

1.2.4. Geochemical Cycles of Fe and Bioessential Metals

Major Questions: An important characteristic of Archean and early Proterozoic sedimentary rocks is the abundance of rocks comprising of mostly silica (quartz, SiO_2), iron oxides (hematite, Fe_2O_3; and magnetite, Fe_3O_4), and/or iron carbonates (siderite, $FeCO_3$). Depending on the relative abundance of these minerals, the rocks are classified as chert, ferruginous chert or jasper (see Fig. 1.3), oxide-type BIFs, or carbonate-type BIFs. Cherts are the most favorable for a search of microfossils due to their resistance to thermal and chemical modifications. Some important astrobiological questions concern: (1) the depositional environment of cherts, jaspers, and BIFs (shallow continental shelf or deep submarine hydrothermal fields); (2) the sources of SiO_2 (continental weathering or submarine hydrothermal fluids); (3) the source of Fe^{2+} (continental weathering, hydrothermal fluid from the mid-ocean ridges, or local submarine hydrothermal fluids); (4) the source of O_2 (locally produced or atmospheric); (5) the source of CO_2 and HCO_3^- (atmospheric and oceanic or hydrothermal); (6) the mechanisms for precipitation of the primary minerals (evaporation, photochemical reactions, microbial precipitation, or mixing of hydrothermal fluids and seawater); and (7) the biogeochemistry of heavy metals in the Archean oceans. These questions will be answered from the following two types of investigations (1.2.4.1-1.2.4.2).

1.2.4.1. Geochemical analyses of cherts and shales (Ohmoto)

We expect our mineralogical and geochemical investigations of the Marble Bar chert-jasper sequence (DDH 1) will help settle the controversies on the processes and environment of formation for cherts, Fe-oxides, and Fe-carbonates in the Archean oceans. Their geologic settings (e.g., close association with pillow lavas, absence of clastic materials) suggest they formed in deep (>1 km) submarine environments, where the precipitation mechanisms of these minerals are quite limited. Beginning with detailed petrographic and mineralogical investigations, we will conduct studies on fluid inclusion, Ge/Si ratios (e.g., Hamade et al. 2003), and $\delta^{18}O$ values of cherts (Karhu & Epstein, 1986), along with rare-earth element ratios (especially Eu and Ce anomalies: Kato et al., in press).

The solubilities of major host minerals in igneous and sedimentary rocks for Mo (molybdenite and pyrite: MoS_2, FeS_2), U (feldspars and uraninite: UO_2), W (sheelite: $CaWO_4$), and V (V-oxides) generally decrease with decreasing Eh, while those of host minerals for Fe, Ni, Cu, and Zn (ferrous silicates and sulfides) increase with decreasing Eh; most of these minerals increase their solubility with decreasing pH (Stumm & Morgan, 1996). We will investigate the contents of these bioessential metals in the Archean oceans and lakes by normalizing trace element abundances to Al and Ti (i.e., clastic components) in shales from DDH 2-5, and by comparing these data with those in Phanerozoic shales. Therefore, if the Archean atmosphere was anoxic and the oceanic pH was similar to today, we would expect the contents of Mo, W, U, and V in the Archean oceans to have been higher and those of Fe, Ni, Cu, and Zn to have been lower than those in the modern oceans. Such differences may have created Archean biota quite different from today because the types of metal requirements greatly vary among different microbes (Madigan et al., 2000).

1.2.4.2. Radiogenic and Fe isotope analyses (Stewart and Capo)

Recent advances in the measurement of Fe isotopes (Johnson & Beard, 1999; Mandernack et al., 1999; Anbar et al., 2000; Bullen et al., 2001) have opened a new avenue for studying this essential, redox-active element. The mass difference between isotope pairs is sufficient to allow for measurable mass fractionation during low-temperature (earth surface) geologic processes (Urey, 1947; Polyakov, 1997; Schauble et al., 2001; Anbar, 2001; Matthews et al., 2001). Samples to date show a δ^{56}Fe range of about –2 to +1‰, with a typical uncertainty of 0.1-0.2‰ (summarized by Anbar, 2001). Beard and Johnson (1999) suggested that biological processes should produce the strongest isotope fractionation effects. However, subsequent experiments have demonstrated biologically mediated reactions with no fractionation effects (Mandernack et al., 1999; Brantley et al., 2001), as well as the abiotic fractionation of Fe isotopes in laboratory experiments (Anbar et al., 2000; Matthews et al., 2001; Johnson et al., 2002; Skulan et al., 2002) and field studies (Bullen et al., 2001).

Currently, limited BIF data suggest small (<1‰), but systematic and significant positive shifts in δ^{56}Fe in certain BIF bands, which were interpreted as reflecting a biogenic origin (Beard et al., 1999). However, these shifts could also reflect abiotic fractionation processes (e.g., Schauble et al., 2001; Zhu et al., 2002), or Fe from different sources, such as hydrothermal fluids (Sharma et al., 2001) or continental weathering (e.g., Zhu et al., 2000). Iron isotopes have promise as a provenance indicator for Fe, especially when combined with other isotope systems such as Sm-Nd or Rb-Sr. For example, a Nd isotope investigation of the Hamersley Group carbonate demonstrates similar sources of REE for both shallow and deep water carbonates (Stewart et al., 2002). In our investigations of Precambrian BIF petrogenesis, we will apply Fe isotopes as a tracer for Fe provenance in conjunction with other radiogenic isotopes. Our studies will also include analysis of marine carbonates and cherts (see *1.2.1.1*) to obtain a more complete picture of variations in ocean Fe chemistry with depth.

1.2.5. Evolution of the Terrestrial Biosphere: Archean and Proterozoic Paleosols (Capo, Stewart, and Ohmoto)

Paleosols are a direct link between the lithosphere and atmosphere, and thus are essential for interpreting paleo-redox conditions on early Earth. They are also about the only geologic samples providing remnants of organisms and chemical signatures of life on the Precambrian land surface. Identification of geochemical signatures for the existence of organisms on the land surface (e.g., Fe^{3+}/Ti and Fe^{2+}/Ti ratios: Ohmoto, 1996; Beukes et al., 2002) will be extremely useful for the detection of life (past and/or present) on other planets, such as Mars. We have been conducting detailed investigations of organic matter, mineralogy, Fe chemistry, and REE chemistry of selected paleosols. Over the past five years, we have discovered: (a) the oldest (2.6 Ga) remnants of life on land (microbial mats composed mostly of cyanobacteria) at Schagen, South Africa (Watanabe et al., 2000), and (b) the oldest (2.3 Ga) lateritic soils in very large areas in the Kaapvaal Craton (Beukes et al., 2002). Because the formation of a laterite sequence requires abundant organic acids to leach Fe from rocks and abundant O_2 to fix the dissolved Fe as ferric oxides, our discovery suggests that most land surface was covered by microbial mats (probably of cyanobacteria) and the atmosphere was already oxygenated by 2.3 Ga.

We have recently demonstrated that Sm-Nd and Rb-Sr isotopic analyses can, in some cases, determine the absolute ages of soil formation and post-depositional metamorphism of Archean and Proterozoic paleosols (Stafford et al., 2000, 2002). Application of Fe isotopes to Precambrian paleosols from critical periods in Earth's history will provide a new tool to evaluate paleo-redox conditions. This work will be closely integrated with studies of modern soils (Section 5), which will allow us to better understand Fe isotope behavior during weathering and pedogenesis and also to evaluate the effectiveness of usefulness of Fe isotopes in the search of present/past life on Mars.

We recently obtained preliminary REE data on paleosols from Kalkkloof, South Africa, which formed between 2.6 and 2.4 Ga ago. The REE data are exciting because they exhibit distinct Ce anomalies, which are excellent indicators that the soils formed under an oxygenated atmosphere. This paleosol profile may be the oldest soil section with unquestionable Ce anomalies. We have also obtained C isotope data on kerogen extracted from these paleosol samples. The data are also interesting because their $\delta^{13}C$ values (ca. -24‰) are quite different from those of the 2.6 Ga Schagen paleosols (ca. -15‰) and those of typical marine organisms of that time (i.e., ca. -28‰). We will continue detailed geochemical investigations of the Kalkkloof paleosols. Research will also continue on the following paleosol sections: (a) the 2.4 Ga paleolaterites at Hallavaara, Finland; (b) the 2.4 Ga paleosols at Pronto and Stanleigh mines, Ontario, Canada; (c) the 2.7 Ga "paleosols (?)" at Mt. Roe, Western Australia; (d) the 2.9-3.0 Ga paleosols at Steep Rock, Ontario, Canada; and (e) the 3.4 Ga Cooterunah Unconformity. We have already acquired a considerable amount of geochemical data on surface samples from (a), (b), (c), and (d). Our new initiatives will include deep drill core samples, which will be recovered from (c) in 2003 and from (e) in 2005 under the NAI drilling projects.

In addition to investigating the organic geochemistry, mineralogy, and bulk-rock inorganic geochemistry of the above paleosols, we will study the following new topics on paleosols: (a) weathering rates of pyrite crystals; and (b) the MIF of S isotopes. Because we have good kinetic laws for the dissolution of pyrite as a function of pH and pO_2 (e.g., Williamson & Rimstidt, 1994; Kamei & Ohmoto, 2000), we will be able to place constraints on the pO_2 and pCO_2 levels of the Archean and early Proterozoic atmosphere from the changes in the amount and grain size of the original pyrite crystals in paleosol profiles. If the uppermost soil zone was not eroded away, we could find "atmospheric pyrite" that formed from the S^0 generated by photochemical reactions of volcanic SO_2 in the atmosphere.

1.2.6. Extraterrestrial Influence on the Earth's Early Biosphere (Ohmoto and Kasting)

The scarcity of sedimentary rocks on Earth older than ~3.5 Ga has been attributed to the combination of plate tectonics and heavy bombardments by meteorites, which could have destroyed the early-formed proto-continents and oceanic islands (e.g., Windley, 1995a; Ward, 2000). The heavy bombardments could have also completely destroyed life on early Earth or at least hindered its early evolution (Maher & Stevenson, 1988; Sleep et al., 1989). However, the abundance of organic carbon in shales of the ~3.8 Ga Isua Formation in Greenland (Rosing, 1999) and in all sedimentary rocks in the Pilbara district (<3.56 Ga) (see Section 1.1.2) suggests the Earth may have experienced repeated histories of emergence, evolution, and extinction during the period of heavy bombardment (e.g., Windley, 1995b; Arrehenius et al., 1998; Nisbet & Sleep, 2001); post-3.6 Ga organisms may only represent those evolved from the last common ancestor (e.g., Nisbet & Sleep, 2001). The ~100 m *continuous section* of chert/jasper from the ABDP drill core (DDH 1) offers a unique opportunity to investigate variations in the flux of cosmic material (meteorites and cosmic dust) over time and its effects on marine life during the waning period of heavy bombardment; it is analogous to a >1 km deep polar ice core. The identification of layers enriched in volcanic ash, extraterrestrial materials, and/or organic matter occurs in a background of mostly white-colored chemical precipitates (i.e., silica, goethite, and siderite precipitates from the ocean water ± hydrothermal fluids) is much easier than finding them in rocks dominated by clastic minerals (i.e., shales and sandstones). Using NAI and PSU funds, we have purchased an ideal instrument for our investigations, the Horiba XGT-5000 x-ray chemical microscope. This recently developed instrument is capable of chemically mapping ~30 elements simultaneously with a spatial resolution of 10 μm on a hand specimen-sized rock (or biological) sample under air and without a pre-treatment; chemical mapping of a 2"x3" rock chip can be performed in 0.5–2 hours, depending on the concentrations of elements of interest. In contrast, microchemical analyses by other instruments are typically performed on small, pre-treated (e.g., gold coated) chips placed in a vacuum chamber, and chemical mapping of the same area takes more than 100 times longer. Using this instrument, we will map the distributions of layers rich in cosmic materials by identifying enrichments in platinum group and siderophile elements (Fe, Ni, Co, Cr), and their relations to organic carbon-rich layers, in the ~100 m continuous section. An example of a Cr-rich layer in a 3.8 Ga Isua BIF sample is shown in Fig. 1.7. In order to identify whether a Cr-rich layer is composed of volcanic ash or extraterrestrial materials, other elements must also be analyzed. After identifying layers possibly enriched in extraterrestrial materials, we will search for enrichments of iridium using the neutron activation analysis. Organic-rich layers may be absent in the layers immediately following a Cr-rich layer but may become more abundant toward the next Cr-rich layer, if meteorite impacts affected the marine (and terrestrial) biosphere on early Earth.

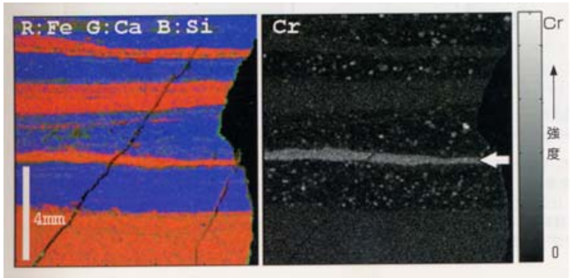

Fig. 1.7. Chemical maps of Fe, Ca, Si, and Cr on a 3.8 Ga Isua BIF sample, using the Horiba XGT-2800 X-ray chemical microscope (Takano, personal communication, 2002).

1.3. MODELING OF COUPLED S-C-O-FE GEOCHEMICAL CYCLES

(Kump and Arthur)

The experimental and analytical/observational approaches described above provide critical information on the evolution of Earth's surface environment and the intensity of biogeochemical cycling of the elements during 2.5 billion years of Earth history. We use box models to translate these observations into a quantitative assessment of the rates of biospheric processes, including weathering of the continents, biological productivity of the ocean (and land surface), burial of organic carbon and pyrite sulfur (and thus the fluxes of O_2 to the atmosphere), and make estimates of the atmospheric and oceanic composition for times in the distant geologic past **(**Kasting, 1993; Hotinski et al., 2000; Kump, 1991; Kump & Arthur, 1999; Beerling et al., 2002**).** More sophisticated models that provide spatial resolution (1-D atmospheric photochemical/radiative convective (e.g., Pavlov et al., 2001a) and oceanic diffusion models; 2-3D climate and ocean circulation models) are also used to address important questions concerning the coupling of atmospheric, oceanic, and biotic evolution. For example, such models must be used to deconvolve atmospheric evolution from geochemical records preserved in marine sediments (e.g., Hotinski et al., 2001): biological processes in the ocean can create reduced (or oxidized) marine conditions that may not reflect the redox state of the atmosphere. Numerical models can be used to explore the conditions under which such decoupling occurs. Modeling focuses will be on the following two topics:

1.3.1. Deep Sea Anoxia

We will continue work begun under current funding to constrain the necessary and sufficient conditions for deep-sea anoxia. We are particularly interested in the interval from 1.8 Ga to the onset of Snowball Earth (0.75 Ga), when sulfidic waters may have existed in the deep sea (Canfield, 1998). Important issues include (1) the nutrient- (e.g., phosphate) and sulfate content of the ocean required to maintain euxinic conditions; and (2) the degree to which issue (1) depends (quantitatively) on the efficiency of the biological pump (see Section 2), atmospheric oxygen level, and climate-sensitive ocean circulation. Although we continue to develop improved models for paleo-applications, existing models (e.g., Hotinski et al., 2001) can be used to answer this question.

1.3.2. Carbon Isotopes of Paleoproterozoic Carbonates

What are the possible explanations for the very heavy $\delta^{13}C$ values of Paleoproterozoic (2.2-2.0 Ga) carbonates (Melezhik & Fallick, 1994; Melezhik et al., 1999; Karhu & Holland, 1996; Bekker et al., 2003)? The traditional explanation of isotopically heavy carbonates is that they reflect high rates of organic carbon burial, and thus rapid increases in atmospheric oxygen levels. However, the duration and magnitude of this event imply the establishment of O_2 levels many times the present. Kump (1989) has argued in the past that large positive $\delta^{13}C$ excursions occur at times when overall rates of carbon cycling are anomalously low. Such conditions would be realized during times of supercontinental stasis, when the degree of continentality is maximized, and thus rates of continental runoff (and weathering rates) are minimized. We propose to investigate this alternative hypothesis for the Paleoproterozoic event. The modeling we perform will be aided by new data collected in collaboration with Victor Melezhik of the Norwegian Geological Survey.

II. PHOTOCHEMICAL REACTIONS OF SULFUR AND IRON IN THE EARLY EARTH

Kasting (Leader), Castleman, Schoonen, and Arthur

The first part of research in this Section (2.1) deals experimental and theoretical investigations on mass independent fractionation of sulfur isotopes during photochemical reactions of SO_2 in order to provide the fundamental data necessary for geochemists to correctly interpret the sulfur isotope record in rocks (see Section 1.2.2). Specific focus of the experimental investigations (Section 2.1.1) will be placed on: (a) the details of gas-phase reactions that lead to mass independent fractionation of sulfur isotopes; and (b) to identify the specific sets of atmospheric conditions that match the sulfur isotope record in pre-2.3 Ga rocks. Section (2.1.2) presents modeling of atmospheric photochemical reactions that link the experimental results (Section 2.1.1) with the rock record from Section (1.2.2). Photochemical experiments on ferrous hydroxide mineral and pyrite are proposed in Section (2.2) in order to investigate the possible mechanisms to form ferric hydroxide minerals in banded iron-formation and aqueous sulfate in the early oceans. Sections 2.3 proposes to identify the oxygen and sulfur isotope signatures of SO_4^{2-} that was formed: (i) by photochemical reactions SO_2 in Section (2.1.1), (ii) by photochemical reactions of pyrite in Section (2.2), and (iii) in carbonate minerals during precipitation and diagenesis; these experimental data are necessary in order to relate the chemical and isotopic compositions of Precambrian carbonates (Section 1.2.2) to those of the Precambrian oceans.

2.1. MASS INDEPENDENT FRACTIONATION OF SULFUR ISOTOPES

2.1.1.Background

One of the most exciting developments in the past several years has been the discovery of mass-independently fractionated (MIF) sulfur isotopes in rocks of Archean and Early Proterozoic age, 2.3 Ga and older (Farquhar et al., 2000, 2001). (See Fig. I in "Introduction and Overview" of this proposal). Farquhar et al. (2000) interpreted the presence of MIF of S isotopes (Fig. I) as indicating that atmospheric O_2 concentrations were low enough prior to 2.3 Ga to allow SO_2 to be photolyzed. This gives an upper limit on pO_2 of ~0.01 PAL (times the Present Atmospheric Level). Following publication of their results, our PSARC group used an atmospheric photochemical model to place even tighter constraints on O_2 (Pavlov & Kasting, 2002). We showed that in order for MIF to be preserved in sediments, S must exit the atmosphere in a variety of different oxidation states. Today, virtually all of the S that leaves the atmosphere is oxidized to sulfate and combined in the oceanic sulfate reservoir. According to the model of Pavlov and Kasting (2002), this remains true down to O_2 concentrations of 10^{-5} PAL. Thus, Archean pO_2 must have been less than this value--probably significantly so. Theoretical considerations predict that pO_2 should be $<10^{-13}$ PAL as long as the volcanic reduced gas flux exceeds the photosynthetic source of O_2 (Walker, 1977; Kasting, 1993). This appears to have been the case prior to 2.3 Ga.

Although photochemical models have provided this important constraint, their further usefulness is limited by the lack of kinetic data on isotope fractionation within the atmosphere. When SO_2 is photolyzed at 190 nm, the following reactions are expected to occur (Yung & DeMore, 1999):

$$SO_2 + h\nu \rightarrow SO + O \qquad (2\text{-}1)$$
$$SO + h\nu \rightarrow S + O \qquad (2\text{-}2)$$
$$SO + SO \rightarrow SO_2 + S \qquad (2\text{-}3)$$

Studies of MIF in oxygen isotopes during ozone formation (Gao & Marcus, 2001) suggest that symmetry is a key factor. We suspect that that symmetry may also be involved in MIF of S isotopes.

Farquhar et al. (2001) measured highly positive $\Delta^{33}S$ in elemental sulfur, S_8, which forms from S atoms via sequences such as: $S + S + M \rightarrow S_2 + M$, $S_2 + S_2 + M \rightarrow S_4 + M$, $S_4 + S_4 + M \rightarrow S_8 + M$. MIF could arise during sulfur chain formation by these or related reactions. A third possibility is that MIF occurs during the reaction of SO with itself (reaction 2-3). The reaction apparently proceeds by way of an unstable, excited dimer state, $(SO)_2^*$ (Herron & Huie, 1980) which splits apart if it is not stabilized by a collision with another molecule. Symmetry of this excited dimer species may be an important factor.

In light of the uncertainty about the fractionation mechanism, we propose to examine all of the key steps leading from SO_2 to elemental sulfur either experimentally or theoretically. This work is described in the next section.

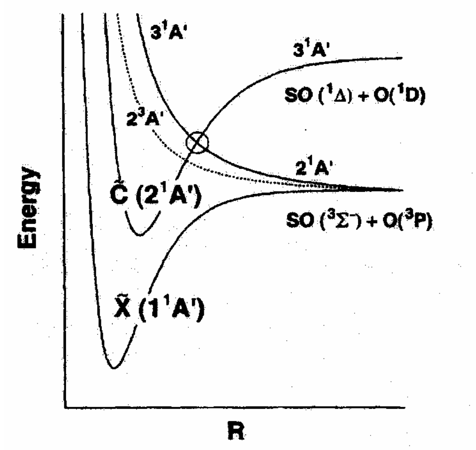

Fig. 2.1. Potential energy surface of SO_2 showing the C ($2^1A'$) state as well as other excited states. (From Katagiri et al., 1997).

2.1.2. Photochemical Experiments (Castleman)

2.1.2.1. SO_2 photolysis

We will begin with the photolysis of SO_2 because this is the initial step and is the most straightforward of the experiments. When SO_2 absorbs a photon at wavelengths near 190 nm it is raised to the excited $C(2^1A')$ state (Fig. 2.1). Once in the excited state, the SO_2 molecule can do one of two things: 1) It can transition spontaneously to the unbound state, SO + O; or 2) it can collisionally deactivate to give ground state SO_2. Which of these two things happens depends on the lifetime of the excited state compared to the collision time. Collision times can be estimated from kinetic theory, given a known (or hypothesized) distribution of atmospheric pressure and temperature. Thus, the key measurement that is required is the lifetime of the excited state. We propose to measure this directly for ^{32}S, ^{33}S, and ^{34}S. How we will do

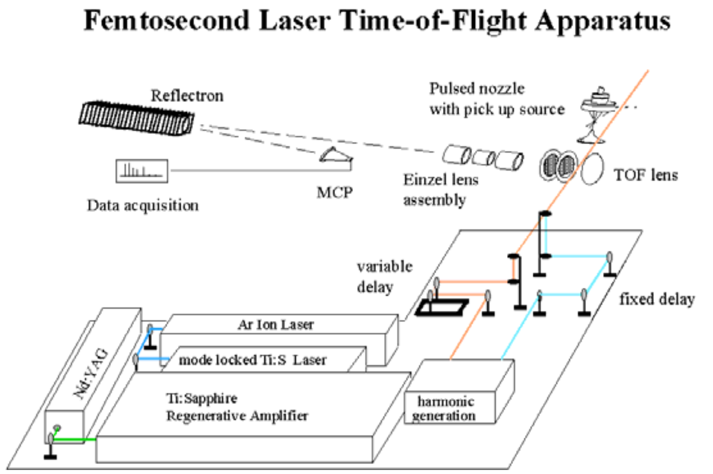

Fig. 2.2 Setup of the femtosecond pump-probe time of flight mass spectrometer.

this is described below.

A reflectron time-of-flight mass spectrometer (RETOF-MS) and femtosecond laser system coupled with the pump-probe technique will be utilized to study the dynamics of SO_2 and related sulfur species (Fig. 2.2). The laser system used in these experiments consists of a mode-locked Ti:Sapphire oscillator (Spectra Physics Tsunami) pumped by a 10W argon ion laser (Spectra Physics 2060) which generates an 82 MHz pulse train. Pulse amplification is carried out with a regenerative Ti:Sapphire amplifier pumped by the second harmonic of a 10Hz Nd:YAG laser (Spectra Physics GCR 150-10). Typical output from the amplifier is a beam with a wavelength centered around 795 nm, having a pulse-width of 100 fs and a pulse energy of about 3 mJ. The fundamental wavelength can be frequency doubled with a BBO crystal to generate the second harmonic. The third harmonic (~266 nm) of the fundamental wavelength can also be generated by frequency mixing of the fundamental and second harmonic with a BBO crystal.

