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University of Colorado Center for Astrobiology

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EXECUTIVE SUMMARY

The University of Colorado Center for Astrobiology proposes a coherent and integrated plan for research, teaching, and community development in astrobiology. We are located at a major research University, and our approach is to build on the substantial institutional commitments across the entire breadth of astrobiology, to assemble a team of scientists who together span the entire range of disciplines that comprise astrobiology, and to use this group to leverage resources via participation from a larger group of scientists who do research in astrobiology. Our Co-Investigators were included based on their cutting-edge research and their desire to help develop a larger program in astrobiology.

Research. Our research efforts are divided into three distinct themes that provide a structure under which the individual research tasks are organized, spanning the entire range of astrobiology disciplines. In addition, we propose a fourth theme based on the need for astrobiology technology development connected to NASA flight missions.

Theme 1: The origin and evolution of life on Earth. The evolution of life on Earth can be broken down into five qualitatively different processes: (i) Creation of appropriate conditions for a habitable world; (ii) the origin and early development of a genetic code; (iii) the evolution of biochemical pathways; (iv) the evolution of microbial life; and (v) the origins and evolution of multicellular life. We have designed our biology theme to cut across this diverse spectrum in order to examine these fundamental issues in the development of life on Earth. This will provide a more subtle understanding of how life elsewhere in the universe might develop. Our goal is to understand the major processes that governed the development of life on Earth, so that we can provide guidance as to the constraints on environments that will govern our search for habitable planets and life elsewhere in our solar system or on planets outside of our solar system.

The specific research tasks that we are proposing are: (i) Laboratory studies of the origins of an RNA world (led by Co-I M. Yarus). (ii) Evolution of biochemical pathways (led by Co-I S. Copley). (iii) Re-tracing steps towards a habitable world via the biogeochemical evolution of sulfur on the early Earth (led by Co-I S. Mojzsis). (iv) Molecular survey of extremophile microbial diversity in hypersaline ecosystems (led by Co-I N. Pace). (v) Origin of multicellularity and complex land-based ecosystems (led by Co-I W. Friedman).

Theme 2: The origin and evolution of habitable planets. Understanding the potential for life beyond Earth requires applying our knowledge of what allows an environment to be capable of supporting life to our discoveries regarding the various environments that exist within our solar system and beyond. In doing this, we can look at the different environments that exist on planets (and satellites) in our solar system and ask which ones are capable of supporting life, what factors control their habitability, and what processes are responsible for the evolution of suitable characteristics. To understand what makes a planet habitable and what makes a planet non-habitable, we need to look at specific characteristics of individual planets as well as at general characteristics that might apply to all planets. We will approach the problem of understanding planetary habitability by looking in detail at the two objects in our solar system other than the Earth that are most likely to be habitable (Mars and Europa) and by examining more general physical and chemical processes that control habitability. In addition, we will examine the astrophysical processes that govern the formation of planets to begin with.

The specific research tasks that we are proposing are: (i) Biogeochemical cycling and resources on Mars (led by P.I. B. Jakosky). (ii) Geological evolution and habitability of Europa (led by Co-I R. Pappalardo). (iii) The impact of atmospheric aerosols on life (led by Co-I O.B.

Toon). (iv) Geochemical-microbe interactions in chemolithoautotrophic communities on Earth (led by Co-I T. McCollom). (v) Astrophysical constraints on the origins of planetary systems (led by Co-I Bally).

Theme 3: Philosophical and societal issues in astrobiology. Astrobiology is set apart from many other scientific disciplines by its relevance to understanding the ways in which the science interacts with the broader society. Ranging from using astrobiology as a way to better understand the nature of science to exploring the public's fascination with the topic and the societal implications, astrobiology provides a powerful way to better understand these interactions. Our group is one of the few that from the beginning has explored the connections between the sciences and the humanities, and we will continue to do so during the coming five years.

The specific tasks that we will explore are: (i) The nature of historical science and the elucidation of biosignatures from the perspective of philosophy of science (led by Co-I C. Cleland). (ii) Societal issues in astrobiology (led by P.I. B. Jakosky). (iii) The emergence of astrobiological thought from evolution (led by Co-I W. Friedman).

Each of these three research themes will coordinate its activities through a discussion group involving the key research personnel and other interested persons. The purpose of each group will be to bring together the individual research tasks into a coherent program and to provide cross-task interaction that integrates the individual tasks.

Theme 4: Astrobiology technology development. The NASA Astrobiology Institute has little formal connection to the NASA flight missions other than through the activities of its individual members. This is especially problematic with regard to the need to develop technology for instruments and missions that will allow upcoming missions to explore astrobiological problems. We propose to create a technology consortium with nearby Ball Aerospace Corporation and Lockheed Martin Astronautics (LMA) that will bring together scientists and technologists from academia and industry to work together to define the technology needs for astrobiology. This activity will help to create a coherent astrobiology community, will bring the astrobiology science community up to speed on how to design, develop, and implement flight programs, and will serve the goals of the NAI and of NASA by helping to integrate the technology programs into a coherent program. We will hold workshops, run short courses, and write white papers on the technology and science needs, and communicate the results to the community and to NASA Headquarters. Participants who will form the core of a community steering group include scientists from the CU program (led by P.I. Jakosky), senior technologist from the LASP Engineering Division that oversees instrument, spacecraft, and mission development and mission operations (Co-I M. McGrath), senior personnel from Ball (Harold Reitsema and Steve Kilston) and from Lockheed Martin (James Crocker and Ben Clark), and a leader from the biotechnology development area (Collaborator Andrew Steele from Carnegie Institution of Washington).

Education and public outreach. We propose three distinct yet complementary efforts that together comprise a comprehensive program of education and public outreach: (i) We will continue our public symposia in astrobiology at the University of Colorado in order to interact with the local university community and the Boulder-Denver public. These will address diverse aspects of astrobiology, and will bring cutting-edge research to the public along with thoughtful discussion of the broader societal significance. Speakers will be selected from nationally known scientists. (ii) We will take public symposia involving CU astrobiologists to communities and colleges that typically are not able to interact with leading scientists. We will visit 2- and 4-year colleges that do not have funds to bring in outside speakers, as well as colleges attended predominantly by historically underrepresented groups. These programs, provided at no cost to the receiving institution, will allow students and faculty to interact with leading astrobiologists in both small groups and large venues. We will do three of these per year, with a "warm up" symposium planned for April 2003 at Ft. Lewis College (Durango, Colorado), and have planned tentatively our next symposium for Hampton University in Virginia. (iii) We will educate and inform the public via a series of workshops intended to provide background material to science journalists on issues in astrobiology. The goal is to inform the writers of the deep background of the science issues, so that they can better report on the issues. Our first workshop will be held in December 2003, and will deal with the Mars landings to take place in December and January. Emphasis will be on the missions, the science to be done, and the astrobiological connections.

Astrobiology community development. We are extremely active in helping to develop the astrobiology community. Activities include leadership and participation in the NAI Focus Groups, in senior-level committees that have involvement in and oversight of astrobiology research activities, on the editorial boards of astrobiology-related journals, in NASA flight missions pertinent to astrobiology, and in national and international conferences devoted to astrobiology.

Training. CU has created a number of undergraduate and graduate level classes in astrobiology and its component disciplines. We have instituted a Graduate Certificate in Astrobiology that provides training and recognition for students who are emphasizing one of the component disciplines. We are active in training graduate students and postdoctoral research associates in astrobiology and its component disciplines.

Collaboration and networking. The CU Center for Astrobiology will continue to participate in activities designed to take advantage of the "virtual" aspect of the NAI. We will participate in videoseminars, videoconferencing, and ongoing technology development and implementation activities led by the NAI. We will utilize the videoconferencing equipment for point-to-point discussions and research collaborations with other NAI teams.

Institutional commitment. CU has a commitment to developing the discipline of astrobiology, as seen for example in its hiring of several new faculty in astrobiology during the past four years. In addition, it is committing resources of funding from state funds, matching funds on equipment, Graduate Teaching Assistant support to enhance interactions between astrobiology research and teaching, IT/Tech personnel support, and faculty time to participate in programmatic and scientific issues in astrobiology. Ball and Lockheed Martin are both providing matching support in kind. In total, the dollar and equivalent dollar value of resources contributed by CU, Ball and LMA sum to approximately $1.3 million over five years.

Management. Each Co-I will have authority over funds allocated to them, with oversight provided by the Laboratory for Atmospheric and Space Physics and the University of Colorado. Subcontracts will be made to Ball and LMA to participate in our collaborations. The P.I. has final authority and responsibility over all expenditures, subject to the rules and regulations of the University of Colorado, the State of Colorado, and NASA.

Research and Management Plan

1. INTRODUCTION

1.1. The discipline of astrobiology

Astrobiology as a suite of disciplines has taken on a life of its own (so to speak) in the last few years, driven by major discoveries that point to the potential for life to exist beyond the Earth. On the Earth, these discoveries include the nature of the early environment and the earliest evidence for life, our understanding of diverse life functions, and the wide range of environments that can support life. Within our solar system, we have learned about the occurrence of similar environments on other planets and satellites, the processes responsible for producing the architecture of our solar system, and their implications for formation and evolution of other planetary systems. And discoveries beyond our solar system, of course, include the discovery of planets orbiting other stars and the implications for Earth-size and Earth-like planets.

Astrobiology brings together these otherwise disparate fields into an integrated approach to understanding the boundary conditions surrounding the existence of life. As such, it not only cuts across the components of space science and biological science, but integrates them together in support of addressing a common theme. In this sense, the issues surrounding the possible existence of life elsewhere have become, *de facto*, the intellectual centerpiece of the NASA space science efforts. This can be seen, for example, in the recent National Research Council Decadal Survey on Solar System Exploration, in which understanding the potential and actual distribution of life in our solar system is included as one of the highest level goals of the planetary program, and in the recent NASA Space Science roadmaps produced by Code S, which emphasize the connections of each discipline to astrobiology.

The strength of astrobiology, and of the NASA Astrobiology Institute (NAI), lies in its ability to address the interdisciplinary aspects of the field. In order to make advances in astrobiology, we need to understand not just whether there is life on a particular planet, for example, but also why and how the planet evolved to be able to support life or not, how life interacts with the local and global environment on a planet (including the Earth), and how these same governing processes might play out on other planets and in other planetary systems. This way, we can begin to understand the boundary conditions surrounding life's occurrence, and use them as a guide to determining what the distribution of life throughout the universe is likely to be (e.g.: Drake and Jakosky, 2002). In this context, the major components of astrobiology center on understanding the following (as described, for example, in the recent roadmap for astrobiology):

- The environment on the early Earth and its ability to support life;
- The processes involved in the origin of life on Earth, and the earliest evidence for life on Earth;
- The biological record of early life on Earth, the evolution of life, and the origin of complex life;
- The origin and evolution of our solar system and the nature of the governing processes;
- The environments and potential for life on other planets and satellites in our solar system, and whether life actually exists on any of them;
- The characteristics of extrasolar planetary systems and the implications for understanding the origin and evolution of planetary systems and Earth-like planets; and
- The habitability of extrasolar planets and implications for life beyond our solar system.

Thus, astrobiology requires not only in-depth investigation of problems within each relevant discipline but also interdisciplinary collaboration in order to relate each set of issues to the others.

1.2.. University of Colorado approach

As an educational institution, the University of Colorado plays an important role in the broader program. We are in a position to bring together expertise in each of these science areas to focus on the common problem of understanding the nature and distribution of life in the universe, and to create a program of training the next generation of citizens, scientists, and astrobiologists through undergraduate, graduate, and postgraduate programs. Our approach in addressing the science questions in astrobiology is as follows:

- To support research from the breadth of astrobiology, by including as Co-Investigators researchers who, together, span the entire range of disciplines that comprise astrobiology;
- To promote cross-disciplinary interaction, both formally and informally, among the Co-Is and their research groups, and between Colorado participants and other researchers both within the NAI and outside of it, in order to address interdisciplinary and multidisciplinary questions; and
- To use the Co-Is and the program as a means of connecting to a larger group of faculty and researchers both locally and nationally who are involved in astrobiology-related research, in order to leverage the NAI support into a much larger program.

Using these concepts as broad guidelines, we have developed a research program to take best advantage of the university setting and expertise. Our astrobiology program was initially built five years ago on the cornerstones of the existing University strengths that also are cornerstones in astrobiology — RNA chemistry and evolution, planetary science, and astrophysics. We added expertise (including new faculty hires) in biochemistry of metabolism, microbial ecology and life in extreme environments, the geological record of ancient terrestrial life, and the humanities. In addition, for this proposal we are adding expertise in the complementary areas of the outer solar system and terrestrial and planetary biogeochemistry. Our approach of breadth rather than focusing our resources on addressing a single problem takes advantage of the strength of being in a university environment, and is complementary to the approach taken by individual research groups elsewhere or at national laboratories.

Our approach to developing our proposed research is as follows:

- To focus research on distinct science themes in order to maximize cohesiveness and collaboration;
- To "evolve" our program, rather than automatically continuing the same activities, by including new Co-Is and new research tasks (e.g., five of our eleven science Co-Is have been added since we began our efforts five years ago); and
- To include an additional theme, one that deals with astrobiology technology development in support of upcoming space flight missions, in order to help the NAI and the astrobiology community as a whole develop a stronger connection to the flight programs.

Through this approach, the University of Colorado will continue to build a substantial program in astrobiology that recognizes the unique, coherent, and integrating nature of astrobiology while at the same time fostering the connections to a much broader research program than can be funded through the NAI. We expect that we will continue to contribute in significant ways to the overall efforts within the NAI and to the broader national effort in astrobiology.

1.3. Science and technology themes

We are proposing tasks that take advantage of the expertise of each individual Co-Investigator and that fit together into distinct science themes. These themes link closely related tasks, allow each group to address complex problems that span a range of topics, and provide the guiding structure to our research program. In addition, we propose a fourth theme involving technology development that allows us and the NAI to address the central issue of the connections between astrobiology and the flight programs. These themes are described briefly here and then expanded on in the remaining research portion of the proposal.

The four themes are:

(i) The origin and evolution of life. Life on Earth is the only example we have of life in the universe, and we use it to understand the nature of life, its history, its interactions with its local environment that drive ecosystem evolution, and how it can inform our view of what makes a planet habitable or non-habitable. We propose five distinct tasks that fall under this theme and make substantial contributions to our overall understanding. These tasks address the behavior of RNA as a catalytic agent and the nature of the RNA World that likely preceded our present DNA world; microbial diversity and ecology in a complex ecosystem (the Guerrero Negro hypersaline ecosystem in Baja California) in order to understand the general character of interactions between organisms and their environment and the processes of ecosystem evolution; the nature of evolution at the biochemical level and, in particular, gene duplication and protein functions in archaea; evolution at the macroscopic level and implications for the development of complex ecosystems either here on Earth or around other stars; and the characteristics of the Earth's early environment and biosphere and of their mutual interplay.

(ii) The origin and evolution of habitable planets. With an understanding of the characteristics that an environment must have in order to be able to support life, we can ask where in our solar system these conditions might be met and how the processes that control planetary formation and evolution operate, both in our own solar system and in other planetary systems. From the more specific to the more general, the tasks within this theme include determining the extent to which Mars has had habitable environments and the geochemical constraints that we can place on possible martian life and ecosystems; the extent to which Europa has evolved over time, and implications for the habitability of its (probable) global subsurface oceans; studying the physical, chemical, and radiative effects of atmospheric aerosols and their ability to control the evolution of climate on terrestrial planets (including the early Earth); exploring the biogeochemistry of environments on Earth that can support chemolithoautotrophs, with implications for the potential for life to exist on Mars, Europa, or elsewhere; and determining the ability of planetary systems to form in "hazardous" stellar environments, using direct observations of forming systems.

It is not surprising that some of the tasks proposed do not separate cleanly into either the "biology" or the "planetary" theme, but overlap both. This is apparent in the tasks dealing specifically with interactions between terrestrial organisms and their environments. It results from the fact that the Earth is a planet, too, and that we need to understand terrestrial environments and their interactions with microbes in order to understand how planetary environments might be capable of supporting life.

(iii) Philosophical and societal issues. One aspect of astrobiology that separates it from many other science disciplines is the tremendous public interest in it, and this interest allows us to use astrobiology to explore the connections between science and society. This includes various philosophical and societal issues, and our group is one of the few groups that has been

exploring these themes by including participation from the humanities as an integrated component of our program. We are proposing three tasks that explore these issues: the philosophy of science issues in astrobiology, the nature of defining life, and the implications for life-detection strategies; the societal impact of astrobiology, including in particular the reasons behind the tremendous public interest in life elsewhere; and the history of evolutionary thought ("the evolution of evolution") and the connections between early evolutionists and astrobiological thought.

(iv) Astrobiology technology development initiative. The NAI exists in part to "provid[e] scientific and technical leadership on astrobiology investigations for current and future space missions and for ongoing research programs" [quoted out of the NAI mission statement]. However, there has been minimal connection between the NAI and advance planning for flight programs except through the separate efforts of individual members. We are proposing an astrobiology technology development initiative, in which we will involve scientists and technologists from academia and industry to provide leadership to the astrobiology community in this area. We propose to create an oversight group on astrobiology technology development within the structure of the NAI focus groups, and to use this as a vehicle by which to organize community workshops on technology, to offer short courses for astrobiologists and technologists, and to work with NASA to galvanize the community and provide direction to technological development. In order to reach beyond the existing players in astrobiology technology and to increase "P.I. savvy", we are involving members of industry as key participants in our efforts, and bringing together within a single forum the necessary science, instrument, spacecraft, mission, and biotechnology leadership.

2. THEME 1: ORIGIN AND EVOLUTION OF LIFE

The evolution of life on Earth can be broken into discrete and qualitatively distinct processes: (i) Creation of appropriate conditions for a habitable world; (ii) the origin and early development of a genetic code; (iii) the evolution of biochemical pathways; (iv) the evolution of microbial life; and (v) the origins and evolution of multicellular life. We have designed our biology theme to cut across this diverse spectrum in order to examine these fundamental issues in the development of life on Earth. This will allow us to gain a more nuanced understanding of how life elsewhere in the universe might develop. Our explicit goal is to understand the major processes that governed the development of life on Earth, so that we can provide guidance as to the constraints on environments that will govern our search for habitable planets and life elsewhere in our solar system or on planets outside of our solar system. We will focus on broad aspects of the problems faced by life on Earth at a variety of different times in its development. This will provide the greatest potential to elucidate key hurdles that life had to "overcome" as well as to provide details on how these key evolutionary transitions might have taken place on Earth or elsewhere in the universe.

Our specific tasks are as follows, with each being described in detail in the remainder of this section.

(i) Laboratory studies of the origins of an RNA world (led by Co-I Yarus). We will experimentally explore the properties of the RNA molecule that might have allowed it to develop the ability to catalyze chemical reactions and thereby led to an RNA world. To do this, we will determine the utility of short sequences of RNA that might have allowed much smaller numbers of molecules to serve as the original source for chemically reactive molecules, explore the nature of the active region of a particular ribozyme, and examine the interactions of RNA molecules with mineral surfaces that

might have acted as efficient concentrators to increase reactivity.

(ii) Divergence of protein families to provide novel functions (task led by Co-I Copley). A key process that enabled the evolution of the complex biochemistry typical of modern microbes was gene duplication, followed by mutations that allowed development of a new function in the protein encoded by one copy. Thioredoxins are ancient proteins that are found in all forms of life and therefore probably existed in the last common ancestor. They provide an excellent system for studying the recruitment of ancient proteins to serve new functions via gene duplication and evolution. We will examine thioredoxins in a hyperthermophilic, sulfate-reducing member of the domain Archaea, in order to understand the evolution of multiple proteins from a single ancestral protein.

(iii) Re-tracing steps towards a habitable world: The biogeochemical evolution of sulfur on the Early Earth (task led by Co-I Mojzsis). We use the geological record of the Earth to understand the evolution of planetary habitability, focusing in particular on the information that can be derived from sulfur isotopes as a constraint on biogeochemical processes. Sulfur isotopic measurements provide detailed information on mass-independent fractionation processes involving anoxic atmospheres. We will continue our analysis of sulfur from key locations in Precambrian terranes, in order to provide key details on the abundance of oxygen at the time that the sulfur-bearing minerals were deposited. This history of oxygen is important for understanding the evolution of planetary climates and habitability.

(iv) Molecular survey of extremophile microbial diversity in hypersaline ecosystems (task led by Co-I Pace). Because of their relative stability and comparative simplicity, hypersaline mats have become models for studies of chemical and energetic factors that support photosynthesis-driven communities. The principles derived from such studies are pertinent to planetary ecology, which is the summation of many such systems, and to the nature of life in extreme environments. We will continue our detailed study of the Guerrero Negro hypersaline mats as a model extreme ecosystem, surveying the microbial makeup and organization of selected hypersaline mats using culture-independent molecular methods based on cloning and sequence analysis of ribosomal RNA (rRNA) genes.

(v) Origin of multicellularity and complex land-based ecosystems (task led by Co-I Friedman). We plan to significantly enlarge the scope of our molecular investigations to investigate the role of symbioses associated with the establishment of photosynthetic multicellular life on land. Recent paleontological evidence suggests that the one-time colonization of land-based environments by plants may have required a key symbiosis between plants and fungi (in essence a cooperative relationship between photoautotrophs and heterotrophs). Our goal is to sample the fungal symbionts of representative members of all living plant lineages whose origins can be traced to the first one hundred million years of multicellular plant life on land. The results will provide a better understanding of the establishment of complex ecosystems as they have evolved here on Earth and that might populate Earth-like planets around other stars (and thereby be suitable for detection from Earth).

2.1. Laboratory Studies of the Origins of an RNA World (led by Co-I M. Yarus)

2.1.1. Introduction and premise.

There is only one current idea for the succession from an earlier Earth biota that commands significant experimental evidence. That idea is the RNA world — the notion that the present kind of life on Earth was preceded by simpler creatures that used only RNA (ribonucleic acid or some molecule that resembles it) for everything that modern cells do using protein, DNA, and RNA. While there is much data supporting these biological events (despite the fact that they probably occurred ca. 4 x 10^9 years ago), data that would apply to the beginnings of life in other settings also, the main difficulty lies in seeing how RNA molecules for the RNA cells (ribocytes) could themselves have originated. Below, we summarize several areas we will pursue during the period of this proposal which bear on the origins of the primordial ribocytes and its RNAs. A successful conclusion to these experiments would define what sorts of RNA molecules must have been present in an early-life setting, how the catalysts needed for RNA cells could have been found among them, and in particular how the RNAs needed for the first translation system might have arisen.

2.1.2. Studies of the minimal evolutionarily useful RNA populations.

RNAs are the simplest molecules currently known to catalyze reactions and replicate themselves. This makes them logical candidates for catalysis and replication in early cells. However, RNAs are large and their synthesis is difficult, particularly under prebiotic conditions. The relevant question then is: how much of this rare species is actually needed? In other words, what is the smallest RNA population that can serve for the isolation of new RNA activities? Clearly the smaller the magnitude of the answer, the earlier during life's history on Earth and the more likely it is that RNA organisms could have become possible. Our first potentially realistic calculations of the amount of RNA (the number of molecules of arbitrary sequence) needed to evolve particular ribozymes like those that would have been required by the early RNA cells (ribocytes) suggested that the number of random-sequence RNA molecules required is anti-intuitively small (Yarus and Knight, 2002; Knight and Yarus, 2003). Zeptomoles of RNA molecules (from 1 zmol = 602 molecules to 1000 zmol [1 amol], 602,000 molecules), less than in a modern bacterium, might suffice to begin accumulating RNA activities (the "zeptomole world" hypothesis). This range begins 12 orders below the nanomole levels used in modern selection experiments. Thus, an RNA world could be up to 12 orders more accessible than would have been thought from modern selection experiments. Alternatively, we might express the result by saying that the problem of prebiotic synthesis of nucleic acids could be up to 12 orders less severe than might have been thought from a survey of modern experiments.

Our proposed work will extend these findings, which bear significantly on the initiation of an RNA world. First, we will extend our mathematical and statistical studies by performing experiments intended to confirm or correct them. We necessarily predict that the methods of selection-amplification, in which RNA active sites are isolated from experimental populations of molecules of randomized sequence, can be used to isolate RNA catalysts from zmol of independent sequences instead of the nmol usually used. This would confirm the existing analytical calculations (two independent methods come to similar numerical conclusions; see Yarus and Knight, 2002, and Knight and Yarus, 2003) as well as the computer simulations (searching *in silico* through short computer-generated randomized sequences) that agreed with the theory.

The experiments are selection-amplifications or SELEX protocols, using decreasing numbers of independent sequences to isolate a given RNA structure. We propose to isolate the isoleucine-binding RNA we have called the UAUU motif (Majerfeld and Yarus, 1998), which is simple, well characterized, and has an interesting relationship to the code for isoleucine (reviewed in Yarus, 2002). The goal of the experiment is to show whether the isolation of the isoleucine binding site continues down to zmol of RNA molecules. One thousand zmol however, is so few molecules (6.02 x 10^5)

that it is difficult to handle such pools of individual molecules without having them disappear, say by irreversible adsorption onto the walls of the plastic tubes used for experiments. Therefore, the pools will contain normal total numbers of RNA molecules (e.g., 10^{14}) but with each sequence replicated 10^8 times; thus one can have an initial amount of RNA that is practical to handle though it only contains zmol of different sequences.

Successful experiments would make an RNA world a much more plausible event in the Earth's history and in the possible history of life elsewhere. These experiments might require the full attention of a postdoc and graduate student for 2 years.

2.1.3. Poisoning.

How is it possible that no one has noticed that modern selections employ 12 orders of magnitude too many RNA molecules? There may be an unappreciated 'poisoning' phenomenon that has hidden the true nature of selection. The essence of this is that molecules with too many randomized positions actually inhibit the folding of potential active sites within themselves. Imagine a small sequence (the potential active site) in the midst of a much larger arbitrary nucleotide sequence (the rest of the randomized region). The small active site can be poisoned by the accidental ability of the surrounding nucleotides to pair with it and thereby alter the folding of the potentially active sequence. Thus, many possible active RNAs are not detected, and the larger the randomized sequence, the worse the problem. Accordingly, an immense number of molecules must be used to survey many that have a potential active sequence, in order to find the rare example that has an active site that will fold unmolested. The result is that experiments have greatly overestimated the numbers of molecules required in a selection, and thereby greatly underestimated the capabilities of RNA. This effect was noticed by Sabeti et al. (1997), but we believe was greatly underestimated.

We therefore propose computational experiments in which RNAs containing the subsequences required for an active isoleucine binding site are mostly composed of arbitrary sequences to see how often folding of the isoleucine site is preserved. Some such sequences can be synthesized by transcription and experimentally tested for isoleucine binding by isoleucine-Sepharose affinity chromatography, in order to confirm the validity of the conformational calculations. However, if the calculations are shown to be relatively accurate, cases can be computed much faster than experiments can be done, and a more refined understanding of the magnitude and conditions of poisoning can be reached. Active sequences become more probable if there is a bigger sequence neighborhood to search for them; however, bigger random sequences are more likely to poison their contents. Thus, it is likely that there is an optimal size for the randomized molecule used to search for a new activity. Therefore, these experiments should illuminate the size of the randomized sequence required for selection of primordial RNAs as well as the modern practice of SELEX.

These computations and related experiments are the work of about 6 months to one year for two persons, postdoctoral or graduate student.

2.1.4. New transition state analogues for the peptidyl transferase.

During the previous funding cycle, our lab helped Nissen et al. (2002) to establish that the ribosome is a ribozyme, making peptide bonds using an RNA catalyst that probably dates to the RNA world itself (Nissen et al., 2000). Our contribution was in devising a transition state analogue, CCdApPuromycin, which bound specifically to the ribosome's active site. CCdApPuro thereby identified the ribosomal elements involved in making peptide bonds. To better understand this RNA patrimony from the Earth's early history, new transition state analogues will be tested and supplied to crystallographers studying the structure of ribosomes and, particularly, peptidyl transferase. In particular, there is evidence that the dA of CCdApPuro, which is an unnatural deoxyribose sugar right at the point of reaction, would be better replaced by a ribose in CCApPuro.

We therefore propose the synthesis and characterization of CCApPuro, particularly with regard to whether it fits the peptidyl transferase active site better than does the original transition state analogue. These experiments would involve studies of binding to the peptidyl transferase center, as well as crystallography to define the position of the compound within the ribosome. Synthesis and biochemical characterization of CCApPuro will require one to two work years of effort by a postdoctoral level bioorganic chemist.

2.1.5. RNA interactions with mineral surfaces.

Having reduced the likely magnitude of the RNA abundance problem by up to 12 orders, we propose to virtually eliminate it as a salient concern. Our hypothesis is that the final step of the solution to RNA abundance is chromatography on natural minerals. That is, we expect that certain plausible Archean minerals (obtained and manipulated in collaboration with our geochemical colleague Steve Mojzsis) will adsorb and release RNA under different likely early Earth conditions. This will produce the well-known effects of RNA chromatography — concentration, stabilization and purification.