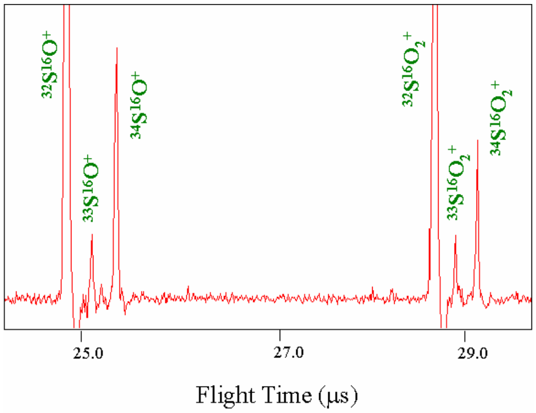

Fig. 2.3 Mass spectrum of SO_2, SO, and their isotopes.

The ^{33}S and ^{34}S isotopes of SO_2, seen in previous experiments by Hurley and Castleman, could be studied in a similar fashion. Figure 2.3 shows a mass spectrum of SO_2, SO, and their isotopes. Further experiments on the isotopes using the pump-probe could produce useful temporal information.

A typical experiment will be performed as follows. The second harmonic will be used in a two-photon excitation to populate the C ($2^1A'$) state of SO_2. The third harmonic can also be used to excite SO_2 to the coupled $^1A_2$, $^1B_1$ state. Figure 2.4 shows a pump-probe trace of SO_2 with 2 photons of the second harmonic as the pump and 4 photons of the fundamental as the probe. Temporal data extracted from traces of the SO_2 isotopes (^{33}S, ^{34}S) will be useful in our model. With further modifications to the apparatus discussed above, pump-probe traces of the isotopes of SO_2 could be taken. These include a higher resolution detector with minimal ringing characteristics. Eventually, an OPA (optical parametric amplifier) will be required to select a wide range of wavelengths and, hence, molecular states.

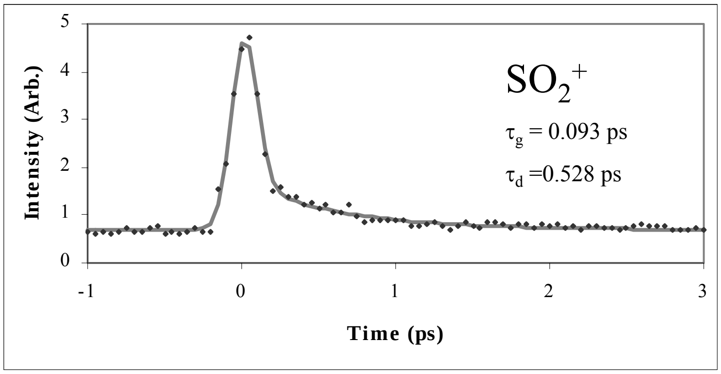

Fig. 2.4 Pump-probe trace of SO_2+, where τ_g represents a growth and τ_d represents the decay of the excited state.

2.1.2.2 Sulfur chain formation

The next set of reactions that we propose to study experimentally are those leading to sulfur chain formation. We are especially interested in the reactions:

$$S + S + M \rightarrow S_2 + M \quad (2\text{-}4)$$

$$S + S_2 + M \rightarrow S_3 + M \quad (2\text{-}5)$$

Reaction 2-4 is the simplest S recombination reaction. Symmetry is clearly involved because the S_2 molecule can either contain two identical S atoms or two different isotopes of S. Reaction 2-5 is analogous to the ozone formation reaction ($O + O_2 + M \rightarrow O_3 + M$). Hence, we can anticipate *a priori* that this reaction should involve MIF.

The sulfur clusters will be formed by the following procedure: Laser ablation of solid sulfur will produce gas phase sulfur atoms. These sulfur atoms, seeded in helium backing gas, will under go an expansion through a nozzle resulting in cooling and cluster formation. The isotopic composition of these clusters can then be studied using mass spectrometry and laser techniques.

2.1.2.3. $SO + SO \rightarrow SO_2 + S$ Reaction

Techniques exist for the generation of SO and S_2O_2 by thermal decomposition of sulfur-containing organic precursors. S_2O_2 and clusters of SO such as $(SO)_2$, which can be formed by supersonic expansion, can be studied by the photolysis technique described in the proposed work. As with SO_2, photolysis experiments will measure the time dynamics of the chemical process, in this case the formation of SO_2, and elucidate the influence of the sulfur isotopes on the time scale of the chemical process.

2.1.3. Photochemical Modeling of Sulfur Isotopic Fractionation (Kasting)

Once kinetic data become available for the primary process(es) causing MIF in sulfur isotopes, we can return to the photochemical model of Pavlov and Kasting (2002) and use it to derive more accurate estimates for the isotopic compositions of the various atmospheric sulfur gases and particles that can exit the atmosphere. When combined with new analytical data on the sulfur isotopic composition of Precambrian rocks, some of which will be collected here at Penn State (see Section 1.2.2), this should provide a powerful tool for constraining both the nature of the Archean/Early Proterozoic atmosphere and the processing of sulfur species that must have occurred within the marine ecosystem at that time. Several other NAI groups (Carnegie Inst., Harvard, UCLA) have also been collecting new multi-S-isotope data. We have been working with the first two of these groups, and these efforts have already resulted in one joint publication (Ono et al., submitted).

A brief description of what we hope to learn from this modeling follows: In the Ono et al. paper, we present data from the 2.7-Ga Jeerinah

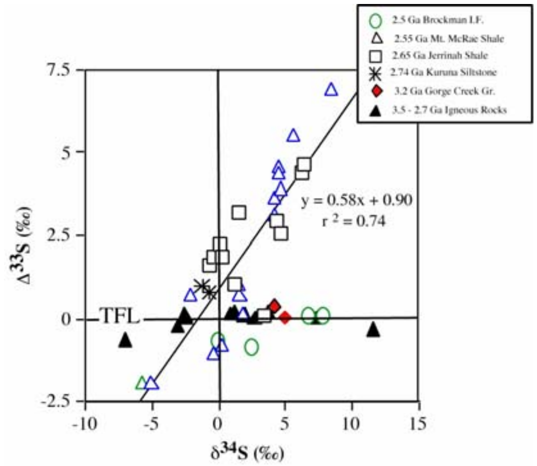

Fig. 2.5. S isotope data on sulfides from sedimentary and igneous rocks (3.2-2.5 Ga in age) from the Pilbara-Hamersley districts, W. Australia. Data from Farquhar et al. (2000), Onot et al. (submitted), and Ohmoto et al. (in prep.).

formation and Lewin shale (Hamersley Basin, Australia) which show $\Delta^{33}S$ values of +4.7‰ and +8.2‰, respectively (see Fig. 2.5). These values may be compared with the maximum value of +2.2‰ in the original paper by Farquhar et al. The first question that comes to mind is: what is responsible for these very high $\Delta^{33}S$ values? Based on the measurements of Farquhar et al., we suspect that this signal is coming from elemental sulfur (S_8) particles created photochemically within the atmosphere. These particles are formed preferentially in the upper troposphere of our model and, hence, can in theory preserve the strong MIF signature created by the types of reactions described above. Gaseous sulfur species, by contrast, exchange their isotopes with other sulfur gases during their voyage through the lower atmosphere and are much less likely to have strong MIF signatures. We relate the $\Delta^{33}S$ values and deposition rates of S_8 particles to the CH_4 content of the atmosphere and the volcanic outgassing rate of sulfur. S_8 formation varies roughly as the 8th power of the atmospheric SO_2 concentration and, so, is a strong function of the sulfur outgassing rate.

Once in the ocean, we suggest that elemental sulfur was used as an electron acceptor by sulfur reducing bacteria. Reduction of elemental sulfur is thought by some researchers to have been among the oldest forms of metabolism (Canfield & Raiswell, 1999). We can potentially test this idea by looking as the sulfides that these organisms produced. We also see evidence in our data of mass-dependent S isotope fractionation, which we attribute to bacterial sulfate reduction. Three-isotope plots should eventually allow us to determine what types of sulfur-utilizing bacteria were present in the Archean ecosystem, as well as what types of atmospheric conditions prevailed at that time. None of these detailed questions can be answered, however, until the primary fractionation processes are understood. Thus, the measurements described in Section 1.2 are critical to making further progress with our model.

2.2. PHOTOCHEMICAL AND CHEMICAL PROCESSES INVOLVING IRON

(Schoonen)

Iron is the most abundant redox-active, metallic element on Earth, a major constituent on all other planetary bodies in our inner solar system, and is thought to be a major component on any Earth-like planet elsewhere. Hence, attempts to describe the evolution of the redox state of a planetary body such as the Earth must account for redox processes involving iron. The objective of this task is to investigate photochemical and thermal redox processes involving iron that may have been relevant in the evolution of the redox state of the oceans through time. Some of the processes that will be considered in this task may have also been relevant in prebiotic synthesis on the early Earth. The relative stability of Fe(III)-O-H and mixed Fe(II)-Fe(III)-O-H solid phases with respect to pure $Fe(OH)_2$ is the driving force for many of the processes that will be investigated here. Hence, $Fe(OH)_2$ in aqueous environments serves as an electron donor. In the early oceans, water, dinitrogen, carbon monoxide, and possibly carbon dioxide may have been the chief electron acceptors. Reduction of these compounds could have produced some of the precursors to the building blocks of life, such as ammonia and formaldehyde. Experimental studies of redox processes involving iron in aqueous environments may also provide constraints on the role of abiotic processes in the precipitation of iron-containing minerals that are a major component in the BIFs that formed up to 1800 Ma ago (with a reprise in the Neoproterozoic).

For the purpose of designing a research strategy it is convenient to consider the fate of dissolved ferrous iron (Fig. 2.6). Ferrous iron, predominantly derived from submarine hydrothermal exhalative systems (Kump et al., 2001), may be removed from the water column via redox and non-redox precipitation processes, assisted or unassisted by light. Out of the processes shown in Fig. 2.6 we have selected a few for further study. The selected processes have the potential to have been important in regulating the redox state of the early oceans.

Photo-oxidation of dissolved ferrous iron leads the formation to Fe(III)-OH or Fe(III)-Fe(II)-OH phases. A seminal experimental study by Braterman and collaborators (Braterman & Cairns-Smith, 1987; Braterman et al., 1983; Braterman et al., 1984) has shown that UV-irradiation of a near-neutral ferrous solution devoid of dissolved molecular oxygen leads to the precipitation of γ-FeOOH . In this process, protons act as electron acceptors producing molecular hydrogen. We propose to experiment over a wider range of solution compositions than deployed in the original work by Braterman et al. (1987.). In particular, we wish to establish if the quantum yield of the reaction is affected if the composition of the electrolyte solution is varied. This is of importance because hydrogen formed in this reaction will tend to escape Earth, leading to an overall oxidation of the ocean/atmosphere/crust. Better constraints on the quantum yield of the reaction will provide improved constraints on the hydrogen escape rate. In addition we wish to evaluate if CO and N_2 could act as electron acceptors in this process. Some of the experiments in the original work were conducted under nitrogen gas, but no attempt was made to analyze for ammonia. Formation of reduced N or C compounds would limit or eliminate the formation of hydrogen and the net oxidation state of the ocean would not be affected (cf. loss of H_2).

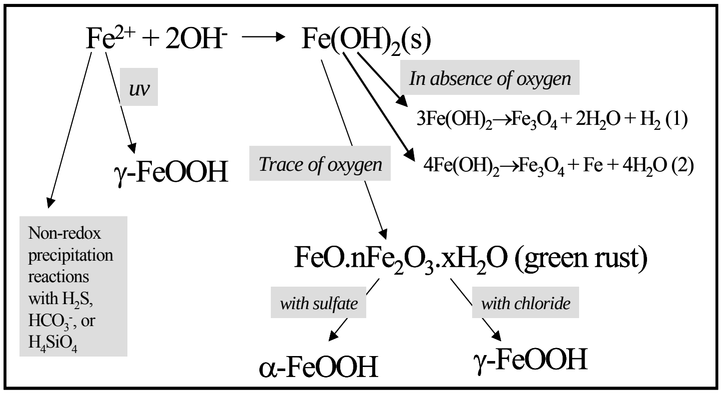

Fig. 2.6. Fate of Fe^{2+} in solution, including decomposition of $Fe(OH)_2$.

The second process of interest is the conversion of $Fe(OH)_2$. $Fe(OH)_2$ is a white solid that will undergo a spontaneous conversion to magnetite and metallic iron in systems devoid of oxygen, while it converts to mixed double-layer hydroxides if as little as a trace of oxygen is present (Blesa & Matijevic, 1989). The conversion under strictly anoxic conditions proceeds via two competing reactions, reactions 1 and 2 in Fig 2.6. As demonstrated by Schrautzer and Guth (1976) , metallic iron formed in reaction 2 is very reactive and capable of reducing water (Evans & Wanklyn, 1948), dinitrogen and carbon monoxide. Experiments with dissolved CO led to the formation of methane (Schrauzer & Guth, 1976). The state of the metallic iron produced in this process is of great interest. It appears to be in the form of small clusters, possibly nanoscale particles, dispersed in a magnetite matrix. The work by Schrauzer and Guth (1976) was conducted under conditions that are far from those that are relevant for the origin of life. We propose to explore the fate of $FeOH_2$ under strict anoxic conditions in NaCl solutions and vary the composition (including addition of CO, CO_2, N_2 and other plausible trace metals). The results will constrain the rate of H_2 production, methane formation, and formation of precursors to the building blocks of life via the processes associated with the conversion of $Fe(OH)_2$.

If a trace of oxygen, nitrate, or nitrite is present, $Fe(OH)_2$ convert readily to layered iron hydroxides (i.e. green rust). This class of materials contains structural anions that are derived from the solution (Brindley et al., 1976; Bessiere et al., 1999; Gehin et al., 2002; Hansen & Poulsen, 1999; Benali et al., 2001; Legrand et al., 2001)). While the mechanisms of the conversions are not fully understood (Blesa & Matijevic, 1989; Domingo et al., 1993; Hansen & Poulsen, 1999), green rust has been shown to be able to reduce nitrite, nitrate, selenate, chromate, and possibly dinitrogen (Denisov et al., 1991; Hansen et al., 1994; Myneni et al., 1997; Refait et al., 2000; Williams & Scherer, 2001). We propose to conduct experiments to explore if the conversion of green rust can reduce water, CO and dinitrogen. It is also noteworthy that mixed layer metal oxides may have been important in the prebiotic synthesis (Kuma et al., 1989).

While not the primary focus of research proposed in this task, it is logical to also study the nature of the Fe-O-H products formed as conditions are changed in these experiments. Not only is it of interest to study the structure of the products, but also their composition. For example, magnetite formed via the conversion of $Fe(OH)_2$ under strict anoxic conditions may contain metallic iron, while magnetite and hematite formed in the presence of a trace of oxygen may inherit a significant amount of sulfate or chloride as a result of the conversion of the intermediate green rust. If we can establish that there are indeed significant differences in chemical composition depending of the formation path, it is a logical next step to study natural magnetites from BIFs through time. While untested, the potential payoff, an independent constraint on seawater composition (Cl/SO_4 ratio) and a constraint on O_2 levels, is high. Changes in the nature of the Fe-O-H phases formed in the oceans as well as conversions from one form to another may have also affected the fate of phosphate and REE in the oceans as the surface chemistry (particularly the surface charge) of the various iron phases differs for a given ocean composition. The interaction of REE with the iron phases formed via the processes outlined here is poorly understood and will be addressed in a joint study by Kump and Schoonen.

The proposed experiments can be readily conducted in Schoonen's laboratory at Stony Brook. The lab is equipped with the necessary UV-photochemical equipment, GC and IC, a cryogenic Mossbauer spectrometer for the characterization of Fe phases, Raman, FTIR, and expertise and equipment to conduct research on systems devoid of molecular oxygen. Given that CO can be reduced by $Fe(OH)_2$ as it converts to magnetite and possibly also forms as green rust converts, the research proposed in this task may provide a constraint on the rate of abiotic methane production of methane.

2.3. SULFUR AND OXYGEN ISOTOPES OF SULFATE (Arthur)

2.3.1. Oxygen Isotope Signatures of Sulfate Produced by Photochemical Reactions

With the likely absence of large-scale oxidative weathering of sulfur on land during the Archean, sulfate-sulfur in seawater could have been derived from: i) the production of SO_4^{2-} from volcanic sulfur accompanying atmospheric photochemical reactions and disproportionation of SO_2 in surface seawater; ii) the SO_4^{2-} produced by photo-oxidation of pyrite utilizing H_2O_2 in shallow seas and on land; and iii) sulfate ion formed by bacterial sulfide oxidation in possible surface-water oxygen "oases." We are interested in distinguishing sulfate from each source, and suggest that oxygen isotopes might constitute a way to do so. Photochemical experiments conducted in Schoonen's laboratory (see Section 2.2) indicate substantial peroxide formation from water in seawater under visible light illumination, using Fe^{+3}, and that this peroxide oxidizes pyrite. However, there are no experimental or theoretical data to evaluate the O isotopic composition of type-(ii) SO_4^{2-}. The objective of this study is, therefore, to experimentally determine the $\delta^{18}O$ characteristics of type-(ii) SO_4^{2-}. Such experimental data, together with the $\delta^{18}O$ values of trace SO_4^{2-} in Archean carbonates (see Section 2.3.2.3) may allow us to evaluate the relative importance of the two sources of SO_4^{2-} in the Archean oceans.

We will conduct a series of pyrite weathering experiments in an anoxic chamber, with visible (solar spectrum) illumination to examine the production rate of peroxide and the oxidation rate of pyrite in the absence of oxygen. Experiments will utilize different pyrite precursors, from fine-grained framboids to crystalline pyrite at a range of temperatures. We will examine the sulfur and oxygen isotopic signals of reactants and products in the experiment. In particular, we will analyze the oxygen isotopic composition of peroxide and the $\delta^{18}O_{sulfate}$ values in order to document oxygen isotopic discrimination resulting from peroxide oxidation and resulting dissolved sulfate production. Experiments conducted in Schoonen's laboratory indicate substantial peroxide formation from water in seawater under visible light illumination, using Fe^{+3}, and that this peroxide oxidizes pyrite (Borda et al. 2001; Borda 2003; Borda, et al. 2003). This process could conceivably have been important in terrestrial environments even under an anaerobic atmosphere. Such conditions probably prevailed prior to 2.7 Ga, and may have existed intermittently through the Proterozoic.

2.3.2. Sulfate Partitioning during Precipitation and Diagenesis of Carbonates

It is well known that the original isotopic compositions of Precambrian carbonates have often been modified by later alteration as the result of long-term exposure, prolonged contact with groundwater, or exchange with fluids during deep burial. Since groundwater typically contains much less sulfate than seawater and the oxygen isotope exchange rates between SO_4^{2-} and H_2O are extremely slow (Lloyd, 1968), it is likely that the $\delta^{34}S$ and $\delta^{18}O$ values of the trace sulfate in the Precambrian carbonates may have retained the values of the Precambrian seawater; however, we are concerned that sulfate contents of the carbonates might have been altered during diagenesis in pore waters that have experienced extensive bacterial sulfate reduction. If the sulfate content of carbonate is not readily changeable during reactions with groundwater, it may be used to estimate the sulfate content of Precambrian oceans. Therefore, the main objective of this research is to investigate the chemical and isotopic partitioning between trace sulfate in carbonate and sulfate in solutions (a) during precipitation of carbonates (calcite and aragonite) and (b) during recrystallization of carbonates in the presence of aqueous solutions.

2.3.2.1. Experimental precipitation of aragonite and calcite

There are no published data that allow quantification of the relationship between seawater sulfate concentration and concentrations in carbonate minerals. We will investigate sulfate incorporation in carbonates at range of known sulfate/carbonate ion ratios over a range of temperatures in both low-Mg calcite and aragonite. Because sulfate is strongly complexed by magnesium in seawater, we will also utilize Mg-free artificial seawater solutions in some experiments. In addition, we will conduct precipitation-rate experiments in order to gauge the extent to which supersaturation with respect to carbonate minerals and their rate of precipitation affects the distribution coefficient for sulfate.

2.3.2.2. Experimental diagenesis

We will study sulfate gain or loss and accompanying isotopic changes during mineralogic inversion (conversion of aragonite to calcite) in hydrothermal solutions at low temperature. We will also examine the effects of fluid interaction at a range of temperature in hydrothermal vessel experiments; we will add ^{34}S-enriched sulfate to some solutions in order to detect isotopic exchange of sulfate, independent of concentration changes, during diagenesis.

2.3.2.3. Analysis program

δ^{34}S analyses will be performed on SO_4^{2-} in solution and in carbonates, and δ^{18}O analyses on (a) SO_4^{2-} and H_2O in the experimental solutions and (b) SO_4^{2-} and $CaCO_3$ in the precipitates. In addition, we will use FTIR (FTIR microscopy) to examine spatial distributions of sulfate incorporation in carbonate minerals, for example as a function of precipitation rate. Raman spectroscopy (and/or Raman microscopy) will be used to explore patterns of bonding and inclusion of sulfate in interlattice sites or fluid inclusions. XAFS (non-routine) will be conducted on well-characterized samples to examine the potential lattice effects (distortions, etc.) of sulfate incorporation in order to understand how this might impact carbonate mineral solubility and mobility of sulfur under post-depositional conditions. XAS and possibly micro-XAS, available at Brookhaven National Laboratory, will be conducted on well-characterized samples to determine the mode of incorporation on the basis of the local structure around sulfate while incorporated into the carbonate host. Carbonate mineral solubility and mobility of sulfur under post-depositional conditions may depend on the mode of incorporation.

III. GENOMIC RECORD OF THE EARTH'S EARLY BIOSPHERE

Hedges (Leader), House, and Patzkowsky

3.1. Introduction

Typical prokaryote genomes contain between 1,000-6,000 genes and approximately 1-6 million nucleotides, which represent a great resource for astrobiological studies. Here, we will conduct research to improve analytical tools of genomic analysis and apply them to evolutionary questions concerning organisms that may have impacted the biosphere of the Archean and Proterozoic. For example, when did certain microbes evolve that were responsible for producing or consuming important gases (e.g., oxygen, methane) and minerals (e.g., sulfur, iron) in the early Earth environment? What are the evolutionary relationships among geologically significant microbial lineages (e.g., S-reducers, fermenters, acetogens, methanogens, sulfate-reducers, sulfide oxidizers, and oxygenic microbes)? What was the extent of lateral gene transfer in early Earth history and how did the transfer of genes affect the organisms and their environments? Clarifying the relationships and times of origin of such organisms will help to constrain models for the evolution of Earth's atmosphere, temperature, and biosphere in general.

We will use genomic data to construct evolutionary trees, estimate times of divergence, and determine whether genes and their functions were inherited vertically (e.g., speciation) or horizontally through genetic transfers between species. We will use sequence data from complete genomes and transcriptomes available in the public databases. The analytical tools have themselves evolved as data sets have grown; therefore existing tools will be evaluated through computer simulations, and new tools will be developed where applicable. The synthesis of evolutionary and environmental information will be facilitated by the development of a web database documenting the history of the biosphere. It will integrate evolutionary trees and divergence times with chronologically arranged panels containing geological, geochemical, atmospheric, and astronomical data.

3.2. Evolutionary Genomic Methods

Genomic data analysis continues to increase in complexity as sequence databases grow and tools are developed and refined. In general, protein sequences are desirable for the most ancient events because DNA sequences are usually too divergent in those cases to make accurate estimates on the basis of patterns of nucleotide substitutions, with the exception of some very conserved genes (e.g., rRNA). All phylogenetic methods minimize sequence change, but this can be accomplished in different ways, including maximum likelihood, minimum evolution, parsimony, and Bayesian methods (Li, 1997; Nei & Kumar, 2000). When sequences have not changed greatly over time, most methods produce the same results. However, the sequences that will be examined here are considerably divergent and therefore the methods of analysis take on greater importance. We will continue to use a diversity of methods, refine existing tools and develop new ones, especially through automation (where feasible), and conduct computer simulations to test the efficiency of different methods. This includes methods for detection of lateral gene transfer (Brochier et al., 2002; Brown et al., 2001).

Initial assembly of sequences is accomplished with the use of BLAST tools (Altschul et al., 1997). We use a local 48-cpu (24 gigabytes memory) dedicated parallel BLAST server housing the latest versions of public sequence data (from GENBANK). To further increase efficiency, we have designed tools to automate the sequence assembly procedure, including BLAST analysis, sequence alignment, and initial tree construction. However, the automation only serves to speed the initial assembly of data and not to evaluate and select groups of orthologous sequences (those reflecting organismal rather than gene evolution). As the public databases grow and taxon sampling improves, orthology determination will become more easily automated. Also, we use a tool (Hedges et al., 2001; Shigenobu et al., 2000) that combines global (Thompson et al., 1994) and local alignment algorithms to better ensure alignment of homologous sites, which can be a problem with highly divergent sequences (Hansmann & Martin, 2000).

The most complex models of molecular evolution available for phylogenetic analysis, such as those that incorporate amino acid composition and rate variation among sites, do not account for all of the substitutional biases encountered in real data (Fitch & Markowitz, 1970; Nei & Kumar, 2000). For example, it appears that eukaryotes evolve faster than prokaryotes on average, across many genes (Hedges et al., 2001; Kollman & Doolittle, 2000). We will conduct simulations to determine how differences in parameters such as rate variation among sites affect phylogenetic inference to help improve methods. With complete genomes available, it is also possible to use the presence and absence of genes (genome content) to build phylogenies, and this general approach has proven useful (Fitz-Gibbon & House, 1999; Snel et al., 1999; Tekaia et al., 1999; Wolf et al., 2002; Wolf et al., 2001). We will continue to refine methods of gene content analysis and apply them to evolutionary questions.

Methods of molecular time estimation are important for understanding the early evolution of life because the Precambrian fossil record is relatively sparse. In all methods, the rate of sequence change inferred with reference to splitting events of known time (calibrations) is used to estimate the time of other divergences. The diverse array of sophisticated methods that exist today can be grouped into four categories by the way the data are handled (genes considered separately or combined in a supergene) and the allocation of rate variation among the lineages (global versus local clock) (Hedges & Kumar, 2003; Wray, 2001). Individual divergence times have been estimated with large numbers of genes (Feng et al., 1997; Gu, 1998; Heckman et al., 2001; Hedges et al., 2001; Kumar & Hedges, 1998; Wang et al., 1999; Wray et al., 1996) and such studies have indicated that the reliability of single gene estimates is low, emphasizing the need for multigene analyses (regardless of the specific method used). Fortunately, large data sets are appearing more frequently and permit greater precision in time estimation.

Global clock methods use genes and lineages that are not rejected in rate tests. Multigene time distributions are usually symmetric and have a strong central tendency (Kumar & Hedges, 1998). The supergene implementation involves concatenating all relevant genes (or gene segments) of a species to form a single alignment for time estimation (distances also can be averaged) (Nei & Glazko, 2002; Nei et al., 2001). This approach avoids the reduced statistical power of small data sets and potential statistical artifacts of the multigene method (Nei et al., 2001; Rodriguez-Trelles et al., 2002). Local clock methods, including the Bayesian and Likelihood smoothing methods, allow rate to vary in "local" parts of the tree and can be used with multigene and supergene approaches (Aris-Brosou & Yang, 2002; Hasegawa et al., 1989; Sanderson, 2002; Schubart et al., 1998; Thorne & Kishino, 2002). They offer the advantage that all genes and taxa can be used, and not just those that pass tests for rate constancy. Also, time constraints can be included in these analyses. However, the various methods differ considerably in their approaches, and it is not yet clear which method is optimal. Therefore, we will conduct computer simulations designed to reveal these differences so that users of the different methods will be better informed, and to provide a guide for our own analyses. Publications, supplementary data, and tools will be made available on the NAI Evogenomics Focus Group web site (www.evogenomics.org).

3.3. Evolutionary Genomic Applications

Oxygen and methane are two components of the atmosphere that can be produced by microbes and which figure prominently in models describing the evolution of the atmosphere. To obtain better constraints on these models, we will use genomic data from the diversity of prokaryotes and eukaryotes in the public databases to estimate phylogenies and divergence times for lineages and groups relevant to the generation of those gases in particular, and to other biogeochemical reactions (e.g., sulfate reduction, sulfide oxidation). The origin of oxygenic photosynthesis can be constrained biologically on the early end by the time of divergence of cyanobacteria from other Bacteria and on the later end by the earliest divergence among living, oxygen-producing, organisms (cyanobacteria and plastid-bearing eukaryotes). Such time constraints can be refined by considering, in addition, the geologic record of the rise of oxygen (Holland, 2002) and biomarker evidence (Summons et al., 1999). Similarly, the origin of biological methane production can be constrained on the early end by the time of divergence of methanogens from other Archaea and on the later end by the earliest divergence among living methanogens (members of Euryarchaeota). In both cases, it must be assumed that oxygenic and methanogenic species now unavailable (e.g., extinct) would not substantially change the results. For cyanobacteria, this is probably a valid assumption because they form a monophyletic group, are relatively derived phylogenetically among Bacteria (Hedges, 2002; Wolf et al., 2002), and they appear to have a derived biochemical system for producing oxygen (Raymond et al., 2002; Xiong et al., 2000). It is also a reasonable assumption for methanogens, even though they are not monophyletic, because they are nonetheless restricted to one subgroup of Archaea (Perry et al., 2002; Wolf et al., 2002) and not scattered among prokaryotes. Also, additional genome sequences of methanogens are in progress (public projects) and this increased taxonomic sampling will better define the reference points constraining the origin of methanogenesis.

Our work will involve evolutionary analyses, using methods as described in the previous section, of all prokaryotic and eukaryotic genomes (>100 publicly available, with many more to be released over the next few years). Rate calibrations will come from the fossil record of eukaryotes (Butterfield, 2000; Hedges, 2002; Knoll & Carroll, 1999; Kumar & Hedges, 1998). Time estimation methods, as described in the previous section, are themselves complex, and rate differences between eukaryotes and prokaryotes, and among prokaryotes, bring additional complexity. Initial evaluation of methods with the use of computer simulations will help determine their usefulness (and weaknesses), suggest refinements, and provide a guide for their application. Lateral gene transfers will be identified using available methods (Brochier et al., 2002; Brown et al., 2001), and the function of genes transferred will potentially provide information for reconstructing ancient environments and communities.

3.4. Database of Biosphere History

Recently, we began the design and development of a public database of molecular clock and fossil divergence times, supported by the National Science Foundation (DBI 0112670; Hedges, PI). Dr. Sudhir Kumar (Department of Biology, Arizona State University) is a PSARC associate member and collaborator on this project. The database will compute and visualize evolutionary trees scaled to time (timetrees) using data from published molecular clock studies and the fossil record. The timetrees will be hyperlinked in the sense that nodes and branches will be linked to additional trees (scaled up or down) and other information. For example, an initial tree might show the basic structure of the tree of life, whereby clicking on the Archaea branch will show the evolutionary history of that group, with greater taxonomic resolution. Clicking on a node in the tree will reveal error of the molecular time estimate, the number of genes or proteins, number of nucleotides or amino acids, PubMed publication link, and information on the earliest fossils pertaining to that node. Our initial funding has supported research and design of the database structure, and initial entry of fossil and molecular divergence time data from tetrapod vertebrates (for release to public later in 2003).

We propose here to extend the taxonomic scope of the database to microbes and early evolutionary events (Precambrian) and to incorporate geological, geochemical, atmospheric, and astronomical data in this database. The goal will be to visually integrate the biological (evolutionary) and non-biological histories of the planet. Most molecular phylogeneticists would have difficulty obtaining data on carbon and sulfur isotopes, and most geochemists would have difficulty in locating evolutionary trees. A database that integrates these diverse data in a common thread (time) will assist astrobiologists in better understanding how the biosphere as a whole evolved.

The non-biological data will be presented in the form of horizontal panels, scaled to time, appearing above or below the timetree. Each panel will show the temporal history of a different measurement (see figure). For example, one will present carbon isotope data while others will show sulfur isotope data, solar luminosity, continental breakup patterns, and extraterrestrial impact events. The database user would have control over the number and type of panels shown at any one time. Retrievable literature references will be linked to the displayed data (evolutionary and geological). Each panel will represent a separate database in itself, derived from the published literature and consultation with members of the astrobiological community, at Penn State and elsewhere. Information from existing, related databases (e.g., Tree of Life, Paleobiology) will be incorporated, and in some cases, linked. Penn State Co-I Patzkowsky has been a major contributor to the Paleobiology database (http://www.paleodb.org) (Alroy et al., 2001) and will assist with the assembly of the paleontological data. The most complex aspect of the overall database will be in accounting for differences of opinion (in all fields involved), when they exist. Our goal is to have a data-driven rather than authority-driven database that includes alternative viewpoints.

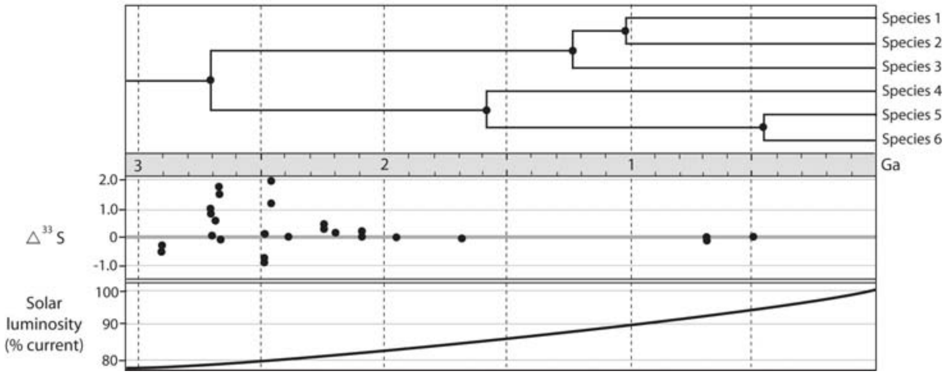

Figure 3.1. One possible presentation of the biosphere database illustrating a timetree of species relationships and divergence times (hypothetical) with panels showing deviation of delta 33-S from the normal mass fractionation line (middle) (Farquhar et al., 2000) and solar luminosity (lower) (Gough, 1981).

IV. LABORATORY MICROBIAL SIMULATIONS: ASTROBIOLOGICAL SIGNATURES

House (Leader), Arthur, Freeman, Ferry, Brantley, and Ohmoto

4.1. INTRODUCTION AND OVERVIEW

In order to understand the evolution of a habitable planet, it is essential that research be directed toward determining biosignatures and also toward understanding the interplay between microorganisms and their environment. We propose to investigate microbial biosignatures and to investigate microbe-environment interaction using a series of different culturing techniques coupled with an array of geochemical, biochemical, and genetic approaches.

First, we propose to use laboratory microbial culturing techniques, genetic analysis, and geochemical approaches to explore biosignatures produced by diverse microorganisms in pure culture and in mixed ecosystems. In particular, we will study the production of biogenic gases, the synthesis of lipids, the signatures recorded by carbon and sulfur isotopic fractionation, and the trace metal content of cells. The results will provide new data on how the geochemical and biochemical impact of microorganisms can be used to detect traces of life in the geological past, on Mars or Europa, and in the atmospheres of extrasolar planets.

Second, we propose to use laboratory microbial culturing to investigate microbe-environment interaction. A significant effort will be directed at investigating the response of anaerobic organisms (including sulfate-reducers and methanogens) to O_2. The goal of this research is to document the relative sensitivity of geologically significant anaerobes to O_2, and identify proteins essential for adaptation to low levels of O_2 by ancient anaerobic lineages. The project is expected to provide data on O_2 sensitivities, reveal responses to O_2 stress including biomarkers for microaerobic conditions, and possibly reveal novel facultative aerobic metabolisms in species traditionally thought of as strict anaerobes. Understanding these innovations will provide important insight into how Precambrian microbial ecosystems responded to the rise of O_2 during the evolution of our habitable planet. In addition to studying the response of microorganisms to O_2, we also propose to use microbial microcosms to study how O_2-producing microorganisms deal with high methane levels and/or sulfidic conditions, and how microbes respond to changes in nutrients, temperature, salinity, pH**,** and varying sulfate concentrations.

Approaches to the problems

In order to advance our understanding of microbial innovation, and the response of microorganisms to environmental impacts, we will draw upon all of the expertise in PSARC in order to apply a myriad of different techniques. This interdisciplinary work will entail genomic and genetic analyses, gas analyses, isotope geochemistry, lipid analyses, gene expression studies (using microarrays and 2D-protein gels), and DNA sequencing. While the integration of these various types of data will be an important challenge, the results will be a comprehensive new view of the interaction of microbes with their environment, and when integrated with other PSARC studies will help create a new understanding of the evolution of our habitable planet.

4.2. LABORATORY SYSTEMS

Specific experiments require different laboratory growth systems ranging in size from small serum bottles to large-scale microbial microcosms. Currently, PSARC is set up for routine anaerobic microbial culturing in serum bottles of up to 0.5 L volume at temperatures of 0°C to 110°C with continuous orbital shaking, and from 10°C to 60°C with continuous orbital shaking and programmable (i.e., day/night) illumination. PSARC also has a 60-L anaerobic fermentor that can be used at temperatures up to 80°C. This project will use these microbial culturing systems for the cultivation of a wide range of microorganisms in pure culture or in simple artificial and natural consortia. Additionally, we propose to build a set of 0.5-L bench top chemostats and four identical large-scale mesophilic bioreactors to allow for pure-culture, simple consortia, or complex microcosm-style experiments with multiple experimental controls. Each proposed bioreactor will be constructed using a vertical 6-inch diameter, 5 foot long clear plastic pipe surrounded by a removable aluminum pipe to block ambient light (but allow for the viewing of any stratified microbial ecosystem). With illumination from above, these vertical bioreactors will allow for the simulation of some water column microbial ecosystems in a relatively inexpensive and reproducible design. Along the side of the structure, there will be a series of input/output ports for injection or withdrawal of gases and solutions and for sensors of Eh, pH, DO, and T. While we recognize that it will be difficult to control all conditions in such a bioreactor, we are confident that short term (up to a month) microbial culturing experiments will prove valuable for understanding modern microbial ecosystems (such as that of Fayetteville Green Lake), as well as for simulating conditions not presently found of on Earth (such as a low sulfate permanently stratified marine water column).

4.3. RESEARCH ON LABORATORY MICROBIAL COMMUNITIES

4.3.1. Chemical and Isotopic Signatures of the Laboratory Ecosystems

Here, we propose to advance several important areas of astrobiology by using microbial culturing to search for chemical and isotopic biosignatures. The work will answer the following key questions: (1) What were the biogenic gases in the Precambrian atmospheres? (2) What are the carbon isotope biosignatures of lipids and kerogen components of individual organisms and consortia growing under different environments? (3) What are the sulfur isotope biosignatures of pyrite, organic S, and sulfate during sulfate reduction in different ecosystems? (4) How do microbes extract essential metals from minerals? (5) Can cellular metals be used as a biosignature?

4.3.1.1. Biogenic gases (Ohmoto)

An important method for the detection of life (present or past) has been (and will be) to search for the presence of biogenic gases in planetary atmospheres. One approach is to search for a non-equilibrium assemblage of methane (CH_4) and ozone (O_3) (Schindler & Kasting, 2000) in which the CH_4 may have been produced by methanogenic microbes and the O_3 by photochemical reactions of the O_2 molecules generated by oxygenic photosynthetic organisms (e.g., cyanobacteria, eukaryotic algaes, and plants). However, a planet with anaerobic microorganisms, but without a large population of O_2-producing organisms (a condition that persisted on the early Earth) would fail this CH_4/O_3 detection test. Therefore, for the detection of life on such planets, we must identify the characteristics of gases generated by anaerobes (± minor aerobes) under dominantly anoxic conditions where entirely (or mostly) anoxic oceans are overlain by an anoxic atmosphere.

From a large number of analyses made by previous researchers on gases (and aqueous species) in anoxic environments (e.g., within sediments, euxinic seas, marshes, and ponds) (e.g., Hunt, 1974; Whelan et al., 1980; Oremland et al., 1980; Claypool and Kvenvolden, 1983; Ueda et al., 2000), it has become apparent that microbes generate the following products: CO_2 (and HCO_3^-), methane (CH_4), ethane (C_2H_6), propane (C_3H_8), isobutene and n-butane (C_4H_{10}), ethylene (C_2H_4), N_2O, H_2S (and HS^-), and H_2. Typical concentration ratios of these gases in pore waters of anoxic sediments are $CO_2/CH_4 \approx 0.5 – 1$ at the methane maximum, $CH_4/(C_2H_6 + C_3H_8) \approx 100 - 5000$, $(CO_2 + HCO_3^-)/(H_2S + HS^-) \approx 2$. The concentration ratios of these gases, however, vary greatly in the overlying anoxic and oxic water bodies because of variable productions/consumptions of these gases by anaerobes and aerobes in the water bodies. The ratios of these gases that escaped the atmosphere also differ greatly from those in the water bodies because of reactions with O_2 and the differences in Henry's law constants.

Although the information of biogenic gases in modern anoxic environments is useful in predicting the types of gases we could expect in an entirely (or mostly) anoxic world, the ratios of these gases in the Archean oceans (and atmosphere) may have been quite different. In particular, modern anoxic environments are typically overlain by oxygenated water bodies with abundant aerobes. Compared with modern anoxic settings, ancient ecosystems were potentially exposed to different organic substrates and decreased availability of SO_4^{2-} (Habicht et al., 2002) and other oxidized electron acceptors.

In order to predict the nature of biogenic gases in the Archean world (and also other planets with an early biologic history), we propose to create similar conditions in the laboratory and systematically investigate the relationships among the compositions of the atmosphere, oceans, microbial communities, and the production rates of biogenic gases. We must understand: (a) how a specific type of organism (e.g., methanogens and sulfate reducers) in a variety of microbial consortia responds to a change in a specific environmental parameter (e.g., atmospheric concentrations of CO_2, H_2, SO_2; SO_4^{2-} content and pH of oceans; types and flux of nutrients); (b) how the change in the nature of microbial communities will change the production/consumption rates of various biogenic gases; and (c) how the change in biogenic gases will change the chemistry of the atmosphere and oceans and climate.

The microbial consortia in these experiments will be primarily composed of anoxygenic photoautotrophs (green sulfur bacteria, green non-sulfur bacteria, purple sulfur bacteria, purple non-sulfur bacteria), fermentative microbes, methanogens, and sulfate reducing microbes. In some experiments, cyanobacteria, iron oxidizing bacteria, and methanotrophs will be added to the system.

For this work, we will investigate different principle atmospheric gases (N_2, CO_2 and H_2 of various mixing ratios) with small amounts (< 1%) of SO_2, H_2S, and/or O_2 added in some cases. The artificial seawater will be composed of mixtures of major salts (NaCl, $CaCl_2$, $MgCl_2$, and KCl), $NaHCO_3$ (together with the atmospheric pCO_2) to control the pH, Fe^{2+} (varied from 1 to 100 μM), and SO_4^{2-} (0.05 – 30 mM). We will also add various mixtures of phosphate, nitrate, and ammonia with trace amounts of some bioessential metals (e.g., Ba, Mo, Ni, and Zn).

Samples of the artificial seawaters and sediments will be periodically withdrawn from the bioreactor, and analyzed for: (a) the abundance of various types of microbes; (b) carbon isotopic composition of the major individual microbial species; (c) chemical composition of solution and dissolved gases; and (d) carbon and sulfur isotopic compositions of solution and dissolved gases. Samples of the air will be periodically withdrawn for chemical and isotopic analyses.

4.3.1.2.Carbon isotopes (House and Freeman)

Evidence for past life is often based either on the carbon isotopic composition of bulk sedimentary organic mater or on the structures and carbon isotopic composition of preserved extractable lipids. The objective of this investigation is to continue past research identifying controls on microbial carbon isotopic fractionation. The proposed project will explore the carbon isotopic composition of bulk biomass or specific compounds produced by pure cultures and artificial microbial ecosystems produced in the laboratory. Previous research has identified the primary carbon fixation pathway as an influence on carbon isotopic fractionation from CO_2 with lower fractionation for the reductive TCA and the 3-hydrohxypropionate cycles (Sirevåg et al., 1977; Holo & Sirevåg, 1986; Preuß et al., 1989; House et al. 2003), and larger fractionations observed in biomass produced from the reverse pentose phosphate cycle and the acetyl-CoA pathway (Preuß et al., 1989; House et al., 2003).

However, it has been observed that the magnitude of isotopic fractionation observed in methanogen cultures is also related to the particular growth status that had been attained by the various cultures, with increasing fractionation as growth proceeded (House et al., 2003). Botz et al. (1996) obtained a similar result when they studied isotopic fractionation from CO_2 to methane. In order to understand carbon isotopic fractionation in methanogens, we will cultivate several different *Methanococcus* species in 500-mL bottles with a large headspace of gas, and in flow-though chemostats. We propose to investigate various different H_2 / CO_2 gas ratios, as well as the carbon isotopic composition of individual compounds as these experiments proceed to test whether changing degrees of isotopic fractionation are related to changes in the relative amount of different biomolecules in the bulk cell mass as the culture grows.

Recent advances in molecular biology and isotopic geochemistry have made the carbon isotopic analysis of nucleic acids, such as rRNA, possible (MacGregor et al., 2002, Pearson et al., 2001; Pearson et al., 2002). In principle, taxon-specific rRNA from a diverse microbial ecosystem can be extracted and analyzed to link microorganisms with their natural isotopic composition in a similar fashion as has been done by direct ion microprobe analysis of identified cells (Orphan et al., 2001; Orphan et al., 2002). However, important laboratory studies are necessary to understand what such results on the natural isotopic composition of RNA could reveal. We propose, therefore, to investigate the carbon isotopic relationships between whole cell biomass, lipids, and nucleic acids within a diverse set of methanogen pure cultures as a principle step toward understanding the isotopic relationship between these different biomolecules within methanogens. The work will provide a basis for using the carbon isotopic composition of natural nucleic acids and lipids of methanogens to study metabolism in a mixed environment.

In a separate set of experiments, we propose to investigate artificial mixtures of cultures created to explore the various different biospheres that could produce extremely ^{13}C-depleted carbon. The motivation for this work is that that extremely ^{13}C-depleted kerogen is an astrobiological signature observed in the Earth's record 2.7 Ga ago and potentially preserved in other planetary settings such as on Mars. A global ecosystem of methanogens and aerobic methanotrophs could be the cause of extremely ^{13}C-depleted kerogen at 2.7 Ga ago (Hayes, 1994). It is not, however, clear that extremely ^{13}C-depleted kerogen requires a global ecosystem of methanogens and aerobic methanotrophs. The conditions for producing such a signature may have occurred in restricted basins. It may also be possible to produce such a signature with acetogens that produce large carbon isotopic fractionations (Preuß et al., 1989) or with anaerobic methanotrophy under low sulfate conditions (Hinrichs, 2002). For these reasons, we propose to study artificially produced mixed microbial ecosystems containing methanogens and aerobic methanotrophs, while also studying carbon produced by an ecosystem of acetogens and acetotrophs in the absence of methangens.

The results will be integrated with previous data on the carbon isotopic composition of marine anaerobic methane oxidizing microbes (Orphan et al., 2001; Orphan et al., 2002) to give reasonable constrains on how large a ^{13}C-depleteion might be expected from a world of methanogens, of methanogens and aerobic methanotrophs, of acetogens and acetotrophs, and of methanogens and anaerobic methanotrophs (considering different levels of sulfate).

4.3.1.3. Sulfur isotopes (Ohmoto)

Sulfur isotopic composition (δ^{34}S value) of pyrite in marine sedimentary rocks, or more precisely the difference in the δ^{34}S values between pyrite and sulfate minerals (anhydrite and barite) of the same geologic age, has been used by many investigators as an important guide for the activity of sulfate-reducing microbes and the sulfate content of coeval seawater (e.g., Hattori & Cameron, 1986; Ohmoto & Felder, 1987; Ohmoto et al., 1993; Canfield & Teske, 1996; Kakegawa et al., 1998; Shen et al., 2001). This is because the magnitude of sulfur isotope fractionation during biological reduction of SO_4^{2-} to form H_2S (and HS^-), which is expressed as $\Delta_{SO4-H2S} = \delta^{34}S_{SO4} - \delta^{34}S_{H2S}$, may depend on the SO_4^{2-} content of water and various other parameters, including the types of sulfate-reducing microbes (genera and families), types and concentrations of electron donors (e.g., H_2, lactate, acetate, alcohols, and CH_4) and temperature (Kaplan & Rittenberg, 1964; Kemp & Thode, 1968; Habicht & Canfield, 1997; Canfield, 2001; Brüchert et al., 2001). For example, Habicht et al. (2002) have concluded that the sulfate content of the Archean oceans must have been less than 1/100 of the modern oceans, because the small $\Delta_{SO4-H2S}$ values of <10 ‰, which may be required to explain the sulfur isotope characteristics of Archean sedimentary rocks, were produced only when the SO_4 contents of their experimental solutions were below ~200 μM. However, a serious problem in such an argument is that all previous laboratory experiments, including those of Habicht et al., were conducted using only one type of electron donor, most commonly lactate, in their experimental systems. In contrast, sulfate reducing microbes in natural environments always occur in consortia with various other microbes that produce and/or consume simultaneously a variety of electron donors. The principles governing the sulfur isotope fractionations in natural environments may be quite different from those in simplified laboratory systems.

The main purpose of our investigations is, therefore, to understand the relationships between the magnitude of sulfur isotopic fractionation and the nature of microbial consortiums, specifically the types of organisms and the rates of production and consumption of H_2, lactate, acetate, alcohols, CH_4 and other hydrocarbons. We will also focus our attention on the sulfur isotope fractionation between ^{33}S and ^{32}S isotopes (i.e., δ^{33}S value), as well as that between ^{34}S and ^{32}S (δ^{34}S value), in order to understand the magnitude of mass independent fractionation (MIF) of sulfur isotopes during microbial sulfate reduction (see Section 1 and 2 of this proposal).