Concentration — RNA can be adsorbed from a large volume and released in a small volume, thereby harvesting the RNA synthetic efforts of a bigger natural reactor. This is the primary effect of adsorption of RNA, that it can be concentrated from a large volume and made more available. However, this primary effect is probably supported by other consequences of chromatographic adsorption. For example, Stabilization — adsorbed RNA can be much more stable than RNA free in solution because internucleotide bonds of adsorbed RNA cannot rotate into conformations favorable to hydrolysis, again boosting the concentration of available RNAs. RNA could therefore have appeared over a long period and been preserved on a mineral ‘sink’. Purification — Some RNAs should adsorb better or elute better or both, thereby partially purifying the necessarily heterogeneous products of prebiotic chemical nucleic acid synthesis. Clearly, a potential combination of these three chromatographic effects could deliver unexpectedly concentrated and somewhat purified populations of RNA to hypothetical early ribocytes.

Therefore we propose the laboratory synthesis of pure samples of the following common cationic clays:

Kaolinite, $Al_4[Si_4O_{10}]\,(OH)_8$
Illite, $K_{1\text{-}1.5}\,Al_4[Si_{6.5\text{-}7}Al_{1\text{-}1.5}O_{20}]\,(OH)_4$
Smectite, $Ca_{0.5}\,Na_{0.7}\,(Al, Mg, Fe)_4\,[(Si, Al)_8\,O_{20}]\,(OH)_4.nH_2O$

and the study of the adsorption and elution of RNA molecules therefrom, and of the stability of bound RNA. Methods for elution should be simple and potentially prebiotic, such as temperature, high ionic strength (e.g., via evaporation), low ionic strength (e.g., via rainwater) or chelation (e.g., by oligophosphates). An elaboration of this project would be to use selection-amplification to isolate particular RNA molecules that bind and/or elute from the synthetic clays to see if particular sequence classes or structural types might have been accumulated in this way.

These studies have an inevitable uncertainty because of the exploratory element, in that synthesis of the materials must be optimized and a list of clays and chemical possibilities for adsorption and elution must be explored. Therefore we are estimating that this may require 3 person-years to reach an interesting point, with a postdoctoral and a graduate student as possible experimentalists. A clear success would illustrate how infrequent, dilute natural synthesis may have nevertheless yielded concentrated RNA populations that would have supported the emergence of a ribocyte. In addition, such experiments might help design an effective search for biological or protobiological materials in other planetary environments; e.g., on Mars.

2.2. Divergence of Function in an Ancient Protein Family: Thioredoxin-like Proteins in *Archaeoglobus fulgidis* (led by Co-I S. Copley)

2.2.1 Introduction

The diversification of microbial life on the early Earth involved an extraordinary expansion of the rudimentary capabilities of the first living organisms. Catabolic pathways evolved that allowed organisms to exploit a variety of sources of carbon and other nutrients. Biosynthetic pathways evolved that allowed synthesis of building blocks for cellular macromolecules. Elaborate control mechanisms evolved to allow rapid responses to changing environmental conditions. A key process that enabled the evolution of the complex biochemistry typical of modern microbes was gene duplication, followed by mutations that allowed development of a new function in the protein encoded by one copy. The extent of this phenomenon is vast. While there are over 18 million gene sequences in GenBank, the estimated number of distinct protein folds is only 10,000 (Koonin, 2002). Clearly, the adaptation of existing protein folds to serve new functions has been a dominant theme in the evolution of life.

Thioredoxins (Trxs) are ancient proteins that are found in all forms of life and therefore probably existed in the last common ancestor. The Trx fold is very versatile and has been adapted for many purposes as life has become more complex. Extant organisms contain multiple Trxs and Trx-like proteins. Although some of these proteins have very specific functions, others have retained overlapping functions. Thus, the divergence of Trxs has provided new functions that contribute to the metabolic and regulatory complexity of the organism, while maintaining some functional redundancy that protects organisms from deleterious mutations in critical genes.

The functions of Trxs have been studied primarily in bacteria and eukaryotes. Trxs serve as reductants for several enzymes involved in metabolic pathways and in defense against oxidative stress. These include ribonucleotide reductase, arsenate reductase, 3’-phosphoadenylyl-sulfate reductase, methionine sulfoxide reductase, and peroxiredoxin (Follman and Häberlein, 1995/1996). Eukaryotes have recruited Trxs to serve regulatory and signaling functions, as well (Follman and Häberlein, 1995/1996; Nishiyama et al., 2001). In chloroplasts, Trxs activate enzymes involved in the Calvin cycle (which incorporates CO_2 into carbohydrates) in response to light. Trx regulates several transcriptional regulators that control gene expression in response to changes in redox conditions. Trx also interacts with apoptosis-regulating kinase, which is involved in the signaling cascade that initiates programmed cell death. In multicellular organisms, Trxs have been recruited to serve as cytokines (chemical messengers that allow cells to communicate with each other) in the extracellular milieu.

Trxs provide an excellent system for studying the recruitment of ancient proteins to serve new functions as life became more complex and organisms diversified to occupy different ecological niches. An enormous piece of the puzzle is missing, however, because we know almost nothing about the functions of Trxs in Archaea. Studies of Trxs in Archaea will help us identify functions that are shared with bacteria and eukaryotes, and thus may have derived from the last common ancestor, and will also identify functions that have developed within the Archaeal domain in response to selective pressures experienced by Archaea in their particular environmental niches, which include some of the most extreme environments on the planet. We propose to study the functions of Trxs in *Archaeoglobus fulgidus,* a hyperthermophilic, sulfate-reducing Archaeon that is capable of growth on a variety of carbon and energy sources, as well as autotrophic growth on hydrogen, thiosulphate, and carbon dioxide (Klenk, et al., 1997). Homologues of several enzymes that are reduced by Trxs exist in *A. fulgidus*. However, we expect that Archaea in general and *A. fulgidus* in particular may have recruited Trxs to serve functions that have not been observed in bacteria or eukaryotes.

The aims of the proposed work are:

1. To identify protein targets that are reduced by the six Trx-like proteins in *A. fulgidus*;
2. To use bioinformatics coupled with an understanding of the physiology and metabolism of *A. fulgidus* to suggest functions for these target proteins; and
3. To purify the target proteins, test their functions *in vitro*, and confirm that the Trx-like proteins can reduce the target proteins.

2.2.2. Experimental Plan

Aim 1. Trxs use two active site cysteine (Cys) residues to transfer reducing equivalents to a variety of targets. These include a) enzymes in which a disulfide bond formed between two Cys residues during catalysis must be reduced in order to complete the catalytic cycle, b) proteins that are activated by reduction of a disulfide bond, and c) disulfides that are formed as a consequence of oxidative damage. The mechanism of these reactions is shown in Figure 2.2-1. A clever approach to identifying the targets of Trxs has recently been described (Motohashi et al, 2001). This approach uses immobilized mutant proteins that lack the second Cys required for completion of the thiol-disulfide exchange reaction to trap target proteins (see Figure 2.2-2). The target proteins become covalently attached to the Trx because the second Cys is not available to release them. The target proteins can be released by treatment with a small thiol reagent. We will use this procedure to separate target proteins from cell extracts of *A. fulgidus*. The released target proteins will be separated by gel electrophoresis. The bands containing each target protein will be cut out and the proteins will be digested with a protease in the gel slice (Rosenfeld et al., 1992). The masses of the resulting peptide fragments will be determined using MALDI-TOF (matrix-assisted laser desorption ionization – time-of-flight) mass spectrometry. Comparison of the fragments produced with those expected from proteins in the *A. fulgidus* genome will allow us to identify the genes encoding each of the target proteins. We will carry out these studies for each of the six Trx-like proteins in *A. fulgidus*.

Aim 2. The next step in our analysis will be prediction of function for the target proteins identified in Aim 1. *A. fulgidus* contains 2420 protein-encoding genes; functions have been predicted for about 45% (Klenk et al., 1997). Thus, we are likely to find target proteins for which no function has yet been predicted. For these proteins, we will attempt to predict function using a combination of clues. Information available at the National Center for Biotechnology Information (NCBI) includes a) assignments to groups of orthologous proteins in the COGS (clusters of orthologous groups) database (1930 proteins); b) identification of conserved domains (1531 proteins); and c) sequence similarity to proteins of known structure (778 proteins). We will supplement this information with searches for distant relatives with known functions using the Shotgun algorithm (Pegg and Babbitt, 1999) and motif analyses for identification of conserved regions using the MEME algorithm (Bailey and Elkan, 1994). We will incorporate knowledge of the genomic context of the gene, since genes involved in similar functions are often clustered in *A. fulgidus*. Finally, we will use Northern analysis (Ausubel et al., 1995) to measure mRNA levels observed in cells grown under various conditions to reveal the circumstances under which the gene is expressed. We have previously predicted and verified the function of several bacterial genes, so this effort is within our area of expertise. We expect to be able to predict functions for approximately 80% of the target proteins identified in Aim 1.

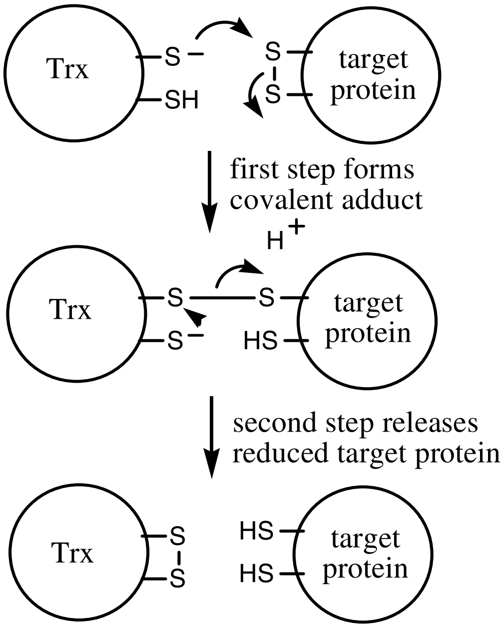


Figure 2.2-1. Transfer of reducing equivalents from the sulfhydryls (-SH) of the active site cysteines of thioredoxin (Trx) to oxidized target proteins. The oxidized Trx is subsequently reduced by Trx reductase.

Aim 3. Our next step will be to purify target proteins and verify both their functions and the nature of their interactions with Trx-like proteins. Each target protein will be over-expressed in *E. coli* using standard techniques (Ausubel et al., 1995). Proteins from hyperthermophiles can often be purified from *E. coli* cell extracts simply by heating the cell extract, causing the *E. coli* proteins to denature and precipitate. After removal of the denatured proteins by centrifugation, the desired *A. fulgidus* protein can be further purified if necessary by standard procedures such as ion exchange chromatography and size exclusion chromatography. (If necessary, we will use affinity-tagged proteins. However, affinity tags sometimes interfere with catalytic activity and protein-protein interactions, so we prefer to avoid them.)

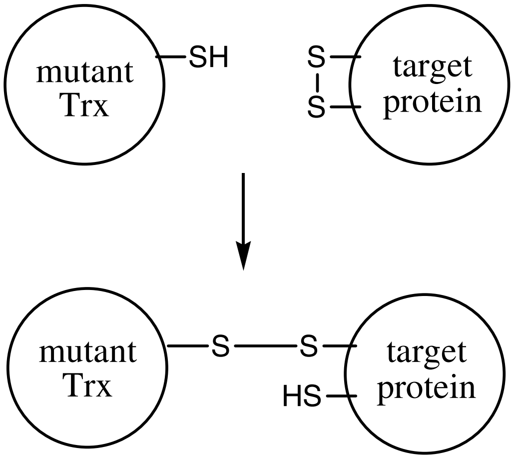


Figure 2.2-2. Trapping of target proteins by mutant Trxs that lack the second Cys at the active site.

For each target protein, we will set up an assay to quantify activity and the effect of Trxs. We will use literature procedures when available, and devise our own procedures when necessary. Dr. Copley has 20 years of experience in designing and executing enzyme assays, so we do not expect this step to be a problem.

Trxs are expected to play a role in either activation of the target proteins (by a one-time reduction of a disulfide that converts the protein from an inactive to an active form), or in substrate turnover (by providing reducing equivalents during every catalytic cycle). We will differentiate between these possibilities by carrying out assays in the presence of a) stoichiometric amounts of reduced Trxs, and b) catalytic amounts of Trxs and a regenerating system consisting of Trx reductase and NADPH (which will convert oxidized Trx to reduced Trx capable of interacting again with the target protein). If time permits, we will compare the abilities of different Trx proteins to interact with the target proteins so that we can assess both the specialization of certain proteins and the degree of functional redundancy in the system.

2.2.3. Relevance to Astrobiology

These studies are relevant to astrobiology for several reasons. First, they will flesh out the emerging picture of how Nature has modified the simple and ancient Trx-fold to serve a variety of metabolic and regulatory purposes as life became more complex. Second, greater understanding of the functions of Trxs in Archaea will supplement existing knowledge of their functions in bacteria and eukaryotes and consequently provide insights into the biochemical and physiological properties of the last common ancestor. Finally, these studies will enhance our understanding of metabolic pathways, gene regulation, and response to oxidative stress in *A. fulgidus*, a thermophilic sulfate-reducing Archaeon that is a valuable model for life in extreme environments.

2.2.4. Plan of Work

Experimental work will be carried out by a graduate student and a post-doctoral associate. Dr. Copley will supervise and train the graduate student and post-doc and carry out the bioinformatic tasks.

Year 1 – Clone the 6 Trx-like proteins from *A. fulgidus*; use site-directed mutagenesis to change the second active site Cys to Ser; express, purify, and immobilize each mutant protein

Year 2 – Isolate target proteins from *A. fulgidus* and characterize by mass spectrometry; use bioinformatics and transcriptional profiling to predict their functions

Year 3 – Clone and express genes for target proteins in *E. coli* and purify target proteins

Year 4 – Develop assays for approximately 5 target proteins; quantify activity and define role of Trx-like proteins

Year 5 – Continue Year 4 activities for an additional 5 proteins; if time permits, examine abilities of all 6 Trx-like proteins to interact with target proteins to identify overlapping target ranges

2.3. Re-tracing Steps Towards a Habitable World: The Biogeochemical Evolution of Sulfur on the Early Earth. (Research task led by S.J. Mojzsis)

2.3.1. Introduction.

A key element in the further success of astrobiology will be to use the terrestrial geologic record as a baseline for understanding the origin and evolution of a habitable world. Indeed, this knowledge is critical in developing plans for astrobiology-focused space missions such as the search for Earth-like planets elsewhere in the universe. Towards this aim, our research group seeks to constrain the duration and magnitude of the switchover from an essentially anoxic ($pO_2<10^{-5}$ present atmospheric level; PAL) to oxygen-rich (>0.1% PAL) planet between ~2.4 and 1.9 billion years ago (2.4–1.9 Ga) using the mass-independent behavior of sulfur isotopes. This pivotal era in Earth history is termed the “Great Oxidation Event” (GOE; Holland, 1984). What were the biogeochemical mechanisms for its advent? How did life respond to such a marked rise in free O_2? The rise in free oxygen on Earth led to the rise of a biosphere dominated by aerobic multicellular life. This life ultimately developed the ability to investigate its own origins. How long does it take an Earth-like world to reach this state?

To explore the record of the GOE, we exploit the fact that sulfur isotopes can exhibit mass-independent (as opposed to mass-dependent) fractionation behavior, thereby revealing unique information about past atmospheric oxygen levels and past metabolic styles of sulfur-utilizing organisms. We will undertake an extensive program of high-resolution multiple sulfur isotope measurements of sulfides preserved in rocks from the GOE; the new sulfur isotope technique described herein has been developed specifically for this study (Mojzsis et al., 2003).

2.3.2. Sulfur isotope variability in nature.

Sulfur isotopes (^{32}S, ^{33}S, ^{34}S and ^{36}S) have long been recognized as a powerful tool for deciphering geobiological processes on Earth (Thode et al., 1949). Sulfur isotopes can be used to follow the evolution of sulfur metabolisms (Monster et al., 1979; Canfield and Raiswell, 1999; Shen et al., 2001) and to explore fundamental interactions between chemical reservoirs from the dawn of the rock record, such as the rise of free oxygen in the atmosphere (Berner and Petsch, 1998; Farquhar et al., 2000, 2001).

Fractionation of sulfur isotopes ($^{34}S/^{32}S$) and ($^{33}S/^{32}S$) is expressed in the conventional δ notation (per mil; ‰) as:

$\delta^{34}S_{CDT}$ (‰) = $[(R^{34}_{\text{sample}} / R^{34}_{\text{CDT}}) - 1] \times 1000$

$\delta^{33}S_{CDT}$ (‰) = $[(R^{33}_{\text{sample}} / R^{33}_{\text{CDT}}) - 1] \times 1000$

where $R^{34} = {}^{34}S/^{32}S$, $R^{33} = {}^{33}S/^{32}S$, and the standard is Cañon Diablo troilite (CDT). On Earth, these values exhibit a range near 0‰ for igneous (0±5‰) and hydrothermal (0±10‰) rocks and ore bodies (Ohmoto and Rye, 1979). In contrast, biological effects have been invoked to account for the >150‰ range in $\delta^{34}S$ observed in sediments (Nielsen, 1979).

Plots of $\delta^{33}S/\delta^{34}S$ yield a highly correlated and mass-dependent array defined by the relationship $\delta^{33}S = 0.515\ \delta^{34}S$ (Bigeleisen et al., 1947; Hulston and Thode, 1965). The slope (0.515) in $\delta^{33}S$ vs. $\delta^{34}S$ arises from the mass dependence of vibrational frequencies, velocity terms, kinetic effects, diffusion, evaporation/condensation and metabolic fractionations. These processes fractionate according to the relative mass differences of the isotopomers such that $\delta^{33}S/\delta^{34}S$ variations define a slope of ~0.5 because of the ratio of the mass difference between ^{33}S and ^{32}S (1 amu) and ^{34}S and ^{32}S (2 amu) or about 1/2.

2.3.3. Mass-independent sulfur isotope effects – atmospheric and biological implications.

Some mass-*independent* fractionation (MIF) processes exist that result in anomalous 3-isotope values that do not describe the slope $\delta^{33}S/\delta^{34}S = 0.515$. MIF in sulfur is expressed as: $\Delta^{33}S = \delta^{33}S - 0.515\ \delta^{34}S$ when $\Delta^{33}S \neq 0$. Only certain gas phase reactions in the atmosphere, or nuclear processes as recorded in meteorites, produce MIF (Thiemens, 1999). UV SO_2 photolysis experiments in the gas phase result in both anomalous $\Delta^{33}S$ values and fractionations in $\delta^{34}S$ rivaling bacterial sulfate reduction (Farquhar et al., 2000; Farquhar et al., 2001), but only under *very low* pO_2 conditions.

2.3.4. Hypothesis – MIF can be used to constrain ancient terrestrial atmospheric oxygen levels.

Quantitative constraints on Archean pO_2 levels have long been sought. The current paradigm is that the free oxygen levels were much lower ($pO_2 \ll 10^{-2}$ PAL) before *ca.* 2.3 Ga (Cloud, 1972; Walker, 1977; Walker et al., 1983; Holland, 1984, 1994, 1999; Kasting, 1992, 1993, 2001; Rye et al., 1997; Rye and Holland, 1998; Farquhar et al., 2000; Pavlov and Kasting, 2002). An alternative view argues that the atmosphere was relatively oxygen-rich throughout the Archean (Dimroth and Kimberley, 1976; Towe, 1990; Ohmoto, 1996, 2001; Watanabe et al., 1997). A major review of the geological record of atmospheric evolution (Holland, 1984) noted several pieces of evidence as important for establishing the case for an anoxic atmosphere before ca. 2.3 Ga, highlighting in particular the study of detrital gold, uraninite and pyrite grains in ancient sediments (cf. Barnicoat et al., 1997). Other geochemical indicators such as (Fe^{2+} vs. Fe^{3+}) in paleosols (Holland, 1994; Rye and Holland, 1998; Rasmussen and Buick, 1999), arguments regarding the origin and possible mode of deposition of banded irons (Cloud, 1968), of isotopically light carbon isotope signatures in Precambrian organic matter (DesMarais, 1997) and isotopically heavy carbon in carbonates (Karhu and Holland, 1996) have been used to support models for an early anoxic atmosphere (cf. Ohmoto, 1999).

MIF behavior of sulfur provides three independent constraints on past pO_2.

(1) Separate mass-dependent arrays (*slope* ≈ 0.5) within Archean MIF $\delta^{33}S/\delta^{34}S$ data argue against effective pyrite oxidation and homogenization of both reduced sulfur and (oxidized) sulfate reservoirs either by biological cycling or inorganic processes. This supports conclusions based on paleosol and detrital mineral data cited above that pO_2 was $\leq 10^{-4}$ PAL and marine sulfate was of limited availability prior to ~2.3 Ga.

(2) Experiments delimiting the range of conditions for 190- to 220-nm (UV) SO_2 photolysis in the lower atmosphere and the lifetime of this reaction under various parameters succeed in producing large MIF signatures (Farquhar et al., 2001) mimicking those observed in ancient sulfides. This poses a problem for interpreting large sulfur isotope fractionations as exclusively due to biology. Experiments imply that atmosphere column abundances of O_3 and O_2 were so low that 190–220 nm UV could penetrate deep into the pre-2.3 Ga atmosphere. Anomalous $\Delta^{33}S$ can thus be used to place firmly quantitative upper limits to Archean pO_2 of ~ 10^{-1}–10^{-2} PAL.

(3) The only fractionation mechanism that appears to be consistent with the pre-2.3 Ga MIF sulfur data involves atmospheric reactions such as SO_2 photolysis by hard UV. Hence, chemical models must be developed to provide mechanisms for transferring MIF to the surface in early anoxic atmospheres. Theoretical work (Pavlov and Kasting, 2002) shows that MIF in sulfur aerosols are preserved from homogenization in oceanic sulfate reservoirs only in atmospheres with pO_2 well below 10^{-5} PAL. Models suggest it is not possible to create anomalous $\delta^{33}S/\delta^{34}S$ in high pO_2 atmospheres; the cessation of MIF signatures is complete before the $pO_2 = 10^{-5}$ PAL threshold is reached.

In both the experimental (1, 2) and theoretical (3) studies cited above, magnitudes of $\Delta^{33}S$ anomalies may be proportional to the residence time of reactive sulfur species (e.g., SO_2) in the atmosphere, balanced with the surface deposition of reaction products, including elemental sulfur (Pavlov and Kasting, 2002). The signature of this unusual $\Delta^{33}S$ sulfur chemistry can be transferred to and preserved in the geologic record, probably via sulfate and elemental sulfur (*e.g.* S^8) aerosols from the atmosphere into surface minerals, sediments, waters and ices (Farquhar et al., 2000; Thiemens et al., 2001).

2.3.5. Techniques: Measuring MIF in Precambrian sedimentary sulfides.

A new ion microprobe technique developed by our group and collaborator K. D. McKeegan (UCLA) can quickly resolve $\Delta^{33}S$ anomalies *in situ* at the intra-grain scale (<30 μm) of individual sulfides (pyrite, pyrrhotite, etc.) in rocks, facilitating exploration of the magnitude and distribution of MIF sulfur in a wide range of natural samples.

In a recent feasibility study (Mojzsis et al., 2003), we identified $\Delta^{33}S$ in several Precambrian sulfides and compared our results with $\Delta^{33}S$ data obtained by conventional bulk rock or mineral separate methods (Farquhar et al., 2000). More than 150 separate analyses were made on standards interposed with a suite of sulfide samples collected from high- to low-grade metamorphic sedimentary rocks spanning a large range of ages (Figure 2.3-1).

It is apparent from Figure 2.3-1 that data track a major change in sulfur geochemistry sometime between 1.9–2.4 Ga related to the demise of the $\Delta^{33}S$ effect. We ascribe this change to the rise of oxygen: $\delta^{33}S/\delta^{34}S$ compositions of samples older than ~2.4 Ga display MIF with a total range between $\Delta^{33}S$ = -1.72±0.34‰ (2σ) and +2.02±0.08‰ (2σ).

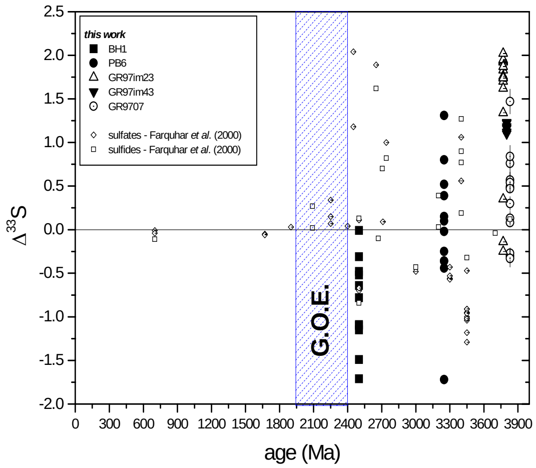


Figure 2.3-1

These values can only be explained by the presence of a global anoxic atmosphere facilitating the mass-independent fractionation of sulfur isotopes by UV-driven photolytic reactions in the gas phase. Samples younger than ~1.9 Ga fall within a relatively narrow range about $\Delta^{33}S \approx 0$‰ indicating that MIF sulfur was absent just as it is in the present O_2-rich atmosphere.

2.3.6. Hypothesis – Ancient sulfur metabolisms can be investigated by MIF.

Sulfur metabolism is probably an ancient biological phenomenon (Stetter, 1996). Many hyperthermophilic organisms in Bacteria (Thermotogales, Proteobacteria) and in Archaea (sub-Domain Crenarcheota, *Pyrococcus* and *Thermoplasma* in sub-Domain Euryarcheota) perform elemental sulfur (S^0) reduction (ESR) to H_2S using both H_2 and organic compounds as an electron donor (**bold** in Figure 2.3-2). Bacterial and Archaeal lineages share this metabolic style (Stetter and Gaag, 1983; Stetter, 1996; Canfield and Raiswell, 1999; Pace, 2001) underscoring the antiquity of this metabolic pathway and ESR may even be an inherited trait from the last common ancestor(s) of all life. Rapid cooling of volcanogenic sulfurous gases where $2SO_2 + 2H_2S \leftrightarrow 4S^0 + 2H_2O$, shifts the equilibrium in favor of S^0 (Grinenko and Thode, 1970) which is metabolically available for reduction to H_2S.

A consequence of anomalous $\Delta^{33}S$ sulfur aerosol formation is that sedimentary pyrite forming via ESR to H_2S would retain <u>positive</u> $\Delta^{33}S$ signatures, while pyrite forming from sulfate reduction would preserve <u>negative</u> $\Delta^{33}S$ values (Farquhar et al., 2001). The latter case may be seen in our data for *BH1,* a ~2.5 Ga BIF (Banded Iron Formation), where $\Delta^{33}S$ in sulfides is consistently negative (Figure 2.3-1). The pres-

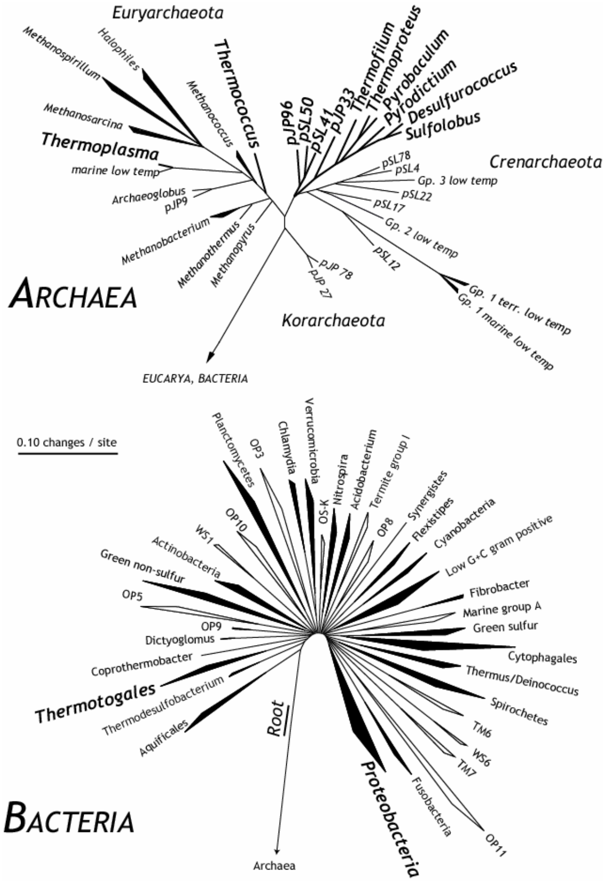


Figure 2.3-2

ence of positive $\Delta^{33}S$ in pre-2.4 Ga rocks and dominantly positive $\Delta^{33}S$ in pre-3.6 Ga rocks makes a compelling case that sulfur in these systems was cycled through the atmosphere and introduced to the oceans in the Archean/early Proterozoic. That MIF is preserved in the sediments clearly means that the sulfur was isolated from rehomogenization by volcanic and hydrothermal (non-anomalous) sources of sulfur. Moreover, sulfur aerosols derived from atmospheric reactions and deposited into the oceans would provide a ready source of sulfur to a plethora of microbial environments at the global scale.

To explore the significance of these results for the co-evolution of the atmosphere and metabolic styles requires us to obtain much more data on a variety of ancient sediments through an intensive project of fine-scale sampling of sediments spanning 1.9–2.4 Ga to track the disappearance of $\Delta^{33}S$.

2.3.7. Work Plan – MIF in sulfur traces the rate and duration of the oxidation of Earth.

Current data (Figure 2.3-1) are consistent with low pO_2 levels ($<10^{-5}$ PAL) and a photochemically active atmosphere before the mid- to early Proterozoic. However, data that we seek to generate in this project specifically aims to fill in the time period 1.9–2.4 Ga when the Earth went from anoxic to oxic (Figure 2.3-3) using samples collected from fieldwork with collaborator J.A. Karhu (University of Helsinki). How might the GOE correlate with the demise of a resolvable $\Delta^{33}S$ signal? Existing MIF data for this time period as plotted in Figure 2.3-3 underscore the urgent need for detailed sampling of rocks across this time interval. We will undertake collecting trips in northern Finland (Rovaniemi district) for samples containing pyrite that have been well characterized by geochronology (Perttunen and Vaasjoki, 2001) and stable isotope (carbon and oxygen) studies (Karhu, 1993). Pyrite (FeS_2) for MIF measurements will be selected for this study on the basis of textural and chemical evidence indicative of a syngenetic origin and analyzed by the ion microprobe methods cited here (see Greenwood et al., 2000; Mojzsis et al., 2003). All sample preparation, archival, microscopy and trace element analyses will be done at the University of Colorado.

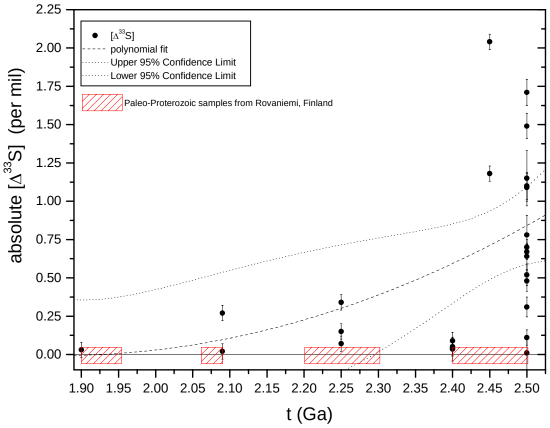


Figure 2.3-3

Collaborator J.A. Karhu (Univ. Helsinki) will provide samples for these studies in Yrs. 1-3. Cores stored in the Geological Survey of Finland and in open pit quarries of the Peräpohja schist belt will be sampled and sent to University of Colorado for petrographic, chemical and isotopic analyses on a regular basis. Mojzsis' lab will concentrate efforts on the sulfides from all field areas and will carry out MIF measurements; the isotope measurements will be made at UCLA and funded by subcontract from CU as described in the budget. In years 1-3 of the proposed research, team members will concentrate on preparing samples and gathering MIF data. Years 3-5 will be focused on finishing the analytical work and expanding the MIF search to other localities that potentially provide significantly improved or enhanced temporal resolution of the GOE. For instance, there is much potential in the Paleoproterozoic (1.9–2.4

Ga) sediments of the nearby Nash Fork Fm. in southern Wyoming (e.g., Bekker et al., 2003).

Budget justification of proposed work – The project requests support of a Ph.D. graduate student for their dissertation, as well as support of analytical expenses for Years 1-5. Collaborator K.D. McKeegan (UCLA) will assist with data collection by ion microprobe during the project. Collaborator J.A. Karhu (Helsinki) will undertake fieldwork and sample collection in Finland (Yrs. 1-3). Data analysis and interpretation will be shared by all project members and published in peer-reviewed journals. Exploration of unusual sulfur isotope chemistries in the Precambrian is an intensive analytical task on many hundreds of samples and will require the full 5 years of support requested in this proposal.

2.4. Molecular Survey of Microbial Diversity in Hypersaline Ecosystems (led by Co-I Norman R. Pace)

2.4.1. Overview.

Hypersaline microbial mats are complex, integrated ecosystems that occur worldwide, wherever evaporation results in a stable, high-salinity setting. Because of their relative stability and comparative simplicity, hypersaline mats have become models for studies of chemical and energetic factors that support photosynthesis-driven communities (Oren, 1999, for reviews). The principles derived from such studies are pertinent to planetary ecology, which is the summation of many such systems, and to the nature of life in extreme environments. The NAI Ecogenomics Focus Group (Sogin, DesMarais, co-Chairs) has undertaken study of selected Guerrero Negro hypersaline mats as a model extreme ecosystem. The combined goal of the Group is to define the relationships between microbial diversity, complex gene expression patterns, and biogeochemical gradients in shaping ecosystems and, ultimately, to predict the behavior of planetary systems.

The role of the Pace laboratory in this effort is to survey the microbial makeup and organization of selected hypersaline mats using culture-independent molecular methods based on cloning and sequence analysis of ribosomal RNA (rRNA) genes. This molecular census of microbial diversity is a critical component of the Ecogenomics Group effort, but also stands alone as an independent task. The results will provide a basis for relating microbial activities to the chemical properties of the system. The survey also is a substantial venture in microbial discovery. Remarkably little is known about the makeup of microbial communities in general and we have already encountered a wide breadth of novel diversity. Some of the results call into question past assumptions about the driving organisms in these systems.

2.4.2. Introduction.

Studies of the biological and geochemical properties of hypersaline ecosystems have been conducted worldwide, notably at Guerrero Negro, Mexico, in the context of the NAI Ecogenomics Group effort. Detailed measurements with microelectrodes of gas and sulfate fluxes (e.g., Jorgensen et al., 1989; Revsbech et al., 1989; Canfield and DesMarais, 1993) have painted many details of mat metabolism. Some groups of microbes have been studied, for instance cyanobacteria (D'Amelio et al., 1989) and sulfate reducing bacteria (Risatti et al., 1994). The mats are metabolically dynamic on the daily scale, driven by photosynthesis and oxygen metabolism during the daytime, and by sulfate metabolism at all times. Mats also are thought to be structurally dynamic, as organisms migrate in the search for favorable chemical zones created during the photocycle. Seasonal and ephemeral variations in local conditions undoubtedly influence the composition of mats, as well.

The outlines of the metabolic themes that drive these ecosystems are emerging, but our understanding of the organisms responsible for the chemical themes is rudimentary. The communities are expected to be highly complex. The main energy input to hypersaline mats is photosynthesis. Beyond photosynthesis, the general occurrence of high concentrations of molecular hydrogen (presumably a metabolic product) and sulfate fuel lithotrophic cycles; and, of course, organisms with heterotrophic metabolisms are supported by the overall primary productivity in the system. Hypersaline mats are highly stratified on the sub-millimeter scale. Oxic metabolism at the surface (ca. 1 mm) grades into anaerobic metabolism to depths of several cm. Organisms representing those various physiologies have been observed by culture or microscopy, but their actual contribution to the overall biomass could not be determined until recently. The actual makeup of the community could not be assessed with the traditional culture-based methods because most (>>99%) naturally occurring microbes can not be cultured using the usual techniques. Thus, any characterization of the microbiota of Guerrero Negro hypersaline mats that is relevant to large-scale chemical fluxes must use culture-independent techniques.

DNA sequence-based methods provide ways to identify and study organisms without the requirement for culture (Pace, 1997, for review). The phylogenetic types of organisms (phylotypes) that make up these communities are identified by ribosomal RNA (rRNA) gene sequences. The sequences also are the basis of molecular methods with which to study *in situ* the properties of organisms detected by the sequences. rRNA genes most often are obtained by cloning amplified products of the polymerase chain reaction (PCR). The rRNA sequences are specific identifiers of organisms independently of morphological or physiological variation. Thus, comparison of the sequences with known rRNA sequences reveals phylogenetic relationships of organisms in the community to known organisms. Furthermore, fluorescently labeled oligonucleotide hybridization probes that bind selectively to rRNA can be used with confocal microscopy for study of the physical distributions of particular organisms in environmental settings.

It is possible to infer some properties of an unknown organism based on the properties of its relatives because the organism, as a member of the particular phylogenetic group, is expected to have the properties that are common to the group. Beyond focus on particular organisms, the phylogenetic results identify novel biodiversity, previously unknown organisms, and potential sources of interesting or useful genes. Molecular studies of environmental diversity have expanded our view of microbial life dramatically, in bacterial, archaeal and eucaryotic lineages. Results have shown that our knowledge of the types and distributions of microorganisms that occur in the environment is rudimentary. There is a genuine need for aggressive sequence-based surveys of microbial community-composition in general. Our ongoing molecular study of the Guerrero Negro hypersaline mats is a leading example of how the methods (and philosophy) of culture-independent analysis are extending and revising our perception of microbial ecosystems.

2.4.3. Preliminary results.

The Pace laboratory has been involved in the analysis of hypersaline mats for about two years, in the context of the Ecogenomics Group. Our results in microbial diversity stand alone, however, independently of the success of other members of the Group. Based on extensive previous studies, efforts focus on a specific site, “Pond 4 near 5” at the Guerrero Negro salt works, Baja California, Mexico. We have made three collecting trips in concert with other members of the Group and have sufficient materials under various modes of storage for the introductory surveys. DNAs have been purified from different locations within the study site, and from different strata in mat cores. Our effort currently is on the analysis of rRNA sequences, which will constitute a phylogenetic census of the specific site under study by the Group. This work currently is conducted by Collaborators Drs. Ruth Ley and John Spear, postdoctoral fellows in the laboratory, with a few undergraduates. (Ley has been supported in part by an NAI fellowship. This will end 09/03 and further support will be from the proposed budget. Spear has been supported in part by an NSF fellowship, which ends 07/03. His further participation in the project will be as an Agouron Institute fellow. As these postdoctoral workers obtain academic positions, they will be replaced with other postdoctoral or graduate students.) We have so far accumulated a few thousand rRNA sequences from various strata in the mat, and analyses are ongoing. We have just obtained through a collaborative effort with the Sogin sequencing facility (MBL) another several thousand sequences. Preliminary analyses of the sequences indicate important and sometimes unexpected findings. A few highlights include:

1. The rRNA sequences document a vast extent of novel microbial diversity, including new candidate divisions (main phyla) of Bacteria. Surprisingly, the main contributors to the rRNA libraries from the photic portion of the mats, and presumably the main biomass of that portion of the mat, are not those of the classically expected, morphologically conspicuous cyanobacteria. Rather, the dominant bacterial rRNA sequences in clone libraries, even if made with different sets of PCR primers, are those of "green nonsulfur" (GNS) bacteria. GNS sequences outnumber those of cyanobacteria 6-7:1. If this result is validated (below), it will change fundamentally the conception of primary productivity (carbon flow) in these photic ecosystems. For instance, carbon incorporation currently is thought due mainly to the cyanobacterial Calvin Cycle, which posits the use of the enzyme Rubisco for carbon fixation. Only a few GNS bacteria are known, and they do not use Rubisco, but rather, conduct carbon fixation by "reductive carboxylation," a relatively little-known pathway. This finding potentially has impact on the interpretation of biogeochemical studies, for instance measurements of isotope fractionation in the mats.

2. We (John Spear with an undergraduate) have completed analysis of the microbial makeup of a laminated endolithic community in brine-saturated gypsum precipitates associated with the Guerrero Negro salt works. Such communities have been reported, but the microbiology had not been studied. We have documented several hundred novel species (rRNA sequences) and currently are microscopically evaluating the distribution of different organisms in the laminated structure.

3. The archaea and microbial eucaryotes of hypersaline mats have not been studied and essentially are unknown. We have so far characterized a few thousand archaeal rRNA gene clones from selected strata of the Guerrero Negro mat and find, again surprisingly, that crenarchaeota outnumber classic halophilic archaea by 10- to 100-fold. No hypersaline crenarchaeota previously were known, so the finding raises substantial future research opportunities with these kinds of organisms. Similarly, studies of microbial eucaryotes that are underway are revealing a wealth of novel diversity, including organisms that are not related to known microbial eucaryotes at the kingdom level.

2.4.4. Proposed Studies.

We propose to continue the community analysis of the Guerrero Negro mats. This ecosystem is proving to be a spectacular source of novel biodiversity and results so far pose many questions. The complexity of the system, while not unexpected, is daunting. Specific first-order efforts proposed for the immediate future are the following:

1. Sequence-survey of current samples will be continued, to analyze the bacterial, archaeal, and eucaryal constituents of these mats, and to obtain diurnal and annual comparative information. (We have June and October, day and night samples; and a February sample needs to be acquired.) Some sequence analyses will be conducted at different sites with similar chemistry in order to test the uniformity of the mats. The complexity of the communities demands higher sequencing capacity than our current facility. Consequently, we have recently acquired a capillary sequencer that will expand our capacity >10x. We anticipate the determination of 20-30,000 rRNA sequences during the course of the study. This analysis of the Guerrero Negro mats will be a landmark study in microbial discovery.

2. An early goal is to validate the PCR-based results that indicate the major role (abundance) of the GNS bacteria in the system. This was an unexpected result and shakes a number of microbiological shibboleths. Validation of the results will come from analysis of selected samples with alternative methods to PCR. These will include use of fluorescence *in situ* hybridization (FISH) with specific nucleic acid probes and direct microscopic enumeration; bulk hybridization (slot blot) analysis of *in situ* rRNA concentrations, a measure of biosynthetic capacity; and selected pigment analyses. Additionally, we are developing real-time PCR approaches for quantification of particular phylotypes that are deemed important by group-abundance (e.g., GNS bacteria) or unusual character (Crenarchaeota). These methods will be used to map the distribution of selected phylotypes in the mats, for instance across and along gradients of salinity, more efficiently and less expensively than direct sequence analysis.

3. The microscopic distribution of selected organisms in mat samples will be determined using FISH with confocal microscopy. The distributions of different phylotypes will lend clues as to their roles and the workings of the community, and are likely to indicate novel symbioses (intermixed co-occurrence of particular phylotypes). One potential such symbiosis under study involves "trichomes" (sheath-enclosed bundles) of *Microcoleus*, a conspicuous cyanobacterium in the mats. Microscopy shows, however, that the sheaths enveloping the *Microcoleus* cells are crowded with other morphotypes besides *Microcoleus* filaments (possibly the enigmatic GNS bacteria?). These and other apparent consorts will be studied by harvesting (micromanipulation) morphological units for PCR-based molecular identification and FISH analysis to understand the fine-scale structure of the units.

4. As a high priority, representatives of the apparently abundant GNS bacteria will be cultured for further study. Only a few examples of GNS bacteria (one of about 40 main relatedness groups of bacteria) have been cultured. The rRNA sequences will be useful tools with which to track the organisms into culture. The abundance of the GNS sequences in clone libraries indicates that they engage in primary productivity, so presumably derive energy from photosynthesis. Culture conditions, therefore, will focus on photoautotrophic organisms under both oxic and anoxic states. Long-term goals will include physiological characterization and determination of the genome sequences of cultivars that represent the dominant Guerrero Negro microbiota.

5. Comparative studies in hypersaline sites other than Guerrero Negro will be important to generalize the currently developing molecular survey results. Therefore, analysis of other photosynthetic hypersaline communities will be continued in concert with efforts of the EcoGenomics group. Potential comparative ecosystems for study include Great Salt Lake, Utah, and selected tidal sediments. We propose to begin during the later portion of the program (yrs. 3-5) to conduct selected sequence analyses in these environments. The Great Salt Lake set-

ting, although inland, is generally similar to marine hypersaline settings, including the presence of relatively high concentrations of sulfate (Spencer et al., 1985). Consequently, we predict that similar suites of organisms and physiologies will be encountered at Great Salt Lake as at Guerrero Negro. The general makeup of Great Salt Lake hypersaline mats is unknown at this time. Tidal sediments occupy a substantial fraction of the terrestrial surface and must significantly influence global gas fluxes. It is likely that the same phylogenetic kinds of organisms encountered in the Guerrero Negro mats also occur in the outer centimeters of photic sediments. We propose to begin during the late stages of the program, with lowest priority, analysis of selected tidal sediments (e.g., Bolinas, CA).

2.4.5. Astrobiological relevance.

There are two particular ways in which the program fits into the larger scope of Astrobiology. One area of contribution by the program is pertinent to the charge to the NAI to understand the course of evolution on Earth. Through the discovery of new major lines of descent, the survey expands our knowledge of biodiversity. The new sequence results of the study also enhance the accuracy with which we can infer the deepest branches in the universal Tree of Life. As a theoretical side, the Co-I (Pace) has long been engaged in inference and interpretation of large-scale phylogenetic trees. Those efforts will continue into the proposed period, and are expected to refine and extend our view of the evolutionary topology of life.

The program additionally contributes to our growing understanding of what it takes to comprise an ecosystem. The spectroscopic search for life outside our Solar System is not for particular organisms, but for the products of ecosystems. The places to search for life in our own planetary system are dictated by the chemical niches occupied by terrestrial life. For what do we look? The terrestrial examples of life are our only models. Microbial ecosystems offer the best chance for understanding the interplay between organisms and biochemistry that results in ecosystems, be they small or large. The proposed research will provide rich new perspective on an extreme ecosystem dependent on metabolisms that are likely to be encountered any place there is life. The results of studies such as these provide the experimental fodder and intellectual basis for speculation on potential ecosystems elsewhere than on Earth, past and future.

2.5. Origin of Complex Land-based Ecosystems. (Led by Co-I W. Friedman.)

The most significant evolutionary events in the history of life (on Earth) are the development of a genetic code (RNA/DNA), the origin of unicellular life, the 30 or 40 separate origins of multicellularity, and the migration of multicellular life forms from water-based environments to land. This last event began with the migration of aquatic green algae onto land some 475 million years ago (Shear, 1991; Edwards and Selden, 1993; Raven, 1997) and resulted in a veritable explosion of evolutionary innovation and consequent diversification of terrestrial ecosystems. Thus, after nine-tenths of the history of life on Earth to date had transpired, land-based environments were finally colonized by diverse multicellular life forms - a radical change from pre-Ordovician microbial crusts (Raven and Edwards, 2001). The centrality of photosynthetic life to the diversification of ecosystems with large multicellular heterotrophic organisms (such as animals) is related to the obvious need for energy (from light) to be channeled (via plants) into the organisms that comprise an ecosystem.

Current molecular and paleontological data agree that evolution onto land by complex multicellular algae occurred just once in the 4 billion year history of life on Earth (Gray, 1985; Kenrick and Crane, 1997; Karol et al., 2001).

This suggests that this one-time transition (from an aqueous environment to a terrestrial one) may not have been simple for photosynthetic organisms and that the precise evolutionary innovations required for life out of water were (and are) not easily acquired.

Prior to the first significant weathering of surface rocks and subsequent buildup of soils in the late Silurian and Devonian (a consequence of the evolution of land plants and their rooting structures; Raven and Edwards, 2001), land surfaces on Earth were comparatively nutrient-poor with respect to the diverse mineral nutrients required for plant growth. Thus, the transition from a water-based environment (in which the organism is continuously bathed in a nutrient-rich medium) to a nutrient-poor land environment may have been constrained by the inability of multicellular photoautotrophs to gain sufficient access to scarce inorganic nutrients that are required for basic biological activity (Pirozynski and Malloch, 1975; Smith and Read, 1997).

Currently, over 70% of land plants develop a critical symbiosis with fungi (termed a "mycorrhizal association") that is essential for normal plant growth. The fungus, which lives within the plant body and whose filaments (hyphae) ramify into the soil, receives organic carbon from the plant, while the plant acquires increased access to various "limiting" mineral nutrients through the fungus' significant surface area contact with the soil (Smith and Read, 1997).

It has long been hypothesized that a mycorrhizal symbiosis with fungi enabled the first terrestrial photoautotrophs to gain sufficient access to scarce micronutrients in the environment (Pirozynski and Malloch, 1975; Remy et al., 1994; Redecker et al., 2000; Brundrett, 2002); and that this association was the key to the one time transition from aqueous environments to land. Remarkably, despite a century of (largely ecological and physiological) study of this important and widespread (mycorrhizal) symbiosis, virtually nothing is known of the specific identity of the fungi that were involved in the one-time transition of multicellular photosynthetic life forms from water to land, and the subsequent evolutionary radiation of these plants into diverse environments.

During years four and five of the currently funded astrobiology effort, we began to identify (in essence, discover) some of the fungal species that grow symbiotically within the bodies of ancient lineages of plants. Until now, molecular identification of the fungal symbiotic partners of land plants had been restricted to groups of plants with extremely recent origins (less than 100 million years ago).

Using molecular identification techniques, we are beginning to piece together the story of a long and complex symbiotic relationship between two major types of life on Earth: plants and fungi. Identification of fungal symbionts in plant tissues can be accomplished with standard and newly emerging DNA amplification and sequencing protocols (Bridge, 2002) using carefully targeted fungal specific primers (Abbas et al., 1996; Redecker et al., 1997; Schüssler et al., 2001). BLAST analysis (against sequences in GenBank) of amplified and sequenced fungal DNA from living representatives of ancient plant lineages can then be used to determine the phylogenetic identity of the specific fungi (Strumer and Morton, 1997; Morton and Redecker, 2001).

We have exploited genomic small subunit ribosomal DNA (18S rDNA) and genomic ribosomal internal transcribed spacers (ITS) sequences to identify the biotypes of the fungal symbionts of early land plant lineages. Preliminary identification and phylogenetic analysis of these fungal symbionts has yielded exciting results. Based on comparisons to databased sequences, all of the sequenced mycorrhizal fungi in ancient lineages of land plants we have studied to date (Psilotales and Ophioglossales) belong to the genus *Glomus*, the same group of fungi that form symbiotic associations in most other (and recently evolved) lineages of land plants. This is the first time that mycorrhizal symbioses in early lineages of land plants have been documented on the basis of the DNA sequence identity of the fungal symbiont. Our preliminary data provide some of the first concrete molecular evidence that the extremely widespread mycorrhizal symbiosis found in extant plants may have arisen during (and potentially enabled) the earliest phases of the colonization of land by plants (Figure 2.5-1).

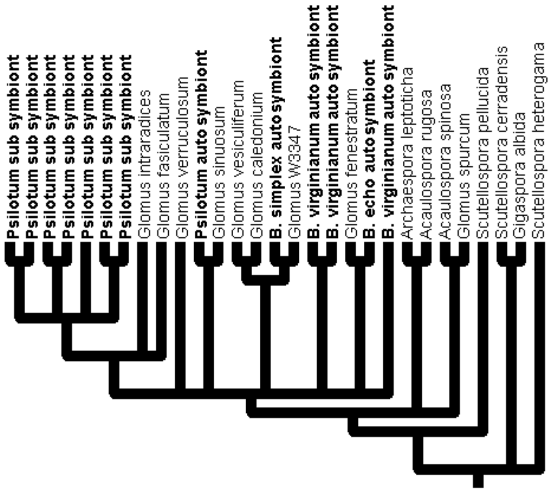


Figure 2.5-1: Molecular identification of fungal partners of ancient land plant lineages. Neighbor-Joining bootstrap phylogenetic tree using 560bp of 18S rDNA . All fungal symbionts of Psilotum nudum subterranean phases ("sub") are found in one well supported monophyletic group. This clade may represent one biotype or several different species. Fungal symbionts in the photosynthetic phases ("auto") of Psilotum and Botrychium are found in many different fungal lineages and represent many potential biotypes/species. All taxa in bold are newly discovered in our lab; sequence identities of taxa not in bold are from known fungal species listed in GenBank.

Recently, we have detected strong evidence (unpublished data – see Figure 2.5-2) that approximately 75 million years after the original colonization of land by plants, and concurrent with the first increase in land plant stature from centimeters to meters, part of the life cycle of land plants became entirely subterranean (and strictly heterotrophic). This extreme alteration in the ecology of early plants appears to have been accomplished through an alteration of the symbiotic relationship of plants to their fungal partners. In essence, the flow of organic carbon from the plant to the fungus was reversed, and the plant became a parasite upon the fungus and its carbon (ultimately derived from fungal connections with other plants that are photosynthesizing).

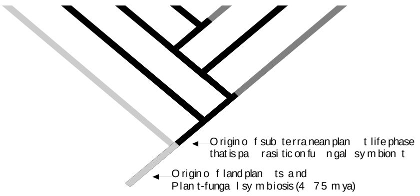


Figure 2.5-2: Evolutionary tree (phylogeny) of land plants with grey bars indicating a "typical" mycorrhizal symbiosis (photosynthetic plant obtains scarce mineral nutrients from fungus and fungus receives organic carbon from plant). Black bars indicate lineages of plants with a subterranean phase that is parasitic on the fungal symbiont for both its mineral nutrients and its organic carbon.

This type of subterranean habitat during a major portion of the life cycle of plants still exists today and is widely found in ancient lineages of plants: lycopods, ferns, and psilophytes (see Figure 2.5-3). In these lineages of extant plants, the entirely subterranean heterotrophic phase of the life cycle may be as long as 15 years. A major unanswered (and until now, unasked) question is what kinds of fungi were involved in bringing plants onto land, and then shortly thereafter, "driving" them underground?

We plan to significantly enlarge the scope of our molecular investigations to discover the identities of the fungal partners of ancient lineages of plants. The goal is to sample the fungal symbionts of representative members of all living plant lineages whose origins can be traced (based on the fossil record) to the first one hundred million years of multicellular plant life on land (Table 2.5-1). During years one through three of the proposed effort, we will specifically extract fungal DNA from diverse species of ferns and lycopods whose current life cycles include an extended subterranean phase and subsequent above-ground photosynthetic phase. These plants will serve as a proxy for examining the key symbiotic relationships of land-based photoautotrophs during the first major radiation and significant increase in plant stature during the Silurian and Devonian.

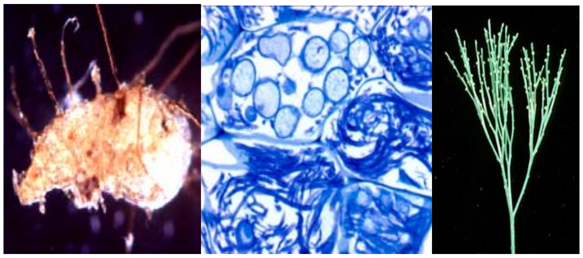


Figure 2.5-3. A. Subterranean phase of extant Psilotum plant. B. Microscope section of subterranean phase of Psilotum showing massive invasion of fungal partner in each cell of the body. We have discovered the DNA-based sequence identity of these fungi. C. The above-ground photosynthetic phase of extant Psilotum.

During years three through five of the proposed grant activities, our research will focus on the evolution of the mycorrhizal symbiosis in the most ancient lineage of land plants, the liverworts. These extremely primitive plants (still living) have long been known to associate with fungi, potentially in a symbiotic relationship. To date, the fungal partners of liverworts have not been identified (Duckett et al. 1991; Read et al. 2000). In light of the fact that liverworts are the most ancient lineage of land plants, and appear to have evolved shortly after the colonization of land by aquatic green algae, these organisms will serve as a direct proxy for investigating the role of one of the most critical symbioses in the history of life on Earth, that of ancient land plants and their fungal partners.

Table 2.5-1

Target Plant Taxa Years 1-3	Collection Sites
Psilotum (Psilotales)	Australia*, Georgia, Hawaii, Florida
Tmesipteris (Psilotales)	Australia*
Botrychium (Ophioglossales)	South Carolina*, Missouri*, Idaho*
Ophioglossum (Ophioglossales)	Australia*, Florida
Lycopodium (Lycopsida)	Colorado, Florida,
Huperzia (Lycopsida)	Florida, Washington
Target Plant Taxa Years 4-5	
Marchantia (Liverworts)	England, U.S.
Frullania (Liverworts)	Hawaii, Georgia,

* denotes where taxa have already been collected

The relevance of this proposed research to the NASA Astrobiology Program derives from the prospect of gaining a far more nuanced understanding of the evolution of complex ecosystems that might populate planets around other stars. For example, while life throughout the universe may be common, the issue of the relative organismic structural and ecosystem level complexity (i.e., unicellular vs. multicellular life forms; water-based ecosystems vs. land-based ecosystems) of life that might be expected to evolve elsewhere in the universe remains largely unaddressed. This, in turn has important implications for choices that will be made vis à vis detection of extrasolar life. What kinds of life and ecosystem signatures will be looked for? In land-based environments, should subterranean complex ecosystems be searched for? Finally, the proposed research will have a major impact on understanding how major evolutionary and ecosystem transitions have occurred during the history of life on Earth.