4.3.1.4. Trace metals (Brantley and House)

All organisms need essential trace metals for active sites in enzymes, as well as for coenzymes and cofactors. While the relative proportion of metals needed by microorganisms roughly parallels their concentrations in seawater (Schlesinger, 1991; Frausto da Silva & Williams, 1991), there are notable exceptions. The concentration of Fe in oxic seawater is very low, making necessary the microbial production of high-affinity Fe-specific ligands (siderophores) by marine microorganisms. The problematic need to extract micronutrients from earth materials and natural solutions may have created bottlenecks in evolution.

Of the ~10 or more bioessential trace metals found in rocks, however, only Fe minerals are known to be the target of metal-specific ligands secreted by environmental microbes (Hersman, 2000; Liermann et al., 2000). Although no metal-ophores (here defined as high affinity metal-specific ligands secreted by organisms for metal uptake) other than siderophores have so far been fully identified and documented, several lines of evidence, including findings based upon PSARC-funded research, suggest that these ligands may be important in natural systems. Several researchers have documented that strongly bound ligands found in seawater show great specificity for Fe, Cu, Zn, Cd, and Co (e.g. Coale & Bruland, 1990; Bruland, 1989, 1992; Saito & Moffett, 2002). Saito and Moffett (2002) have inferred the presence of organic cobalt ligands for the uptake of Co by a marine phytoplankton. Moffett has suggested that Cu-complexing compounds may be secreted by some organisms as a detoxification mechanism (Moffett & Brand, 1996; Croot et al., 2000). A variety of compounds secreted by microorganisms have also been identified that show strong and relatively metal-specific chelation capability with metals other than Fe (Stolworthy et al., 2001; Cortese, et al., 2002); however, the utility of these ligands for metal uptake by organisms has not been demonstrated. Thus, if metal-ophores other than siderophores are produced in natural systems, the nature and effect of these ligands is at present not known.

It has been hypothesized that the diazotroph *Azotobacter vinelandii* may produce a "molybdophore" to bind and transport Mo. *A. vinelandii,* a Gram-negative, obligately aerobic soil diazotroph (Page & von Tigerstrom, 1982; Duhme et al., 1996) produces the dicatecholate azotochelin, which acts as a tetradentate ligand ideally suited to bind the four vacant co-ordination sites of the MoO_2^{2+}-unit (Duhme et al., 1998). However, in tests with azotochelin incubated with a Mo- and Fe-containing glass in Co-PI Brantley's laboratory, no enhancement of Mo release to solution was observed. In contrast, incubation of cell-free supernatants from *Azotobacter* culture with the glass causes significant enhancement of the release rate of Mo, and we have identified the responsible ligand: the monocatecholate aminochelin.

A report of this research has been submitted for publication, and we seek further funding now to investigate whether such molybdophores are generally produced by diazotrophs. In particular, we plan to investigate Mo extraction from Mo-silicate and Mo-oxide by cyanobacteria grown under nitrogen-fixing conditions. We will investigate Mo uptake by *Anabaena*, a cyanobacteria which we are already growing in our laboratory, to determine whether this organism secretes a molybdophore.

Our first five years of work has also focused on whether methanogens also produce metal-ophores. Specifically, Ni is essential for many enzymes necessary for methanogenesis. By growing *Methanobacterium thermoautotrophicum* in Ni-depleted medium we have now shown that this methanogen can preferentially extract Ni from a Ni-containing glass. We are working to identify the nature of the ligand secreted. Now, we seek funding to use high performance liquid chromatography, nuclear magnetic resonance, and mass spectrometry to determine the mass and structure of the compound. Under favorable conditions, these techniques in combination can permit the full structural elucidation of novel natural products from crude extracts (Albert, 1999; Lommen et al., 2000; Bringmann et al., 2002). For example, we have previously used HPLC-MS to identify iron-chelating agents (Kalinowski et al., 1998).

When we have more information about the compound, we can continue to test our hypothesis by analyzing for it or seeking evidence of its importance in water or soil samples. To further the search for biosignatures, we also propose to continue to use x-ray photoelectron spectroscopy

(XPS) to search for depletion of Ni and Mo on silicate surfaces after incubation with Ni- and Mo-extracting bacteria. In our earlier work, we identified the development of Fe-depleted layers on hornblende crystal and glass after incubation with a siderophore-secreting soil microorganism (Kalinowski et al., 1998). We seek to search for other similar chemical signatures on earth materials including exploring using trace metal isotopes as possible biosignatures (Brantley et al., 2001).

Despite being less sensitive to redox conditions, Se and W are scarce in basaltic environments. However, these elements are necessary for the growth of many microbes including hyperthermophiles (Adams, 1994; Kletzin & Adams, 1996). As part of the research funded by a supplemental NAI grant, we have observed W-extraction from basalt by *Pyrobaculum aerophilum* under hydrothermal conditions. This is our first evidence that microbial trace metal extraction may be necessary under certain hyperthermophilic conditions. We have sought NSF funding to continue this work on hyperthermophiles and extend it test whether certain hyperthermophiles extract Se as a trace metal from basalt. As part of PSARC's effort to understand the evolution of a habitable planet, we propose to now explore Se- and W-scavenging in non-hyperthermophilic marine microbes as W is necessary for the growth of methanogens (Kletzin & Adams, 1996) and Se is a growth requirement of a wide range of mesophiles as it is required by any organism that uses selenocysteine as an amino acid (Gladyshev & Kryukov, 2001; Kohrl et al., 2000; Rother et al., 2001).

The varying microbial needs for trace metals suggests that metabolisms that use one or more of the metal-specific enzymes may require elevated cellular concentrations of the cofactor metal. Previous work indicates that *E. coli* exerts tight genetic control over cellular metal concentrations (Outten & O'Halloran, 2001). These studies suggest that microbes contain a background metal content, termed the "metal quota" that is conserved regardless of the metal content of the media. This research seeks to investigate whether or not the trace metal quota of different cultured microorganisms varies with the metabolic capabilities of the species, and thus with the species particular trace metal needs for enzymes and cofactors. A variety of microorganisms will be grown in media of variable trace metal concentrations to correlate their cellular metal quota with their different metabolisms. Alternate culturing strategies will be used to minimize mineral precipitation (such as the use of Ti(III) citrate as a reducing agent in anaerobic media), and trace metal concentrations of microbial biomass will determined by ICP-MS, and tied to cell counts to produce per cell concentrations.

The validity of measured trace metal concentrations of cultured organisms will be examined by direct comparison of cultured bacteria with environmental microbes. This will be accomplished through the development of culture-independent techniques for determining microbial metal content in environmental samples. Fluorescent in situ hybridization has previously been coupled with secondary ion mass spectrometry (FISH-SIMS) to measure carbon isotopic ratios of single cells of environmental organisms (Orphan et al., 2001, 2002). In collaboration with other NAI member institutes, this technique will be modified and performed on contaminant-free polished silicon wafers to measure trace metal content of microbial cells from environmental samples (Fayetteville Green Lake, discussed in Section 5).

In sum, the results of this research will have important implications for astrobiology because (1) the results will reveal ligands for the leaching of metals other than Fe (e.g., Ni, W, Se, and Mo) for a variety of organisms, (2) the results will shed light on the extent to which common microorganisms are altering the chemical and isotopic compositions of mineral surfaces, (3) the results will provide measures of the lower limits of trace metals required by particular microorganisms, and (3) the results will reveal potential biosignatures of microbial life. In addition to providing microbial biosignatures, this research will help us understand if, when, and how trace metals may have limited ocean productivity (Anbar & Knoll, 2002).

4.3.1.5. Archaea cell membrane lipids (Freeman)

The structures and abundance of microbial membrane lipids are known to vary with properties of the growth environment such as pH and temperature. The fatty acids of Eubacteria and the fatty acids and other waxy compounds (like alkenones) of Eukaryotes are most commonly studied in this context. Lipid structures of Archaea are also known to vary with environmental conditions, most notably temperature. For example, higher growth temperatures are associated with a greater number of cyclic rings in tetra-ether linked diphytanyl structures (DeRosa & Gambacorta, 1988; Gliozzi et al., 1983; Uda et al., 2001).

Recent analytical advances now permit relatively routine analyses of large lipid structures including tetraether compounds from the cell membranes of Archaea (Hopmans et al., 2000). Initial efforts reveal structural diversity among Archaea lipids, and provide an exciting opportunity to understand relationships between changes in membrane lipids and properties of the environment. The membrane lipids from both Crenarchaeota and the Euryarchaeota are distinctive and preserved in the sedimentary record. The two basic types of ether lipids, glycerol diphytanyl glycerol tetraethers (GDGT) and diphytanyl glycerol diethers (DGD), are produced found in a wide variety of environments as well as ancient sediments (Schouten et al., 2002). A new temperature proxy, TEX_{86} (TetraEthers indeX), uses the linear relationship ($TEX_{86}= 0.015T + 0.28$ ($r^2=0.92$)) between the relative concentration of cyclic GDGT structures and sea surface temperature (T°C) (Schouten et al., 2002). TEX_{86} has the potential to provide paleotemperature information that may be used in conjunction with other proxies to fine tune ocean reconstructions.

Cyclic GDGTs create a densely packed membrane structure to protect Crenarchaeota living at high temperatures (40-102°C). Mesophilic Crenarchaeota add a six-member ring to create a “kink” that ultimately increases membrane fluidity at lower temperatures (Damste et al., 2002). This type of GDGT is called Crenarchaeol because it is the dominant membrane lipid of pelagic Crenarchaeota. Two major types of DGD, archaeol and hydroxyarchaeol, are chemotaxonomic markers of Euryarchaeota. Two isomers of hydroxyarchaeol appear to be specific to methanogenic genera (Koga et al., 1993; Nishihara & Koga, 1991; Sprott et al., 1993). Carbon isotope analysis of these compounds often confirms that the methanogenic Archaea are active in anaerobic oxidation of methane (Hinrichs et al., 1999, 2000; Nauhaus et al., 2002; Orphan et al., 2002; Schouten et al., 2001). A new macrocyclic DGD containing cyclopentane rings has been attributed to methanogens in the Black Sea (Stadnitskaia et al., 2002). Finally, we note that halophilic Euryarchaeota produce only DGD (Kamekura, 1993; Kamekura & Kates, 1999; Kates, 1997). DGD have been found to document halophiles in ancient evaporite deposits (Barbe et al., 1989; Benali et al., 1995; Grice et al., 1998; Teixidor et al., 1993). Recent work by Schouten et al. (2002) has focused on calibrating the TEX_{86} proxy against annual sea-surface temperature. However, the relationships between lipid structures and salinity are essentially unexplored in both the field and in culture experiments.

We propose to construct a systematic study of lipid structures in a diversity of Archaea cultures, including both a range of growth temperatures and salinities. Experiments will include Euryarchaeota, and Crenarchaeota cultures, as available. In addition, we will evaluate lipid properties in suspended and sinking particles from a range of oceanic localities, including the

Black Sea, the Cariaco Trench, the Mediterranean Sea, the northwestern Atlantic (near Bermuda), and from coastal waters of Virginia and Maryland. These sampling localities will enable us to evaluate relationships between Archaea diversity (via selected analyses using FISH or sequencing methods), lipid chemistry and growth-environment properties, including specifically a range of salinities.

The cultures and field samples will be evaluated using methods that target both the diether and tetraether structures. We have optimized a LC-APCI-MS (liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry) method for evaluation of both types of structures, and we use this method for both structure identification and evaluation of the relative concentrations of both forms. We are also able to isolate diether structures by gas chromatography, coupled with mass spectrometry for structure determination. Our existing, state-of-the-art stable isotope facilities include numerous continuous flow inlets for C, D, O and N analyses on individual compounds and on microgram quantities of solid samples. Coupling molecular isotopic analyses (^{13}C, D) with structural analyses by LC-MS and GC-MS, allow us to link insights to controls on lipid properties with element flow (especially H and C) within cultures and natural ecosystems.

4.3.2. Microbial Response to Environment

By applying microbial culturing to investigate microbial response to differing environments, we propose to address the following important astrobiological questions: (1) How do anaerobes deal with O_2? (2) When did oxygen detoxification enzymes evolve? (3) Are there any biomarkers for microaerobic conditions? (4) How do aerobes (e.g., cyanobacteria and eukaryotes) deal with an anaerobic atmosphere or a sulfidic medium?

4.3.2.1. Oxygen metabolism in anaerobic microorganisms (Ferry and House)

Anaerobic prokaryotes, by definition, are sensitive to O_2; however, accumulating evidence suggests they evolved mechanisms for the utilization of O_2 in energy metabolism and response to oxidative stress. While free O_2 is not likely to have been abundant until the evolution of oxygenic photosynthesis, recent studies by PSARC have revealed that hydroxyl radical is produced at the surface defect sites of pyrite when pyrite is added to O_2-free water (Borda et al., 2003). This leads to the formation of H_2O_2 in a process that is expected for the early Earth (Borda et al., 2001, 2003). The goal of this research is to utilize geochemical, genetic, genomic, proteomic, and biochemical approaches to document the relative sensitivity of geologically significant anaerobes to O_2 and H_2O_2, and identify and characterize enzymes and proteins essential for adaptation to

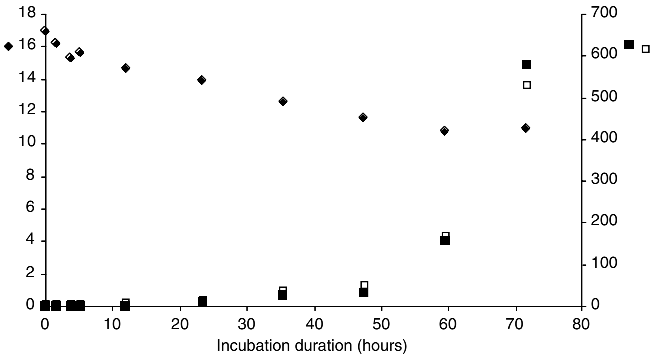

Figure 4.1. Headspace oxygen and methane partial pressures of a "microaerobic" culture of *M. acetivorans* grown in a 125-mL serum bottle. The 30 mL of media contained cystine (S source), but lacked sulfide. Decreasing oxygen is likely due to a reaction with cystine. The results show that exponential growth is obtained within hours of inoculation despite >1% O_2 in the headspace.

low levels of O_2 by anaerobic Archaea.

First, the geomicrobiological implications of O_2-metabolism in anaerobes will be explored. We will document the relative sensitivity of geologically significant anaerobes to O_2 and H_2O_2. Preliminary culturing results show that *Methanosarcina acetivorans* (a methane-producing microbe) will grow in bottles with >1% O_2 in the headspace (Figure 4.1). Using microbial cultivation, gas analysis, HPLC, and numerical modeling, we propose to determine the O_2 concentration in the media and reveal any products of growth that might lead to a biosignature for these conditions (such as a lipid modification or the production of a novel cofactor). For example, quinones are good oxygen scavengers and there may be novel archaeal quinones that are synthesized under oxidative stress that could serve as a biomarker for microaerobic conditions. Furthermore, our preliminary results suggest that the lipid ratio of hydroxyarchaeol to archaeol increases when *M. acetivorans* is grown in the presence of O_2. Additionally, growth in the presence of O_2 and H_2O_2 will be investigated for other organisms that were thought to be strict anaerobes (e.g., acetogens; Boga & Brune, 2003). The ability of oxygen-reducing Prokaryotes to metabolize H_2O_2 for energy will be explored as an explanation for the widespread phylogenetic distribution of this metabolism. Overall, this integrated project will reveal new modes of growth for prokaryotes previously considered strict anaerobes, provide data for considering the microbial implications of the oxidation of the oceans, and may reveal preservable biomarkers indicative of microaerobic conditions.

In contrast to enzymes from aerobic organisms, enzymes from strict anaerobes that reduce O_2, H_2O_2, or O_2^- do not produce O_2 as a product (Ward et al., 2001; Jenney et al., 1999; Gomes et al., 1997; Chen et al., 1993; Coulter et al., 1999; Lumppio et al., 2001) suggesting that these enzymes evolved as a response to free O_2. We propose to investigate this adaptive response to O_2 with genetic, genomic, and proteomic approaches using the model organisms *Archaeoglobus fulgidus* (a sulfate-reducing anaerobe) and *M. acetivorans*. We will exploit the genomic sequence of *M. acetivorans* (Galagan et al., 2002) to identify proteins specifically involved in the adaptive response to O_2. Cells will be cultured in the presence and absence of either O_2 or H_2O_2 to induce the synthesis of proteins expected for reduction and/or dismutation of oxidative species (O_2, H_2O_2, O_2^-), electron transport, and gene regulation. DNA microarray analyses and protein separation/identification methods (LC/MS-MS in collaboration with D. Jones, Penn State) will be employed to identify the proteins that are up-regulated in the O_2-challenged cells. Approximately 40 percent of the ORFs of *M. thermophila,* have no significant percent deduced identity to any known proteins; thus it is expected that novel proteins and enzymes will be discovered and that their characterization will uncover novel biochemical principals. The induced genes will be characterized by transcriptional mapping and the gene sequences will be analyzed to provide preliminary information regarding their potential function. To validate and further investigate the role in the oxidative stress response, the identified genes will be disrupted by established methods (Metcalf et al., 1997) and the mutant cells characterized. These experiments will also lay a foundation for understanding the molecular mechanisms of regulation that apply not only to *M. thermophila* and the methanoarchaea but also to the Archaea domain.

Additionally, we will characterize the newly discovered, and previously hypothesized, proteins involved in the O_2-adaptive response for *A. fulgidus* and *M. acetivorans*. We will ask if they either interact directly with oxidative species (i.e. catalyze reduction/dismutation to less toxic products) or function to supply electrons to the reductive enzymes. It is also planned to utilize biochemical and molecular approaches to determine if any of the up-regulated proteins are redox sensors that could function to regulate the expression of genes involved in the adaptive response. The proteins will then be characterized kinetically and biochemically to determine the function and catalytic mechanism. The structures of proteins will be determined in collaboration with PSARC associate Dr. Cathy Drennan (MIT). The structural information together with sequence analyses and biochemical/physiological characterization will be used to predict the evolutionary history of ancient enzymes and metabolic pathways.

4.3.2.2. Response of a microcosm to changes in environment (Arthur and House)

As described later in the proposal (Section 5), Fayetteville Green Lake (FGL) is an ideal natural setting that in some ways approximates a Proterozoic ocean. In order to gain insight into the microbial biosphere of the Proterozoic and Archean, we propose to use samples from FGL to create microbial microcosm that can be perturbed in the laboratory. In parallel with field studies (described in Section 5), we will create microbial microcosms using the various microorganisms collected from FGL These relatively large-scale bioreactors will, in essence, be living box-models with-which to ground truth numerical modeling studies, as well as environments on which experiments can be conducted.

Our first objective will be to compare the geochemical attributes (dissolved oxygen, nitrate, ammonia, phosphorous, etc.) of such a microcosm as a function of time with those of FGL to assess how similar these artificial microcosms can be to a natural setting. After this initial work, we propose to study the effects of major perturbations to the microcosm including creating conditions that do not exist presently on Earth. One such experiment will be to alter the headspace to be consistent with postulated atmospheres of the early Earth (see Section 4.3.1.1 Biogenic gases) in order to investigate the response of cyanobacteria when exposed to high dissolved methane concentrations, an anoxic atmosphere, and/or a sulfidic medium. Experiments will also include observing the microbial response to changes in nutrients provided – such as nitrogen sources, phosphorous, and trace metals (Beck et al., 2002; Flemer et al., 1998). These experiments will provide important information with which to interpret the biogeochemical cycles of FGL, as well as with which to address the hypothesis that different nutrients have limited the productivity of the photic zone at various time in Earth history (Anbar & Knoll, 2002). Additionally, these FGL laboratory microcosms will be used to observe the effects of that microbial ecosystem when sulfate concentrations are greatly reduced. The importance of this research stems from the fact that the sulfate concentrations of Precambrian oceans are thought to have been significantly lower than those of FGL. The microcosm experiments coupled with the field experiments proposed later in this proposal will allow us to accurately extrapolate results (including sulfur isotopes) from FGL to allow geochemical biosignatures to be used for the examination of Precambrian ecosystems.

As with the work on biogenic gases described previously, samples of the artificial seawaters and sediments will be periodically withdrawn from the bioreactor, and analyzed for: (a) the abundance of various types of microbes; (b) carbon isotopic composition of the major individual microbial species; (c) chemical composition of solution and dissolved gases; and (d) carbon and sulfur isotopic compositions of solution and dissolved gases. Samples of the air will be periodically withdrawn for chemical and isotopic analyses. Additionally, genomic approaches (such as microarrays) will be employed as a means to monitor changes in species composition, as well as changes in gene expression during experiments.

V. MODERN ANALOGUES OF PRECAMBRIAN MICROBIAL ECOSYSTEMS

Kump (Leader), Arthur, Brenchley, Freeman, House, Capo, and Stewart

5.1. INTRODUCTION AND OVERVIEW

A strong motivation for conducting the microbial laboratory cultivation experiments of Section 4 is that the pervasiveness of eukaryotic life in natural environments makes modern ecosystems different than those of the Precambrian. With rare exceptions, only extreme modern environments (hydrothermal springs, highly evaporative lagoons, arid desert soils) provide modern analogues of Precambrian ecosystems that existed before the rise and spread of metazoans. Other NAI groups have been investigating these extreme environments, including the Ecogenomics Focus Group and the microbial mat studies being performed at NASA Ames. We propose to complement their investigations with numerical modeling of mat biogeochemistry (work in progress) and field studies of other modern settings where archaea and eubacteria dominate biological and biogeochemical processes. One such setting is a meromictic (permanently stratified) lake that supports a diverse microbial community of cyanobacterial, green and purple sulfur bacteria, sulfate-reducing bacteria, and methanogens and may provide a useful analogue for Precambrian pelagic ecosystems, of interest to the Mission to Early Earth Focus Group. Our studies of microorganisms in deep glacier ice cores will help meet the NASA objective of learning about the environmental limits of life, provide insight into how organisms could survive extended cold periods during the Snowball Earth, and serve as analogues to extraterrestrial cold habitats that might exist on Mars or Europa (Kerr, 2001; McKay et al., 1992). Finally, we will continue our investigation of modern soils forming in desert environments and developed on mafic and ultramafic parent materials to aid in the evaluation of early Earth land-atmosphere interaction and in the identification and interpretation of Precambrian terrestrial environments.

Through these investigations we will address the following major questions of Precambrian biospheric evolution:

1) What was the efficiency of the “biological pump” in Precambrian pelagic marine ecosystems? Was it sufficient to sustain a chemically stratified global ocean?
2) Were essential trace elements available to planktonic ecosystems living in an ocean with sulfidic deep waters?
3) What are the mechanisms of metabolism, long-term survival, and recovery of viable microbial cells in glacial ice?
4) Can we detect any organism or groups of organisms that could serve as biomarkers for specific geological or climatic events?
5) Are there diagnostic criteria for soils developed under anoxic atmospheres that are distinct from those of soils that are devoid of oxygen because they are water saturated?

5.2. A MODERN ANALOGUE OF PRECAMBRIAN PLANKTONIC ECOSYSTEM: FAYETTEVILLE GREEN LAKE, NEW YORK, USA

The primary focus of most research on Archean and Proterozoic life has been on microbial mats (including stromatolites), but it is likely that the pelagic oceans were productive, and some form of “biological pump,” the transfer of biological material from surface ocean to the deep through particle settling or advection, existed in the ancient ocean (e.g., Hotinski et al., submitted). The efficiency of the biological pump is uncertain: absence of fecal pellets and eukaryotes such as diatoms that enhance coagulation of small (non-settling) particles may have slowed the transfer of organic material to the deep sea, affecting the oxidant demand that presently creates the O_2 minimum in the ocean (Logan et al., 1995). However, microbes play a fundamental role in the formation of marine aggregates (Decho, 1990). Moreover, cyanobacteria are known to stimulate and perhaps regulate (Thompson & Ferris, 1990) the precipitation of $CaCO_3$, which serves as an effective ballast material in the modern ocean (Armstrong et al., 2002). The establishment of an anoxic and sulfidic deep ocean in the Proterozoic (Canfield, 1998), when atmospheric oxygen levels were significant, requires an efficient biological pump. We can only interpret the Archean and Proterozoic redox history of the atmosphere/ocean system from marine sedimentary rocks if we understand the effect that planktonic life had on the deep sea.