3. THEME 2: ORIGIN AND EVOLUTION OF HABITABLE PLANETS

Understanding the potential for life beyond the Earth requires applying our knowledge of what allows an environment to be capable of supporting life to our discoveries regarding the various environments that exist within our solar system and beyond. The environmental requirements for being able to support life come directly from our knowledge of terrestrial life, as we have no overriding theoretical understanding independent of our one example of life. In doing this, we can look at the different environments that exist on planets (and satellites) in our solar system and ask which ones are capable of supporting life or not, what factors control their habitability, and what processes are responsible for the planet having evolved so as to have suitable characteristics. In order to generate an understanding of what makes a planet habitable and what makes a planet non-habitable, we need to look at specific characteristics of individual planets as well as at general characteristics that might apply to all planets.

To do this, we are taking a multi-faceted approach. No single planetary object and no single suite of processes that occur on an individual planet can provide the information we need to understand habitability. Instead, we will look across the range of the most interesting objects and the most interesting processes, in order to bring multiple approaches to the problem.

We will approach the problem of understanding planetary habitability by looking in detail at the two objects in our solar system other than the Earth that are most likely to be habitable (Mars and Europa) and by examining more general physical and chemical processes that control habitability (radiative transfer and chemistry of atmospheric aerosols, and geochemistry of aqueous geological systems that might support life). In addition, we will look at the astrophysical issues evident in planetary formation by trying to understand what processes govern the formation of planets to begin with. By looking at a range of objects and processes, we can avoid getting locked into thinking that will lead us down blind alleys. And by using a group of tasks that address a common problem from multiple perspectives, each task will benefit from the results obtained by the others.

The tasks that we propose are as follows, with each one being discussed in more detail in the remainder of this section.

(i) Biogeochemical cycling and resources on Mars (task led by Co-I Jakosky). We will constrain the habitability of Mars by estimating the energy available from geochemical sources to support metabolism in hydrothermal and aqueous systems and by using measurements from a variety of sources combined with theoretical models to better understand the history of martian volatiles and climate as a boundary condition to the existence of life.

(ii) Geological evolution and habitability of Europa (task led by Co-I Pappalardo). The habitability of Europa depends largely on whether there has been and still is a large quantity of liquid water (i.e., an ocean) beneath the surface covering of water ice. We will use digital mapping and modeling of surface stress fields to determine whether the patterns recorded by Europa’s numerous lineaments are indicative of a steady-state level of activity, or whether they indicate a decrease in activity level through time. This will strongly constrain the geological evolution of the surface, and thereby the history of liquid water.

(iii) The impact of atmospheric particles on life (task led by Co-I Toon). Atmospheric aerosols affect planetary climate and habitability by their radiative effects, which depend on their composition and structure. We will model the

effects of aerosols on climate using theoretical models, and we will use an aerosol mass spectrometer to measure the composition and physical properties of aerosols formed under various conditions that might have obtained on the early Earth.

(iv) Geochemical-microbe interactions in chemolithoautotrophic communities on Earth (task led by Co-I McCollom). We will examine the habitability of terrestrial environments that are likely to be significant for life but have been all but ignored—moderate- and low-temperature environments that occur on the margins of hydrothermal systems and in aqueous systems involving ophiolite sequences that contain very reducing environments. We will determine the nature of geochemical weathering and alteration in these environments and the implications for their potential to support microbial communities.

(v) Origins of planetary systems (task led by Co-I Bally). We wish to understand the formation of planetary systems themselves, in light of observations of astrophysical processes that can destroy protoplanetary disks very effectively before they can form planets. We will combine theoretical models with observations at many wavelengths to understand the destructive processes in disks, with the corollary of understanding what combinations of processes allow planetary systems to form successfully.

3.1 Biogeochemical Cycling and Resources on Mars (task led by Co-I Jakosky)

3.1.1. Introduction

The potential for life on Mars depends on the availability of geochemical resources. Key factors include the presence of liquid water, access to the biogenic elements, and the availability of energy to support metabolism (Jakosky, 1998, and references therein). Abundant geological and geochemical evidence suggests the availability of liquid water. Geochemical reactions involving water and rock and capable of releasing energy that could support metabolism likely have occurred on Mars. This may have been either in high-temperature environments such as hydrothermal systems or low-temperature (ambient) conditions at or near the surface. The spatial and temporal availability of geochemical energy from these sources is not well understood, however. The amounts of usable energy have been estimated in broad terms, but those results are too general to assess particular environments that can be explored by spacecraft.

Further, even the limited data available indicates that there is complex coupling between the atmosphere and crust. Volatile exchange affects the composition of the atmosphere with implications for greenhouse warming, the nature of the climate, and the availability of volatiles within the subsurface that can drive geochemical weathering and alteration. Our ability to define and later to interpret measurements that can be made *in situ* or on returned samples depends on understanding these complexities.

We propose to investigate the potential for biogeochemical cycling on Mars and the implications for evolution of the biogenic elements and availability of geochemical energy to support metabolism. We will examine the geochemical energy available from alteration of martian surface and near-surface materials, the coupling to atmospheric and subsurface volatiles, and the implications for volatile cycling and evolution of the atmosphere-and-crust system throughout time. We will combine measurements made by spacecraft and using the martian meteorites with numerical models to determine the implications for the system as a whole.

Specific tasks will address:

(i) The geochemical energy that can support metabolism via mineral alteration and fluid-rock interactions in martian hydrothermal systems;

(ii) The geochemical energy that can support metabolism via chemical weathering at low temperatures in aqueous environments; and

(iii) The exchange of volatiles between the martian atmosphere, surface, and subsurface, as a constraint on martian volatile and climate evolution and possible biogeochemical cycling.

The results will tell us about the availability and history of resources that can support life and the overall biological potential for Mars. Although they will be specific to Mars, they also will allow us to understand the general behavior of terrestrial planets, with implications both for our solar system (including the early Earth) and for planets around other stars. In addition, they will be key to defining measurements that can be made by upcoming Mars missions to better understand the potential for life and its geochemical context.

3.1.2. Energetics of martian hydrothermal systems.

Hydrothermal systems probably are present on Mars, based both on the geological evidence for water and volcanism and on the widespread occurrence of impacts that have intensely heated the subsurface (Walter and Des Marais, 1993; Carr, 1987; Newsom, 1980; Brakenridge et al., 1985). The martian meteorites also contain minerals indicative of alteration by water at elevated temperatures (Romanek et al., 1994; Treiman et al., 1993). Energy derived from water-rock reactions in similar systems supports metabolism of some microorganisms on Earth and may have supported the earliest life (Brock et al., 1991; Baross and Hoffman, 1985; Shock, 1990); martian systems may support similar thermophilic and hyperthermophilic life. In earlier work, we examined the alteration of volcanic rocks under martian conditions and estimated the energy available and the amounts of biota that it could support (Jakosky and Shock, 1998). We also used the known mineralogy of martian meteorites and the inferred composition of martian groundwater to refine these energy estimates (Varnes et al., 2003; see Fig. 3.1-1).

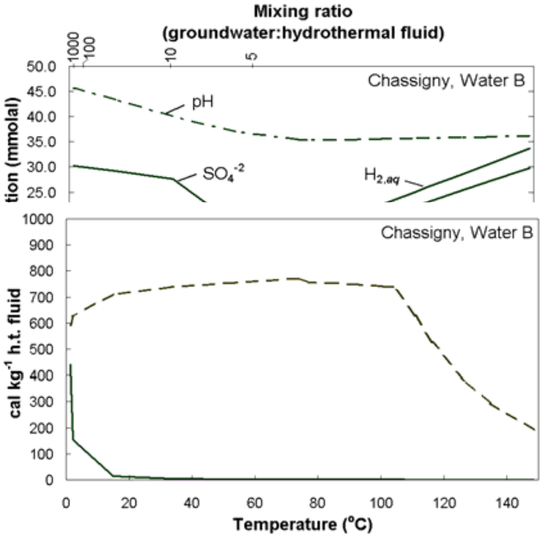


Figure 3.1-1. Energy yields and chemical species concentrations for mixing of fresh "martian" groundwater with hydrothermal fluid derived from the interaction of a Chassigny-composition rock with groundwater. Methanogenesis shown with a solid line, sulfate reduction with a dashed line.

A more realistic calculation of the energetics would incorporate the evolution of hydrothermal systems and the resulting spatial and temporal variations of the alteration reactions. We will construct coupled models of the physical and chemical evolution of martian hydrothermal systems, in order to model the alteration within a single system. In this way, we can determine the alteration and the energy available to support metabolism at a single point, the regional behavior of a single hydrothermal system, and the total energetics of the martian crust through time.

The physical and chemical histories of a hydrothermal system can be separated to a large extent. The physical behavior involves the two- or three-dimensional history of temperature and fluid flow through the system subsequent to volcanic intrusion or crater formation. Calculations of these have been carried out for both terrestrial (e.g., Norton, 1984; Norton and Taylor, 1979) and martian systems (Gulick, 1998), and can be easily modeled or parameterized.

Chemical alteration is driven by the rock and fluid compositions, the temperature, and the prior history of the water and rock. We will calculate the 2- or 3-D history of alteration by tracking individual parcels of water and rock separately and calculating the resulting chemical interactions. The chemical models will use the standard EQ3/6 geochemical modeling software that we used in our earlier analyses. The EQ3/6 software package includes an equilibrium aqueous speciation code, EQ3NR, and a reaction path code, EQ6 (Wolery, 1992; Wolery and Daveler, 1992), which we use in conjunction with a customized database of thermodynamic values (McCollom, 1999). These programs are used to determine the distribution, activities, and saturation states of species in solution and the fugacities of gases, allowing us to model processes such as fluid mixing, mineral dissolution and precipitation, and heating or cooling of fluid-rock systems. Reaction path codes model a succession of equilibrium states over a progress variable that corresponds with relative time, and will depend here, for example, on the spatial and temporal variation of water-rock ratio (see Griffith and Shock, 1995, 1997). Rock composition will be based on the mineralogy inferred from remote-sensing observations and derived from the martian meteorites; initial groundwater composition will be constrained by the composition of the martian crust and atmosphere (see Varnes et al., 2003).

The results will provide detailed information on the extent of alteration and the availability of energy, resolved both temporally and spatially, and will represent a significant advance over the representative calculations done previously. We will be able to determine the energy available, for example, at locations where temperatures are low enough for life to exist. We anticipate that our results will substantially modify our earlier analyses and will provide strong constraints on the ability of martian hydrothermal systems to support life.

3.1.3. Energetics of martian ambient-temperature aqueous systems.

Recent spacecraft results also suggest the possible occurrence of near-surface liquid water at or near $0^{o}C$ on Mars. Near-surface aquifers or surface deposits could be the sources of water for carving small-scale gullies (Malin and Edgett, 2000; Mellon and Phillips, 2001; Christensen, 2003); thin films or outright melting of polar and high-latitude ground ice may occur at moderate or high obliquities (Jakosky et al., 2003); outflows may debouch from beneath the polar ice deposits (Payne and Farmer, 2002); or transient lakes may exist within craters (Cabrol and Grin, 1999). We wish to understand the resulting geochemical environment, in which biota might exist. We will determine the weathering products that will result, using numerical geochemical modeling methods similar to those used at high temperatures and using terrestrial systems as analogs, and we will quantify the chemical energy potentially available to organisms. Although addressed in our preliminary model (Jakosky and Shock, 1998), we have not applied this approach systematically.

Geochemical modeling of low-temperature aqueous environments will be carried out using the same EQ3/6 software package used for geothermal systems. These systems can include both subsurface and surface water, such that systematic models of these environments could include weathering at various temperatures and water and rock compositions, formation and evolution of brines, and evaporation of water (Ridley et al., 1997; Ridley and Plumlee, 1995; Marion, 2000). As described by them, weathering products of basalt in a freezing or sub-zero environment depend upon the composition of the fluid altering it, which in turn depends on the composition of materials with which the fluid has been in contact as well as the prior history of reaction, evaporation, or freezing. Terrestrial systems can be used as analogs, based on the observed composition of weathering products. For example, the weathering of basalt near $0^{o}C$, buffered by the present martian atmosphere, can produce a range of minerals including hematite, goethite or ferrihydrite, smectites clays, Mg-silicates, carbonates and sulfates (Ridley and Plumlee, 1997). Most of the thermodynamic data for aqueous species are available at relevant temperatures, and databases exist to deal with brines and freezing solutions (see Wolery, 1992). These will allow calculation of the energy available from these weathering reactions.

We will explore a range of water and rock compositions, determine the assemblages of minerals that will result by calculation and by analogy with Earth systems, and quantify energy available from weathering reactions for use by microorganisms. We will combine our results with current estimates of the distribution of surface and subsurface liquid water on Mars based on interpretation of morphology in order to derive an estimate of the potential ambient-condition biota both in specific locales and globally. We anticipate that the results will be of direct relevance to estimating the biological potential of the martian surface and subsurface in non-hydrothermal regions that can support liquid water.

3.1.4. Biogeochemical cycling of volatiles.

Exchange of volatiles can occur between the martian crust and atmosphere. Exchange of water depends on the climate, ongoing geological processes, and mineral reactions; CO_2 can dissolve in and be carried by the water, with carbonates forming by mineral reactions or releasing CO_2 again as a result of heating (e.g., Kahn, 1985; Pollack et al., 1987). Evidence for exchange comes from the martian meteorites, with the isotopic composition of C, H, and O in mineral weathering products reflecting fractionation that most likely resulted from atmospheric escape processes (Watson et al., 1994; Romanek et al., 1994; Jakosky and Jones, 1997). The history of the partitioning of water and CO_2 between crustal reservoirs and the atmosphere has important implications for Mars' climate history, volatile inventory, and crustal evolution, and will provide important constraints on Mars' biological potential. Understanding this exchange will help define martian geochemical cycles, which in turn allows us to understand the roles that biota might play in possible biogeochemical cycling and to identify key measurements that can be made from spacecraft that will constrain this role.

To address these issues, we wish to track current reservoirs where volatiles might be stored, exchange rates between reservoirs (including rates of loss to space), and how these vary with time. We will address these issues as follows:

(i) We will assemble a current understanding of martian cycles for C, O, and H. We will track CO_2 and H_2O in atmospheric, polar, and crustal inventories, including loss to space, exchange between the atmosphere and crust, and supply of juvenile gases from the deep interior. We will include exchange of O between CO_2 and H_2O and with crustal minerals. We also will track nitrogen; however, N_2 is not a climatically active gas, and mineral forms such as nitrate or nitrite have not been identified in martian surface materials or meteorites.

(ii) We will describe and, where possible, quantify the nature and sizes of non-atmospheric reservoirs of gases and their rates of exchange. Constraints will come from models of the related processes and measurements of the modern system. These will incorporate escape of atmosphere to space (Luhmann et al., 1992; Yung et al., 1988; Krasnopolsky, 2000), isotopic measurements within different reservoirs (Jakosky, 1991; Jakosky et al., 1994; Jakosky and Jones, 1997), the geological history inferred from images (Greeley and Schneid, 1991; Tanaka et al., 1988) and the martian meteorites (McSween, 1994), and from models of volatile-related processes (Pollack et al., 1987).

(iii) We will utilize our current understanding of martian geology, geophysics, volatiles, and history, as based on measurements from spacecraft and of the martian meteorites. In particular, we will pay attention to the history of water at the surface (Carr, 1996; Phillips et al., 2000; Jakosky and Phillips, 2001) and the constraints that it imposes on both the crustal inventory of water and the history of its release, exchange, and availability to the surface.

We will include the nature of the weathering reactions that can occur at both ambient and elevated temperatures to understand the ability of volatiles to exchange with the atmosphere. The output of our analysis will be a "box model" of the martian volatile system, showing reservoirs, exchange between reservoirs, and rates, of the type that has been so useful in understanding terrestrial biogeochemical cycles. The result will be stronger constraints on the nature of the martian biogeochemical system and the movement of volatiles within it. This will have important implications for understanding the history of the atmosphere (and thereby of climate), the availability of volatiles within the regolith and crust, and the possible coupling between biological processes and geochemical processes.

3.1.5. Work plan and personnel

The three tasks are distinct and will be carried out in parallel. Results from each will be integrated with the others as appropriate. Work will be done by a graduate student (not yet identified) in collaboration with and under the supervision of Co-I Jakosky. Co-I Tom McCollom will consult on geochemical issues.

Collaborator Raymond Arvidson (Washington Univ. St. Louis) will provide input into the geochemical cycles task based on geological and mineralogical analysis from the MER rover missions to be launched in 2003, on which he is Deputy Principal Investigator. Collaborator David Des Marais (NASA/Ames Res. Ctr.) will consult on the geochemical cycles task based on his experience with analogous terrestrial processes.

3.2. Geological Evolution and Habitability of Europa (task led by Co-I R. Pappalardo)

3.2.1. Background and relevance

A subsurface ocean may be able to support life within Europa today. Understanding the astrobiological potential of Europa requires knowledge of its geological history, including the origin of its surface features and possible changes in the satellite's level of geological activity over time. If Europa's internal activity is robust today, its potential to harbor life is much greater than if the satellite's activity and heat sources have dwindled through time. We propose to use digital mapping techniques and modeling of candidate stress fields to determine whether the patterns recorded by Europa's numerous lineaments are indicative of a steady-state level of activity, or whether they indicate a change in activity level through time.

Imaging data from the Galileo spacecraft provide strong evidence for a subsurface ocean within Europa today (Pappalardo et al., 1999; Hoppa et al., 1999; Kivelson et al., 2000). Moreover, models of impactor dynamics and the observed paucity of craters suggest a surface age of only ~10 to 250 Myr (Zahnle et al., 1998; Pappalardo et al., 1999), implying that Europa is probably still geologically active today. However, it is unknown whether Europa's activity level has remained constant through time or dwindled in the recent past.

Possible energy sources for life on Europa are hydrothermal vents at its deep rock/water interface (Jakosky and Shock, 1998; McCollom, 1999), or oxidants created by charged particle bombardment of the surface and carried into the ocean by geological activity (Chyba and Phillips, 2001). A fundamental issue in understanding the current habitability of Europa is whether there is a great enough supply of chemical energy to the ocean to sustain a significant biosphere today (McCollom, 1999; Chyba and Phillips, 2001). Thus, understanding whether the satellite's level of internal activity has changed through time is critical to understanding the satellite's potential habitability.

It has been suggested that Europa may be in a steady state, with the rate of formation of its geological features constant through time (Greenberg et al., 1999, 2002; Hoppa et al., 2001a, 2001b). On the other hand, geological mapping suggests that there may have been a change in Europa's geological style from ridge- and-band-dominated to chaos-dominated over time (Prockter et al., 1999; Greeley et al., 2000; Figueredo and Greeley, 2000; Spaun et al., 2003). A change in geological style could be indicative of cooling and thickening of Europa's ice shell, potentially in response to episodic or secular changes in orbital eccentricity and the corresponding rate of tidal heating (Ojakangas and Stevenson, 1986; Showman and Malhotra, 1997). The record of Europa's geology, specifically its ridges and bands, is the key to unraveling this evolution.

Deciphering the geological evolution of Europa's surface is a task that has confounded researchers to date because of the spaghetti-like intricacy of the satellite's lineaments and the complexity of the several stressing mechanisms that may have operated through time. We will undertake a study that combines digital mapping, computer-based stratigraphic analysis, and sophisticated stress modeling to determine the formational history of Europa's structures. Our integrated, state-of-the-art approach will be able to unravel the satellite's geological evolution through time.

3.2.2. Stressing mechanisms

The complexly lineated pattern of Europa's surface may have been shaped over time by as many as three different types of tidal distortion, each of which induces a characteristic stress pattern. First, "diurnal" stresses result from the raising and lowering of tides as Europa orbits, including resultant libration of the ice shell, on the rapid time scale of Europa's 3.55 day orbit about Jupiter (Greenberg et al., 1998). Diurnal stresses are probably responsible for Europa's unique cycloidal shaped features and may also produce other cracks (Hoppa et al., 1999).

Second, Europa's ice shell probably rotates slightly faster than the tidally locked interior on a very long time scale ($>10^4$ yr period). This "nonsynchronous rotation" sets up a stress pattern that sweeps easterly across the surface over time, leaving a distinctive structural pattern in its wake (Helfenstein and Parmentier, 1985; McEwen, 1986; Leith and McKinnon, 1996; Geissler et al., 1998). Early studies suggested that the surface records a total of ~60° of nonsynchronous rotation (McEwen, 1986; Leith and McKinnon, 1996; Geissler et al., 1998). However, more recent studies suggest that the surface may record 1-2 full rotations of the ice shell (Figueredo and Greeley, 2000; Kattenhorn, 2002) and perhaps hundreds or thousands of rotations (Hoppa et al., 2001a).

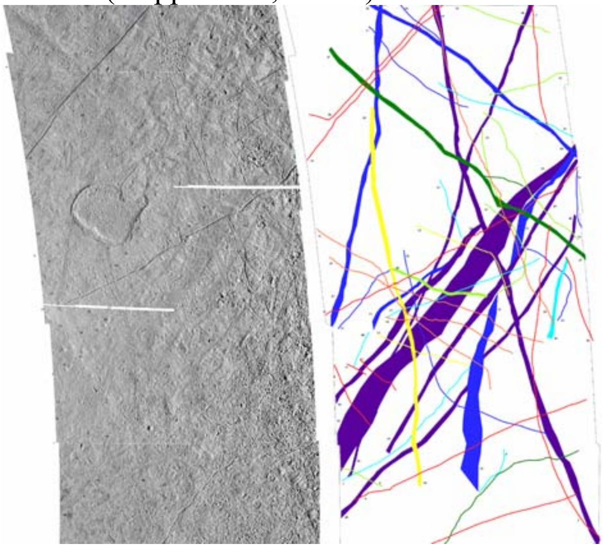


Figure 3.2-1. Image and corresponding map of ridges and bands near the leading point of Europa's orbital motion (0°, 90°). Approximate stratigraphy is mapped through the spectrum from violet (oldest) through red (youngest) (Stempel and Pappalardo, 2002). Preferred NE and NW trends are consistent with lineament formation in a zone of compressive stress pre-

dicted along Europa's equator due to nonsynchronous rotation. The lack of E-W structures suggests that the visible surface records <90° of nonsynchronous rotation. Reconciling these observations with the locations of cycloidal ridges (cf. Hoppa et al., 2001a) could indicate whether Europa's geological activity is in a steady state or has waned over time.

Third, Europa may have undergone polar wander, in which the rotation axis of the ice shell shifts with respect to the axis of orbital motion (Ojakangas and Stevenson, 1989). This would also set up a distinctive stress and structural pattern as a record of the event (Leith and McKinnon, 1996). There is plausible though limited geological evidence that polar wander has taken place (Leith and McKinnon, 1996; Sarid et al., 2002). These three candidate stress mechanisms (diurnal, nonsynchronous, and polar wander) can combine. To date only the combination of short-term diurnal stresses and secular nonsynchronous stress has been studied (Greenberg et al., 1998).

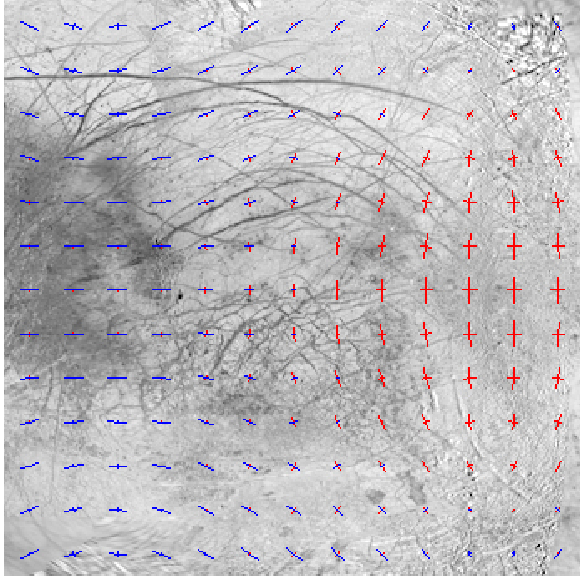


Figure 3.2-2. Superimposed on a map of Europa (135-265° lon., ±65° lat.) are the direction and magnitude of tensile (red) and compressive (blue) stresses at one point in the daily tidal cycle (45° before apojove). Integration of these stresses through the diurnal cycle, and addition to nonsynchronous and polar wander stresses, will allow for quantitative comparison to global lineament patterns, and inference of Europa's evolutionary history.

If Europa has experienced a steady-state level of activity, then combinations of diurnal and nonsynchronous stresses and perhaps polar wander stress are expected to have produced geological structures that overlap like a ball of string, with some roughly constant rate of structure formation as the ice shell rotates nonsynchronously. This has been the assumption (implicit or explicit) of several geological studies (Figueredo and Greeley, 2000; Kattenhorn, 2002; Greenberg et al., 1999; Hoppa et al., 2001a, 2001b). On the other hand, if Europa's level of activity has declined though time, then the surface should record a change in the level of activity as reflected in the number and/or style of geological structures formed during each nonsynchronous rotation, and the surface might record few (perhaps <1) complete nonsynchronous rotation(s) of the ice shell (Stempel and Pappalardo, 2002).

3.2.3. Approach

Existing analyses of Europa's structural history adopt three simplifying assumptions regarding Europa's stratigraphy that are probably incorrect. All of these problems are remedied in our approach.

First, existing stratigraphic analyses (Prockter et al., 1999; Figueredo and Greeley, 2000; Kattenhorn, 2002; Spaun et al., 2003) group Europa's structures into discrete stratigraphic intervals in a subjective manner. That is, individual lineaments deemed to have formed in a generally similar time interval are grouped together without objective criteria for determining the precise relative age of individual structures. This approach is necessary in manual stratigraphic mapping of Europa because the cross-cutting relationships are so numerous and intricate. This can lead to oversimplification of the region's stratigraphy and misinterpretation of the stress that formed the structures.

We will employ Geographic Information Systems (GIS) techniques to map Europa's structures. For each intersection of two lineaments, we will record which lineament overlays which. Like a child's game of Pickup Sticks, our software will be able to peel back and decipher a *lineament-by-lineament* stratigraphy of each mapped area. Any ambiguous intersections will be keyed and flagged as such in the output, potentially indicating simultaneously active structures. The resulting objective and precise stratigraphy can then be compared to calculated stress models, making it clear whether or not there are systematic variations in lineament orientation (for example, as predicted by the sweeping of nonsynchronous rotation stresses across the surface), or more complex patterns (as discussed next).

Second, with the exception of the earliest such analysis (Helfenstein and Parmentier, 1980), published studies have assumed that all Europa's structures are fractures that formed perpendicular to the local least compressive stress direction. This assumption does not consider the possibility that shear failure has occurred in portions of Europa's equatorial region, where stress modeling suggests that it could occur (Helfenstein and Parmentier, 1985; Leith and McKinnon, 1996). In contrast to previous assumptions, shear failure can create simultaneous structures of two conjugate orientations oblique to the principal stress directions (as is inferred in the region mapped in Figure 3.2-1) (Stempel and Pappalardo, 2002; Spaun et al., 2003).

We will account for the possibility of shear failure and near-simultaneous formation of conjugate structures in Europa's equatorial regions. Unlike past analyses that assume lineament orientation is perpendicular to the least compressive stress direction, our lineament-by-lineament stratigraphy will allow us to decipher any back-and-forth swinging of lineament orientation, with potential implications of near-simultaneous formation of conjugate structures.

Third, existing studies have compared structural orientations to either the nonsynchronous or polar wander stress patterns alone, or to a combination of nonsynchronous and diurnal stresses at a single specific point in Europa's orbit. Existing studies have considered neither combinations of these stresses with polar wander stresses, nor the integration of diurnal stress over an entire orbit.

Our study will calculate stress at each point on Europa's surface based on any combination of diurnal, nonsynchronous, and polar wander stresses, comparing the results to observed structures to find most reasonable fits. Moreover, we will examine the pattern of combined nonsynchronous and diurnal stresses where the relevant least compressive stress is integrated over Europa's entire orbit. In this way, we determine the least compressive failure stress that is most likely to occur at each surface location during the course of the Europan orbit. This is the most appropriate pattern to compare lineament orientations, rather than any arbitrary moment in the diurnal stress cycle.

Our study will calculate surface stresses in a manner that is mathematically rigorous yet easily adaptable. Previous investigators have assumed deformation of a thin ice shell and have calculated stresses as arising from a discrete distortion of the ice shell (e.g., Leith and McKinnon, 1996). Some other researchers have not provided the mathematical foundation for their stress predictions (Greenberg et al., 1998; Hoppa et al., 1999). Working with Collaborator John Wahr, we will calculate stress by determining the vector components of displacement at Europa's surface as functions of the degree 2 Love numbers for any ice shell thickness. We calculate the stress tensor components, then diagonalize to obtain its eigenvectors and eigenvalues, which are the directions and magnitudes of the principal surface stresses. An example of the diurnal stresses derived from this method is shown in Figure 3.2-2. The resultant Matlab software that calculates and outputs stress vectors across Europa will be distributed freely to interested researchers.

3.2.4. Implications for geology, convective transport, and astrobiology

Researchers currently disagree as to whether stratigraphic relationships suggest that Europa's activity level is in steady state or has decreased over time. The proposed study will unravel the history of Europa's complexly lineated surface through improved stress calculations and GIS-based stratigraphic mapping, techniques that will define a new state of the art. In this way we will determine whether Europa is in a steady state or if its level of geological activity has changed through time, while determining the sources of stress that have affected the surface and thus the satellite's geological evolution through time. These results are fundamental to assessing Europa's potential to harbor life.

This study will be complementary to (but will not overlap with) two other studies currently underway by Dr. Pappalardo at the University of Colorado. One study funded through NASA's Planetary Geology and Geophysics Program examines the morphology and nature of tectonic structures on Europa, with comparisons to its neighbor satellite Ganymede. There is fundamental synergism between that study and new modeling of the stress regimes in which structures may have formed.

Another synergistic study is funded through NASA's Exobiology Program. That study examines the nature and style of solid-state convection on Europa, concentrating on the implications of this vertical transport mechanism for movement of nutrients and perhaps organisms within Europa's ice, and for planetary protection. The stress models developed in the proposed study have direct bearing on modeling of convection within Europa. To date we have provided an initial assessment of the effects of non-linear ice rheology on the thermal structure and deformation style of Europa's ice if it is convecting (Barr and Pappalardo, 2003). However, in order to best consider the effects of non-linear ice rheology in a geophysically self-consistent manner, one must consider the tidal strain on Europa's ice shell as a driver of convection. This is because the thermal structure within the shell due to tidal heating and convection are linked to the applied tidal force through the ice viscosity. The convective and tidal effects must be treated simultaneously, and modeling the stresses that act on Europa will permit this. Collaborator Shijie Zhong will assist with this integration.

Understanding the processes that have operated on Europa and the manner in which they have changed through time is fundamental to understanding the satellite's present-day habitability. This study develops innovative techniques to achieve that goal.

The proposed work will be carried out by Dr. Pappalardo and a postdoctoral researcher, emphasizing the GIS-based analysis of Galileo images. Collaborator John Wahr (CU Physics Dept.) will carry out stress calculations using models that he will develop for ice shells of varying thickness and properties. Collaborator Shijie Zhong (CU Physics Dept.) will develop analytical and numerical models of coupled convective and tidal stress fields. Wahr and Zhong are funded under other grants, and no funding for their time is requested here.

3.3. The Impact of Atmospheric Particles on Life (task led by Co-I O. Toon).

3.3.1. Introduction

Two outstanding problems in astrobiology are to understand the environments in which life originated, and to determine how to detect places where life did or could arise. While much work has been done to understand the gases that compose atmospheres conducive to the origin of life and their spectroscopic signatures, relatively little work has been done to understand the clouds and aerosols in such atmospheres. This is an especially important gap since aerosol particles and cloud droplets provide unique physico-chemical environments that may provide a path to some critical biochemical reactions. For example, atmospheric aerosols have been recently postulated as playing an essential role in the origin of life on Earth (Dobson et al., 2000; Donaldson et al., 2002).

In the oxygen poor Archean atmosphere (Pavlov and Kasting, 2002), reduced atmospheric gases (such as methane and elemental sulfur) could have been polymerized (Pavlov et al., 2001) as happens in the present Venus and Titan atmospheres. The presence of a haze layer, or smog, would have affected the paleoenvironment in several ways. Both organic and sulfur aerosols are effective UV absorbers and could have provided necessary UV shielding for ancient biota. On the other hand, aerosols would have absorbed solar radiation affecting ancient climate. Whether aerosol forcing was important is a strong function of aerosol optical properties, which in turns depends on aerosol chemical composition and size/number density distribution.

We propose a combined theoretical and laboratory investigation to shed further light on these issues. We discuss our planned theoretical studies and then the laboratory work. We finish with our work plan. Our goal is to understand the aerosols and clouds in the atmosphere of early Earth, how these particulates influenced the UV radiation reaching the surface and the climate, and how they may have served as food for the biota.

3.3.2. Theoretical studies of aerosols

We plan to investigate three coupled issues. First we will simulate the formation of organic aerosols and sulfur aerosols in the early methane rich atmosphere of Earth. Second we will explore the dependence of haze formation on the changing atmospheric chemistry. Third we will investigate the role of these particulates in the climate and chemistry of the atmosphere.

We have adapted the NASA Ames/University of Colorado Community Aerosol and Radiation Model for Atmospheres (CARMA, Toon et al., 1988) to the problem of haze “evolution” in the atmosphere. Once haze has been formed at the top of the atmosphere (through photochemical reactions), it is subject to coagulation, gravitational settling, rainout etc. These processes change the particle size/number density distribution with height, and therefore affect both UV-shielding and the “antigreenhouse” effect of haze.

Pavlov et al. (2001) predicted that the rate of production of organic aerosols was a strong function of the CH_4/CO_2 ratio in the Archean (this result has been confirmed experimentally by our group as discussed below). A major constraint for the abundance of hydrocarbon aerosols comes from climate. Hydrocarbon aerosols absorb visible radiation and are relatively transparent to outgoing IR radiation creating an “antigreenhouse” effect. Therefore, if haze abundance is high, the surface temperature drops below the freezing point of water most likely shutting down methane production.

Organic haze is not the only haze that would have been generated in the anoxic Archean environment. Elemental sulfur would also polymerize, forming S_8 particles and other sulfur allotropes (as in the present Venus atmosphere). We have also developed a one-dimensional isotopic photochemical model to simulate isotopic composition of sulfur particles. Pavlov (in Ono et al., 2003) proposed that anomalously high mass independent fractionation in sulfur isotopes in the Archean rocks is direct evidence for sulfur aerosols, as opposed to sulfates which now occur in the atmosphere.

We will perform the following tasks:

First, we will include the hydrocarbon chemistry up to C_8 in a 1-D photochemical model (Pavlov et al., 2001) to get an initial profile of hydrocarbon and S_8 chemical production under different CH_4/CO_2 atmospheric ratios and different SO_2 volcanic outgassing rates. We will then input those production rates to the 1-D aerosol transport model (CARMA), which will allow the calculation of particle size distributions at all atmospheric levels. We will then perform simulations of hydrocarbon and sulfur aerosol abundance with modified atmospheric vertical transport, because if the haze layer was present it would stratify the atmosphere and dramatically decrease the tropopause height (little rainout).

Second, we will conduct radiative transfer (climate) simulations using “realistic” optical properties of the Archean haze. (Optical properties of the haze have not been measured yet and this is an essential part of the lab work we will discuss below.) We will also concentrate on the magnitude of the UV shield from haze particles for ancient biota (since there was no ozone UV shield).

We will use the preceeding two steps to build up a conceptual model of an Archean biosphere without photosynthetic primary production. Effective production of high-altitude organic haze from the atmospheric methane could provide enough food for the methanogenic community so they would have kept resupplying atmospheric methane.

S_8 aerosols form relatively low in the atmosphere (6-10 km) so their removal time is short (rainout) and S_8 could not build up in the atmosphere to substantial levels. However, if organic haze was also present in the Archean, the Archean atmosphere would have been stratified down to the ground level (Pavlov et al., 2001). Therefore, S_8 aerosols (potentially) could have accumulated in the atmosphere to substantial levels and affected climate and photochemistry.

The presence of a haze layer, or smog, would have affected the paleoenvironment in many important ways (photochemistry, climate, “food” source, etc.). We hope to get quantitative answers to the following “key” questions for the Archean paleoenvironment: 1) What were the rates of haze production? 2) How much UV-shielding was produced? 3) How strong was the climatic impact of the haze layer and what limited its thickness in the atmosphere? 4) How digestible were haze particles for the ancient biosphere and was it possible to maintain the primary production without photosynthesis?

While the work we have just described is theoretical in nature, further progress is dependent on lab studies to determine several critical parameters. These include not only the optical properties of aerosols, but also their rates of production in various atmospheres, and their chemical properties.

3.3.3. AMS studies of particles on early Earth

While the Toon research group is primarily theoretical, we recognized that further progress would be limited without an adequate laboratory program to measure the parameters needed by the theory. We therefore developed a collaboration with Professor Margaret Tolbert and Professor Jose Jimenez in the CU Chemistry Department. So far this collaboration has produced several important new results, and led to the involvement of several chemistry graduate students in astrobiology research.

To make progress on understanding clouds and aerosols in planetary and early Earth atmospheres, new laboratory methods must be developed and employed. Recently, a new technique has been developed that has the capability of quantitatively analyzing the size and chemical composition of freely floating aerosol particles in real-time (down to 10 ms time resolution). This aerosol mass spectrometer (AMS) is sparking a revolution in understanding particulate matter in Earth's current atmosphere, with 19 research groups starting to use it in the US, Canada, Germany, the UK, and Japan (Jimenez, 2002). While not yet exploited, this technique also has the potential to make major, new contributions relevant to laboratory studies of astrobiology

Current research in the Tolbert group represents the first attempt to utilize an AMS to perform detailed studies of particles that may have been present on early Earth. Future work in the Jimenez research group will expand these studies to longer timescales to follow particle evolution under realistic atmospheric conditions. Prof. Jimenez was heavily involved in the development of the AMS and thus has a high level of expertise in its use.

We are performing laboratory experiments to probe the formation and composition of Titan-like particles that might have formed on the early Earth (Sagan and Chyba, 1997). Previous laboratory investigations of this nature have involved either collection of aerosol samples for later analysis or study of gas phase products. Both of these methods have limitations in the ability to accurately determine aerosol composition and particle characteristics at formation. In order to improve on those studies, we are using a novel analysis technique based on detection of particles using an AMS. Using the AMS, we are able to determine the number, size and chemical composition of the particles in real time without first collecting and concentrating them, a procedure known to be riddled with artifacts.

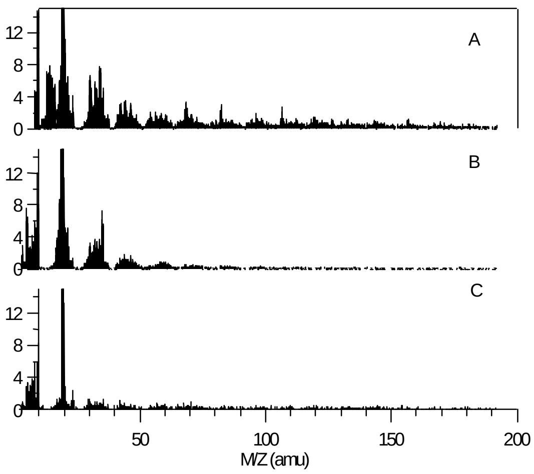
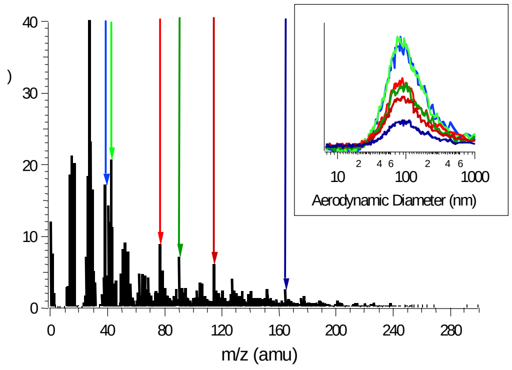


Figure 3.3-1. *(A) 10% CH_4 in N_2 (B) 1% CH_4 (C) 1000 ppmv CH_4*

Our studies focus on characterizing the particles as a function of input trace gas composition, and to date we have looked at methane (CH_4) and carbon dioxide (CO_2) in a background of nitrogen. We have varied CH_4 concentrations from 10% CH_4 in N_2 to less than 1000 ppmv CH_4, a relevant mixing ratio for the early Earth (Kasting et al., 2001a). The mass spectra resulting from these experiments are shown in Figure 3.3-1., and indicate the definite presence of long hydrocarbon chains, as well as aromatics and other hydrocarbon structures previously described (Sagan and Khare, 1979). The spectra also show how the particle composition changes with a decrease in CH_4 concentration; as less methane is added to the reactant mixture the size of the hydrocarbon chains decreases and the structure is altered.

The AMS allows us to measure aerodynamic diameters of aerosols of a particular molecular composition. Since each mass spectrum represents an average composition of the aerosol population, it is not obvious whether the particles in question are internally or externally mixed. Figure 3.3-2. shows the results from an experiment performed to determine whether ions in the spectra were produced from molecules in the same particles. We can show that the aerosols that produce each ion in the spectra have the identical size distributions, as seen in the inset graph. This is consistent with the haze aerosols being internal mixtures of a complex mixture of organic molecules. The size distributions shown in Figure 3.3-2. also indicate that the haze aerosols produced in our experiments are unimodal and are approximately 90 nm in diameter, which is in the size range seen in previous studies of such particles.

An important factor in whether or not these organic haze aerosols could have formed on the



.Figure 3.3-2 Size distributions for several ions in spectrum

early Earth is the C/O ratio of the atmosphere (Kasting et al., 1997). The inclusion of oxygen atoms in the gas mixture is expected to oxidize organics and terminate the synthesis of long chain hydrocarbon molecules, thus altering both the composition of the hazes as well as particle size and concentration. We have studied the effects of adding CO_2 to the N_2/CH_4 mixture and the resulting mass spectra are shown in Figure 3.3-3. The CH_4 concentration in N2 is held constant at 1%.

As more CO_2 is added to the starting gas mixture, the appearance of long hydrocarbon chains diminishes, and ion peaks that are indicative of oxidized products, such as m/z 44 and 58, begin to appear. Once the C/O ratio begins to approach its lowest value, it appears that the aerosol production is greatly reduced, which is consistent with the expected behavior. We are now in the process of determining if the new products formed could be sources of food for the Earth's earliest life.

There are several important lines of research that we will pursue using the continuing access to an AMS that we are proposing. Our first priority is to alter our photolysis source from discharge excitation to UV irradiation to better simulate conditions on early Earth. The particle characteristics such as size and chemical composition may change dramatically with energy source, so our first goal would be to repeat the above experiments with the UV source. Our next goal is to add other trace gas species that might have been available on early Earth. Specifically, we would like to add trace sulfur gases such as H_2S and SO_2 to see how the particle properties were affected.

In addition to determining particle size and composition, climate models require accurate knowledge of the optical properties of the resulting particles. We propose to make optical constant measurements of the particles and correlate those properties with the chemical composition of the particles determined using the AMS. We have previously measured the optical constants of a number of materials over a wide range of temperatures and have considerable expertise in this area.

Finally, there will be new data from the Aerosol Collector and Pyrolyser (ACP) on the Huygens probe of Cassini on the chemical composition of the tholin particles on Titan. The ACP uses pyrolysis GC/MS characterization of the molecules making up the particles, a technique that should yield mass spectra very similar to those of the AMS. We will compare the Titan data with AMS data we obtain in the laboratory. We will then vary conditions in the laboratory to unravel the formation mechanism of the Titan particles and learn more about how polymerization and growth processes control the particle characteristics. The AMS is ideal for these studies because it provides a quantitative measure of the various components in the particle in a short period of time. An AMS can do studies quickly and economically that might take years of effort, and cost vast sums of money to analyze by electron microscopy or other chemical techniques.

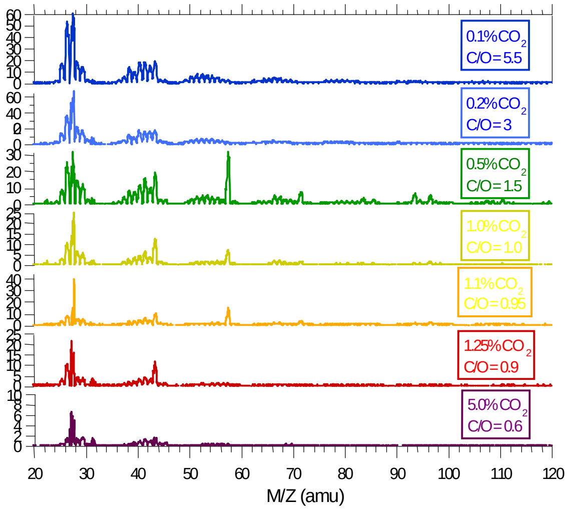


Figure 3.3-3 *Mass Spectra for Haze Aerosols Produced in Varying C/O Mixtures*

3.3.4. Studies of early Earth in a "bag"

A new environmental chamber facility being constructed in the Jimenez research group will be used to study the photochemical evolution of laboratory-generated particles over long time periods (several hours). We will use this facility to study the formation, growth and aging of early Earth tholin particles. These studies complement those described above, but utilize longer periods of time with more complex processes possible. We will use the AMS to probe the chemical composition and size of the particles over time. In addition, the large size of the chamber (30 m^3) will allow us to optically probe the particles to determine optical properties of the aerosols in-situ. The Jimenez and Tolbert groups are co-located so time-sharing the AMS will be readily accomplished.

3.3.5. Work plan

The theory and lab work proposed will be done in parallel. As we learn more from lab studies, for instance the optical constants of particles, the information will be added to climate models and calculations done.

Brian Toon will be responsible for the overall conduct of the research. With his students and postdocs, he will conduct the theoretical studies, and provide guidance to the laboratory work. Professor Tolbert will be responsible for the AMS and optical constants studies. Professor Jimenez will provide access to the aerosol chamber, and provide advice on operating the AMS.

Analysis to date has been done using an Aerosol Mars Spectrometer essentially borrowed from another research group. The proposed funding will allow us to obtain one that will be used jointly by the Toon, Tolbert, and Jimenez groups and which will be much more readily available for the research proposed here. As described in detail in the equipment portion of the proposal (vol. 2), Professor Jimenez will cost share buying the AMS, which he will use separately for experiments in the aerosol chamber.

3.4. Geochemical-Microbe Interactions in Chemolithoautotrophic Communities on Earth (led by Co-I T. McCollom)

3.4.1. Introduction

Our current understanding of the early evolution of life on Earth suggests that the complex machinery of photosynthesis evolved well after life had become established, implying that the first biological communities probably relied on inorganic chemical energy sources rather than sunlight for biomass synthesis (a process known as chemolithoautotrophy). The hostile surfaces of Mars and Europa also suggest that any biological communities that currently exist there probably inhabit subsurface environments where chemical sources would supply the energy for metabolism. There are several environments on the modern Earth where geological processes supply the chemical energy to support chemolithoautotrophic microbial communities with little or no input of photosynthetically derived organic matter. Examples include deep-sea hydrothermal systems, subsurface aquifers in terrestrial and oceanic basalt, and acid mine drainages.

These kinds of chemolithoautotrophic environments may provide the best analogs for biologic habitats on the early Earth and for habitats that might now support life, or have supported life in the past, on other planetary bodies. The more we know about how microbial communities thrive in these environments on the contemporary Earth, the better equipped we will be to interpret biological processes on the early Earth and to evaluate potential biological habitats on extraterrestrial bodies such as Mars and Europa. Therefore, we propose to conduct research that will increase our understanding of the structure and function of biological communities that thrive on geologically-derived sources of chemical energy.

3.4.2 Proposed Research

The unique character of the biological communities that occupy geologically-based chemolithoautotrophic habitats has led to extensive scientific study of these systems in recent years. However, most of this research has focused on isolation and characterization of the novel microbial populations that inhabit these environments and in elucidating their internal metabolic pathways. Largely missing from previous efforts has been corresponding research to understand the underlying geological processes that allow these communities to exist and to evaluate how geochemical processes influence the abundance and distribution of microbial activities. Information of this sort will be required, however, to fully describe the microbial ecology of these habitats. Furthermore, this type of information will be required to extrapolate from modern ecosystems in order to infer the biological potential of analogous habitats on the early Earth and on other planetary bodies where geochemical conditions differ substantially from those on the current Earth.

Accordingly, we propose to conduct research that will focus on the connections between geochemical processes and microbial activities in chemolithoautotrophic environments. Among the issues we will address are: Where and how do sources of chemical energy arise from geological processes? What is the abundance and diversity of chemical energy sources in these environments? To what extent do these energy sources control the composition and spatial distribution of the microbial population? How do variations in geological processes, fluid chemistry, etc., influence the metabolic diversity and abundance of microbial populations? How do chemical energy sources in a particular environment evolve over time? How do microbes alter the chemistry and mineralogy of the environment, and are characteristic chemical and mineral biomarkers produced in the process?

Our effort to address these issues will primarily involve the development of quantitative geochemical models of chemolithoautotrophic environments. Since chemolithoautotrophic microbes gain their metabolic energy by exploiting disequilibria in oxidation/reduction reactions, a primary objective of the models is to identify and quantify sources of redox disequilibria that develop in geological systems as a consequence of ongoing physical and chemical processes (McCollom and Shock, 1997; McCollom, 1999, 2000). This information about chemical disequilibria can then be used to infer the abundance, spatial distribution, and metabolic characteristics of microbes that can live in different habitats within the geological environment (e.g., Fig. 3.4-1).

Initially, the research will concentrate on mid-ocean ridge habitats and ophiolite-hosted alkaline springs, as described below:

Mid-ocean ridge hydrothermal and subsurface habitats – Nearly all efforts to study the

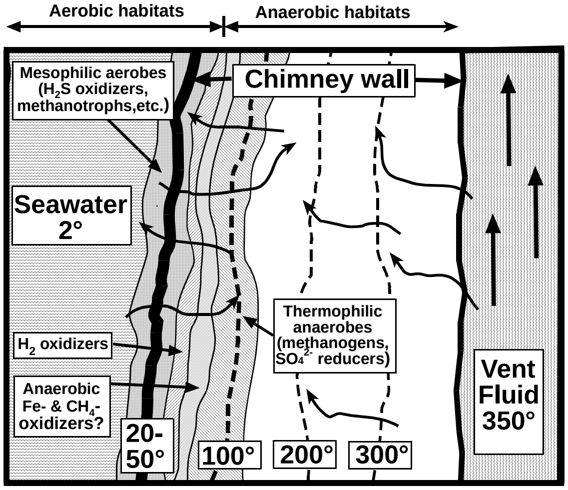


Figure 3.4-1. Distribution of chemolithoautotrophic habitats across a deep-sea hydrothermal chimney wall as inferred from geochemical modeling (McCollom and Shock, 1997). The model indicates that thermophilic microbes should represent only ~1% of the total primary biomass production in this environment. The model results agree with what is known of the microbial population in chimney environments, providing a framework for understanding the distribution of microbes (Karl, 1995; Kelley et al., 2002).

microbial community of deep-sea hydrothermal systems to date have focused on symbiotic and free-living microbes living on dissolved compounds in the direct vicinity of high-temperature, "black smoker" vents. Yet, the high temperature vents and chimneys represent only one of many potential habitats for chemolithoautotrophic microbes that are created by the formation of ocean crust at mid-ocean ridges. Other habitats with a large potential to support chemolithoautotrophic microbes include widespread diffuse subsurface mixing zones where hydrothermal fluids mix with seawater beneath the seafloor, downflow zones on the flanks of ridges where relatively oxidized seawater circulates into the crust on the downward limb of hydrothermal circulation cells, ultramafic-hosted hydrothermal systems where seawater interacts with tectonically-displaced rocks from the upper mantle, and off-axis sites where low-temperature fluids (<~50°C) interact with aging basaltic crust. The proposed models will explore microbial activities in these other habitats. Topics of particular emphasis will be the utilization of solid substrates (basalt, metal sulfide minerals, etc.) as energy sources by chemolithoautotrophs, and the microbial role in mineral alteration and biogeochemical cycling of carbon, iron, and sulfur.

Ophiolite-hosted alkaline springs – Ophiolites represent sections of the ocean crust and upper mantle that have been tectonically transported onto a continent. Several ophiolites occur along the western coast of the U. S. Peridotite, an ultramafic rock composed almost entirely of the mineral olivine, is a predominant rock type in ophiolites. Alteration of peridotite produces strongly reducing conditions through the "serpentinization" reaction: Olivine + H_2O → Serpentine + Magnetite + Brucite + H_2, and dissolution of brucite [$Mg(OH)_2$] generates alkalinity. As a result, fluids emanating from ophiolites are frequently alkaline and highly enriched in reduced gases such as H_2 and CH_4. These reduced compounds could support populations of methanogens, hydrogen oxidizers, and other chemolithoautrophs, but the microbiology of the springs has received very little attention to date. Since rocks of similar composition are widespread on the seafloor and in Archean terranes, and are also represented in martian meteorites (e.g., LEW88516), these systems may have direct parallels to environments on the early Earth and Mars.

We will investigate the chemolithoautotrophic potential of the springs through geochemical models, with particular attention on understanding the source of reduced compounds in the subsurface and describing environments for chemolithoautotrophs within the springs. This work will be performed in collaboration with scientists at NASA Ames (David Blake and Mitchell Schulte) who are presently initiating a comprehensive field and laboratory study of these springs. Their study will collect data on the petrology, fluid chemistry, and microbiology of the springs, and these data will be provided to us as constraints on the models. In turn, the models will provide a framework for interpreting the field and laboratory data.

Models will be constructed by combining thermodynamic and kinetic constraints on chemical reactions with information on geological systems (mineral and fluid compositions, fluid flow, etc.) to develop an understanding of geochemical conditions throughout the system. Construction of the models will be facilitated through the use of available computer packages including EQ3/6 (Wolery, 1992) and PHREEQC (Parkhurst, 1995) that model geochemical processes such as fluid mixing, heating and cooling, fluid-rock interactions, and reactive transport. These programs can be modified to account for kinetically inhibited redox reactions (e.g., McCollom and Shock, 1997; McCollom, 1999, 2000). Input parameters such as rock and fluid compositions, water/rock ratios, fluid fluxes, etc., will be derived from published descriptions or obtained from collaboration with researchers working on the environments under study. Thermodynamic and kinetic parameters will be compiled from published sources as in previous studies. Additional constraints will be derived from ongoing laboratory measurements of geochemical reaction rates conducted by Co-I McCollom, including studies of redox reactions like the reduction of CO_2 to formate and methane (e.g., McCollom and Seewald, 2001, 2003).

It is anticipated that the models will both improve current interpretations of the distribution of the microbial habitats within chemolithoautotrophic ecosystems, and provide new directions for future investigations. For instance, geochemical models of fluid mixing in deep-sea hydrothermal systems have provided a framework for interpreting the distribution of microbes across the walls of hydrothermal vent chimneys (Fig. 3.4-1). In addition, results from previous models indicating that as much as 40% of the chemical energy generated in hydrothermal vents is available in the form of solid substrates rather than as dissolved compounds provided the impetus for new research that led to the discovery of several novel species of chemolithoautotrophic microbes capable of living on sulfide minerals as their source of metabolic energy (Fig. 3.4-2).

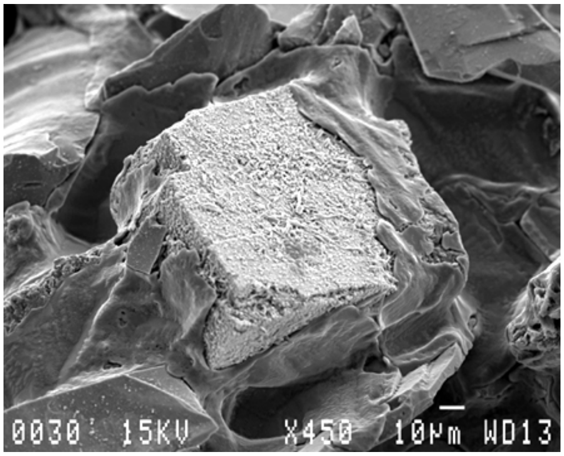


Figure 3.4-2. Axenic culture of an Fe-oxidizing microbe growing chemolithoautotrophically on a pyrite crystal. Unlike previously cultured microbes from deep-sea hydrothermal habitats that are only known to live on dissolved compounds, this species derives metabolic energy from solid substrates such as basalt and metal sulfides. Note thick biofilm and the stick-like Fe-oxide precipitates formed by the microbe where the surface is exposed. This strain was isolated by Co-I McCollom and collaborators K. Edwards and D. Rogers at Woods Hole Oceanographic Institution from samples collected near a deep-sea hydrothermal system (image courtesy of K. Edwards).