We will use a variety of approaches, including direct observation, manipulation using limnocorrals (e.g., Schindler, 1999), and numerical modeling to understand the controls on biological pumping in a modern, microbially dominated ecosystem. We have selected an ideal natural observatory: Fayetteville Green Lake (FGL) in upstate New York. In FGL surface waters, planktonic cyanobacteria dominate primary production and facilitate a seasonal precipitation of $CaCO_3$ in the water column (a “whiting”). Because it is meromictic and receives sulfate-rich groundwaters, FGL exhibits a strong chemocline (Figure 5.1) replete with the full suite of autotrophic and heterotropic sulfur bacteria so important in microbial mats and other anoxic, sulfidic environments (e.g., the Black Sea). The lake’s small size (500m diameter) allows us to sample it comprehensively both in space and time, a situation not afforded by oceanographic studies of such basins as the Black Sea. However, in comparison to the microbial mat, the spatial scale of variation is large, so that we are able to detail its structure without having to resort to microelectrode techniques. In fact, although the focus of our study will be on the pelagic ecosystem, FGL has its own well-developed carbonate bioherms and microbial mats (Thompson et al., 1990) which allow us to directly examine ecosystem function at a variety of spatial scales, from the millimeter scale of the mat to the meter scale of the water column. We will monitor intensive variables over the course of multiple seasonal cycles using a multiprobe sonde (YSI), a remote video camera for plankton observations, and a spectral irradiance monitor. Water and particle samples will be recovered by peristaltic pumping through the tubing system.

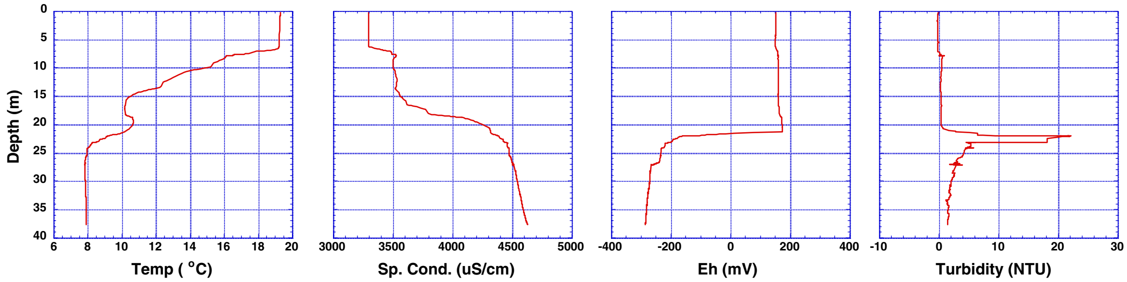

Figure 5.1. Characteristics of FGL on September 30, 2002. Temperature and conductivity profiles indicate that a pycnocline extends from 15-25m, while the Eh and Turbidity profiles reflect a strong chemocline at 21.5 m. Turbidity maximum results from high cell densities of purple and green phototrophic sulfur bacteria.

Samples will be processed and/or fixed immediately on board the pontoon vessel and transferred to the shore-based lab for analysis or frozen for later analysis.

5.2.1. **Nitrogen and Phosphate Cycling** (Kump and Arthur)

Phosphate is often the limiting nutrient in lacustrine systems, and it has been proposed as a limiting nutrient for the Precambrian marine biota as well (Bjerrum & Canfield, 2002). However, in meromictic lakes such as FGL, there is substantial recycling of phosphate that results from both sorption-desorption reactions on particles (especially Fe and Mn oxides) and organic phosphorus remineralization (Taillefert et al., 2000; Hongve, 1997). High concentrations of dissolved phosphate in the anoxic monimolimnion supplies phosphate by mixing and diffusion across the chemocline. Blooms of *Synechococcus* and their associated calcite precipitation may be effective in controlling phosphate concentration. Phosphate is known to have an affinity for calcite surfaces, and a high flux of micron-sized calcite particles from surface waters may limit phosphate availability and productivity during blooms (Otuski & Wetzel, 1972).

Little is known regarding these rates and processes of phosphate cycling in layered microbial systems. Our research will examine the role of particle fluxes, including organic particles, calcite grains and iron oxyhydroxides (which undergo reductive dissolution, perhaps mediated by bacteria) in phosphate transfer from shallow- to deep waters using sediment traps. We will monitor alkaline phosphatase activity and attempt to relate it to fluxes of organic phosphorus from grazing and transport from the chemocline.

Most sediments of ancient anoxic basins have average nitrogen isotopic values of +1‰. This relatively low N isotopic value has led some workers to propose an active role for cyanobacteria in fixing nitrogen within surface waters in these basins with substantial transfer of this carbon to sediments. However, there is little evidence for N fixation in oxic surface waters of modern anoxic basins, such as Framvaren Fjord (Velinsky & Fogel, 1999). It is more likely that particulate organic fluxes to sediments in such basins are dominated by production within the chemocline where N can be fixed by phototrophic anaerobic sulfide oxidizing bacteria and/or a reservoir of isotopically light ammonia is available. Depleted carbon and nitrogen isotopic values in sediments of anoxic basins probably indicate the presence of a chemocline within the photic zone, as shown for Framvaren Fjord by Velinsky and Fogel (1999). We need to understand the relative importance of carbon flux from oxygenic photosynthetic organisms vs. anaerobic phototrophic organisms in such environments. Thus, analyses of the nitrogen (and carbon) isotopic composition of DIN and particulates in the water column and sedimentary nitrogenous phases in Green Lake may provide a picture of Proterozoic planktonic ecosystems.

5.2.2. **Trace Metal and Sulfur Cycling** (Kump, Arthur, Capo, and Stewart)

Under euxinic conditions (including, perhaps, the deep oceans of much of the Proterozoic; Canfield, 1998) concentrations of redox-reactive metals, particularly Fe, Cu, Zn and Mo, can be limiting to productivity in the photic zone because of direct growth limitations and suppression of nitrogen fixation (Anbar & Knoll, 2002). Thus, it is important to examine the cycling of these trace metals through incorporation into organic matter, sorption onto particles, transport to the chemocline, particle dissolution and decomposition, metal desorption, and precipitation of metal sulfides (e.g. Suits & Wilkin, 1998; Wilkin et al., 1997). We will trace this cycling using large-volume pumping and filtering, sediment traps and by analysis of sediment metal accumulation rates. We will also determine total dissolved reduced sulfur concentrations in the deep lake. The FGL water column will be sampled at discrete intervals and samples will be analyzed for dissolved and particulate Fe, Mo and S. To track particulate fluxes and Fe cycling processes, Nd and Fe isotope ratios will be measured on selected samples and on lake input sources. FGL sediments and particulate matter will be sequentially extracted to verify the dominant form in which the metals and S are sequestered.

The sulfur isotopic composition of dissolved sulfate and sulfide (and individual reduced sulfur species when possible), organic sulfur, and sulfide minerals such as pyrite and/or monosulfides will allow characterization of sulfur species in the water column and sediments (Fry, 1986). We also plan to track the isotopic composition of sulfate in surface waters via analyses of sulfate found in calcite precipitated in the mixolimnion. The latter analyses will help validate the technique for tracking ancient sulfur cycling.

5.2.3. **Genetic Approaches** (House in collaboration with D. Bryant, PSU)

Complete sequencing of microbial genomes has dramatically altered the approaches that are available for analyzing the total responses of organisms to their environment. An important part of our research is to identify gene products that are associated with these biogeochemical processes. To this end, we will develop and employ several different environmental genetic approaches.

Polymerase-chain-reaction (PCR)-based amplification of 16S rRNA sequences will be used to identify closest relatives of the phototrophic and heterotrophic organisms of the lake community that have been well-studied in the laboratory. Complete genomic sequences are already available or in progress for cyanobacteria (*Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120, *Nostoc punctiforme*, *Prochlorococcus marinus*, *Synechococcus* sp. PCC 7002, *Synechococcus vulcanus*, and *Synechococcus* sp. PCC 7942), a green sulfur bacterium (*Chlorobium tepidum*) and purple non-sulfur bacteria (*Rhodobacter capsulatus*, *Rhodobacter sphaeroides*). This sequence information, as well as that from other bacteria, can be used to identify specific genes that can serve as reporters of nutrient status or activity status for various biochemical pathways. The activation of these targeted reporter genes will be detected using DNA microarray methods (Taroncher-Oldenburg et al., 2003; Dennis et al., 2003), and thus the nutritional status and biochemical activities of the major photoautotrophs in FGL will be monitored throughout the year. These season-long microarray experiments will (1) provide complete seasonal profile for the genetic regulation of the genes identified as being important to the biological response to the seasonal cycle, and (2) provide adequate time dependent data for analysis of the statistical significance of the genetic results identified during this project. The available sequence information for model organisms will also be used in leveraged sequencing approaches to identify genes conferring unique capabilities to the organisms of FGL.

5.2.4. **Numerical Modeling** (Kump and Arthur)

Our goal here is to adapt a simple, one-dimensional model of the physical mixing, temperature and density structure of the lake, and to then add biogeochemical and stable isotopic components to the model to investigate the interplay between the biota and their physical environment and its isotopic signature. The model will incorporate our understanding of the couplings among system components. Simulations will be performed for each of the limnocorral experimental conditions and for the annual cycle of light and temperature. Toward this end we will begin with the lake model developed by Jewell (1992). Adequate treatment of trace-metal cycling, however, requires that the water-column model be modified. It must account not only for active trace-metal uptake by the biota, but for the nutrient or toxic effects of some metals and their tendency to be scavenged onto small particles, both of which are dependent on their speciation (e.g., Clegg & Whitfield, 1990, 1991).

5.3.MICROBIAL DIVERSITY IN GREENLAND GLACIAL ICE (Brenchley)

5.3.1. Introduction

Compared to other cold environments, glacial ice sheets are special habitats that have preserved microbial life (including *Proteobacteria, Cytophaga/ Flavobacterium/ Bacteroides* and Gram-positive bacteria) chronologically for geologically significant times (Abyzov, 1993; Christner. et al., 2000; Christner, 2002). These results suggest that microorganisms survive for long periods in ice and may be metabolically active at subzero temperatures, possibly in proposed liquid veins along three-grain boundaries in ice (Price, 2000; Junge et al., 2001). In order to investigate microbial survival during these extreme conditions of low temperature, nutrients, oxygen, and desiccation, we will expand our investigation of the abundance, viability, diversity and recovery of microorganisms in Greenland glacier ice cores. The proposed research addresses the following questions: (1) Do different physiological groups predominate at different depths in the ice sheet? (2) What are the mechanisms of long-term survival and recovery of viable microbial cells?

Our previous investigation demonstrated the presence of an abundant viable (10^7 cells/ml) microbial community trapped for over 100,000 years in the basal portion of a Greenland glacier (GISP2) ice core (Sheridan et al., in press). Clone libraries of 16S rDNA were obtained from anaerobic psychrophilic enrichment cultures started from the ice core. Approximately 60 Bacterial inserts were screened and 24 representative sequences compared. Diverse Bacterial sequences represented major phylogenetic groups including *Alpha-, Beta-* and *Gamma-Proteobacteria* as well as relatives of the *Thermus*, *Bacteroides*, *Eubacterium*, and *Clostridium*. Seven sequences may reflect new genera and one was over 12% distant from its closest relative and may represent a novel Order (Sheridan et al., in press). In addition, nearly 800 isolates were recovered from the melted ice and aerobic and anaerobic enrichments. The phylogenetic and physiological analyses of the isolates showed a diverse range of physiological groups, dominance of psychrophilic and pigmented organisms, and a higher representation of low G+C Gram positive bacteria as

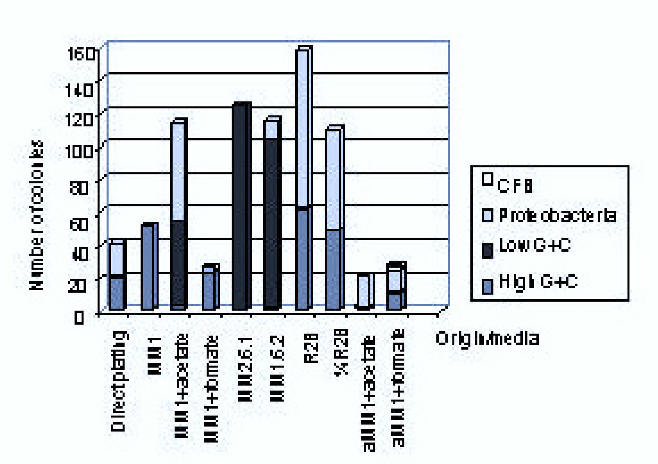

Fig. 5.2 Chart showing the number and phylogenetic distribution of colonies obtained after directly plating a sample from the ice core and after enrichments in a variety of anaerobic media.

compared to the clone libraries (Figure 5.2). Only three isolates had 16S rRNA gene sequences corresponding to those from the clone library. These results demonstrated the value of using both culture-dependent and culture-independent approaches to obtain a more comprehensive view of the ice core community. Of particular importance was the discovery of many dwarf cells (< 1 micron) so small they would have been excluded by the standard methods for concentrating cells. Because this finding is significant to detecting microorganisms in all environments, including extraterrestrial habitats, one of our future goals includes developing methods for examining and identifying these ultra-small cells.

5.3.2. Objectives, Goals, and Approaches

In order to address questions relevant to our NASA mission, we will investigate both the Bacterial and Archaeal diversity of Greenland ice cores and other glacial environments. Our objective will be to reveal their community structure, identify the ultra-small microbial population, and test proposed mechanisms of survival and recovery of damaged and stressed cells. Future success in the recovery and cultivation of environmental isolates will largely depend on our knowledge of the mechanisms these organisms use to deal with the multiple stress factors associated with life in one of the most extreme environments on Earth.

Goal one: Examining the community diversity. Ice core sections will be treated to remove contaminated layers and aseptically sampled using procedures developed in our laboratory. The morphological diversity will be examined microscopically and the ratio of live/dead individual cells determined by epifluorescence microscopy. We will also use flow cytometry to estimate cell abundance and cell size and FISH (fluorescent in situ hybridization) to determine the *in situ* presence/absence of *Bacteria* and *Archaea* and of key physiological groups, especially ones of geochemical interest in other sections of this proposal. The prokaryotic diversity in these new ice core samples will then be analyzed using both culture independent and culture dependent analyses to obtain a comprehensive view of the populations. The first approach will include extracting DNA directly from different sections of the ice core followed by PCR amplification, cloning, and sequencing the 16S rRNA genes for phylogenetic comparisons. The culture dependent examination will recover and isolate organisms from different ice samples and enrich for physiological groups targeted in the microscopic and culture independent studies. Novel isolates will be characterized physiologically and phylogenetically.

Goal two: Studying ultra-small microorganisms. Extremely small cells dominate oligotrophic terrestrial habitats (Torrella & Morita, 1981), it has been proposed that they represent a novel group of organisms (Schut et al., 1997). The presence of small but viable cells in the melted ice and other oligotrophic environments is also consistent with the concept of adaptation to stress, including cell size reduction, cytoplasm condensation, permeability loss, RNA content decrease, inability to multiply, and a metabolic reduction leading to viable but not culturable (VBNC) state (McDougalda et al., 1998). Our work will separate and characterize these dwarf cells and determine whether they represent one dominant type or a heterogeneous population.

Goal three: Determining factors that affect survival, recovery and culturability of microorganisms. Our observations suggest that microorganisms trapped in the ice are dormant or injured and require special conditions for resuscitation. Others suggested that stressed cells undergo oxidative suicide after transfer to a rich media (Bloomfield et al., 1998). We will design media and incubation conditions to examine the critical factors needed to recover stressed cells. We also observed that over 75% of the isolates were pigmented, as seen in other cold environments (Bowman et al., 1997; Christner et al., 2000; Christner, 2002; Dancer et al., 1997; Smith et al., 2000). We have obtained a non-pigmented variant of one isolate and will test the hypothesis of Fong et al. (2001) that pigments enhance survival. The pigmented isolate and its variant will be subjected to freeze-thaw treatments, UV irradiation, and desiccation and the survival of the cells compared. Our work will answer questions about life that survives *under extreme conditions and explore the biochemical strategies that push the physical-chemical limits of life.*

5.4. MODERN SOIL ANALOGUES FOR PRECAMBRIAN TERRESTRIAL ENVIRONMENTS (Capo and Stewart)

Soils harbor primitive life forms on the early Earth and other planetary bodies. Preserved ancient soils (paleosols) also offer one of the few records of direct lithosphere-atmosphere interaction, and have often been used to evaluate the composition of Earth's early atmosphere (e.g., Holland, 1984; Ohmoto, 1996). We propose to investigate modern soil profiles, with a particular emphasis on those aspects that are relevant for interpretation of Precambrian paleosols and extraterrestrial weathering processes. In particular, the problems to be addressed include: (1) the behavior of redox-sensitive elements in soils developed under differing Eh-pH conditions; (2) the relationship between bulk soil chemistry and the evolutionary history of the soil; (3) the impact of multiple sources of material (e.g., fluvial, eolian) on the geochemistry of a soil profile; and (4) radiogenic and Fe isotope systematics of modern soils and implications for soil chronology and evolution.

In collaboration with soil scientists and R. Graham and O. Chadwick, (PSARC Associate Members), we will focus on soil profiles that allow the best comparison with the pedosphere of early Earth and that of other terrestrial planetary bodies (e.g., Mars). Soils developed on mafic and ultramafic substrate would be appropriate analogues for many early Precambrian terrestrial paleosols, and common on extraterrestrial surfaces; soils developed on ultramafic rocks such as serpentinite also tend to be sparsely vegetated and thus better analogs for Precambrian soils. Pedogenic carbonate has been documented in Precambrian paleosols (e.g., the Schagen paleosol, South Africa; Martini, 1994; Watanabe et al., 2000) and could be a significant component of near surface deposits on Mars (Knauth, 2001). Profiles developed under locally anoxic conditions provide insight into the geochemistry and texture of soils that formed in an anoxic atmosphere vs. those that formed under water-saturated conditions. Candidate sites for detailed field, micromorphologic, elemental and radiogenic isotope tracer studies study include well characterized weathering profiles developed on serpentinized ophiolite units in Northern California (e.g., Alexander et al., 1990; Graham et al., 1990), hydromorphic, anoxic soils developed on basalt in Hawaii (Stewart et al., 2001), and desert soils containing calcite and dolomite from Hawaii (Capo et al., 2000; Whipkey et al., 2002) and New Mexico (Capo and Chadwick, 1999).

VI. PLANETARY HABITABILITY AND LIFE DETECTION

Sigurdsson (Leader), Wolszszan, Kasting, Freeman, Capo, and Stewart

INTRODUCTION

The earlier sections of this proposal focus on efforts to understand the earliest stages of the evolution of the atmosphere, the role of biochemical processes in modifying the Archean surface, and the characterization of the early evolutionary processes that lead to the development of life, and the interaction of life and planetary geochemical processes. In this section we propose to initiate a new effort to make some additional progress in understanding how astrophysical processes give rise to planets elsewhere, the dynamical evolution of planetary systems, and investigations both to improve our understanding of prospects for the detection of habitable planets, and the actual detection of biosignatures. This involves integrating ongoing research in the Penn State astronomy department with the existing Astrobiology effort, and expanding astronomical research into new areas, resources permitting. We note that the Astronomy department has made an offer of a tenure-track position to a planetary astronomer who is a member of the Terrestrial Planet Finder (TPF) science working group.

The research proposed in this section complements work at other NAI institutions and ongoing research which is beyond the current scope of the NAI. With the proposed formation of a new NAI Astronomy Focus Group, it is important to have a local astronomical focus at PSARC and to integrate astronomical research into complementary ongoing research in planetary sciences and the evolution of the early Earth. PSARC provides a valuable nexus for cross-disciplinary communication, and through its student support it provides a basis for the long term presence of a group of astronomers with formal education and network connections in the planetary sciences, geosciences, chemistry and biology. It is the establishment of such informal networks that will lead to long term synergy between the currently somewhat disconnected fields.

A major focus of our research efforts over the next five years will be to investigate the chances of finding habitable planets around different types of stars and to aid in the search for life on such planets and on planets in our own Solar System. This work will involve a combination of theoretical, observational, and laboratory-based research. The following section describes those efforts.

6.1. TESTING THE "RARE EARTH" AND PERSISTENCE IN THE HZS (Sigurdsson)

Is the Earth Rare? Relatively little theoretical consideration has been given to the question of planet formation around non-solar like stars, although a few authors have discussed this problem (Sigurdsson, 1992; Lineweaver, 2001; Zinnecker, 2002). This lack of attention is in part because our understanding of the formation of the Solar System itself has been limited and has absorbed much effort. The older stars in the Galaxy are systematically more metal-poor than the Sun. Considering the length of time it took for multi-cellular life to develop on Earth, and that for 98% of the age of the Earth evolution was not at a stage where intelligent life could plausibly have developed, long term prospects for detection of complex life outside the solar system must be sensitive to how late in the history of the Galaxy the opportunities for terrestrial planet formation in the habitable zone around main-sequence stars became possible. This tests some of the key assumptions of the "Rare Earth" hypothesis put forward by Ward and Brownlee (2000). This hypothesis suggests that complex life (defined as multicellular animal life) is rare because the conditions required for its evolution are rare. One aspect of this hypothesis concerns stellar metallicities. We would like to understand whether Earth-like planets could have formed around the earlier generations of stars.

6.1.1. Role of Stellar Metallicity in the Formation of Habitable Planets

Planet formation does not necessarily take place exclusively in solar, or near-solar, composition protoplanetary disks. How does stellar metallicity, particularly carbon and oxygen abundances, affect terrestrial planet formation and primordial atmospheric composition? It has been argued that anomalously high metallicity is critical to planet formation in the habitable zone (Gonzalez, 1999; Gonzalez et al., 2001; Lineweaver, 2001), but other considerations suggest that planet formation may generically take place over a wide range of initial composition of the proto-planetary disk (Gaidos, 2000; Sigurdsson, 1992; Miller & Hamilton, 2001). There is little observational evidence for planets around non-solar like stars (cf. Zinnecker, 2002; Gilliland et al., 2000; an exception is the planet in M4 [Thorsett et al., 1999]). If top-down formation of Jovian planets, through a disk gas instability, is a common formation mechanism, then Jovian planet formation may be insensitive to metallicity, but terrestrial planet formation may be sensitive to it (cf. Boss, 2002). Gaidos (2000) noted the importance of C/O abundance ratios, which correlate with mean metallicity, but with large stochastic scatter for individual stars. This determines the oxidation state of metals in the protoplanetary disk and the water abundance. We plan further investigation of the impact of different ice abundances and oxidation states as initial conditions for the formation of terrestrial planet mantles, and the late bombardment enrichment of the surface. The prospects for life and early evolution of the atmosphere will depend on the initial composition, and some exploration of the range of possibilities will make an interesting student project.

We have two fundamentally conflicting lines of evidence: On the one hand, some authors argue that anomalously high metallicity may be critical to planet formation and persistence within the habitable zone (but see Heiter & Luck, 2002). On the other hand, the Copernican Principle would suggest that planets form easily and persist robustly in the most extreme range of metallicity environments probed. Resolution of this issue is critical to our understanding how common terrestrial planets with liquid water are, how stably they remain in habitable zones, and hence the number of planets on which life may evolve and persist for significantly long times. To investigate this issue we propose to first extend modeling of the "branching ratio" for solar impact of planetesimals and planetary embryos during the dynamical evolution of young model planetary systems. Even if the degree of pollution is inadequate to explain the observed planet-metallicity correlation, we still expect potentially quantifiable accretion of metals onto the star; this may also provide a signpost for searching out planetary systems. The mechanism described in Debes and Sigurdsson (2002) can selectively enhance stellar metal accretion, providing a signpost for multi-planet systems that may be especially interesting as sites for life bearing terrestrial planets (cf. Chambers, 2002). Secondly, we will model the formation and evolution of, and look for planets around, non-solar like stars. Most of the observing effort involved in this will be supported by independently of NAI (HST proposal, submitted), the students supported by this grant are expected to take part in the observing effort and in modeling and analysis of the data.

6.1.2. Geochemical Initial Conditions and Atmospheres

What is the probability distribution of observing the atmosphere at different stages, and hence the probability of detecting given presumed biomarkers? If terrestrial planets formed early in galactic history, then this both affects future strategies for which stars to search for habitable planets, and the duration that has been available for a "typical" habitable planet to evolve life and imprint biosignatures on its atmosphere. We will model probability of seeing an atmosphere at different stages (oxidizing, reducing, inert, etc.) depending on formation scenarios and the composition of the planetary nebula. This investigation is planned to form a major part of A. Mandell's PhD thesis.

6.1.3. Spectroscopic Studies of Parent Stars

Are the spectroscopic "smoking gun" signatures of planet formation in the photospheres of host stars? We have been carrying out simulations of asteroid scattering due to jovian planet migration and evaluating rates of photospheric contamination by planetesimal and planetary collisions. The resultant anomalous metallicity can provide a spectroscopic signature of a planetary system and a diagnostic of past planetary dynamical evolution (Figure 6.1).