The results of this research are expected to have several direct benefits to astrobiology. The models can be readily modified to reflect conditions in analogous habitats on other planetary bodies or on the early Earth (e. g., McCollom, 1999; Varnes et al., 2003). In this respect, this effort will complement studies of potential biological habitats on Mars described elsewhere in this proposal. By providing a rigorous means of evaluating potential microbial habitats on Mars and Europa, the models will be useful in planning future exploration. For instance, the models can be used to define extraterrestrial habitats with the greatest likelihood for supporting life, help evaluate mineralogical and chemical signatures of life in rock samples, and to estimate the likely abundance of biomass in particular environments. In addition, extrapolation of the models to conditions on the early Earth will provide a basis for evaluating the potential for biological activity and resulting biosignatures in ancient rock samples, as well as to provide quantitative estimates of microbial processes for studies of carbon cycling and atmospheric evolution.

Work on this task will be led by Co-I McCollom, who will be responsible for defining model inputs, overseeing model development and execution, and interpretation of the results. Dr. McCollom will be aided by undergraduate student research assistants who will construct computer databases, operate programs, plot results, etc., under his supervision. Collaborators

David Blake and Mitchell Schulte (NASA Ames) will share results from their field studies for use in the alkaline springs models. Co-I McCollom will also assist in the collection and chemical analysis of the field samples. Work on this task will be initiated in year 2, with modeling of mid-ocean ridge and alkaline spring environments performed concurrently.

3.5. Origins of Planetary Systems (task led by Co-I J. Bally)

3.5.1 Introduction

How common are planets beyond the Solar System? How common are terrestrial planets? How diverse are planetary system architectures? How are the materials needed for life processed and accumulated? How do planetary systems form? What hazards do forming and mature planetary systems face? These are key questions in the *"astro"* side of astrobiology. Investigations within our own solar system, studies of nascent planetary systems, searches for and characterization of extra-Solar planets, and *investigation of the environment in which planetary systems form* will eventually provide answers. Our astrobiology research during the last five-years, and the work proposed for the next five, is focused on the planet-forming environment.

3.5.2 Background and highlights of recent research

Planet formation is a byproduct of star formation. Most stars form from the gravitational collapse of *giant molecular clouds* (GMCs) that typically spawn thousands of stars on a time-scale of about 10 million years within regions having dimension of one to tens of parsecs (see reviews in Mannings et al., 2000).

There are many hazards to planet formation. Most low-mass Sun-like stars form in the vicinity of more-massive stars that produce torrents of ultraviolet radiation, powerful winds, and explosions; the typical environment in which most stars are thought to form is violent. The Orion Nebula region, which contains several thousand low mass stars younger than one million years, is the nearest site of ongoing star formation in which both low- and high-mass stars are forming (O'Dell, 2001). The Hubble Space Telescope (HST) has shown that hundreds of these stars are surrounded by protoplanetary disks (the so-called "*proplyds*" – externally illuminated protoplanetary disks). Recently, we found a large population of proplyds in the even harsher environment of the Carina nebula (Smith et al., 2003). These proplyds are being destroyed by the intense UV light emitted by nearby massive stars. Mass-loss rates from disks in the Orion nebula range from 10^{-8} to 10^{-6} Solar masses per year (e.g., Henney and O'Dell, 1999; Bally et al., 2001), implying that a "minimum solar nebula" disk survives only for 10^4 to 10^6 years (Johnstone et al., 1998; Bally et. al., 1998a, b; Bally et al., 2000). If planets form from such disks, they must do so before the disk is destroyed.

Recent observations indicated that most stars are born in groups in which low-mass members can be ejected (e.g., Reipurth, 2000) or cannibalized by their companions (Bonnell et al., 1998). Thus, the star-forming environment may also pose *dynamical* hazards to protoplanetary disks. The jets and outflows produced by young stars during birth provide fossil records of violent dynamical histories (e.g., Reipurth and Bally, 2001). Infrared and radio wavelength observations indicate that over 90% of stars are born in multiple star systems (Reipurth et al., 2001; Reipurth et al., 2002). Thus, the outer parts of most proto-planetary disks surrounding low-mass stars may be destroyed either by UV radiation or by encounters with sibling stars.

Planets *may* form in such hostile environments, if they can do so rapidly by gravitational condensation from the disk (Boss, 2002), or sufficiently close to their parent stars to avoid the radiation or dynamical hazards of the star-formation environment. Indeed, we found evidence for large dust grains at the translucent outer edge of a large protoplanetary disk seen in silhouette against the Orion Nebula (Throop et al., 2001). The attenuation of background light was found to be "gray" below a wavelength of 2 μm, indicating that most dust grains responsible for attenuating background light are an order of magnitude larger than typical interstellar particles (Shuping et al., 2003). These results are consistent with the predictions of grain evolution models in protoplanetary disks (Throop et al., 2003) and may signal the first steps of planet building. Protoplanetary disks in Orion-like environments may form gas giants rapidly by gravitational collapse (e.g., Boss, 2002). However, there may not be sufficient time for the standard core accretion scenario (e.g., Lissauer et al., 1995). Furthermore, terrestrial planets can form if solids are sequestered into centimeter to meter-sized "gravel" by the time disk gas is removed.

3.5.3 Proposed Research

During the next five years, we will quantify the hazards faced by nascent planetary systems and probe proto-planetary disk structure and compositions at infrared (IR) and radio wavelengths. We will take advantage of new facilities and emerging technologies such as NASA's Space Infra-Red Telescope Facility (SIRTF), adaptive optics on the 8- to 10-meter telescopes, infrared cameras and spectrometers operating at wavelengths from 1 to 30 μm, and emerging new radio wavelength capabilities. These tools will enable us to probe the physical and chemical properties of protoplanetary systems throughout the spectrum with an angular resolution which in some cases will be better than that provided by the HST.

Quantifying hazards to planet formation: Is the Orion nebula region typical of the type of environment in which most stars form? What fraction of proto-planetary disks evolve through a proplyd phase during which they are exposed to harsh and destructive UV radiation fields? What fraction of stars form in multiple systems or larger groups in which protoplanetary disks are truncated or destroyed by star-star encounters? Our own data, and public-domain data sets such as 2MASS (2 micron all-sky survey) and the SIRTF Legacy Science programs, will enable us to study the numbers and distributions of young stars in a representative sample of nearby molecular clouds including Taurus, Ophiuchus, Cepheus, Perseus, Serpens, and Orion (e.g., Ungerechts and Thaddeus, 1987; Dame et al., 2001). We have been investigating these clouds for two decades, first at millimeter wavelengths using carbon monoxide (CO) as a tracer of molecular gas and proto-stellar outflows (e.g., Bally and Lada, 1983; Bally et al., 1987, 1991; Miesch and Bally, 1994; Miesch et al., 1999), and more recently at visual wavelengths using the large-format CCD cameras at the National Optical Astronomy Observatories (NOAO). With our NSF funded "Survey of Nearby Star Forming Clouds" (Walawender et al., 2003; Bally et al., 2003), we obtained narrow-band images in Hα and [SII] to identify young Hα emission line stars and proto-stellar outflows (e.g., Reipurth and Bally, 2001). We propose to combine this optical data with public-domain infrared data (2MASS and SIRTF Legacy) to first identify young stars, and then to study their distribution in a representative sample of nearby clouds.

The NASA 2MASS survey covers the whole sky in three near-infrared bands (wavelength of 1.2, 1.6, and 2.2 μm) to a limiting magnitude of about 14 to 15. Since Sun-like stars at the 460 pc distance of Orion have magnitudes slightly brighter than the 2MASS limits, and most young stars are considerably brighter than "main-sequence" stars, this data-base can be used to identify young (0.1 to a few million year old) Sun-like stars to beyond the distance of the Orion star forming region. NASA's SIRTF

Legacy science program, which will produce a rich harvest of 5 to 240 μm infrared data on many nearby star forming clouds, will enter the public domain in early 2004. Specific data sets which will become accessible include: The Perseus molecular cloud and parts of Orion (Evans et al., 2001), star-forming clouds along the Galactic plane (Churchwell et al., 2002), and several hundred nearby stars with ages ranging from 10 to over 500 million years (Meyers, 2001).

Young stars will be identified using well established criteria including excess near-infrared emission originating from the warm inner part of protoplanetary disks (2MASS, SIRTF), the presence of the 0.656 μm Hα emission line of hydrogen, and the association with an outflow (determined from visual wavelength images, narrow-band infrared data, CO radio maps). This effort will lead to a quantitative estimates of the fraction of stars born in environments where UV radiation destroys disks, and the fraction likely to be affected by dynamical interactions.

Tracing Disk History with Near-IR Observations: Our recent research (e.g., Reipurth and Bally, 2001) has shown that outflows and their symmetries provide an indirect means to probe the recent accretion and dynamical history of forming stars and their disks. We will collaborate with Jon Morse (U. Colorado) to make extensive use of a new instrument being built for the 3.5 meter Apache Point Observatory telescope, the Near Infrared Camera / Fabry Perot Spectrometer (NICFPS; Jon Morse, P.I.). NICFPS, to be commissioned in early 2004, will have field-of-view of 5' and a velocity resolution of 10 km/s, and will acquire three-dimensional data cubes that simultaneously probe both the spatial distribution and velocity field of targets. We will study the structures and velocities of outflows from young stars. However, NICFPS does not have the narrow-band filters needed to carry out our research. Thus, we are requesting funding from this grant to purchase the narrow-band filters required to isolate the spectral lines of H_2, CO, and several bright lines of ions (e.g. [FeII]). (Details on the filters are described in the equipment section in vol. 2.) We will measure the radial velocities of bright emission lines produced by proplyds (such as the 2.12 μm line of H_2) to trace motions of disks and surrounding gas. Disk velocity measurements will enable us to determine the velocity dispersion of the ensemble of young stars to a precision of a few km/s. Combining this with the cluster density constrains the rate of interactions with sibling stars. Are some stars ejected as runaways from multiple star systems? Have such systems retained their disks? NICFPS will be used to study outflow symmetries that trace recent dynamical interactions in clustered star forming environments. “S-shaped” outflows indicate changes in disk orientation; “C-shaped” flows indicate motion of the source through a medium, or a side-wind which deflects the flow.

Measuring Disk Properties with Adaptive Optics Observations: Radiation-induced mass loss rates from proto-planetary disks have been measured only for a few proplyds lying very close to Orion’s most massive stars (Henney and O’Dell, 2000; Shuping et al., 2003). Furthermore, these measurements were obtained at visual wavelengths where resolution is limited to about 1 arcsecond by atmospheric turbulence. We will obtain new near-infrared (λ = 1 to 2.4 μm) measurements using adaptive optics (AO) to compensate for this turbulence. AO-assisted 8- to 10-meter telescopes can deliver images limited in resolution only by diffraction, making them as sharp or sharper than those produced by HST.

Last year, Mark Morris, Ralph Shuping (UCLA), and I used the AO system at the 10-meter Keck telescope to demonstrate these techniques on several proplyds in Orion (Shuping et al., 2003). We will continue collaborating in the acquisition of AO assisted images and spectra (with a spectral resolution of about 50,000) of many more proplyds, extending measurements to the outer parts of the Orion nebula where radiation fields are weaker and more typical. We will measure flow velocities, densities, ionization state, and disk rotation speeds using the many spectral features accessible in this wavelength range such as hydrogen and helium recombination lines, lines of H_2, and the vibrationally excited "overtone" bands of CO (e.g., Greene and Lada, 2000) with ~0.1 arcsecond resolution. These measurements will lead to estimates of central star masses, disk mass loss rates, and survival timescales. AO assisted images of disks seen in silhouette against nebular background light ("silhouette proplyds"; e.g., McCaughrean and O'Dell, 1996; Bally et al., 2000; Throop et al., 2001; Shuping et al., 2003) will establish the wavelength dependence of the attenuation through their translucent parts. In some disks, we will be able to measure the wavelength dependence of reflected (scattered) light from the central star. These measurements will constrain particle size distributions and may provide further evidence of grain growth in protoplanetary disks.

Probing Disk Composition in the Thermal IR: Morris, Shuping, and I have also used a λ= 5 to 24 μm camera at Keck to obtain new images of the core of the Orion nebula and the proplyds located close its bright and massive stars. We were also awarded time to use a new camera on Gemini South for similar measurements in the other parts of Orion. Unfortunately, the Thermal-Region Camera/Spectrograph (T-ReCS) was not ready in time for our observations. We will re-propose to use this facility instrument to extend our studies of Orion's and Carinas's proplyds to a wavelength of at least λ= 24 μm. Thermal spectroscopy will provide data on disk composition as prominent bands of solid silicon, graphite, polycyclic aromatic hydrocarbons (PAHs), various ices (water, CO, CO_2, etc.), and gases such as CO_2 occur in this spectral range. Perhaps these thermal observations may detect warm accreting giant proto-planets forming in the nearest disks.

Probing "Invisible" Disk Components at Radio Wavelengths: We propose to conduct exploratory studies of proplyds using the Very Large Array (VLA) radio telescope in New Mexico to measure of the mass of cold dust, characterize grain sizes in the opaque centers of disks, to search for trace molecules such as SiO and HC_3N, and 21 cm atomic hydrogen. Prior attempts at millimeter wavelength mass determination of Orion's proplyds have only provided limits (e.g., the λ= 1.3 mm study of Bally et al., 1998). However, the new λ = 7 mm system at the VLA will provide an order-of-magnitude improvement in sensitivity. If grain growth models are correct (e.g., Throop et al., 2003), and disks contain a substantial reservoirs of mm-sized (or larger) particles, the detection of λ = 7 mm continuum radiation at the VLA should be easy. While making these continuum measurements, we will also use the VLA's spectral line system to search for molecules whose transitions fall within the observed band such as SiO and HC_3N. Disk photo-evaporation models also indicate the presence of substantial coronae of atomic hydrogen above and below the disk. We will attempt to use the VLA to detect neutral atomic hydrogen in either emission or absorption (against the background nebula) at λ = 21 cm. The detection of strong radio-wavelength spectral lines would open a new window for the future study of proplyds with NRAO's Very-Long Baseline Array (VLBA) which delivers sub-milli-arcsecond resolution (corresponding to a fraction of the Earth-Sun distance for disks in Orion) – an orders of magnitude improvement over present methods.

Personnel and Resources: The work described above consists of two tasks: archival data mining, and the acquisition, reduction, and analysis of data obtained at various observatories. Successful execution will require that we manage between 2 and 4 observing runs per year at several private (Apache Point and Keck) and public (Gemini, and VLA) observatories for the next five years. I have supervised a program having a similar scope during the past 5 years in which we used over a half-dozen facilities, typically, with between 3 to 6 separate observing runs per year. I managed this program with one or two collaborators and one to two students. Our publication record during this period provides concrete evidence that we have successfully reduced, analyzed, and published the resulting data.

Astronomers must propose year by year for telescope time, and time allocations on ground-based facilities come with no funding. I have a strong history of winning telescope time for my research. But, to conduct a coherent program on planet formation in addition to star formation processes, I need funding from astrobiology to support travel, salaries, and data acquisition, reduction, and analysis.

The astrobiology component of this research will be leveraged by support from NASA and NSF via complementary funding which supported research with HST and the investigation of the large-scale aspects of star forming regions. I continue to seek support for ongoing studies with HST, investigations of the large-scale structure of star-forming clouds, and outflows from young stars through NSF and NASA. Funding from astrobiology is needed to support the high-resolution multi-wavelength investigation of proto-planetary disks and planet formation. Funding from this program will be used to support part of my summer salary, a full-time graduate student, travel to various observatories, and the purchase of near-infrared filters essential for our research.

The work proposed here can be conducted by myself, one supported student, and collaborators. Other students, hired on NSF and NASA grants, will be trained on the same instruments and techniques. However, astrobiology funding is essential for the application of these methods to the investigation or proplyds and other proto-planetary systems.

4. THEME 3: PHILOSOPHICAL AND SOCIETAL ISSUES

One thing that sets astrobiology apart from many other scientific disciplines is the ways in which the science interacts with the broader society. Ranging from using astrobiology as a way to better understand the nature of science to exploring the public's fascination with the topic and the societal implications, astrobiology provides a powerful way to better understand these interactions. Our group at Colorado is one of the few groups in the country that from the beginning has exploited the connections between the sciences and the humanities, and we will continue to explore these connections during the coming five years. In particular, we wish to better understand the powerful insights that philosophy of science can provide for our science, to examine the societal interest in astrobiology and the influence on culture, and to explore the long history of the relationship between astrobiology and evolution.

A key goal of the analysis, and of including topics from the humanities along with research tasks in the sciences, is to better understand the connections between science and philosophy of science. We are doing this by approaching many of the same questions from the perspective of the scientist and from the perspective of the philosopher of science. Only by approaching the boundary between the two disciplines from both sides can we better understand the nature of this boundary and of the different disciplines, and thereby promote useful dialog between the two fields.

The specific tasks that we will carry out are as follows, and they are described in more detail in the following text.

(i) The nature of historical science and the elucidation of biosignatures (task led by Co-I Cleland). We will use astrobiology as a means

by which to explore the nature of historical sciences from the perspective of the philosophy of science. We will use case studies of well-known examples (such as ALH84001 or the recent controversy over the fossil microbes studied by Schopf) to explore the nature of science, and will develop a full-blown theory of historical science. Also, we will use our previous work on the difficulty of defining life to better understand the proper interpretation of biosignatures in measurements from other planets, especially with the potentiality of coming across really weird life.

(ii) Societal issues in astrobiology (task led by Co-I Jakosky). We will explore the connections between science and society by improving scientists' understanding of the nature of science, examining the societal significance of astrobiology, and discussing ways in which the science community can enhance the connections between scientists and both philosophers of science and the public.

(iii) The evolution of astrobiological thought (task led by Co-I Friedman). Well known evolutionists explored the origin of life and the potential for life elsewhere and, in fact, anticipated many of the questions now seen as central to astrobiology. We wish to understand the historical context of our field, and will examine the interface between biology and evolution as played out over the last two centuries.

(Note that tasks ii and iii are a formal part of our proposal but do not utilize any funding from the NAI. They are being done at no cost to NASA.)

4.1. The Historical Character of Astrobiology and Circumventing the Problem of Defining "Life" (task led by Co-I C. Cleland)

My proposed research program for the next five years is two-pronged. First, I plan to develop a philosophical theory of historical science with special emphasis on issues pertaining to astrobiology. I will focus on the pivotal role played by (i) "signatures" (faint, indirect traces) as evidence and (ii) computer simulations as surrogate experiments. This is particularly important to astrobiology because (as discussed below) much of the research is historical in character and extensively utilizes both resources. Second, I plan to investigate general strategies for searching for extraterrestrial life in the absence of a definition of "life". This issue is crucial to astrobiology given the insurmountable problems associated with defining "life" (Cleland and Chyba, 2002) and the tendency of astrobiologists to design instrument packages for searching for extraterrestrial life in light of a favored definition of "life".

There are fundamental differences between historical research and experimental research. Historical hypotheses are concerned with particular past events vs. timeless regularities. The pivotal evidence for historical work comes from fieldwork—from searching the messy, uncontrollable world of nature for traces of long past events—as opposed to performing controlled experiments in the contrived setting of a laboratory. Much of astrobiology is historical in character. Research into whether Mars once had standing lakes, Earth's early atmosphere was highly reducing, cyanobacteria existed on Earth 3.5 bya, and meteorite ALH84001 contains fossilized martian life provide salient examples. Moreover, most astrobiological research that is not explicitly historical nonetheless confronts similar problems. The hypothesis that Europa has a large subsurface water ocean is based on indirect evidence (e.g., fracture and tilt patterns observed on its icy crust). Thus, questions about the methodology of historical science and its differences from experimental science are of central importance to astrobiology.

Previously, I showed (Cleland, 2001, 2002) that historical and experimental scientists have access to different evidential relations. The source of this difference lies in an objective and pervasive feature of nature, a time asymmetry of causation. More specifically, localized present events (the ultimate locus of all observation) *over*determine their localized past causes and *under*determine their localized future effects. This means that the present contains many disparate traces (e.g., pumice, ash, masses of basalt) of a past event (volcanic eruption). The work of historical scientists reflects this; they search the present for pivotal traces of past occurrences, and the overdetermination of the past by the present guarantees that such traces are likely to be numerous in both quantity and kind. In contrast, the conditions that experimentalists deliberately bring about in the lab are only partial causes of what subsequently happens; they underdetermine it. One can thus never fully exclude the possibility of a false positive or a false negative. This explains why experimental work is focused on "controlling" for interfering conditions. In short, the differing practices of historical scientists and experimentalists are grounded in an objective asymmetry in the evidential relations at their disposal.

I propose to develop the above framework into a full-blown philosophical theory of historical science. Understanding the nature of the evidential relation between a body of traces and a hypothesis is crucial to historical science, and particularly important to astrobiology given its extensive reliance upon "biosignatures". Most remote and *in situ* robotic investigations are designed to detect faint, indirect traces of life, e.g., isotopic records, biomolecules, geochemical anomalies. Without a clear understanding of what is required for a body of traces to qualify as a reliable evidential foundation for a hypothesis, it will be difficult to reach scientifically convincing conclusions about the presence or absence of life. Although the asymmetry of overdetermination guarantees that the present contains many traces of the past, traces become attenuated with the passage of time. Identifying traces of long past events typically requires sophisticated analytical techniques, and even then it is often difficult to interpret their significance. So much depends upon making correct assumptions about the environment that produced and sustained them until their discovery and subsequent analysis. Inadequate exploration of these assumptions can undermine what would otherwise be solid historical research. A pertinent example is the controversy over the use of carbon isotope ratios (in graphite inclusions from Akilia, Greenland) to infer the existence of 3.8 billion year old terrestrial organisms (e.g., Mojzsis and Harrison, 2002; Fedo and Whitehouse, 2002). In other words, successful historical research requires a stable evidential foundation, but by their very nature, highly indirect and attenuated traces seem unable to provide this.

I propose to investigate the logical, empirical, and social conditions under which indirect traces amplified by sophisticated analytical techniques become scientifically *trustworthy*. This will involve detailed case studies of scientific controversies hinging upon questions about the reliability of various putative "signatures", e.g., the controversy over the interpretation of carbon isotope ratios in quartz-pyroxene rock from Akilia (see above), the controversy over Schopf's interpretation of filamentous structures (found in Australian apex chert) as 3.5 billion year old terrestrial microfossils (e.g., Schopf and Packer, 1987; Schopf, 1999; Brasier et al., 2002; Schopf et al., 2002), and the ongoing debate over the significance of the prismatic magnetite in ALH84001 (e.g., Thomas-Keptra et al., 2000; Freidmann et al., 2001; Golden et al., 2001).

The use of computer models as surrogate experiments is another subject that warrants attention. Computer simulations are common in astrobiology. They are used to "test" conjectures about, e.g., the evolution of the solar system from the solar nebula, the chemical composition of the Earth's early atmosphere, and the structure of Europa beneath its icy crust. To the extent that they manipulate different assumptions about the unobserved causes of observed traces, computer simulations resemble experiments; they yield "predictions" that can be compared with old observations and that suggest new observations. But they also differ from experiments because there is no guarantee that all the relevant conditions and processes operating in a targeted physical system are represented in the model; viewed from this perspective, they more closely resemble specialized theories than experiments. There is thus a pressing need to understand the nature of the support offered by computer models for hypotheses. I propose to address this problem by studying concrete examples (see above) of the use of computer models in astrobiology. My focus will be on the complex logical relations that obtain between a computer model, the evidence upon which it is based, the "predictions" that are derived from it, and the hypothesis that it reputedly supports.

Most remote and *in situ* searches for extraterrestrial life explicitly or implicitly presuppose a definition of "life". The Viking experiments, which were designed to detect carbon metabolism, provide a salient example. In Cleland and Chyba (2002, 2003), I challenged the utility of using definitions of "life" to guide research in astrobiology. Definitions associate concepts with words. They cannot reach beyond our current understanding and tell us about the way things really are. Accordingly, no definition of "life" can provide a scientifically compelling answer to the question "what is life?" What is required to answer this question is a *theoretical identity* (analogous to "Water is H_2O" or "Temperature is mean kinetic energy") grounded in a truly general theory of living systems. Unfortunately, because our theorizing has been limited to life on Earth and all life on Earth shares a common biochemistry, we do not yet have such a theory. This puts us in a position analogous to someone from the seventeenth century trying to define "water" before the advent of molecular theory. No analysis of the seventeenth century concept of water (as a transparent, odorless liquid that is a good solvent) could have revealed that water is H_2O. Yet this is what is required to answer the question "what is water?"

This presents us with a dilemma: Without a definition of life, how will we recognize alien life if we happen to encounter it? This dilemma is exacerbated when one considers the possibility of encountering very simple but really weird alien life. Considerations from the history and philosophy of science suggest possibilities for addressing and ultimately resolving it. Insofar as it emphasizes detecting biosignatures, the search for extraterrestrial life has an historical character. While Ken Nealson and his team recognize the importance of *diversity* of measurement (Nealson and Conrad, 1999, 2001), there has been little discussion of *trustworthiness* (discussed earlier). Moreover, in the absence of *any* examples of non-Earth-like life, abstracting from the biological details of terrestrial life is very problematic. Without a collection of trustworthy biosignatures for *terrestrial* life we are unlikely to reach a scientific consensus regarding the presence of Earth-like extraterrestrial life. Trustworthy terrestrial biosignatures can also play a crucial (albeit different) role in detecting really weird alien life so long as they are not taken as definitive of life. They can help us to discriminate insignificant false positives and false negatives from genuinely ambiguous cases. Genuinely ambiguous cases are the important ones. They provide us with Thomas Kuhn's famous "anomalies" (Kuhn, 1962, Ch. VI). Anomalies are the best grist for the theoretical mill. They alone stand out clearly against the backdrop of our current theories, forcing us to think beyond "the box" and come up with better generalizations about life. It is only in the context of such ideas that we will be able to formulate the necessary *theoretical identities* required for a truly non-Earth-centric approach to the detection of extraterrestrial life.

The above ideas are tentative. They need further exploration and fleshing out. I propose to begin this task by investigating biosignatures for detecting ancient terrestrial life: Which biosignatures are considered most and least trustworthy? On what considerations are these judgments founded; do they reflect appropriate and consistent logical and empirical considerations? I will then compare these biosignatures to those currently being proposed for searching for extraterrestrial life. Can the differences between the two classes be rationally justified? Are they driven by implicit definitions or otherwise unwarranted abstractions from terrestrial life?

Work Plan: My work on the historical science task will be spread out over the full five years of funding, with the first half of this period being devoted to the evidential role of "signatures" in astrobiological research and the second half being devoted to the use of computer simulations in astrobiological research. I anticipate some trips to other NAI institutions for extended discussions with appropriate scientific experts. My work on the "definition of life" task will be carried out in parallel with my work on historical science. Christopher Chyba (SETI Institute and Stanford University) will collaborate on the problem of searching for extraterrestrial life without a definition of "life". He will consult on the scientific aspects of searching for life elsewhere, in the context of the philosophical issues described above; his expertise spans both areas.

4.2. Societal Issues in Astrobiology (led by PI B. Jakosky)

The tremendous public interest in astrobiology is at a level far beyond its practical relevance, and suggests a broader public interest in exploration. We can use this interest as a way to educate the public about science in general and to inform them as to the nature of scientific inquiry (what science is and, of equal importance, what it is not). We will explore the underlying issues regarding the nature of science and its role in society and to encourage activities on the part of the astrobiology community that emphasize these connections (see Jakosky, 2000; Jakosky and Golombek, 2000; Bennett et al., 2002). As our objective is to engage the science community and to understand the nature of their science, this is a "science" rather than an "outreach" task. Specific activities will include:

(i) We will explore how astrobiology as a science operates and contrast the different approaches to doing science from the different disciplines (e.g., historical vs. experimental science). We will do this using specific case examples, with the goal of enhancing connections between disparate disciplines by helping researchers to understand approaches that differ from their own.

(ii) We will investigate the public's interest in astrobiology as a means of exploration, its interactions with the science community, and the role that exploration science plays in society. We will do this by identifying those parts of astrobiology that particularly attract the public's attention, relating them to the components that are most central to the science, and understanding the role of discovery and exploration science in astrobiology.

(iii) We will work with the astrobiology community to enhance the effectiveness of public outreach activities by addressing specifically these underlying issues that are of most interest to the public. We will do this by organizing science forums to discuss the societal connections at astrobiology workshops and conferences and by representing these goals in professional societies and advisory committees. We also will take the ideas directly to the public through various opportunities with the media and by working with students at all levels (e.g., the recently completed undergraduate textbook by Bennett, Shostak, and Jakosky).

Results will be presented at conferences and workshops, in popular science venues, and in a book on astrobiology, science, and society.

4.3. The Evolution of Astrobiological Thought (led by Co-I W. Friedman)

The launch of the NASA Astrobiology Institute in 1997 marked a major initiative to meld the fields of spaces sciences and biology in an effort to gain insights into the question of whether life exists elsewhere in the universe. This interface between biology (particularly the study of the history of life on Earth) and evolution of the universe actually has a remarkably rich intellectual history that goes back at least to the 1840s. Among the major 19th century individuals who can be viewed as the first astrobiologists are Baden Powell (1796 – 1860), Robert Chambers (1802 – 1871) and Alfred Russel Wallace (1823 – 1913). Each of these individuals wrote explicitly about the origin (or origins) of life on Earth and considered the question of the origin and evolution of life elsewhere in the universe (within, of course, the context of 19th century cosmology). What is most impressive is how many of the central questions associated with the NASA Astrobiology program in the 21st century were anticipated by these early thinkers. During the next five years, we propose to explore the intellectual history of early evolutionary and astrobiological thought, and to focus specifically on those who wrote and speculated about the likelihood and requisite conditions for the origin and evolution of life elsewhere in the universe. The goal is to understand the history of intellectual thought about life elsewhere and to relate it to current thinking. Results will be presented as a collection (and analysis) of the writings of these (and other) early evolutionists; negotiations for publication are in progress with the University of Chicago Press.

5. THEME 4: ASTROBIOLOGY TECHNOLOGY INITIATIVE

5.1. Introduction

One of the high-level components of the mission of the NAI is “providing scientific and technical leadership on astrobiology investigations for current and future space missions and for ongoing research programs”. However, the NAI currently does not have a strong connection to the flight programs except through the separate activities of its individual members. We propose to form an NAI Focus Group on Astrobiology Technology (ATFG) that includes representation from the science, instrument technology, and mission technology components of astrobiology. The ATFG would (i) work with the community to define astrobiology science goals that can be addressed by flight missions still being defined, (ii) foster community development of appropriate technological capability to address the science goals, through both individual instrument concepts and mission concepts, and (iii) provide leadership in developing science community awareness and understanding of technology capabilities and advances and of the instrument and mission process within NASA.

Astrobiology instrumentation is in the germination state relative to NASA’s space flight mission opportunities. This is in large part because of the relatively recent increase in the importance and role of astrobiology in space science. As a result, the infrastructure does not exist within the astrobiology community to define, develop, propose, build, and operate instruments and missions that emphasize astrobiology science goals. This problem is apparent, for example, in the current planning within the Mars program; it is not clear that the necessary instruments can be brought to appropriate technology readiness levels in time to propose for the 2009 Mars Science Laboratory mission.

This lack of a strong connection between the NAI and flight missions is of concern because it is through these flight programs that NASA makes many of the astrobiology-related measurements. This is especially the case in that astrobiology science goals have moved to the intellectual center of the space science programs, as described, for example, by both the 2002 NRC Decadal Survey for Solar System Exploration and the recently completed Code S Space Science Roadmaps for all themes. The strongest formal connection at present between the NAI and the flight missions is through the NAI Focus Groups. The Mars and the Europa Focus Groups have emphasized providing short-term advice on missions and instruments. However, even these groups have not been involved in the long-range planning that is necessary for the community to develop specific instruments and missions that could address high-priority science goals. For example, the March 10-11, 2003, NAI-sponsored workshop on Mars life detection technology has been postponed twice in the last year, and now will occur relatively late in the planning for the 2009 MSL mission. And, the new opportunity to develop a Jupiter Icy Moons Orbiter (JIMO) will require strong integration between the astrobiology and flight mission communities in order to develop the scientific ratonale and intrument and mission concepts.

Our efforts will involve stimulation of the community through short courses, workshops, and the preparation of white papers on astrobiology technology that will help to meld the science and technology communities into a coherent group capable of proposing cutting-edge astrobiology investigations. Leadership will be provided by science researchers at CU who span the breadth of relevant disciplines, technologists at CU who have been involved in the development of low-cost space flight instruments and missions, technologists at Ball Aerospace Corporation (in Boulder, CO) who have been heavily involved in space flight instruments and missions that are pertinent to astrobiology, technologists at Lockheed Martin Astronautics Corp. (in Denver, CO) who have been intimately involved in development and operations of Mars orbiters, landers, and Scout mission concepts, and biotechnologists at Carnegie Institution of Washington (in Washington, D.C.) who are involved in state-of-the-art development of biotechnology hardware for space flight opportunities.

5.2. Building community through an astrobiology technology initiative

Building a rapport between scientists, instrument developers, spacecraft engineers, mission designers, and operations specialists has been key to some of the most outstanding success stories for planetary exploration. Such rapport invariably derives from common levels of understanding of the challenges facing each subdiscipline and a willingness to find appropriate solutions to enhance scientific success without compromising mission success. Growth in Principal Investigator savvy occurred long ago in the solar system and the astrophysics communities, enabling both fields to take great advantage of NASA missions. However, this growth has not yet occurred within the astrobiology community.

Despite the now-substantial investment in technology, the astrobiology community has not yet become fully engaged in the process. The NASA Astrobiology Science and Technology Instrument Development (ASTID) program and the Astrobiology Science and Technology in Exploration Program (ASTEP) were created to help address this technology gap. In addition, astrobiology-related instruments are being developed through the Mars Instrument Development Program (MIDP) and the Planetary Instrument Definition and Development Program (PIDDP). Each program is operating without substantial interactions with the community as a whole, and there is little overall community insight into what instruments and concepts are being developed, little community involvement in the directions necessary to achieve scientific goals within astrobiology, little feedback to the community as a whole on how the development is going or what instruments and mission concepts exist, and no public forum for discussion of what directions or concepts are important in instrument development. It is apparent that many of those who are doing technology development do not fully understand the science and many of those who understand the science often do not know how to move forward with the technology. As a result, the efforts as a whole remain relatively immature, although there are a few groups that are able to work effectively. While NASA centers could be providing the oversight and leadership to ensure that necessary community and technology are being developed, this is not happening at present. As a result, there is substantial risk that the necessary technology and instruments will not be developed in a timely or effective manner.

We propose to act as a catalyst to foster and encourage discussion of the technology issues within the broader astrobiology science and technology community, to bring together members of the community for discussion of science and technology goals and issues, and to provide leadership to ensure that necessary and appropriate technology issues are addressed.

It is important to recognize that we are not proposing to, alone, provide direction for the program as a whole. No individual group will have sufficient background and expertise to do this, although we do bring together a combination of science and engineering expertise that may be unique in the astrobiology community. We also are not proposing to the NAI and NASA for funding to develop technology ourselves. Such an investment would not be appropriate for the NAI in general. Finally, we are not asking NASA for funding that we would distribute to other groups to develop technology. That is the purview of the technology programs at NASA and not of one group within the NAI.

By itself, formation of an NAI Focus Group on Astrobiology Technology would not be sufficient to address the technology and flight mission concerns raised here. While such a focus group would provide a forum in which technology issues could be discussed, it would attract interest mainly from those (primarily scientists) who are already members of the NAI, would not provide an opportunity to get participation from industry, and would not provide the leadership from the combination of science and industry perspectives that we envision. By proposing a technology initiative here, we will involve identified leaders from both science and industry right from the beginning who can provide the necessary leadership to promote development of the community as a whole.

5.3. Cross-disciplinary issues to be addressed

Our goal is to stimulate interactions between the communities representing astrobiology science, flight instrument design and operations, spacecraft and mission design and operations, and biotechnology. Below we address technology issues in instrument and spacecraft design and operation and in biotechnology that need to be understood within each group in order to develop appropriate instrumentation.

(i) Space instrument and mission technology

This topic includes a series of activities that are intended to develop the capability of astrobiologists to integrate technology into the discipline of space mission programs. Focus is oriented to maximizing scientific progress. Flight design parameters important to accommodation for spaceflight include not only weight, shape and volume constraints, but also field-of-view and stability requirements such as thermal, pointing, and dynamic mechanical environment. Many instruments are sensitive to condensable volatiles and/or particulate contaminants, and to electromagnetic interference and electrical grounding schemes. A variety of data processing methodologies are now possible, and investigators should become familiar with ways in which to accomplish these, as well as a familiarity with contemporary and advanced approaches to instrument programming, safing, diagnostics, and fault tolerance.

Workshop case studies will include practical examples of "best practices" methods as well as approaches to instrument design and implementation that can be counterproductive. Tutorials will include topics on instrument development scheduling, selection and back-up of advanced technologies, methods in risk mitigation, and use of form, fit, and function engineering models and experiment brassboards in early prototype testing.

Surveys of spacecraft characteristics will include those of flybys, orbiters, landers, rovers, and penetrators. Environments of particular interest to astrobiologists, such as the atmospheres of Venus and the gas giants, and the surfaces of planets and comets will be surveyed, with particular emphasis on Mars, Europa, comets and Titan. Understanding special challenges such as the unique radiation environments of deep space and planetary radiation belts will be essential for certain investigations.

For those instruments which sample the environment directly, whether gases or solids, the nature of sample needs in terms of quantity and quality can be paramount to the success of the investigation. Planetary atmospheres and surfaces are prime candidates for *in situ* investigation, but pose major challenges for reliable sampling. Sample acquisition and processing options are often as critical as the measurements to be made.

It would also be very useful to increase the astrobiology community's awareness of the power of remote sensing technology capabilities, from cameras or spectrometers near solar system targets, all the way to extrasolar observing platforms such as Astrobiology Explorer (characterizing potential biomolecular components in the interstellar environment), Kepler, and Terrestrial Planet Finder.

(ii) Biotechnology

One of the under-exploited areas of the interface between biology, engineering, and space sciences has been the application of biotechnology techniques to solar system exploration. The development of these techniques for space flight would fulfill a unique purpose in space exploration — the detection and characterization of organic and prebiotic chemistry distributed throughout the solar system, with implications for finding life elsewhere in our solar system. The current techniques used for solar system exploration have provided ambiguous results or failed completely to detect indications of life; this may be a combination of inadequate technology and the lack of understanding where and how to look.

Ideally, any organic detection instrument developed for space exploration must be able to detect and distinguish several classes of organic molecules of astrobiological interest. Biotechnology techniques have been used successfully to detect a large majority of the key biomolecules and, with the correct controls, could distinguish between spacecraft biomarker contamination and a true extraterrestrial signal. The obvious exceptions are molecules based on non-terrestrial biochemistries. Current immunoassays, such as Enzyme-Linked Immunosorbent Assay (ELISA), are known to be 150 times more sensitive and a great deal more specific than the best lab-based GCMS instruments (Li et al., 1999), and the Limulus Amebocyte Lysate (LAL) has proven to be a valuable resource for diagnosing extremely small quantities of microbial cell wall material (less than 0.1 picograms of initial biomarker signal) (Wainwright and Child, 1999).

Many of these techniques have been miniaturized for commercial lab applications and hence the marriage of nanotechnology and biotechnology has brought about the birth of "lab-on-a-chip" commercial instruments. BioMEMS and Microfluidics are fundamental to this new paradigm of exploration science, but there is still development and research required to prepare this technology for flight.

These techniques have been developed primarily for clinical chemistry or medical diagnostics carried out in laboratory environments. Hence, although the fundamental technology (e.g., for Agilent/Caliper's RNA assay chip) is small in size, development of the support instrumentation to drive these systems has so far paid little or no attention to volume or mass requirements, or to concerns about the thermal, pressure, gravity or radiation environment to which these systems would be subjected during a space flight mission. Also, since these instruments would be primarily laboratory based, many require some amount of human interaction in the preparation of the sample prior to analysis. Many biotech companies have also developed their technology for a "disposable" society, and there are several systems that are made out of materials that would never survive a long-duration space-flight mission. On the other hand, Biotechnology, BioMEMS and microfluidics are uncharted territory for most aerospace engineers. The design and flight development rules that apply to traditional macro systems simply will not apply when you enter the micro/nano regime. Hence, a collaborative effort or an enhancement of communication between spacecraft engineers and biotechnologists will be crucial for astrobiologists to effectively integrate biotechnology into research and expedition science.

5.4. Specific tasks proposed

We are proposing the following activities in order to address our objectives:

(i) We propose to create an NAI-sponsored Focus Group on Astrobiology Technology. This group would be constituted under the standard NAI rules and procedures for creating focus groups, and would be open to both members of the NAI and non-members. This group would be the formal unit that would organize workshops and short courses (described below), write white papers, etc. The focus group will be integrated with the astrobiology biotechnology focus group that Dr. Andrew Steele (Carnegie Institution of Washington) already has begun to organize. Dr. Steele supports the combination of these into a single effort, and is a formal Collaborator on the present initiative.

(ii) We will sponsor a series of meetings that would provide a forum on astrobiology science and technology issues. As our goal is to stimulate community discussion, there are a number of different types of forums that might be productive, and we would organize the different types as an experiment. These would include:

-Workshop on status of instrument and mission development, in which those who are funded by PIDDP, MIDP, ASTEP, and ASTID, as well as those who are not, would have an opportunity to present their results to and interact with each other and with the broader community;

- Workshop on science and technology needs, in which the community could discuss what the current technology status and development needs are and thereby provide input to the members and to NASA HQ on productive directions. The output product would be a series of white papers aimed at NASA HQ, and the workshops would be repeated regularly with different emphases (e.g., Mars, Europa, Earth-orbiting telescopic, planet-orbiting spacecraft remote sensing, extrasolar missions, etc.);

- Short course on astrobiology for space science technologists, to bring them up to speed on the science issues and directions and to provide guidance as to the needs of this community; and

- Short course on development of flight hardware and missions and mission operations for astrobiologists, to educate the scientists who have good ideas but don't know how to proceed. These workshops will fulfill the needs of astrobiologists for information which enables them to develop investigations which are realistic and compatible with the constraints of space flight.

(iii) We will work with the astrobiology discipline scientist and senior scientist at NASA HQ, the astrobiology integration office at NASA/ARC, and the NAI to identify areas that have specific needs in terms of technology development issues, and we will convene community workshops to address the issues and, where appropriate, write white papers to document the current state of the art and the community needs. White papers and workshop results will be posted on the web for broad dissemination.

5.5. Roles of key participants

Key participants are listed below. Together, they will comprise a working group to oversee the activities described above.

Bruce Jakosky (P.I. of this proposal) and John Bally (Co-I) will lead the science input into the technology development initiative, with involvement as appropriate from the other CU Co-Is.

Michael McGrath (Co-I, Engineering Director, CU Laboratory for Atmospheric and Space Physics) will lead the efforts to integrate flight technology issues from the perspective of small-scale instruments and spacecraft, with participation from other engineering and technical staff. The group he leads has developed and flown instruments, spacecraft components, and spacecraft on earth-orbiting and planetary missions. Through these efforts, LASP has been a pioneer in technology and operations for small space instruments.

Benton Clark (Co-I, Chief Scientist, Lockheed Martin Astronautics Space Exploration Systems) and James Crocker (Co-I, LMA Vice President for Civilian Space Program) will lead the efforts to include spacecraft and mission design and operations. Their group at LMA built and is operating the Mars Global Surveyor and Mars Odyssey spacecraft, has built Mars landers, and is the industry partner for all four Mars Scout proposal finalists currently under consideration.

Harold Reitsema (Co-I, Head, Ball Aerospace Civil Space Systems Space Science Directorate) and Steven Kilston (Co-I, Senior Staff, Ball Civil Space Programs) will lead the efforts to incorporate large and telescopic instrumentation and mission design concepts. Their group has built a large number of instruments relevant to astrobiology, including most of the Hubble Space Telescope cameras, the IRAS and SIRTF infrared telescopes, the upcoming Kepler mission to detect habitable extrasolar planets, and the HiRISE camera for the 2005 Mars Reconnaissance Orbiter.

Andrew Steele (Collaborator, Carnegie Institution of Washington) will lead the efforts to incorporate biotechnology with space science flight opportunities. He is a leader in the pioneering of biotechnology and microfluidics in astrobiology space flight instrumentation development.

Noel Hinners (Collaborator, currently CU/LASP, previously both Vice President for Civilian Space Program at LMA and Associate Administrator for Space Sciences at NASA) will consult on the industrial perspective on technology development.

6. MANAGEMENT PLAN

All research, outreach, education, and other programs supported through the NAI will be coordinated and run through the University of Colorado Center for Astrobiology, which is a Center (operating under the rules and regulations for Centers at CU) within the Laboratory for Atmospheric and Space Physics and reporting through the LASP Director to the Dean of the Graduate School and the Vice Chancellor for Research. The Principal Investigator of this Proposal also serves as the Center Director.

Individual research tasks will be planned and carried out under the supervision of each Co-Investigator, using research facilities provided by or in conjunction with their home departments. While the individual task descriptions present a five-year research plan, it is anticipated that each Co-I will make revisions to their plans in response to discoveries and new results, both within their own group and in coordination with the larger NAI and astrobiology research program. Two new Co-Is are being added to our program; one (R. Pappalardo) will be added in year 1, and the second (T. McCollom) will be added in year 2.

The Principal Investigator and science Co-Investigators together will comprise a steering committee to oversee astrobiology research, education, and other activities at CU. As appropriate, they will make decisions to reallocate funds, if appropriate, to make recommendations to add or delete Co-Is, to invest resources in specific activities that are a part of strengthening the astrobiology community, etc. The steering committee will meet at least once per semester, as it has for the past four years. Each Co-I has reviewed this proposal and endorses their participation (see accompanying letters of endorsement in Volume 2).

The Principal Investigator and technology Co-Investigators together will comprise a steering committee to oversee the technology development activities. They will work in coordination with NASA HQ and the NAI to ensure appropriate participation and representation in these activities on the part of the astrobiology science and technology communities and will make a special effort to reach out beyond the NAI community.

Resources will be managed through LASP, and funds will be allocated for use by each Co-I. LASP will maintain financial oversight of each account and will prepare financial reports and provide them to NAI and NASA as necessary.

Subcontracts will be made to Ball Aerospace Corp. and to Lockheed Martin Astronautics for participation in our technology initiative. These activities will be managed and oversight provided by a steering group comprised of the Principal Investigator, the Co-Is from each organization, and Collaborator Andrew Steele (Carnegie Inst. of Washington).

Education and public outreach activities will be managed and oversight provided by Dr. Emily CoBabe-Amman (LASP Education and Outreach Coordinator), in cooperation with the Principal Investigator.

The Principal Investigator will have final authority on allocation of resources, implementation of the program, and oversight of research and other activities, and responsibility for financial management and reporting to the NAI and to NASA Headquarters on a timely basis, subject to the rules and regulations of the University of Colorado and of NASA. The P.I. or, in his absence, a representative appointed by him will serve as a member of and participate in the activities of the NAI Executive Council.

Science Collaborators are scientific colleagues who have agreed (see letters in Volume 2) to participate in the research to be carried out by the Co-Is, as described in the science task descriptions, at no cost to this proposal unless otherwise articulated in the individual science task descriptions.

Astrobiology Collaborators are scientists who are active in various component areas in astrobiology, who work in the vicinity of CU (at CU or at Southwest Research Institute in Boulder), and who have agreed to participate in the activities of the Center for Astrobiology. They will contribute to the intellectual environment surrounding astrobiology at CU, and they provide a way for us to leverage the activities supported by the NAI into a larger astrobiology research, teaching, and outreach program. Astrobiology Collaborators have provided letters (see Volume 2) stating that they agree to participate in the University of Colorado Center for Astrobiology as described here. The Astrobiology Collaborators and their fields of interest are listed in Table 6-1.

We will foster interdisciplinary interaction and integration across our diverse group by holding regular meetings of various combinations of participants. We envision the following types of interactions: (i) We will form local working groups associated with each of our three research themes, and involving the appropriate Co-Investigators, Collaborators, and members of their research groups. These will meet informally on a regular basis to share results and discuss research directions. (ii) We will have regular meetings of all of the Co-Investigators and senior researchers, to discuss research programs and new results in a small and informal setting. (iii) We will have less-regular informal workshops (e.g., up to several times per year) involving all of the participants in the Center for Astrobiology. (iv) As discussed in the section on strengthening the astrobiology community, we will hold monthly astrobiology colloquia, to bring in keynote speakers from outside of the Colorado group and to hear about the latest scientific results.

We are aware of the separate proposal to the NAI from Southwest Research Institute (David Grinspoon, P.I.). As we understand it, our proposals are complementary to each other. If both are funded, we intend to collaborate and to integrate the research and the education and public outreach plans of the two groups in order to take full advantage of the proximity of the two groups and eliminate possible duplication of education and public outreach activities, while at the same time keeping the programs distinct so that they can each achieve their independent goals. The P.I. of the SWRI proposal has agreed to be a Collaborator on our proposal.

Table 6-1. Center for Astrobiology Collaborators

Star and planet formation and evolution

Thomas Ayres (CASA), evolution of stars and the solar wind
Veronica Bierbaum (CHEM), interstellar environment and planetary formation
Luke Dones (SWRI), planetary evolution
Hal Levison (SWRI), planetary formation, evolution and dynamics
Ted Snow (APS and CASA), interstellar environment and planetary formation
Glen Stewart (LASP), planetary formation and evolution
Brian Wood (JILA), time variability of the solar wind of sun-like stars

Planetary evolution

Dan Baker (LASP and APS), planetary/solar-wind interactions
Clark Chapman (SWRI), planetary impacts and evolution of the solar system
Mike Mellon (LASP), Mars geological evolution
Nick Schneider (LASP and APS), planetary evolution

Planetary habitability

Mark Bullock (SWRI), evolution of planetary atmospheres and exobiology
David Grinspoon (SWRI), planetary evolution and exobiology
Jose-Luis Jimenez (CHEM), experiments related to aerosols in the atmosphere of early Earth
Sara Martinez-Alonso (LASP), Mars mineralogy, remote sensing, and potential environments
Alex Pavlov (LASP), aerosol formation and methane planetary atmospheres
Margaret Tolbert (CHEM), aerosol composition and radiative transfer in planetary atmospheres

Microbial diversity and life in extreme environments

Ruth Ley (MCDB), molecular analysis of microbes in hypersaline ecosystems
Diane McKnight (ENGR), environments and ecology in Antarctica
Steven Schmidt (EPOB), microbiology in psychrophilic environments
John Spear (MCDB), molecular analysis of microbes in hypersaline and hydrothermal ecosystems

Origin and evolution of life

James T. Hynes (CHEM), theoretical studies of peptide bond formation under pre-biotic conditions
Veronica Vaida (CHEM), chemical evolution and origin of life

Societal and philosophical issues

Tom Yulsman (JOUR), origins, science, and society

[Unit abbreviations: APS = Dept. of Astrophysical and Planetary Sciences, CASA = Center for Astrophysics and Space Astronomy, CHEM = Dept. of Chemistry and Biochemistry, ENGR = School of Engineering, EPOB = Dept. of Environmental, Population, and Organismic Biology, JILA = Joint Institute for Laboratory Astrophysics, JOUR = Dept. of Journalism, LASP = Laboratory for Atmospheric and Space Physics, MCDB = Dept. of Molecular, Cellular, and Developmental Biology, SWRI = Southwest Research Institute in Boulder]

7. REFERENCES

Abbas, J. D., B. A. D. Hetrick and J. E. Jurgenson. Isolate specific detection of mycorrhizal fungi using genome specific primer pairs. *Mycologia* **88**(6), 939-946, 1996.

Ausubel, F. M. ,R. Brent ,R. E. Kingston ,D. D. Moore ,J. G. Seidman ,J. A. Smith, and K. Struhl, Ed.; Current Protocols in Molecular Biology; John Wiley & Sons, Inc.: New York, 1995.

Bailey, T. L., and C. Elkan In *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*; AAAI Press, Menlo Park, California: 1994; pp 28-36.

Bally, J. and Lada, C. J. The high-velocity molecular flows near young stellar objects, *Astrophysical Journal*, **265**, 824, 1983.

Bally, J., Stark, A. A., Wilson, R. W., and Langer, W. D., Filamentary structure in the Orion molecular cloud, *Astrophysical Journal*, **L45**, 312, 1987.

Bally, J., Langer, W. D., & Liu, W., Infrared dust and millimeter-wave carbon monoxide emission in the Orion region, *Astrophysical Journal*, **383**, 645, 1991.

Bally, J., Devine, D., Sutherland, R. S., and Johnstone, D., Externally Illuminated Young Stellar Environments in the Orion Nebula: Hubble Space Telescope Planetary Camera Observations, *Astrophysical Journal*, **116**, 293, 1998.

Bally, J., Testi, L., Sargent, A., and Carlstrom, J., Disk Mass Limits and Lifetimes of Externally Illuminated Young Stellar Objects in the Orion Nebula, *Astrophysical Journal*, **116**, 854, 1998.

Bally, J., O'Dell, C. R., and McCaughrean. M., Disks, Microjets, Windblown Bubbles, and Outflows in the Orion Nebula, *Astrophysical Journal*, **119**, 2919-2959, 2000.

Bally, J., Johnstone, D., Joncas, G., Reipurth, B., and Mall\'en-Ornelas, G., Kinematics of Optical Outflows in the Orion Nebula I: The Irradiated Jet HH~502 and the Giant Outflow HH~400, *Astrophysical Journal*, **122**, 1508, 2001.

Bally, J.and Reipurth, B., Herbig-Haro Jets in the Orion Nebula and near NGC 1333, *Astrophysical Journal*, **546**, 299, 2001.

Bally, J., Walawender, J., & Reipurth, B., A Narrow-Band Imaging Survey Nearby Star Forming Clouds: II, Orion, *Astronomical Journal*, in preparation**.**

Barnicoat, A.C., Henderson, I.H.C., Knipe, R.J., Yardley, B.W.D., Napier, R.W., Fox, N.P.C., Kenyon, A.K., Muntingh, D.J., Strydom, D., Winkler, K.S., Lawrence, S.R., and Cornford, C., Hydrothermal gold mineralization in the Witwatersrand basin. *Nature* **386**, 820-824, 1997.

Baross, J.A. and S.E. Hoffman, Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life, *Origins Life*, **15**, 327-345, 1985.

Barr, A. C., and R. T. Pappalardo, Numerical simulation of non-linear convection in ice: Application to Europa, *Lunar Planet. Sci. Conf., XXXIV*, abstract # 1477, Lunar and Planetary Institute, Houston (CD-ROM), 2003.

Bennett, J., S. Shostak, and B. Jakosky, Life in the Universe, Addison-Wesley, San Francisco (391 p.), 2000.

Bonnell, I. A., Bate, M. R., Zinnecker, H., On the formation of massive stars, *Monthly Notice of the Royal Astronomy Society*, **298**, 93, 1998.

Boss, A. P. Formation of gas and ice giant planets, *Earth and Planetary Science Letters*, **202**, 513, 2002.

Brakenridge, G.R., H.E. Newsom, and V.R. Baker, Ancient hot springs on Mars: Origins and paleoenvironmental significance of small martian valleys, *Geology*, **13**, 859-862, 1985.

Bridge, P. The history and application of molecular mycology. *Mycologist* **16** (3), 90-99, 2002.

Brock, T.D. and M.T. Madigan, Biology of microorganisms, Prentice-Hall, 1991.

Brundrett, M. C., Tansley review no. 134 Coevolution of roots and mycorrhizas of land plants, *New Phytologist*,**154**, 275-304, 2002.

Cabrol, N.A., and E.A. Grin, Distribution, classification, and ages of martian impact crater lakes, *Icarus*, **142**, 160-172, 1999.

Canfield, D. E., and D. J. DesMarais. Biogeochemical Cycles of Carbon, Sulfur, and Free Oxygen in a Microbial Mat, *Geochimica et Cosmochimica Acta*, **57**,3971-3984, 1993.

Carr, M.H., Water on Mars, Oxford Univ. Press, N.Y., 1996.

Christensen, P.R., Formation of recent martian gullies through melting of an extensive water-rich snow deposit, *Nature*, in press, 2003.

Churchwell, E. B. et al., GLIMPSE: Plans for a SIRTF Legacy Program to Survey the Inner Regions of the Milky Way, *Bulletin of the American Astronomical Society*, **201**, 5006, 2002.

Chyba, C. F. and C. B. Phillips, Possible ecosystems and the search for life on Europa, *Proc. Nat. Acad. Sci.*, **98**, 801-804, 2001.

Cleland, C. E., Historical science, experimental science, and the scientific method, *Geology* **29**, 978-90, 2001.

Cleland, C. E. and Chyba, C. F., Defining 'life', *Orig. Life Evol. Biosph.* **32**, 387-393, 2002.

Cleland, C. E., Methodological and Epistemic Differences Between Historical Science and Experimental Science, *Philosophy of Science* **69**, 474-496, 2002.

Cleland, C. E. and Chyba, C. F., Does 'life'have a definition?, in *Planets and Life: The Emerging Science of Astrobiology (*W. Sullivan & J. Baross, eds.), 2003 (forthcoming).

Cloud, P.E. A working model of the primitive Earth, *Amer. J. Sci.* **272**, 537-548, 1972.

D'Amelio, D'Antoni, E., Y. Cohen, and D. J. DesMarais. Comparative Functional Ultrastructure of Two Hypersaline Submerged Cyanobacterial Mats: Guerrero Negro, Baja California Sur, Mexico, and Solar Lake, Sinai, Egypt, in Microbial Mats: Physiological Ecology of Benthic Microbial Communities (Y. Cohen and E. Rosenberg, eds.), American Society for Microbiology, Washington D.C., 97-113, 1989.