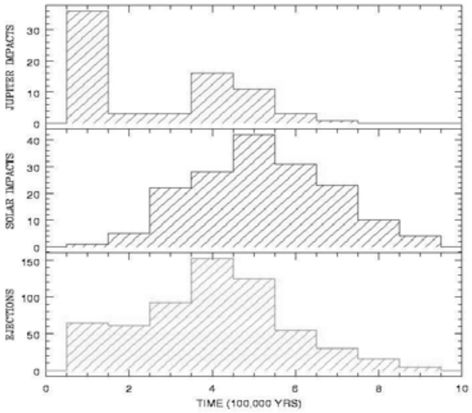

Figure 6.1. Relative frequency of ejection, solar impact and Jupiter impact for a proto-asteroid belt perturbed by a migrating Jovian planet for a solar-like system. The resultant metal accretion onto the parent star can lead to a detectable spectroscopic signature of due to metal contamination of the stellar photosphere, such a signature must be present even if it is not the cause of the observed correlation between metallicity and close Jovian planets (Mandell et al., in preparation).

6.1.4. Habitable Moons and Post-migration Planets

Currently known planetary systems are unlike the solar system, and have probably undergone substantial dynamical modification due to jovian migration. What are the prospects for habitable moons, and habitable planets in post-migration planetary systems? We have been creating models of the dynamical evolution of systems of inner terrestrial planets in response to the onset of rapid migration of outer Jovian planets. Intriguing preliminary results (Figures. 6.2 and 6.3) suggest that there is a significant probability for a terrestrial planet to survive Jovian migration, enabling post-migration circularization of terrestrial planets in

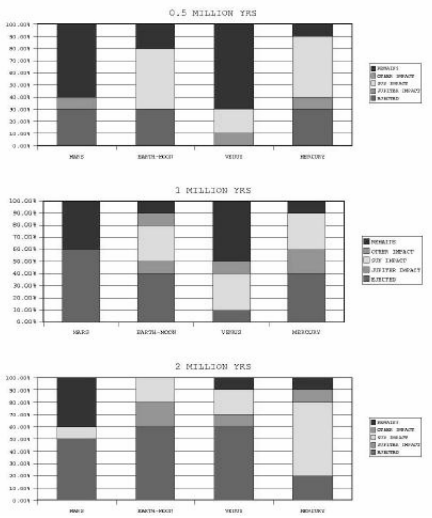

Figure 6.2. Survival probability for terrestrial planets in a Jupiter migration scenario. Note there is a significant probability for survival in a stable orbit. There is also a significant solar impact probability, with possible ensuant detectable photospheric metal pollution (Mandell & Sigurdsson, in preparation).

dynamically stable orbits in the CHZ, outside the now close orbiting "hot Jovian." This suggests that some of the already detected planetary systems may be good prospects for terrestrial planet detection, despite the probable low formation efficiency of terrestrial planets post-migration (Armitage, 2002).

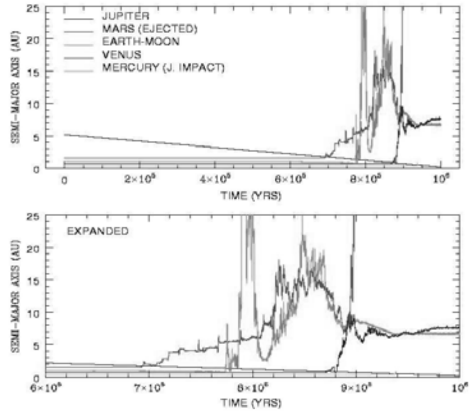

Figure 6.3. Final semi-major axis and eccentricity of a Solar-like planetary system after post-formation Jovian migration. Note that some of the terrestrial planets survive the migration in eccentric, moderately widened orbits (Mandell & Sigurdsson, in preparation).

6.2. SEARCH FOR PLANETS AROUND WHITE DWARF STARS (Wolszczan)

The possible existence of planets around white dwarfs (e.g., Livio et al., 1992) has been seriously considered since the time of the discovery of the first extrasolar planets orbiting the neutron star PSR B1257+12 (Wolszczan & Frail, 1992). Although discoveries of planets around solar-type stars are of more immediate interest (e.g. Marcy et al., 2000), a thorough search for and study of planetary survivors of the post-main sequence evolution of stars of various types is necessary to gain a global understanding of planetary systems and their fate over timescales comparable to the stellar lifetimes.

Possible detections of white dwarf planets would have a number of important consequences. They would provide unique information on planets around stars of spectral types A-F which are difficult, if not impossible to study by means of Doppler spectroscopy. Such planets would also be of great interest from the point of view of planet formation around previous generations of stars. Since it is clear that planets surviving around post-main sequence stars would undergo a significant dynamical evolution (e.g., Debes & Sigurdsson, 2002, and references therein), this process could be studied and understood on the basis of direct or indirect observations. Finally, as there is a possibility that planets can form as a byproduct of white dwarf mergers (Livio et al., 1992), such second-generation planets could be detected by properly designed surveys and, as in the case of neutron star planets, they would provide clues concerning planet formation in circumstellar disks consisting of an evolved stellar material.

Alongside with the studies of various detection methods of planets around solar-type stars, similar approaches have been proposed for the corresponding white dwarf surveys. These include means such as direct detection (e.g., Burleigh et al., 2002), Doppler spectroscopy and astrometry (e.g., Chu et al., 2001; Ignace, 2001), and the timing of stellar oscillations (J. Goodman, 1995, private communication; Provencal, 1997). We are planning to employ our extensive experience in using the pulsar timing as a tool to study various processes in physics and astrophysics in a program of a precision photometry and timing of oscillations in a selected group of white dwarfs that exhibit a sufficient stability of this phenomenon. To our knowledge, the only existing program that seriously exploits this intriguing possibility has been recently implemented at the McDonald Observatory (Ann. Rep. Astro. Obs. Dept., 2002).

We intend to begin this project with a study that will lead to a selection of an appropriate group of target stars using their oscillation stability as a criterion (e.g., Winget, 1998). We will also conduct a search for appropriate telescopes that will be large enough to ensure a << 1 s timing precision of the photometric oscillation curves of >17 mag stars, as white dwarfs are very faint objects. A ~1 s timing accuracy would give us access to Jupiter-mass planets, but our goal is to reach all the way down to terrestrial mass bodies, which will require a millisecond timing precision. Simple calculations show that we should secure access to one or more 4-6 meter-class telescopes, in order to have a chance to achieve this precision goal. We are planning to complete these two initial stages of the project within the first two years and spend the remaining three years on initial observations that should lead to the establishment of a long-term survey that will cover a wide range of orbital periods and masses of the hypothetical white dwarf planets.

6.3. FINDING PLANETS (Sigurdsson)

In collaboration with J. Debes and other PSU students, we are considering alternative detection strategies for planet detection (cf. Gilliland et al., 2000; Debes & Sigurdsson, 2002). Strategies for direct imaging of jovian planets around non-solar like stars using space based infrared imaging, ground based adaptive optics and apodized masks are in progress or proposed. Sigurdsson was part of a previous effort to search for planetary transits around population II stars (HST GO-8267), and further work to consider improved strategies for detection of transits of planets in clusters is planned. New HST data has provided strong evidence for a Jovian planet orbiting a population II star, possibly providing a strong test of Boss's (2002) scenario for Jovian planet formation through top down collapse (Sigurdsson et al., in preparation).The actual observational efforts are beyond the scope of this proposal (funded by a GSRP to J. Debes [Sigurdsson supervising PI] a HST GO proposal [submitted], and other collaborative proposals beyond the scope of the PSARC).

6.4. INDEPENDENTLY FUNDED DEVELOPMENT OF PLANETARY EXPLORATION INSTRUMENTS

6.4.1. Instrumentation for Detection of Biosignatures on Mars (Freeman)

The search for life outside of Earth is at the core of the goals of the field of Astrobiology. Based on research activities employing elemental, molecular and isotopic signatures of organic matter on the early Earth, Penn State researchers have developed expertise in the preservation of life materials on this planet, and these insights are proving useful in the development of strategies for extraterrestrial exploration. In particular, K. Freeman is a member of a research team that is designing a coupled pyrolysis/extraction-gas chromatograph-mass spectrometer system for the study of life signatures on future lander missions to Mars, Titan, Comets and Europa. Continued development of the pyrolysis/extraction and gas chromatography sub-systems were the subject of a recent proposal to the ASTID program. The mass analyzer is a Time-of Flight (TOF) mass spectrometer, along with the sample acquisition delivery sub-systems, are being studied separately in collaboration with Southwest Research Institute and NASA/JPL, respectively. The team is centered at the University of Michigan, and is led by J. H. Waite. The compounds selected for performance characterization come from a parallel laboratory program of organic synthesis and analysis of target organics, led by S. Benner of the University of Florida working with K. Freeman of Penn Sate University and Dr. George Cooper of NASA/AMES.

This parallel laboratory effort provides the intellectual direction for verifying the application of the proposed techniques to future astrobiology missions.

The environment is an important constraint on both the types of organisms that contribute organic matter, and the nature of the reactions that determine their fates. On Earth, more oxidizing environments provide relatively more aggressive means, both chemically and biologically, for the degradation of organic materials. For example, anoxic settings are characterized by higher preservation of organic matter relative to input fluxes. Polymeric compounds such as proteins, nucleic acids and carbohydrates dominate inputs of organic materials from living cells. The oxidation state of carbon in living cells depends on the constituent compound, with carbon in carbohydrates and proteins having average values near zero, while it is above zero for nucleic acids, and about –2 for lipid carbon. Carbon in a living

Table 6.1. Prospective pyrolysis methods for elemental and molecular analyses of organic materials on lander missions.

Pyrolysis Methods		Products			
Technique	Applications	50-300 °C	300-500 °C	> 500 °C	>1000 °C
Inert (N_2): Temperature programmed or Flash-heated to > 500 °C	Widely used, and commonly coupled with direct GC-MS analyses; high-temperature conditions for elemental analyses	Free compounds (held by hydrogen bonds or other weak forces)	Compounds held by lower energy covalent bonds (i.e., C-S, C-O); or single C-C linkages; products can cross-react	Compounds held by multiple covalent bonds	CO, H_2 These are used to quantify total O and H in the organic matrix
Hydropyrolysis (H_2)	Increases yield relative to inert method for lipid biomarkers; product are hydrogenated which protects stereochemistry	Free compounds (held by hydrogen bonds or other weak forces)	Compounds held by lower energy covalent bonds (i.e., C-S, C-O); or single C-C linkages; hydrogenation limits cross-reactions	Compounds held by multiple covalent bonds	
Hydrous Pyrolysis (H_2O)	Increases yield of 'petroleum' like materials; widely used in oil exploration research; water serves as H source	Free compounds (held by hydrogen bonds or other weak forces)	Compounds held by lower energy covalent bonds (i.e., C-S, C-O); or single C-C linkages	Compounds held by multiple covalent bonds	
Oxidative pyrolysis or Combustion (O_2)	High- temp conditions used for elemental and isotopic analyses				CO_2 , NO_x, SO_2 These are used to quantify total C, N and S in organic matrix

cell has an average oxidation state near zero. As fresh biological inputs are degraded in surface environments, the materials delivered to natural sediments becomes increasingly more difficult to analyze with conventional methods due to the removal of dominant compound classes, the formation of oxidized degradation products, and the cross reactions of biochemicals with other compounds and with mineral surfaces. As a consequence, materials that resist degradation, such as lipids and refractory polymers like lignin, dominate organic matter delivered to and preserved in sediments.

6.4.2. Chronology of Planetary Surfaces (Stewart and Capo)

Absolute time scales for planetary processes are difficult to obtain without dated samples from clearly identified features. Such samples are currently available only for the Earth and Moon, while events on other planetary bodies can only be "dated" using the calibrated lunar impact cratering record. Nonetheless, understanding the timing of formation of surface features is critical for our interpretation of planetary history and evolution. Efforts are underway at NASA to develop plans both for sample return and for *in situ* analysis of planetary surfaces. With funding from the NASA PIDDP program and Jet Propulsion laboratory (JPL_ subcontracts, Stewart and Capo are collaborating with G. Cardell (JPL) to design and build an instrument that could ultimately be used to make *in situ* measurements of the crystallization age of rocks on planetary surfaces (Stewart et al., 2001). In addition, Stewart is collaborator on another JPL project (ASTID funding pending) to build a lander-based instrument capable of measuring metal isotope biosignatures. Both instruments are geared toward the astrobiological exploration of the surface of Mars.

cell has an average oxidation state near zero. As fresh biological inputs are degraded in surface environments, the materials delivered to natural sediments becomes increasingly more difficult to analyze with conventional methods due to the removal of dominant compound classes, the formation of oxidized degradation products, and the cross reactions of biochemicals with other compounds and with mineral surfaces. As a consequence, materials that resist degradation, such as lipids and refractory polymers like lignin, dominate organic matter delivered to and preserved in sediments.

Decay, selective preservation, and cross-linking reactions render molecular characterization difficult, despite the rewarding richness of information potentially inferred from structures, isotopic signatures and stereochemistry. A rich array of information can be drawn from analyses of bulk fractions of organic materials, and thus the team has proposed a tiered approach to analyses of planetary surface materials. In particular, bulk characterization of the quantity and elemental abundances (and therefore elemental oxidation state) of organic material (i.e., ratios of C to H, N, O, S, P and other elements) will provide important base information, from which more detailed analyses targeting molecular constituents can be directed. Molecular studies will focus on compounds released by an array of methods, including a solvent extraction and a variety of pyrolysis methods (see Table 6.1), with analysis of the quantity and structures of the products conducted by a TOF mass spectrometer. Isotopic analyses of C and other life-related elements will also be conducted on the bulk and molecular materials.

6.5. THEORETICAL MODELING OF ATMOSPHERIC BIOSIGNATURES ON PLANETS AROUND OTHER STARS (Kasting)

We conclude with a brief discussion of how our efforts here at Penn State relate to the larger NASA mission of searching for life on planets around other stars. One of us (Kasting) has been involved with NASA's Terrestrial Planet Finder (TPF) mission for several years. Although he is not currently a member of the TPF Science Working Group, Dr. Kasting helped to draft the section on atmospheric biosignatures in the TPF book (http://planetquest.jpl.nasa.gov/TPF/tpf_book/index.html) and was a coauthor on the report of the TPF Biomarkers Subcommittee (Des Marais et al., 2001). Kasting is also an (unpaid) member of the JPL NAI group led by Victoria Meadows, whose goal it is to develop a library of synthetic spectra of possible Earth-like planets for future reference by the TPF mission. Kasting and his students have been holding weekly videoconferences with the JPL group and are in the process of writing up the results of one initial investigation (Kasting et al., in preparation). In this study, the Penn State group created coupled photochemical/radiative-convective models of Earth-like planets with various amounts of atmospheric O_2 circling F2V, K2V, and G2V stars. JPL collaborator Martin Cohen from U. C. Berkeley provided help with accessing the stellar UV fluxes from the IUE (International Ultraviolet Explorer) satellite, and Dr. Meadows and her JPL colleague David Crisp calculated synthetic spectra of the model atmospheres that were generated.

We plan to continue the collaboration with JPL over the next five years. As the JPL group gets their own coupled photochemical/climate model on line, our focus will switch—indeed, already has switched—to the difficult "Solid Planet" and "Life" modules that the JPL group hopes to develop. Currently, one of Kasting's graduate students, Pushker Kharecha, is trying to develop better estimates of biotic and abiotic methane fluxes on the early Earth. Methane has already been identified as a key potential biomarker on early-Earth type planets (Schindler & Kasting, 2000; Des Marais et al., 2001), but questions remain as to exactly how much CH_4 must be present in order to constitute a biotic signal. Once we have done what is possible with methane, our focus will likely shift to biogenic sulfur gases (CH_3S, OCS, and DMS) to see if any of these might be possible biomarker candidates.

We are also collaborating with W. Traub at the Harvard Smithsonian Observatory on a similar project. Kasting is an (unpaid) consultant on a recently funded NASA Origins of Solar Systems proposal to perform similar types of spectroscopic studies on hypothetical Earth-like extrasolar atmospheres. Traub's largely independent radiative transfer model will provide a key check on the results generated by Meadows and Crisp at JPL. By involving as many members of the TPF community as possible, we hope to create a robust theoretical groundwork so that we will be ready to analyze the results once the difficult technological hurdles of designing the mission are overcome.

SUMMARY

The goals of PSARC's research activities, described in the preceding six Sections, were developed in coordination with the following goals of the NASA Astrobiology New Roadmap:

Goal 1: Understand the nature and distribution of habitable environments in the Universe.

Goal 2: Explore for past or present habitable environments, prebiotic chemistry and signs of life elsewhere in our Solar System.

Goal 4:Understand how past life on Earth interacted with its changing planetary and Solar System environment.

Goal 5: Understand the evolutionary mechanisms and environmental limits of life.

Goal 7: Determine how to recognize signatures of life on other worlds and on early Earth.

Our group represents a unique blend of scientists whose *coordinated, multi-dimensional, and multi-disciplinary* efforts (Sections I through VI; Fig. II) are directed toward achieving these goals through (1) improving our understanding of the early co-evolution of life and surface environment on Earth, and (2) providing a comprehensive framework for the future recognition of habitable and inhabited planets. We use a multitude of approaches (experimental, observational, and modeling) and state-of-the-art instrumentation drawn from the represented disciplines to tease out the nature of the intimate linkages between the biota and their environment as they interact on a variety of timescales. Although a strong focus of our proposed research, as in the previous funding interval, is on early Earth, we here exploit modern environmental analogues and controlled laboratory experiments to develop a deeper understanding of these interactions. We also avail ourselves of developing collaborations in chemistry and astronomy and astrophysics to extend our research into new areas, including the search for habitable planets outside of our solar system and the detailed mechanisms for the origin of isotopic signatures of environmental evolution.

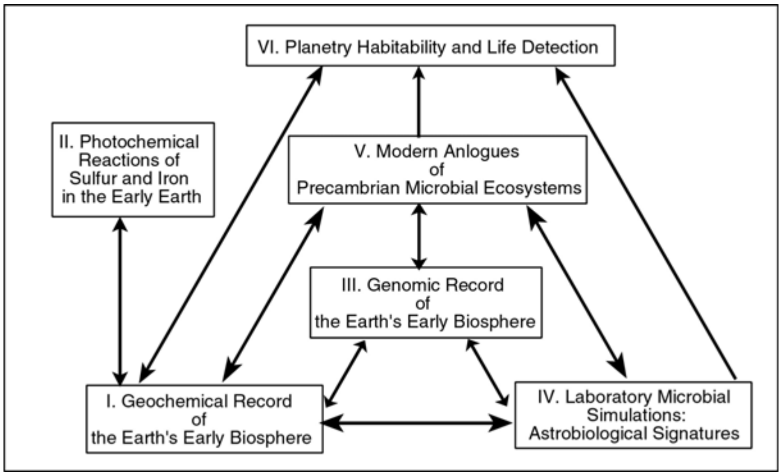

Fig. III Connections of research focuses described in six sections

Executive Committee: Following the administrative structure of the NAI, PSARC has established an Executive Committee, which is composed of the six Section Leaders (Ohmoto, Kasting, Hedges, House, Kump, and Sigurdsson). The Executive Committee will advise the Director on major issues related to the operation of PSARC, including, but not restricted to: (i) the selections of the postdoctoral fellow, graduate fellows, and graduate research assistants who are supported by funds from PSU; (ii) the allocation of undergraduate wages to individual (Co-)PIs; (iii) the allocation of travel funds for Associate Members; and (iv) new initiatives in research, education, and public outreach programs.

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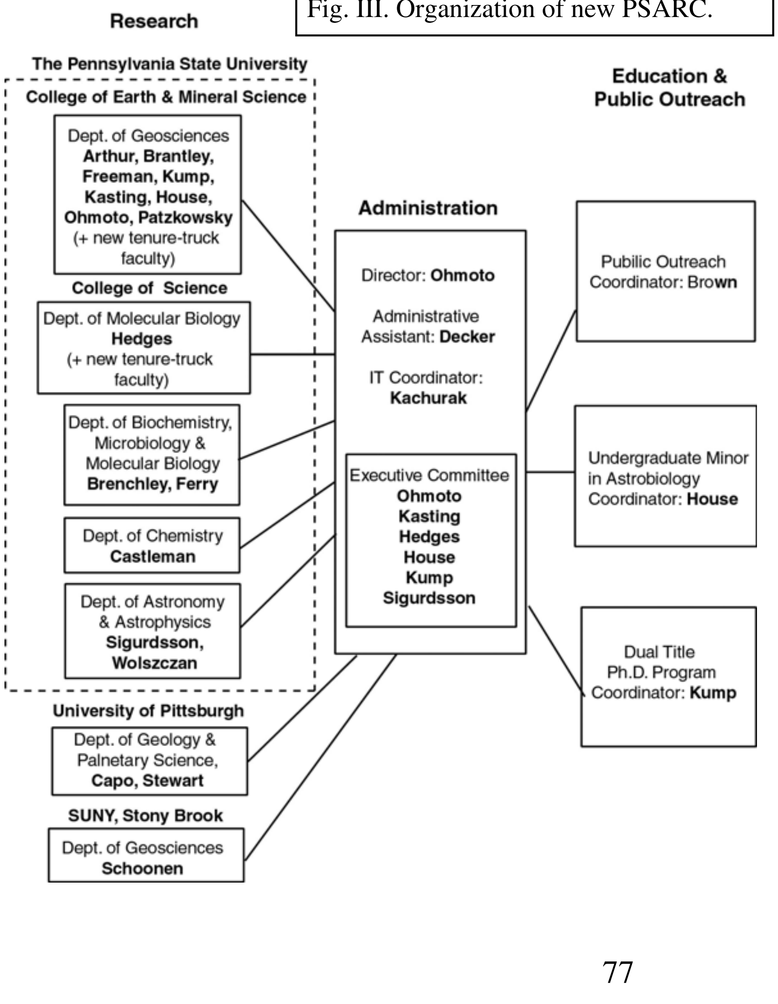
PLAN FOR STRENGTHENING THE ASTROBIOLOGY COMMUNITY

INTRODUCTION AND OVERVIEW

The Penn State Astrobiology Research Center (PSARC) was created in July 1998 by 15 investigators (13 from The Pennsylvania State University (PSU), two from The University of Pittsburgh, and one from SUNY Stony Brook) as one of eleven initial Lead Institutions (Teams) of the NASA Astrobiology Institute (NAI). To support the NAI/PSARC activities to promote astrobiology, ***PSU created a new tenure-track faculty position in the Department of Geosciences to hire Dr. Chris House (astrobiology/geomicrobiology) in 1999.*** The proximity of all members has enabled close interaction and a variety of collaborative research, teaching, and public outreach programs. During the past five years, PSARC has supported all or part of the research/education/PO activities carried out by 142 persons (16 (Co-)PIs, 22 research associates and postdoctoral fellows, five research assistants, two technicians, 49 graduate students, 37 undergraduate students, and five staff in administration/IT/EPO).

With the retirement of two Co-PIs (one in Evolutionary Genomics and the other in Atmospheric Chemistry) and the addition of three new Co-PIs (one in Experimental Chemistry/Physics and two in Astronomy and Astrophysics), PSARC will be operated by 17 (Co)-PIs from two departments of the College of Earth and Mineral Sciences of PSU (Geosciences and Meteorology) and four departments in the Eberly College of Science (Biology, Biochemistry and Molecular Biology, Chemistry, and Astronomy and Astrophysics) at PSU, the Dept. of Earth and Planetary Sciences at the Univ. of Pittsburgh, and the Dept. of Geosciences at SUNY Stony Brook (Fig. III). As part of new commitments to promote Astrobiology, ***PSU will create two additional tenure-track faculty positions***, one in the Department of Geosciences and the other in either the Department of Biology or the Department of Biochemistry and Molecular Biology, pending the funding of this grant proposal (see Section E-5.

Fig. III. Organization of new PSARC.



PSARC remains committed to its efforts to expand and strengthen the astrobiology community and to share the benefits of its research endeavors with students of all grade levels as well as the general public. PSARC activities in education and public outreach programs are described under the following sections: education and public outreach to the K-14 community and the general public (Section E-1); programs directed toward training young scientists and educators in astrobiology (Section E-2); the utilization of information technology (Section E-3); and efforts to strengthen and promote the professional astrobiology community (Section E-4). The University's strong commitments to support the activities of PSARC are presented in Section (E-5).

SECTION E-1. THE K-14 EDUCATION AND PUBLIC OUTREACH PROGRAMS

(Coordinator: Dr. Lisa Brown)

Being the state's Land Grant Institution, PSU has a long tradition of outreach and extension into communities across the state. As such, we have a wealth of education and outreach resources that we can leverage for maximum impact. Key resources include the Pennsylvania Space Grant Consortium (PSGC), Penn State Public Broadcasting, and the Women In Science and Engineering (WISE) Institute.

The PSARC Lead for Education and Public Outreach (E/PO) will be Dr. Lisa L. Brown, Director of PSGC. PSGC is part of the National Space Grant College and Fellowship Program, a national network of colleges and universities working to expand opportunities for Americans to understand and participate in NASA's aeronautics and space programs by supporting and enhancing science and engineering education, research, and outreach programs. Dr. Brown has fulfilled the E/PO Lead role for the past three and a half years. During this time she has been assisted in all programs by Angela Phelps, Assistant Director for the PSGC. Dr. Brown earned her Ph.D. in Geosciences from PSU, working with Dr. Jim Kasting (Co-PI) to study the ancient atmospheres of Earth and Mars. Angela Phelps earned her BS degree in Elementary Education from Southern Illinois University. Over the past three and a half years Dr. Brown and Angela have had extensive interactions and collaborations with the other E/PO teams of the NAI and will continue to do so.

E-1.1. Recruitment and Retention of Women in Science

The future strength of the astrobiology community requires a diverse and talented pool of scientists and engineers. PSARC will make a concerted effort to recruit more women into the field of astrobiology by leveraging an existing program that is sponsored by the PSGC. ***The Women In Science and Engineering Research (WISER) program*** provides research internships to first-year undergraduate women students at PSU. Students will work alongside PSARC faculty, research associates, postdoctoral fellows, and graduate students, receiving a hands-on introduction to academic research. The WISER program is designed to increase the retention rate of women students in science and engineering by intervening during the critical first year of their undergraduate careers, a time when many women students leave the sciences, to provide one-on-one mentoring in a stimulating and supportive research environment. PSARC faculty hosted fifteen WISER students during its first five years; we will work to expand this participation in the next five years.

Research has shown that in order to effectively increase the number of women students in the science and engineering pipeline we need to reach down into the K-12 grades with programs designed to stimulate and nurture girls' interest in science. PSARC will work to engage young women students in astrobiology topics through two existing programs that aim to introduce young women to science and engineering as college majors and careers. The first, ***WISE Camp***, is a one-week residential career awareness program for women in their junior year in high school. The second program, ***Nittany Science Camp for Girls*** is a one-week day camp for students in grades six through ten. During both camps, students participate in a variety of educational and technical activities, including hands-on laboratories in several different science and engineering fields; interaction with role-models from academia and industry; and discussions about educational opportunities at PSU. PSARC faculty and graduate students will introduce students to the field of astrobiology through hands-on investigations and demonstrations. The camps are sponsored and managed by the WISE Institute and by the College of Earth & Mineral Sciences Office of Diversity Enhancement, respectively.

E-1.2. Professional Development for Science Teachers

The PSARC E/PO team has partnered with the teams at NASA Ames, NASA Johnson, and The Marine Biological Laboratory to deliver high quality professional development for science teachers using a variety of existing astrobiology educational products. As an integrated team we are known for consistently modeling science as inquiry and connecting educators to standards-based resources and to the world of professional science. As lead team members that have interactions with NAI researchers we are able to engage astrobiologists directly in our teacher enrichment programs nationwide as speakers, panelists, and discussion facilitators. We each offer ***unique week long programs for educators*** at our sites or in the field, but we have also presented jointly at NSTA, NABT, TOPS, the NAI Executive Council, and the NAI General Meetings. Our E/PO teams include Ph.D. scientists currently involved in the research, university instructors, former school principals, textbook and curriculum publishers, and classroom educators with experience from the elementary to high school levels. This blend of skills gives our integrated team a broad perspective when developing and providing workshops and presentations for educators. All of our professional development programs provide a distribution mechanism for astrobiology-related educational materials, such as the NAI Educator Resource Guide, AstroVenture, Voyages Through Time, the micro-scope online database, as well as other classroom lessons developed by educators and scientists at NASA JSC, NASA Ames, and Yellowstone National Park. We propose ***to expand this multi-team partnership*** to include development of workshops at informal science centers and museums, particularly those that are ***developing new astrobiology exhibits*** (e.g. The New York Hall of Science) or centers that will host traveling astrobiology exhibits.

For the last four years, PSARC has offered a one-week summer workshop for in-service teachers (~24 teachers each year) entitled "Astrobiology: The Origins and Early Evolution of Life". This five-day course is designed to expose middle and high school science teachers to current thinking about the origin and early evolution of life in a way that they can pass on to their students. The course combines lectures by several PSARC faculties, hands-on classroom activities, lab tours, and a field trip to a local bog. PSARC Co-PI, Jim Kasting, is the Lead Instructor and is present throughout the week to facilitate discussion and provide content expertise. In addition to activities created by PSARC members, we have incorporated classroom activities from the NAI Educator Resource Guide “Life On Earth…and elsewhere?” and from TERC's draft High School curriculum. In 2002, a PSARC graduate student in microbiology worked along with a student in Science Education to identify and lead additional activities such as extracting DNA segments from strawberries and collecting and observing magnetotactic bacteria. Evaluations were conducted using daily participant surveys. Following each workshop, teachers are added to our existing listserv discussion group to facilitate and track the application of knowledge from the workshop back to the classrooms. The listserv is also a means for us to continuously disseminate astrobiology-related information and opportunities to the workshop participants. During summer 2003, we will work with a graduate student in Science Education to develop and implement additional evaluation tools. PSARC and PSGC provide funding for the workshop. Housing, meals and some travel support are provided. Teachers are charged tuition for two graduate credits from Penn State; the workshop is approved by the Pennsylvania Department of Education for Act 48 Professional Development credit.

Every year, PSGC exhibits at the Pennsylvania Science Teachers Association convention. Part of the exhibit includes extensive information and resources about NAI and PSARC. Curriculum resources and brochures are distributed. PSGC recruits heavily for all of their Penn State Educator Workshops, including the Astrobiology Educator Workshop. In 2002, Judith Elliott, a middle school educator who taught an Astrobiology unit in her class after attending the Penn State Astrobiology Workshop during June 2001, shared some of her students’ projects as part of our exhibit.

E-1.3. Outreach to K-12 Classrooms

In collaboration with Penn State Public Broadcasting, we have produced two 15-minute television segments on Astrobiology for the Emmy Award winning multimedia current events program, What’s in the News (WITN). The weekly WITN programs reach approximately 5.7 million children nationwide, primarily in grades 4-7, and are distributed nationally via satellite and videotape by International Telecommunications Services, Inc. (I.T.S.). In addition to the video segment, the program employs interactive components to stimulate classroom discussion. The first segment, “Alien Hunting”, featured PSARC faculty members (Hiroshi Ohmoto and Jim Kasting) and gave an introduction to the field of astrobiology and the search for life beyond Earth. The second broadcast, “The Stories Rocks Tell: Learning About Early Earth,” focused on the oxygenation of Earth and how geologists study oxygen clues in rocks to learn how the planet developed an atmosphere that supports life. We will produce two additional segments in the next five years.

Dr. Martin Schoonen, Co-PI at SUNY Stony Brook, has developed a PowerPoint slide show that he has used in presentations to pre-K, K, and first grade students. The topic is planetary comparison. He will be engaged in similar presentations in the future, and continue to improve the PowerPoint slide set, which will be made available to the astrobiological community.

E-1.4. Increasing Public Awareness

PSARC has offered or sponsored numerous lectures and presentations on Astrobiology topics for the general public at PSU, the Carnegie Museum of Pittsburgh, and SUNY Stony Brook. The Earth Explorers Lecture Series at the Carnegie Museum of Natural History offered free public lectures with an earth science theme. The series contained a significant astrobiology component, including presentations by NAI members James Kasting (PSARC) and J. William Schopf (UCLA). The lectures were sponsored by the Museum and the Department of Geology & Planetary Science, University of Pittsburgh, through NSF funding to Co-PI Rosemary C. Capo; plans are underway to continue these activities. PSARC Co-PI Martin Schoonen has been a regular featured speaker in the "Geology Open Night" lecture series at SUNY Stony Brook. These lectures, open to the general public, are held three to four times per semester and draw 100 to 200 people per session.

PSARC will continue to participate in Space Day at PSU, an annual one-day event, sponsored by the PSGC, which is designed to showcase the exciting space-related research being carried out at PSU. PSARC faculty, postdoctoral fellows, and graduate students exhibit posters describing their Astrobiology research and work with students in Science Education to develop engaging hands-on activities that are designed to help translate their research to a general audience, as well as create lesson plans that are aligned with national science education standards and the Pennsylvania Academic Standards for Science and Technology and. We distribute educator kits that include the NAI Educator Resource Guide "Astrobiology in Your Classroom: Life on Earth…and Elsewhere?" as well as the following brochures: NASA Online Educational Resources; How to Access Information on NASA's Education Program, Materials, and Services; NASA – Central Operation of Resources for Education; Space Science Education Resource Directory; PSGC; Penn State Workshops for Science Teachers (one of which is Astrobiology).

E-1.5. Informal Education

PSARC will collaborate with Penn State's College of Earth & Mineral Sciences and the Whitaker Center for Science and the Arts (Harrisburg, PA) to develop a small, interactive traveling museum exhibit on astrobiology. The traveling exhibit will be designed for small museums and science centers, venues that cannot accommodate the large astrobiology exhibits that are currently under development (for example, by the New York Hall of Science). We have an opportunity to take advantage of investments being made by PSU's College of Earth & Mineral Sciences in their newly renovated museum space. ***The College has committed to provide support for a full-time museum Director, to be hired later this year.*** The Director will work with PSARC faculty, who will provide content expertise, and with the staff of the Whitaker Center to develop an exhibit that highlights the astrobiology research focus of the PSU team. Staff from the Whitaker Center will provide expertise to design and fabricate an engaging and educational display. The exhibit will initially be housed in the museum within the College of Earth & Mineral Sciences, but will travel to the Whitaker Center in Harrisburg, PA and to other small museums across Pennsylvania to bring the science of astrobiology to underserved communities outside the major population centers of Pittsburgh and Philadelphia. Likely venues for display include The Discovery Center of Science & Technology, Bethlehem, PA; The North Museum of Natural History and Science, Franklin & Marshall College, Lancaster, PA; The

Reading Public Museum, Reading, PA; The Venango Museum of Art, Science, and Industry, Oil City, PA; and The Erie Historical Museum and Planetarium, Erie, PA.

The Whitaker Center for Science and the Arts and its exhibits and education branch, HARSCO Science Center, have extensive experience in exhibit design and development. They currently maintain over 240 interactive exhibits related to nine discreet scientific fields. They have created in-house exhibits on a range of scientific topics including space exploration and exobiology. Based on their experience and their in-house fabrication capabilities we have budgeted sufficient funds to create an exhibit approximately 1300 square feet in size. However, we plan to partner with the Space Science Institute (Boulder, CO) to pursue additional funding from the National Science Foundation to allow for the development of complimentary educational materials and professional development opportunities for museum staff. We will seek additional partnerships and supplemental funding within the NAI.

One possible element of the traveling exhibit is a replica of the large-scale bioreactors, ***"Simulated Precambrian Biosphere"***, that are described in Research Section IV. The bioreactors could be used to illustrate the structures of microbial ecosystems on the early Earth and of modern ecosystems (e.g., Fayetteville Green Lake, N.Y.: see Research Section V) that PSARC researchers are studying. The bioreactors could also be utilized during the one week teacher workshops, representing sets of 5-foot vertical "Winogradsky-type columns" which many of our workshop participants already utilize in their classrooms. The experimental bioreactors will allow for sampling of various geochemical parameters through the columns, allowing students to understand the connections between microbial consortia and their environments.

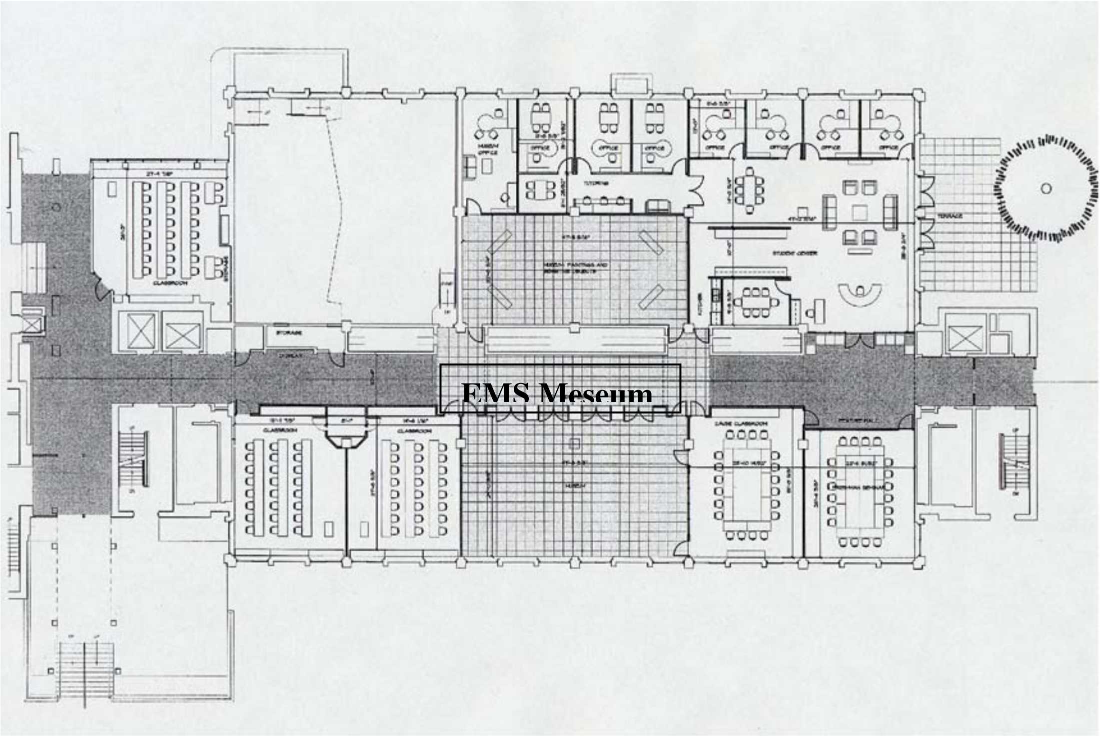


Figure 1: This figure shows a draft blueprint of the groundfloor of Deike Building which will include the new Earth & Mineral Sciences museum at PSU. Our proposed traveling astrobiology exhibit will be displayed in the room at the bottom center of the figure. This exhibit space measures approximately 29 feet by 48 feet.

SECTION E-2. TRAINING YOUNG SCIENTISTS AND EDUCATORS IN ASTROBIOLOGY

The training of new scientists and educators through undergraduate and graduate education and research in astrobiology and related fields has been (and will continue to be) an important objective of PSARC. During the past five years, we have fully or partially supported 49 graduate students and 39 undergraduate students using the funds provided by NASA (graduate assistantships and wages) and PSU (about three graduate fellowships/assistantships and support for undergraduate students). We have graduated ten Ph.D. students and nine MS students who have conducted astrobiology-related research as main parts of their theses. The ten completed Ph. D. students are: R. Hotinski, ’00; A. Pavlov, ’01; B. Borup, ’01; S. Ono, ’01; M. Van Tuinen, ’02; K. Yamaguchi, ’02; Y. Watanabe, ’02; H. Pointkiviska, ’03; M. Borda, ’03; and M. Hurtgen, ’03). The current graduate students who are conducting astrobiology-related research totals 22. PSU has established **an intercollege undergraduate Minor in Astrobiology** (E-2.1); the proposal to create the **Dual-Title Ph. D. Degree Program in Astrobiology** (E-2.2) is currently under review by the Faculty Senate. Other educational programs include the Astrobiology Summer Program at Penn State (E-2.3) and Satelite Courses (E-2.4), and they are also closely connected to The Penn State Biogeochemistry Reserch Initiative in Education (BRIE), which has been largely funded by IGERT (NSF) (Section E-2.5). Collaboration in research and education with Minority Institutions are described in Section (E-2.6).

E-2.1. Undergraduate Minor in Astrobiology (Coordinator: Prof. Chris House)

An inter-college undergraduate program in Astrobiology, headed by Dr. Chris House (PSARC Co-PI), was established in the Fall 2000 semester. The Astrobiology Minor is designed to educate students in this interdisciplinary field covering the varied scientific disciplines that contribute to our general understanding of life, the origin of life, the past history of life on Earth, possible futures for life on Earth, and the possible existence of life on other planetary environments. The principle goal of the minor is to develop student's literacy in astrobiology so that they can critically evaluate claims related to this field that they encounter well after their college education has ended. So far six students from the departments of Astronomy, Microbiology, Biology and Mathematics have completed the requirements and received this BS minor and four students are expected to graduate in May 2003. Student recruitment efforts are in progress to expand this program.

Requirements

To qualify for the Minor students are required to take 18 course credits from the list below, with at least six credits at the 400-level. (A grade of C or better is required for all courses in the minor.)

One of the following REQUIRED Introductory courses (3 credits, semesters 1-6):

EARTH 002: The Earth System (3)

GEOSC 021: Biodiversity and Earth (3)

One course from each group of the following REQUIRED courses (9-10 credits, semesters 5-8):

- ASTRO 140: Life in the Universe (3)
- ASTRO 291: Astronomical Methods and the Solar System (3)
- GEOSC 204: Geobiology (4)
- BIOL 427: Evolution (3)
- BIOL/GEOSC 474: Astrobiology (cross-listed course)

Additional courses (5-6 credits, semesters 5-8):

- ASTRO 475W: Stars and Galaxies (3)
- BIOL 405: Molecular Evolution (3)
- BMB 401: General Biochemistry (2)
- BMB 402: General Biochemistry (3)
- GEOSC 416: Stable and Radioactive Isotopes in Geoscience (3)
- GEOSC 419: Organic Geochemistry (3)
- GEOSC 481: Solid Earth and Planetary Geophysics (3)
- METEO 466: Planetary Atmospheres (3)
- MICRB 201: Introductory Microbiology (3)

E-2.2. Dual-Title Ph.D. Degree Program in Astrobiology (Coordinator: Prof. Lee Kump)

A proposal to create a dual-titled Ph.D. degree program in Astrobiology at PSU is now under review by the Faculty Senate, and we expect the approval shortly. Currently, about 10 graduate students in Geosciences, Biology, Chemistry, and Astronomy & Astrophysics departments are expected to enroll beginning with the Fall 2003 semester in the dual-title program. The Graduate Bulletin at Penn State will have the following section describing this program:

Degree Conferred: Students electing this degree program through participating programs earn a degree with a dual title in the Ph.D., e.g., Ph.D. in (graduate program name) and Astrobiology.

The Astrobiology dual-title degree program is administered by Dr. Lee Kump (PSARC Co-PI) under PSARC. PSARC maintains program definition, identifies courses appropriate to the program, and recommends policy and procedures for the program's operation to the dean of the Graduate School and to the deans of the participating colleges. The dual-title degree program is offered through participating programs in the College of Earth and Mineral Sciences and the Eberly College of Science and, where appropriate, other graduate programs in the University. The program enables students from several graduate programs to gain the perspectives, techniques, and methodologies of Astrobiology, while maintaining a close association with primary program areas of application.

Astrobiology is a field devoted to the exploration of life outside of Earth and to the investigation of the origin and early evolution of life on Earth. For admission to pursue a dual-title degree under this program, a student must apply to (1) the Graduate School; (2) one of the participating primary graduate programs; and (3) the Astrobiology program committee.

Admission Requirements

In addition to the admission requirements set forth by the Graduate School and the primary program, program candidates will be required to take the Graduate Record Examination and, where appropriate, provide a satisfactory TOEFL score. Candidates must also submit a written personal statement indicating the career goals they hope to serve by attaining an Astrobiology dual-title. A strong undergraduate preparation in the basic sciences is expected, with evidence of an interest in multiple disciplines. Participation in research at the undergraduate level is encouraged.

Degree Requirements

To qualify for a dual-title degree, students must satisfy the requirements of the primary graduate program in which they are enrolled, in addition to the minimum requirements of the Astrobiology program. The minimum course requirements for the dual-title in Astrobiology are ABIOL 574 Planetary Habitability (3 credits), ABIOL 590 Astrobiology Seminar (2 credits), ABIOL 570 Astrobiology Field Experience (2 credits), and at least two credits of 400- or 500-level coursework outside of the student's primary program in an area relevant to Astrobiology (through consultation with the advisor). The primary program administers the candidacy examination, but at least one member from the committee must be on the Astrobiology program faculty[1]. Similarly, the doctoral committee should include faculty from the Astrobiology program. In both cases the Astrobiology representative may be the advisor and have an appointment in the primary program of study. The field of Astrobiology should be integrated into the comprehensive examination. A Ph.D. dissertation that contributes fundamentally to the field of Astrobiology is required, as is a public oral presentation of the dissertation.

Financial Aid

Financial aid is generally available through the primary program and through highly competitive University Graduate Fellowships. However, PSARC provides support for students through research assistantships and graduate fellowships. Typically students in Astrobiology are supported 12-months per year on some form of assistantship, fellowship, or summer wages.

Other Relevant Information

Students intrigued by the possibility of pursuing research in Astrobiology should visit the PSARC website (http:// psarc.geosc.psu.edu/) and the NASA Astrobiology Institute website (http://nai.arc.nasa.gov).

Synopsis of the Required Courses

ASTROBIOLOGY (ABIOL)

570. ASTROBIOLOGY FIELD EXPERIENCE (2) Field excursions to sites where the early evolution of life and the environment is revealed in the fossil, geochemical, and sedimentary record or in modern analogue. Possible sites include the Huronian Supergroup of Ontario, Canada, Precambrian rocks of Virginia, West Virginia, or Maryland, Precambrian paleosols of Michigan, modern microbial ecosystems in the Bahamas or at Green Lake, NY State.

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[1] This requirement is waived for departments that do not have individual candidacy committees or where departmental policies preclude this requirement.

574. PLANETARY HABITABILITY (3) This course provides the scientific foundations upon which the search for life on other planets is based, including aspects of Earth history, evolutionary biology, atmospheric chemistry, life in extreme environments on Earth, and planet and life detection.

590 ASTROBIOLOGY SEMINAR (2) Student-led presentations and discussions of current and classic literature relevant to the themes of Astrobiology.

E-2.3. Astrobiology Summer Program (ASP) at Penn State (Prof. Blair Hedges – Coordinator)

An undergraduate summer training program in astrobiology was established at Penn State, with the help of a 3-year NSF REU-Site award (S. B. Hedges, PI). Each summer, it supports 10 students (sophomores, juniors) from colleges and universities other than Penn State to participate in research training under the guidance of Penn State NAI faculty mentors. All expenses associated with the travel, room, and board for each student are covered by the program ($2300 per student), and students are additionally given a stipend of $3500 for the summer. Women and minorities are encouraged to apply. The program has a director (Hedges) and a coordinator (Dr. Carla Hass). The ASP web site provides a full description of the program (http://evo.bio.psu.edu/asp/).

Besides laboratory research, ASP students also have the opportunity to participate in a research excursion to a modern analog of a Precambrian ocean (Green Lake, New York), attend weekly seminars and laboratory tours, stargaze, and participate in a discussion group and a research symposium.

Thirty applications have been received and are under review for the first summer (2003). The applicants represent a diversity of majors (biology, geology, chemistry, physics, astronomy, geology) and are uniformly high in quality. We anticipate renewing this program support from NSF in 2005. Also, we propose here to strengthen this program by using PSARC funds to support two additional students (women or minority) each summer.

E-2.4. Satellite Courses (Coordinators: Prof. Brian Stewart and Rosemary Capo, Univ. of Pittsburgh, PSARC Co-PIs)

Through NAI-provided videoconference facilities, PSARC member institutions the University of Pittsburgh and SUNY Stony Brook have been able to offer successful, full-credit graduate courses in Astrobiology, with real-time guest lectures and discussions from all of the PSARC investigators. Co-PIs B. Stewart and R. Capo developed a graduate course in Astrobiology at the University of Pittsburgh in Spring of 2002. The 3-credit course (GEOL 3953, Topics in Geochemistry: Astrobiology) was taken by eight graduate students, and it featured lectures by Stewart and Capo followed by weekly videoconferences with PSARC researchers at Penn State and Stony Brook. Using the equipment, students from Univ. of Pittsburgh, Penn State and SUNY Stony Brook were able to ask questions and freely interact with the presenters. In Fall of 2002, students from the University of Pittsburgh and SUNY Stony Brook participated by videoconference in an Astrobiology Seminar run by Penn State. We expect to continue offering these courses over the next five years.

E-2.5. The Pennsylvania State University Biogeochemistry Research Initiative in Education (BRIE) (Director: Prof. Susan Brantley, PSARC Co-PI)

An award from IGERT (NSF) was provided in 1999 in order to support cross-disciplinary graduate training in biogeochemistry (S. Brantley, PI; J. Brenchley, K. Freeman, co-PIs). This program funds Ph.D. students who receive training in at least two of the following areas: geochemistry, microbiology, soil science, environmental engineering, meteorology and chemistry. Students are co-advised by faculty in different disciplines, and are provided with research, travel and teaching opportunities that enrich their training experience. Students are engaged in a rich array of research topics, many of which are directly aligned and complementary to the interests of Astrobiology. Many of the BRIE graduate students are working on aspects of the interactions of microbial life with its geochemical environment. This work includes the strategies employed by microbes to contend with salt, oxygen or other stresses in their environment, and the means by which microbes extract of necessary nutrients from minerals. In practical terms there are close links between BRIE and PSARC, as many of the BRIE faculty are also investigators in PSARC.

E-2.6. Teaming with Minority Institutions (Collaborators: Profs. David Shwartzman, Howard University, PSARC Associate; Prof. Warren Gooden, Cheyney University, PSARC Associate)

PSARC faculty have research connections with universities that are predominantly African American (Howard University, Washington, D.C.) and predominantly Hispanic (University of Puerto Rico). Dr. David Schwartzman (Howard U., Biology Department) is an associate member of PSARC and studies the early history of the biosphere (e.g., Life, Temperature, and the Earth; Columbia Univ. Press, 1999). Besides his research collaborations with Penn State, he will bring undergraduate and graduate students from Howard University to annual PSARC meetings for poster presentations and interactions with our faculty and students. Several colleagues of PSARC Co-I Hedges are on the biology faculty at the University of Puerto Rico (J. Ackerman, J. Mutt, R. Thomas) and provide a link for directing interested undergraduates to our Astrobiology Summer Program or PhD program. One such student, Ms. Maraliz Vazquez, participated in our first (trial) 10-week summer program in 2002 and this experience persuaded her to apply to PhD programs in astrobiology this year.

PSARC faculty and staff have also established connections with Cheyney University in Philadelphia, PA; the oldest of the Historically Black Colleges and Universities in America. Cheyney University offers baccalaureate degrees in more than thirty disciplines and the masters degree in Education. Dr. Warren Gooden, Professor of Chemistry, is an associate member of PSARC and has developed an introductory astrobiology course for undergraduate students at Cheyney. The course is offered to science majors and to education students working toward their science teacher certification. Dr. Gooden will collaborate with PSARC faculty to expand this introductory course and to develop a workshop for in-service science teachers modeled after the existing one-week PSARC summer workshop. Virtual connections between Cheyney University and the PSARC campuses can be easily established since Cheyney is a member of the Pennsylvania State System of Higher Education schools, all of which have extensive distance learning technologies. This would give faculty and students at Cheyney an entrance way into the virtual NAI research community.

SECTION E-3. UTILIZATION OF INFORMATION TECHNOLOGY

(IT Coordinator: Frank Kachurak)

Effectively utilizing collaborative technology is one of the key priorities of the Penn State Astrobiology Research Center Information (PSARC) Technology Team. Integrating the technology into educational, research, and administrative functions is the constant goal of the team.

One of the major projects that successfully integrated collaborative technology into the educational aspect of the Research Center is the Astrobiology Seminar Series. A test-pilot supported by the NASA Astrobiology Institute and PSARC, this program deployed a video conference system to Penn State, the University of Pittsburgh, and The State University of New York at Stony Brook. Each Friday during the semester, a professor from one of the universities would give a simultaneously-broadcast one-hour lecture that was followed by a question and answer session. Other than the initial cost of the systems, there was little residual cost as the conference was held via IP. After a full semester of classes, this program is considered to be a success, and both students and professors are looking forward to the seminar class next fall.

Along with larger projects comes the day-to-day support of the members of PSARC. System administration, web design assistance, and IT equipment management are some of the services provided by the IT Team members on a daily basis. A close relationship has also been developed with the NAI Digital Video Expert Center (DVEC) team, and PSARC IT Team members often work with DVEC team members when setting up conferences and scheduling collaboration technology resources.

Future IT plans for the PSARC will be based on a number of factors, including the goal of the NAI to promote collaboration through the use of electronic tools.

Current collaboration takes place mainly via e-mail, video conferences, and telephone calls. Future plans include the implementation of a document sharing system, set up similar to an electronic message board, which will allow asynchronous communication among PSARC members.

Further development of the Astrobiology Seminar Series will include the ability to attend the class from any IP-connected computer. This will enable researchers in the field as well as students who don’t have access to the PolyCom systems to attend and participate in the class activities.

Migration from the current MacOS 9 and Windows 2000 environment to MacOS X and Windows XP is already beginning, but will require additional user training as the changeover takes place. One-on-one user training sessions will provide users the necessary training for this migration.

Finally, the current set-up of the PSARC Communications Center is in the form of a conference room complete with a large table in the center. To enhance the usability of the room for larger video conferences, and to provide a comfortable area for attendees of the NAI Directors Seminar Series, NAI Executive Council meetings, and other popular seminars, the feasibility of redesigning the Communications Center will be investigated. Plans will include the transformation from the standard conference type setup into a miniature auditorium setting, complete with large screen projection of both the video conference and the desktop sharing screens.

See also Section E-2.4 for Satellite Courses.

SECTION E-4. PROMOTING THE PROFESSIONAL ASTROBIOLOGY COMMUNITY

PSARC investigators have played major roles in promoting the new science of Astrobiology through: publications of new ideas and important data in major scientific journals and at major national and international conferences (E-4.1); organizing special sessions related to Astrobiology at major international conferences (E-4.2); serving on editorial boards of major journals related to Astrobiology (E-4.3); serving on national and international committees related to Astrobiology (E-4.4); participation in NAI Focus Groups (E-4.5); and the collaboration with scientists both within and outside the NAI (E-4.6). Our efforts in all these areas will continue to grow in the future.

E-4.1. Research Publications

The scientific impacts of research carried out by the 16 (Co-)PIs of PSARC and their research teams during the last five year period may be best measure by the numbers of papers published in major, refereed journals and books, which are summarized in the following table.

Summary of NAI Publications by PSARC Investigators
(1998-2003)

Journal	Published	In Press	Submitted (in review)	Total
Science	9		1	10
Nature	5		2	7
Proc. Natl. Acad. Sci. USA	9			9
J. Bacteriol.	8			8
Biochemistry	6			6
Mol. Biol. Evol.	5	1	1	7
J. Biol. Chem.	5			5
Other biological journals	26	6	3	35
Astrobiology	4			4
Geology	12		1	13
Geochim. Cosmochim. Acta	8	1	3	12
Chem. Geol.	6			6
Earth Planet. Sci. Lett	2	1	1	4
Other geological journals	23	4	4	31
Ann. Rev. Astron. Astrophys			1	1
Book Chapters	8	3		11
Total	**136**	**16**	**17**	**169**

The full titles of these papers are presented in “List of Publications” in Volume II. Abstracts of papers presented at professional meetings are excluded from the list because there are more than 1,000 entries.

E-4.2. Organizing Special Sessions on Astrobiology

Several PSARC investigators have organized special sessions and symposia at major international scientific conferences to promote Astrobiology, including:

Ohmoto and Schoonen co-organized a special session on “Astrobiology” and House organized a special session on “Genomic meets Geochemistry” at the 11th Goldschmidt Conference of the Geochemical Society at Hot Springs, VA, in May, 2001.

Ohmoto organized a Pardee Symposium and a Pardee Technical Session, “Evolution of the Early Atmosphere, Hydrosphere, and Biosphere 1: Constraints from Ore Deposits”, at the Annual Meetings of the Geological Society of America in Denver, CO, October, 2002. This symposium and technical session were sponsored by the NAI, NASA Exobiology Program, The Geological Society of America, Geochemical Society, and the Society of Economic Geologists. Ohmoto is also a co-convener of a technical session, “Co-evolution of the atmosphere, oceans, and biosphere on early Earth”, at the 13th Goldschmidt Conference of the Geochemical Society in Kurashiki, Japan, September, 2003.

Brantley is President and Schoonen is Scientific Program Chair of the Water-Rock Interaction Conference at Saratoga Spring, NY, in 2004. It will have a Astrobiology-related session.

Program Committee of NAI Annual meetings was served by Ohmoto (2001) and Arthur (2003).

E-4.3. Serving on Editorial Boards of Major Journals related to Astrobiology

Ohmoto: Associate Editors of *Geochimica et Cosmochimica Acta* (through ’02) and *Chemical Geology* (through ’02); Editorial boards of *Resources Geology,* and *Astrobiology*.

Arthur: Editor, *Paleoceanography* (through ’02).

Brantley: Editor, *Chemical Geology* (through ’01).

Castleman: Editor in Chief, new book series devoted to Cluster Sicences/Springer Verlag; Advisory Editorial boards of *Chemical Physics Letters, Advances in Chemical Physics, ResearchTrends,* and *Journal of Cluster Science*.

Freeman: Editorial Board of *Annual review of Earth and Planetary Sciences*.

Kasting: Editorial boards of *Origins of Life and Evolution of the Biosphere, Astrobiology,* and *Geobiology*.

Kump: Editor, *Geology* (through ’00); Editorial boards of *Geochimica et Cosmochimica Acta* and *Chemical Geology*.

House: Editorial boards of *Astrobiology,* and *Geobiology*.

Schoonen: Editor, *Geochemical Transactions*; Editorial bords of *Chemical Geology*.

E-4.4. Serving on National Committees related to Astrobiology

Freeman: Member of the NRC Committee on the Origin and Evolution of Life (COEL).

Hedges: Member of the Astrobiology Roadmap Committee.

Kasting: Chair, the Exobiology review Panel; Member, NRC Committee on the Origin and Evolution of Life (COEL).

E-4.5. Participations in NAI Focus Groups

Missions to the Early Earth: Ohmoto, Arthur, Capo, House, Kump, and Stewart.
Mars: House, Ohmoto.
Evolutionary Genomics: Hedges (Co-chair).
Europa: Brenchley, House.
Ecogenomics: House, Kump.

E-4.6. Research Collaborations

The PSARC created five years ago the Associate Membership Program to enhance collaboration in research, education, and public outreach in Astrobiology with the (Co-)PIs. The monetary rewards for the Associate Members are very small, if any. Occasionally, PSARC has provided support for travel expenses to visit PSU to work with PSARC PIs, to attend workshops and seminars at PSU, to attend NAI meetings, or to conduct fieldwork with PSARC PIs. However, all associate members are registered at the NAI Central and receive almost the same privileges as PSARC graduate students, such as receiving mail/announcements/updates directly from the NAI Central and invitations to attend NAI meetings. Currently, PSARC has the following 29 Associate Members in Research and 4 Associate Members in Education and Public Outreach:

Associate Members of the Penn State Astrobiology Research Center

For research,

Mark Barley (U. of Western Australia): Precambrian geology
Hubert L. Barnes (PSU): hydrothermal geochemistry
Nicolas Beukes (Rand Afrikaans Univ., South Africa): Precambrian geology
Paul Braterman (Northern Texas Univ.): atmospheric chemistry
Oliver A. Chadwick (UC Santa Barbara): pedology
Catherine Drennan (MIT): biochemistry
Charles Fisher (PSU): marine biology
Sorel T. Fitz-Gibbon (UCLA): geonomics
Tracy Frank (U. of Nebraska): Proterozoic geochemistry
Robert C. Graham (UC Riverside): soil geochemistry
Steven Holland (U. of Geogia): sequence stratigraphy
Clark Johnson (U. of Wisconsin, Madison): Fe isotope geochemistry
Takeshi Kakegawa (Tohoku Univ., Japan): sulfur isotope geochemistry
Sudir Kumar (Arizona State Univ.): molecular genetics
Donald R. Lowe (Stanford Univ.): Precambrian geology
Tim Lyons (U. of Missouri): Proterozoic geology
Gwendolyn L. Macpherson (U. of Kansas): geochemistry
Arnold Miller (U. of Cincinnati): paleontology
Robert Minard (PSU): atmospheric chemistry
Hiroshi Naraoka (Tokyo Metropolitan Univ., Japan): organic geochemistry
Munetomo Nedachi (Kagoshima Univ., Japan): paleosols, ore deposits
Thomas Olszewski (Museum of Nature History, Smithsonian Institute): paleontology

Victoria J. Orphan (NASA Ames Research Center): environmental microbiology
Anthony Prave (St. Andrews Univ., Scotland): Neoproterozoic geology
Douglas Rees (Cal Tech): biochemistry
Greg Retallack (U. of Oregon): soil geochemistry
David Schwartzman (Howard University): biology
Kerry Smith (Clemson Univ.): biochemistry
Jennifer Macoledy (Carlton College): geomicrobiology

For public education,
Lisa Brown, Director, Pennsylvania Space Grant Consortium
Kathleen M. O'Toole. Writer/Host, "What's in the News", WPSX
Judi Wakhungu, Director, WISE Institute

In addition to the above Associate Members, the PSARC (Co-)PIs have actively collaborating with many researchers of other NAI Teams and also outside of NAI.

RESEARCH FACILITIES & EQUIPMENT

All of the research outlined in the previous sections can be completed using the existing facilities and equipment except where permanent equipment was requested.

The Materials Characterization Laboratory, a multi-user facility within the College of Earth and Mineral Sciences at PSU provides AFM (Digital Instruments Digital Instruments AFM/LFM Scanning Probe Microscope and Digital Instrument Nanoscope IIIa Dimension 3100 microscope), EM (Cameca SX-50 electron microprobe), ICP-AES (Leeman Labs PS3000UV inductively coupled plasma emission spectrophotometer), ICP-MS (Finnigan Element high resolution inductively coupled plasma mass spectrometry), IR (several models of infrared spectrometers are available for both solution and solid state analysis), SEM (ISI SX 40 secondary electron microscope, ISI SX 40A with energy dispersive xray analysis capability, JEOL JSM-6300F with EDS detector and analysis), SIMS (Cameca IMS-3F secondary ion mass spectrometer/ion microscope), DTA and TGA (DuPont 2100 and Netzsch STA 429 thermal analyzers for thermogravimetric analysis and differential thermal analysis), TEM (Hitachi HF 2000 and Philips EM420ST transmission electron microscopes), XPS/AES (Kratos Analytical XSAM800 pci for x-ray photoelectron spectroscopy and Auger electron spectroscopy), and XRD (Rigaku Geigerflex x-ray diffraction analysis).

The Department of Geosciences provides rock preparation systems, stable isotopes facility, organic and isotopic facility, in-situ laser ablation BrF_5 system at Dr. Peter Deines' laboratory, and the hydrothermal laboratory of Dr. Hu Barnes. The rock preparation systems include rock saws, thin section makers, grinders, and polishers. The stable isotopes facility includes a Finnigan MAT 252 stable-isotope-ratio mass spectrometer equipped with a dual inlet and multiple collector assemblies, a Finnigan Delta-Plus XP mass spectrometer equipped with a GC, two elemental analyzers and micro-volume gas sampling systems for the analyses of C, N, S, O, and H isotopes (under the direction of K. Freeman), a second Finnigan 252 in the same facility (under the direction of M. A. Arthur) is equipped with common-acid-bath automated carbonate analyzer and a third Finnigan 252 (under the direction of T. Sowers) is dedicated to isotopic analyses of trace gases.

The organic and isotopic facility at includes a Rock-Eval pyrolysis unit, a coulometer and an elemental analyzer for the analysis of inorganic and organic carbon in waters, soils, and sediments. The laboratory is well supplied with routine equipment, including glassware, analytical balances, rotary evaporators, solvent-extraction systems, and two GC-FIDs, as well as a HP 5973 GC/MS (with autosampler, EI and CI capabilities), and an HPLC with absorbance detection.

The department of Biochemistry and Molecular Biology provides, as common use equipment: autoclaves, ice machines, ultracentrifuges, scintillation counters, walk-in incubation and cold rooms, etc. Penn State operates a Nucleic Acid Facility which will be used on a fee basis for synthesizing oligonucleotide primers and for nucleic acid sequencing.

The Chemistry Department provides access to a number of mass spectrometers for structure determination, including an HPLC- time of flight mass spectrometer with atmospheric pressure chemical ionization (LC-APCI-MS).

Michael Arthur's facility includes rock crushing and powdering equipment (SPEX mill), a UIC Coulometrics Automated Carbon Coulometer System (new 2002; CM 5200 Autofurnace, CM 5240 TIC device, CM 5014 Carbon Coulometer, and Printer) and computing equipment (HP 2250TN Workgroup printer,5 Sun 10 Workstations with access to MATLAB for modeling, Mac G4 and PCs).

Susan Brantley's laboratory is fully equipped for solution chemistry analysis, mineral preparation, and analysis of rates of dissolution of earth materials. Up to 12 chemical reactors can be run simultaneously. Standard equipment such as balances, pH meters, rotors, water baths, optical microscopes, and other such apparatus is available. For microbiological work, a Coy anaerobic glove chamber, two laminar flow hoods, and an autoclave are all available.

Jean Brenchley's laboratory is equipped for the microbial diversity portions of the proposal. Small laboratory equipment such as balances, pH meter, hot plates, magnetic stirrers, fraction collectors, microcentrifuges, spectrophotometers, high speed refrigerated centrifuge, water baths, incubators, power supplies, electrophoresis equipment for DNA analyses, thermocyclers for PCR reactions, automatic pipetters, general glassware, chemicals and other general supplies are available in the laboratory.

Greg Ferry's laboratory contains anaerobic glove bags and anaerobic fermentation equipment for small and large-scale growth (30 and 80-liter fermentors) of anaerobes. In addition, the laboratory maintains special equipment for anaerobic biochemistry and molecular biology research that includes: three inert atmosphere systems. The laboratory is well equipped with general equipment items. Most of the equipment in the laboratory has a computer dedicated for data analysis and manipulation. Five computers are also available for general use.

Katherine Freeman's laboratory and the Department of Geosciences maintain a large number of glass vacuum lines for the preparation of samples for analysis by dual-inlet stable-isotope mass spectrometry.

S. B. Hedges's bioinformatics laboratory is equipped with 20 personal computers (Pentium IV and Xeon 1.7-2.0 Gigahertz, operating Linux and Windows; also PowerMac), about half of which are workstations with dual processors and 1 Gigabyte of memory for computationally intensive work. There are two servers: one for internal laboratory use and the other for web

databases; each is a Dell Xeon dual processor PowerEdge 4600 with 144 Gigabyte SCSI storage and 1 Gigabyte memory, with RAID mirroring. All are interconnected on the Internet and protected by a firewall. Additional computing resources, including a 140 CPU Linux cluster, are available in the Penn State Center for Academic Computing.

Christopher House's laboratory is equipped with a Nikon E800 photomicroscope set up for brightfield, phase-contrast, epi-florescence (for FISH), or reflected light microscopy. The microscope is connected directly to a cooled high resolution CCD camera with direct real time computer interface (iMac).

The laboratory is also equipped for aerobic or anaerobic microbial culturing at any temperature between 4°C and 110°C with continuous shaking and with light between temperatures of 4°C and 60°C. Equipment includes an anaerobic chamber, a gas station, and six temperature controlled incubators/shakers. The laboratory is well supplied with routine equipment, including glassware, pH meter, analytical balances, and a Beckman J2-HS centrifuge, as well as a laminar flow hood for working in a sterile environment.

Equipment also includes gel electrophoresis chambers, microcentrifuges, a PTC-100 MJ Research PCR thermocycler, an Beckman UV/Vis Spectrometer, -80 freezer, UV transilluminator and gel visualization box with digital camera, and dark box that can be connected to a cooled CCD camera for chemiluminescence detection.

Computing equipment includes a G4 Macintosh computer, a G3 iMac computer, and a unix workstation with dual 933Mhz Pentium III processors. The laboratory has standard phylogenetic software including the following programs that may be of use in this project: Mac Software (PAUP for phylogenetic analysis, MACCLADE for phylogenetic analysis, AUTODECAY for phylogenetic analysis, MACPATTERN for prosite and blocks searches, OLIGO for primer design, DNASTAR package for sequence analysis and manipulation), Unix Software (FASTA3, BLAST (NCBI Toolbox), HMMer, tRNAScan, CLUSTALW for sequence comparisons, PERL for data manipulation, PHYLIP for phylogenetic analysis, ARB for phylogenetic analysis (programs from University of Munich))

James Kasting's laboratory has 3 Sun Spark 20 workstations, 4 PCs, and access to two Cray supercomputers run by the Environment Institute.

Lee Kump's laboratory maintains 6 Sun Ultra 10's with 768 Mbytes RAM and a Sun Ultra 60 Unix workstation with one processor and 512 Mbytes of RAM. These workstations are part of a network of Unix, PC, and Macintosh computers maintained by the Environment Institute (EI) and Department of Geosciences which are available to students, faculty, and postdoctoral associates affiliated with this project. EI's supercomputing facility is also at our disposal, which houses a Cray Research SV1 (16 CPUs, 4GB memory), and an IBM RS/6000 SP with 10 nodes and 4 CPUs per node, on which many of the model codes are already running. In addition, we will have shared use of one existing 2-processor 750-Mhz Sun server in the EI.

Nutrient analyses are performed on a Technicon AutoAnalyzer II according to an approved EPA quality assurance project plan in the laboratory. The laboratory is fully equipped for aqueous and sedimentary geochemical sample collection and analysis. The Department of Geosciences has also recently purchases a YSI multiprobe for water-column profiling of temperature, conductivity, chlorophyll, oxygen, Eh, pH, turbidity, and PAR light.

Hiroshi Ohmoto's laboratory has two mass spectrometers for isotope analyses of H, C, N, O, and S, an in-situ laser ablation system, an elemental analyzer for H, C, S, and N contents of solids and liquid samples, a continuous flow micro-analytical system for S, C, and N isotope analyses, a BrF_5 line for O and S isotope analyses, gas preparation lines for C, S, O, N, and H isotope analyses, including a SF_6 line recently built for the measurement of ^{32}S, ^{33}S, ^{34}S, and ^{36}S, petrographic microscopes with digital camera, video camera, and TV monitor, a cthodoluminoscope, facilities for mineral-solutions-gas reaction systems, and a recently purchased x-ray chemical microscope (Horiba XGT-5000) for chemically mapping up to 30 elements simultaneously with a spatial resolution of 10 µm on hand specimen-sized samples.

Non-Penn State facilities:

Secondary ion mass spectrometry

The CAMECA ims 1270 ion microprobe at UCLA will be used for some of the proposed ion probe hours needed for this work. It is equipped with a multicollection array of detectors, as well as a normal incidence electron gun for charge compensation. This instrument will be used for carbon isotopic analyses on samples where multicollection is needed to help resolve small signals in carbon isotopic signatures or where large fluctuations in secondary ion yield is expected. Additionally, the instrument will be used for the development of other isotopic measurements where the larger magnet is desirable to achieve high transmission at high mass resolving power. A letter is included here indicating the enthusiastic agreement of the UCLA group for the collaborative research proposed. The additional ion probe time needed for the proposed research including the SIMS analysis of trace metals will be purchased from one of the other NAI groups that have a CAMECA 6f. These instruments are monocollection instruments smaller than the 1270, but also equipped with normal incidence electron guns for charge compensation.

University of Pittsburgh, Department of Geology and Planetary Science

Rosemary Capo & Brian Stewart

The University of Pittsburgh Geochemistry Facility includes a clean chemistry laboratory housed in a 490 sq. ft. space, and provided with positive pressure and filtration to a 95% level. It contains 16 lineal feet of hood space as well as ULPA filtered laminar flow stations, HEPA-filtered microbalance room and a Millipore Milli-Q reverse osmosis-filter water system for ultraclean chemical procedures. Nearby sample preparation and acid cleaning laboratories contain fume hoods, muffle furnace, ovens, ultrasonic bath, centrifuges and balances.

Major instruments are housed in a 500 sq. ft. lab filtered to the 95% level with positive pressure and fume hoods. Isotopic analyses are determined on a Finnigan MAT 262 thermal ionization mass spectrometer (TIMS) with eight faraday collectors (seven movable, including high mass cup fixed at ^{208}Pb-^{207}Pb position) and a RPQ+ high abundance sensitivity device. For elemental analysis of Fe and other metals, the laboratory also houses a Spectroflame Modula (FTM 08) axial inductively coupled plasma atomic emission spectrometer (ICP-AES), for major and trace element analysis. The instrument is equipped with an end-on plasma option, UV monochromator (160-480 nm) for sequential elemental analysis, and a seven-channel polychromator for simultaneous analysis of alkalis (Li, Na, K, Rb, Cs) and internal standard and background corrections (Sc, Ar).

The department’s rock sample preparation facilities include mineral separation equipment (motorized sieves, Frantz magnetic separator, density separation equipment), jawcrusher, ball mill, and rock saws. Petrographic analysis facilities include thin section preparation equipment and high quality petrographic microscopes. Additional XRD and scanning electron microscope capabilities are available in the University’s Materials Science Department, including a Philips

X'Pert powder (Cu Ka) and a. a Philips XL 30 field emission gun scanning electron microscope (SEM) equipped with an EDAX energy dispersive system.

A NASA-owned videoconference system (provided by NAI) is available for use by University of Pittsburgh Astrobiology faculty and graduate students. The equipment includes a Polycom videoconference system and monitor, VCR, video projector for simultaneous projection of remote graphics, and an electronic white board. The equipment is housed in a department conference room with full internet, cable and ISDN connections that is now being renovated (scheduled completion in late spring, 2003) specifically to accommodate the NASA equipment.