Dame, T. M., Hartmann, D., & Thaddeus, P., The Milky Way in Molecular Clouds: A New Complete CO Survey, *Astrophysical Journal*, **547**, 792, 2001.

DesMarais, D.J., Long-term evolution of the biogeochemical carbon cycle. *Rev. Mineral,* **35**, 429-448, 1997.

Dimroth, E., and Kimberley, M.M. Precambrian atmospheric oxygen: Evidence in the sedimentary distribution of carbon, sulfur, uranium and iron, *Can. J. Earth Sci.* **13**, 1161-1185, 1976.

Dobson, C.M., G.B. Ellison, A.F. Tuck, and V. Vaida, Atmospheric aerosols as prebiotic chemical reactors, *Proc. of the National Academy of Sciences of the United States of America*, **97** (22), 11864-11868, 2000.

Donaldson, D.J., A.F. Tuck, and V. Vaida, The asymmetry of organic aerosol fission and prebiotic chemistry, *Origins of Life and Evolution of the Biosphere*, **32** (3), 237-245, 2002.

Drake, M.J. and B.M. Jakosky, Narrow horizons in astrobiology, *Nature*, **415**, 733-734, 2002.

Duckett, J. G., K. S. Renzaglia and K. Pell. A light and electron microscope study of rhizoid-ascomycete associations and flagelliform axes in British hepatics with observations on the effects of the fungi on host morphology. *New Phytologist* **118**, 233-257, 1991.

Edwards, D. and P. A. Selden, The development of early terrestrial ecosystems. *Bot. J. Scot,* **46**, 337-366, 1993.

Evans, N. J., II et al., From Molecular Cores to Planet-forming Disks, *Bulletin of the American Astronomical Society*, **198**, 2505, 2001.

Farquhar, J., Bao, H., and Thiemens, M. Atmospheric influence of Earth's earliest sulfur cycle, *Science* **289**, 756-758, 2000.

Farquhar, J., Savarino, J., Airieau, S., and Thiemens, M.H. Observation of wavelength-sensitive mass-independent sulfur isotope effects during SO_2 photolysis: Implications for the early atmosphere, *J. Geophys. Res.* **106**, 32829-32839, 2001.

Figueredo, P. H., and R. Greeley, Geologic mapping of the northern leading hemisphere of Europa from Galileo solid-state imaging data, *J. Geophys. Res.*, **105**, 22,629-22,646, 2000.

Follman, H., and I. Häberlein, Thioredoxins: universal, yet specific thiol-disulfide redox cofactors., *BioFactors*, **5**, 147-156, 1995/1996.

Geissler, P. E., R. Greenberg, G. Hoppa, P. Helfenstein, A. McEwen, R. Pappalardo, R. Tufts, M. Ockert-Bell, R. Sullivan, R. Greeley, M. Belton, T. Denk, B. Clark, J. Burns, and J. Veverka, Evidence for Non-synchronous Rotation of Europa, *Nature*, **391**, 368-370, 1998.

Gray. The microfossil record of early land plants: Advances in understanding of early terrestrialization, 1970-1984. *Philosophical Transactions of the Royal Society of London*, in Evolution and environment in the late Silurian and Early Devonian (Chaloner WG, Lawson JD, eds), **B309**, 167-195, 1985.

Greeley, R. and B.D. Schneid, Magma generation on Mars: Amounts, rates, and comparisons with Earth, Moon, and Venus, *Science*, **254**, 996-998, 1991.

Greeley, R., P. H. Figueredo, D. A. Williams, F. C. Chuang, J. E. Klemaszewski, S. D. Kadel, L. M. Prockter, R. T. Pappalardo, J. W. Head III, G. C. Collins, N. A. Spaun, R. J. Sullivan, J. M. Moore, D. A. Senske, B. R. Tufts, T. V. Johnson, M. J. S. Belton, and K. L. Tanaka, Geological mapping of Europa, *J. Geophys. Res.*, **105**, 22,559-22,578, 2000.

Greenberg, R., P. E. Geissler, G. Hoppa, B. R. Tufts, D. D. Durda, R. Pappalardo, J. W. Head, R. Greeley, R. Sullivan, and M. H. Carr, Tectonic processes on Europa: Tidal stresses, mechanical response, and visible features, *Icarus*, **135**, 64-78, 1998.

Greenberg, R., G. V. Hoppa, B. R. Tufts, P. E. Geissler, and J. Reilly, Chaos on Europa, *Icarus*, **141**, 263-286, 1999.

Greenberg, R., P. Geissler, G. Hoppa, Gregory; B. R. Tufts, Tidal-tectonic processes and their implications for the character of Europa's icy crust, *Rev. Geophys*., 10.1029/2000RG000096, 2002.

Greene, T. P., & Lada, C. J., High-Resolution Near-Infrared Spectra of Protostars, *Astronomical Journal*, **120**, 430, 2000.

Greenwood, J.P., Mojzsis, S.J., and Coath, C.D. Sulfur isotopic compositions of individual sulfides in martian meteorites ALH84001 and Nakhla: Implications for crust-regolith exchange on Mars, *Earth Planet. Sci. Lett.* **184**, 23-35, 2000.

Griffith, L.L., and E.L. Shock, A geochemical model for the formation of hydrothermal carbonates on Mars, *Nature,* **377**, 406, 1995.

Griffith, L.L. and E.L. Shock, Hydrothermal alteration of Martian crust: Illustration via geochemical model calculations, *J. Geophys. Res.,* **102**, 9135-9143, 1997.

Grinenko, V.A., and Thode, H.G., Sulfur isotope effects in volcanic gas mixtures, *Can. J. Earth Sci.* **7**, 1402-1409, 1970.

Gulick, V.C., Magmatic intrusions and a hydrothermal origin for fluvial valleys on Mars, *J. Geophys. Res*., **103**, 19365-19387, 1998.

Harrison, A.G. and Thode, H.G., Mechanism of the bacterial reduction of sulphate from isotope fractionation studies, *Trans. Faraday Soc.* **54**, 84-92, 1958.

Helfenstein, P. and E. M. Parmentier, Patterns of fracture and tidal stresses due to non-synchronous rotation: Implications for fracturing on Europa, *Icarus*, **61**, 175-184, 1985.

Henney, W. J., & O'Dell, C. R., A Keck High-Resolution Spectroscopic Study of the Orion Nebula Proplyds, *Astronomical Journal*, **118**, 2350, 1999.

Holland, H.D. The Chemical Evolution of the Atmosphere and Oceans, Princeton University Press, 1984.

Holland, H.D. Early Proterozoic atmospheric change, in Early Life on Earth: Nobel Symposium No. 84, (S. Bengtson, ed.), Columbia, pp. 237-244, 1994.

Holland, H.D. When did the Earth's atmosphere become oxic? A Reply *Geochem. News* **100**, 20-22, 1999.

Hoppa, G.V., B. R. Tufts, R. Greenberg, and P. E. Geissler, Formation of cycloidal features on Europa, *Science*, **285**, 1899-1902, 1999.

Hoppa, G. V., B. R. Tufts, R. Greenberg, T. A. Hurford, D. P. O'Brien, and P. E. Geissler, Europa's rate of rotation derived from the tectonic sequence in the Astypalaea region, *Icarus*, **153**, 208-213, 2001a.

Hoppa, G. V., R. Greenberg, J. Riley, and B. R. Tufts, Observational selection effects in Europa image data: Identification of chaotic terrain, *Icarus*, **151**, 181-189, 2001b.

Hulston, J.R., and Thode, H.G. Variations in the S^{33}, S^{34}, and S^{36} contents of meteorites and their relation to chemical and nuclear effects, *J. Geophys. Res.* **70**, 3475-3484, 1965.

Jakosky, B.M., Mars volatile evolution: Evidence from stable isotopes, *Icarus*, **94**, 14-31, 1991.

Jakosky, B.M., *The search for life on other planets*, Cambridge Univ. Press, London, 1998.

Jakosky, B.M., Philosophical aspects of astrobiology, in *Bioastronoomy '99: A New Era in Bioastronomy*, (G.A. Lemarchand and K.J. Meech, eds.), Astron. Soc. Pac., San Francisco, 661-666, 2000.

Jakosky, B.M. and M.P. Golombek, Planetary science, astrobiology, and the role of science and exploration in society, *EOS, Trans. Amer. Geophys. Union*, **81**(6; 8 Feb. 2000), 58, 2000.

Jakosky, B.M. and J.H. Jones, The history of martian volatiles, *Rev. Geophys.*, **35**, 1-16, 1997.

Jakosky, B.M. and R.J. Phillips, Mars' volatile and climate history, *Nature*, **412**, 237-244, 2001.

Jakosky, B.M. and E.L. Shock, The biological potential of Mars, the early Earth, and Europa, *J. Geophys. Res.*, **103**, 19359-19364, 1998.

Jakosky, B.M., R.O. Pepin, R.E. Johnson, and J.L. Fox, Mars atmospheric loss and isotopic fractionation by solar-wind-induced sputtering and photochemical escape, *Icarus*, **111**, 271-288, 1994.

Jakosky, B.M., K.H. Nealson, C. Bakermans, R.E. Ley, and M.T. Mellon, Sub-freezing activity of microorganisms and the potential habitability of Mars' polar regions, submitted for publication, 2003.

Jimenez, J.L., Aerosol Mass Spectrometry Web Page, http://cires.colorado.edu/jimenez/ams.html, 2002.

Jimenez, J.L., J.T. Jayne, Q. Shi, C.E. Kolb, D.R. Worsnop, I. Yourshaw, J.H. Seinfeld, R.C. Flagan, X. Zhang, K.A. Smith, J. Morris, and P. Davidovits, Ambient Aerosol Sampling Using the Aerodyne Aerosol Mass Spectrometer, *Journal of Geophysical Research*, 2002, In press.

Johnstone, D., Hollenbach, D., and Bally, J., Proplyds in the Orion Nebula: Destruction of Circumstellar Disks , *Astrophysical Journal*, **499**, 758, 1998.

Jorgensen, B. B. Light Penetration, Absorption, and Action Spectra in Cyanobacterial Mats, in Microbial Mats: Physiological Ecology of Benthic Microbial Communities (Y. Cohen and E. Rosenberg eds.), American Society for Microbiology, Washington D.C., 123-137, 1989.

Kahn, R., The evolution of CO_2 on Mars, *Icarus*, **62**, 175-190, 1985.

Karhu, J.A. Paleoproterozoic evolution of the carbon isotope ratios of sedimentary carbonated in the Fennoscandian Shield, *Geol. Surv. Finland Bull.* **371**, 87p, 1993.

Karhu, J.A., and Holland, H.D. Carbon isotopes and the rise of atmospheric oxygen. *Geology* **24**, 867-870, 1996.

Karol, K. G., R. M. McCourt, M. T. Cimino and C. F. Delwiche, The closest living relatives to plants. *Science*, **294**, 2351-3, 2001.

Karl D. M., Ecology of free-living, hydrothermal vent microbial communities, in The Microbiology of Deep-Sea Hydrothermal Vents (ed. D. M. Karl). CRC Press. pp. 35-124, 1995.

Kasting, J.F. Models related to Proterozoic atmospheric and ocean chemistry, in The Proterozoic Biosphere: A Multidisciplinary Study, (J.W. Schopf and C. Klein, eds). Cambridge, pp. 1185-1187, 1992.

Kasting, J.F. Earth's early atmosphere, *Science*, **259**, 920-926, 1993.

Kasting, J.F. The rise of atmospheric oxygen, *Science* **293**, 819-820, 2001.

Kasting, J.F., Warming Early Earth and Mars, *Science* **276**, 1213-1215, 1997.

Kasting, JF, Pavlov AA, Siefert JL. A coupled ecosystem-climate model for predicting the methane concentration in the Archean Atmosphere. *Origins of Life and Evolution of the Biosphere* **31,** 271-285, 2001.

Kattenhorn, S. A., Nonsynchronous rotation evidence and fracture history in the Bright Plains region, Europa, *Icarus*, *157*, 490 - 506, 2002.

Kelley D. S., Baross J. A., and Delaney J. R., Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Ann. Rev. Earth Planet. Sci.* **30**, 385-491, 2002.

Kenrick, P. and P. R. Crane, The origin and early diversification of land plants: a cladistic study. Washington and London, Smithsonian Institution Press, 1997.

Kivelson, M.G., K. K. Khurana, C. T. Russell, M. Volwerk, R. J. Walker, and C. Zimmer, Galileo Magnetometer Measurements: A Stronger Case for a Subsurface Ocean at Europa, *Science*, **289**, 1340-1343, 2000.

Klenk, H.-P., et. al., The complete genome sequence of the hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus.*, *Nature*, **390**, 364-370, 1997.

Knight R.D. and Yarus M., Finding specific RNA motifs: Function in a zeptomole world? *RNA*, in press 2003.

Krasnopolsky, V., On the deuterium abundance on Mars and some related problems, *Icarus*, **148**, 597-602, 2000.

Kuhn, Thomas S., *The Structure of Scientific Revolutions*. Chicago: University of Chicago Press, 1962.

Leith, A. C., and W. B. McKinnon, Is there evidence for polar wander on Europa?, *Icarus*, **120**, 387-398, 1996.

Li et al., Monoclonal antibody-based ELISAs for part-per-billion determination of polycyclic aromatic hydrocarbons; effects of haptens and formats on sensitivity and specificity, *Anal Chem*, **71**(2), 302-9, 1999.

Lissauer, J. J., Pollack, J. B., Wetherill, G. W., & Stevenson, D. J., authors in Neptune and Triton, (D. Cruikshank and M. S. Matthews, eds.), University of Arizona Press, Tucson, 1995.

Luhmann, J.G., R.E. Johnson, and M.H.G. Zhang, Evolutionary impact of sputtering of the martian atmosphere by O+ pickup ions, *Geophys. Res. Lett.*, **19**, 2151-2154, 1992.

Majerfeld I and Yarus M., Isoleucine:RNA sites with associated coding sequences, *RNA* **4**, 471-478, 1998.

Malin, M.C. and K.S. Edgett, Evidence for recent ground water seepage and surface runoff on Mars, *Science*, **288**, 2330-2335, 2000.

Mannings, V., Boss, A. P., Russell, S. S., in Protostars and Planets IV, (V. Mannings, (A.P. Boss, and S.S. Russell, eds), University of Arizona Press, Tucson, 2000.

Marion, G.M., Carbonate mineral solubility at low temperatures in the Na-K-Mg-Ca-H-Cl-SO_4-OH-HCO_3-CO_3-CO_2-H_2O system, *Geochim. Cosmochim. Acta*, **65**, 1883-1896, 2001.

McCaughrean, M. J., & O'Dell, C. R., Direct Imaging of Circumstellar Disks in the Orion Nebula, *Astronomical Journal*, **111**, 1977, 1996.

McCollom, T. M., Methanogenesis as a potential source of chemical energy for primary biomass production by autotrophic organisms in hydrothermal systems on Europa, *J. Geophys. Res. Planets*, **104**, 30,729-30,742, 1999.

McCollom T.M., Geochemical constraints on primary productivity in submarine hydrothermal vent plumes, *Deep Sea Res. Pt-1*, **47**, 85-101, 2000.

McCollom, T. M. and Shock, E. L., Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems, *Geochim. Cosmochim. Acta*, **61**, 4375-4391, 1997.

McCollom, T. M. and Seewald, J. S., A Reassessment of the Potential for Reduction of Dissolved CO_2 to Hydrocarbons During Serpentinization of Olivine, *Geochim. Cosmochim. Acta,* **65**, 3769-3778, 2001..

McCollom, T. M. and Seewald, J. S., Experimental constraints on the hydrothermal reactivity of organic acids and acid anions: I. Formic acid and formate. *Geochim. Cosmochim. Acta,* in press, 2001.

McEwen, A. S., Tidal reorientation and the fracturing of Jupiter's moon Europa, *Nature*, **321**, 49-51, 1986.

McSween, H.Y., Jr., What we have learned about Mars from SNC meteorites, *Meteoritics*, **29**, 757-779, 1994.

Mellon, M.T. and R.J. Phillips, Recent gullies on Mars and the source of liquid water, *J. Geophys. Res.*, **106**, 23165-23179, 2001.

Meyers, M. R. et al., The Formation and Evolution of Planetary Systems: Placing Our Solar System in Context with SIRTF, *Bulletin of the American Astronomical Society*, **198**, 2506, 2001.

Miesch, M. S., & Bally, J., Statistical analysis of turbulence in molecular clouds*, Astrophysical Journal*, 429, 645, 1994.

Miesch, M. S., Scalo, J. and Bally, J., Velocity Field Statistics in Star-forming Regions Centroid Velocity Observations, *Astrophysical Journal*, **524**, 895, 1999.

Monster, J., Appel, P.W.U., Thode, H.G., Schidlowski, M., Carmichael, C.M., and Bridgwater, D. Sulfur isotope studies in early Archaean sediments from Isua, West Greenland – Implications for the antiquity of bacterial sulfate reduction, *Geochim. Cosmochim. Acta* **43**, 405-413, 1979.

Morton, J. B., S. P. Bentifvenga and J. D. Bever, Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes), *Canadian Journal of Botany* **73** (Suppl. 1), S25-S32, 1995.

Morton, J. B. and D. Redecker, Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. *Mycologia* **93** (1), 181-195, 2001..

Motohashi, K., A. Kondoh ,M. T. Stumpp, and T. Hisabori, Comprehensive survey of proteins targeted by chloroplast thioredoxin., *Proc. Natl. Acad. Sci. USA*, **98**, 11224-11229, 2001.

Mojzsis, S.J., Coath, C.D., Greenwood, J.P., McKeegan, K.D. and Harrison, T.M. (2003) Confirmation of mass-independent isotope effects in Archean (2.5 3.8 Ga) sedimentary sulfides as determined by ion microprobe analysis: *Geochim. Cosmochim. Acta* (in press)

Nealson, K. H. and P. G. Conrad, "Life: Past, present and future", *Phil.Trans. R. Soc. London. B* **354**, pp. 1923-1939, 1999.

Nealson, K. H. and P. G. Conrad, A Non-Earth-Centric approach to LifeDetection, *Astrobiology* **1**, 15-24, 2001.

Newsom, H.E., Hydrothermal alteration of impact melt sheets with implications for Mars, *Icarus*, **44**, 207-216, 1980.

Nielsen, H. Sulfur isotopes, in Lectures in Isotope Geology, (E. Jäger and J.C. Hunziger, eds), Springer, pp. 283-312, 1979.

Nishiyama, A.,H. Masutani, H. Nakamura ,Y. Nishinaka, and J. Yodoi, Redox regulation by thioredoxin and thioredoxin-binding proteins., *IUBMB Life*, **52**, 29-33, 2001.

Nissen P, Hansen J, Ban N, Moore PB and Steitz TA, The structural basis of ribosome activity in peptide bond synthesis, *Science* **289**, 920-930, 2000.

Norton, D.L., Theory of hydrothermal systems, *Ann. Rev. Earth Planet. Sci.*, **12**, 155-177, 1984.

Norton, D. and H.P. Taylor, Jr., Quantitative simulation of the hydrothermal systems of crystallizing magmas on the basis of transport theory and oxygen isotope data: An analysis of the Skaergaard intrusion, *J. Petrology*, **20**, 421-486, 1979.

O'Dell, C. R., The Orion Nebula and its Associated Population, *Annual Reviews of Astronomy and Astrophysics*, **39**, 990, 2001.

Ohmoto, H. Stable isotope geochemistry of ore deposits, in Stable isotopes in high temperature geological processes (J.W. Valley, H.P. Taylor, and J.R. O'Neil, eds), *Rev. Mineral.* **16**. Mineralogical Society of America, pp. 491-559, 1986.

Ohmoto, H. Evidence in pre-2.2 Ga paleosols for the early evolution of atmospheric oxygen and terrestrial biota, *Geology* **24**, 1135-1138, 1996.

Ohmoto, H. When did the Earth's atmosphere become oxic? *Geochem. News* **93**, 12-27, 1999.

Ohmoto, H., Yamaguchi, K. and Ono, S. Questions regarding Precambrian sulfur isotope fractionation, *Science* **292**, 1959a, 2001.

Ohmoto, H., and Rye, R.O. Isotopes of sulfur and carbon, in Geochemistry of Hydrothermal Ore Deposits, 2nd ed. (H.L. Barnes, ed), Wiley. pp. 509-567, 1979.

Ojakangas, G. W. and D. J. Stevenson, Episodic volcanism of tidally heated satellites with application to Io, *Icarus*, **66**, 341-358, 1986.

Ojakangas, G. W. and D. J. Stevenson, Polar wander of an ice shell on Europa, *Icarus*, **81**, 242-270, 1989.

Ono S., Eigenbrode J., Pavlov A., Khareca P., Kasting J., Sulfur Isotopic Constraints on the Archean Atmosphere and Ocean, submitted for publication, 2003.

Pace, N. R. A molecular view of microbial diversity and the biosphere. *Science*. **276**,734-740, 1997.

Pace, N.R. The universal nature of biochemistry, *Proceed. Natl. Acad. Sci.* **98**, 805-808, 2001.

Pappalardo, R. T., and 31 coauthors, Does Europa have a subsurface ocean? Evaluation of the geological evidence, *J. Geophys. Res.*, **104**, 24,015-24,055, 1999.

Parkhurst D. L., User's Guide to PHREEQC- A Computer Program for speciation, reaction-path, advective-transport, and inverse geochemical calculations, *U. S. Geol. Surv. Water Resources Invest. Rpt*. 95-4227, (1995)

Pavlov AA, Brown LL, Kasting JF, UV shielding of NH3 and O-2 by organic hazes in the Archean atmosphere, *J. Geophys. Res.*, **106**, 23267-23287, 2001.

Pavlov, A. A. and Kasting, J. F., 2002, Mass independent fractionation of sulfur isotopes in archaen sediments: Strong evidence for an anoxic Archaen atmosphere, *Astrobiology*, **2**, 27-41,2002.

Pavlov, A.A., M. Hurtgen, J. F. Kasting, and M. A. Arthur, A methane-rich proterozoic atmosphere? *Geology*, **31**, 87-90,2003.

Payne, M.C. and J.D. Farmer, Looking through the ice: Searching for past and present habitable zones in the martian north polar region using MOLA DEMS (abstract), Fall Amer. Geophys. Union meeting, San Francisco, 2002.

Pegg, S. C.-H., and P. C. Babbitt, Shotgun: Getting more from sequence similarity searches., *Bioinformatics*, **15**, 729-740, 1999.

Phillips, R.J., M.T. Zuber, S.C. Solomon, M.P. Golombek, B.M. Jakosky, W.B. Banerdt, D. E. Smith, R.M.E. Williams, B.M. Hynek, O. Aharonson, and S.A. Hauck II, Ancient geodynamics and global-scale hydrology on Mars, *Science*, **291**, 2587-2591, 2001.

Pirozynski, K. A. and D. W. Malloch, The origin of land plants: A matter of mycotrophism, *Biosystems* **6**, 153- 164, 1975.

Pollack, J.B., J.F. Kasting, S.M. Richardson, and K. Poliakoff, The case for a wet, warm climate on early Mars, *Icarus*, **71**, 203-224, 1987.

Prockter, L. M., A. Antman, R. T. Pappalardo, J. W. Head, and G. C. Collins, Europa: Stratigraphy and geological history of the anti-Jovian region from Galileo E14 SSI data, *J. Geophys. Res*., **104**, 16,531-16,540, 1999.

Raven, J, The role of marine biota in the evolution of terrestrial biota: gases and genes, *Biogeochemistry* **39**, 139-164, 1997.

Raven, J.A. and D. Edwards, Roots: evolutionary origins and biogeochemical significance, *Journal of Experimental Botany* **52**, 381-401, 2001.

Raven, P. H., R. F. Evert and S. E. Eichhorn, Biology of Plants. New York, W.H. Freeman and Company, 1999.

Read, D. J., J. G. Duckett, R. Francis, R. Ligrone and A. Russell, Symbiotic fungal associations in 'lower' land plants, *Philosophical Transactions of the Royal Society of London* **355**, 815-831, 2000.

Redecker, D., Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots, *Mycorrhiza* **10**, 73-80, 2000.

Redecker, D., R. Kodner and L. E. Graham, Glomalean Fungi from the Ordovician. *Science* **289**, 1920-1921, 2000..

Redecker, D., H. Thierfelder, C. Walker and D. Werner, Restriction analysis of PCR-amplified internal transcribed spacers of ribosomal DNA as a tool for species identification in different genera of the orders Glomales, *Applied and Environmental Microbiology* 63(5), 1756-1761, 1997.

Reipurth, B., Disintegrating Multiple Systems in Early Stellar Evolution, *Astronomical Journal*, **120**, 3177, 2000.

Reipurth, B., Yu, K., Rodriguez, L.F., Heathcote, S., and Bally, J., Multiplicity of the HH 111 jet source: it Hubble Space Telescope NICMOS images and VLA maps, *Astronomy and Astrophysics*, **352**, L83, 1999.

Reipurth, B. and Bally, J., Herbig-Haro Flows: Probes of Early Stellar Evolution, *Annual Reviews of Astronomy and Astrophysics*, **39**, 403, 2001.

Reipurth, B., Rodriguez, L.F., Anglada, G., and Bally, J., Radio Continuum Maps of Deeply Embedded Protostars: Thermal Jets, Multiplicity, and Variability, *Astrophysical Journal*, 124, 1045, 2002.

Remy, W., T. N. Taylor, H. Hass and H. Kerp, Four hundred-million-year-old vesicular arbuscular mycorrhizae, *Proceedings of the National Academy of Science* **91**, 11841-11843, 1994.

Revsbech, N. P., P. B. Christensen, and L. P. Nielsen. Microelectrode Analysis of Photosynthetic and Respiratory Processes in Microbial Mats, in Microbial Mats: Physiological Ecology of Benthic Microbial Communities (Y. Cohen and E. Rosenberg eds.), American Society of Microbiology, Washington D.C., 153-162, 1989.

Ridley, W.I. and G.S. Plumlee, Reaction path modeling of martian surface alteration through time (abstract), Div. Planet. Sci., Amer. Astron. Soc. 27th meeting, Kona, HI, 1995.

Ridley,W.I. and G.S. Plumlee, On martian alteration as a function of climate and volcanism, *Lunar Planet. Sci. Conf.*, **28**, abstract 1165, 1997.

Ridley, W.I., G.S. Plumlee, J.D. Debraal, and M.H. Reed, Alteration of the martian surface through time: A reaction path modeling approach (abstract), *Meteoritics*, **30**, 565, 1997.

Risatti, J. B., W. C. Capman, and D. A. Stahl. Community structure of a microbial mat: the phylogenetic dimension. *Proc Natl Acad Sci U S A.* **9117**(2100),10173-10177, 1994.

Romanek, C.S., M.M. Grady, I.P. Wright, D.W. Mittlefehldt, R.A. Socki, C.T. Pillinger, and E.K. Gibson, Jr., Record of fluid-rock interactions on Mars from the meteorite ALH84001, *Nature*, **372**, 655-657, 1994.

Rosenfeld, J. ,J. Capdevielle ,J. C. Guillemot, and P. Ferrara, In-gel digestion of proteins for internal sequence analysis after one- or two-dimensional gel electrophoresis., *Anal. Biochem.*, **203**, 173-179, 1992.

Rye, R., Kuo, P.H., and Holland, H.D. Atmospheric carbon dioxide concentrations before 2.2 billion years ago, *Nature* **378**, 603-605, 1997.

Sabeti, P. C., P. J. Unrau, et al, Accessing rare activities from random RNA sequences: the importance of the length of molecules in the starting pool, *Chem Biol* **4**(10), 767-74, 1997.

Sagan, C. and Chyba, C. The Early Faint Sun Paradox: Organic Shielding of Ultraviolet-Labile Greenhouse Gases. *Science* **276**, 1217-1221, 1997.

Sagan, C. and Khare, B. Tholins: Organic Chemistry of Interstellar Grains and Gas. *Nature* **277**, 102-107, 1979.

Sarid, A. R., R. Greenberg, G. V. Hoppa, T. Hurford, B. R. Tufts, and P. Geissler, Polar wander and surface convergence of Europa's ice shell: Evidence from a survey of strike-slip displacement, *Icarus*, **158**, 24-41, 2002.

Schidlowski, M., Hayes, J.M., and Kaplan, I.R. Isotopic inferences of ancient biochemistries. Carbon, sulfur, hydrogen and nitrogen, in Earth's earliest biosphere – Its origin and evolution, (J.W. Schopf, ed.), Princeton University Press, pp. 149-186, 1983.

Schidlowski, M. Evolution of the sulphur cycle in the Precambrian, in Evolution of the global biogeochemical sulphur cycle, (P. Brimblecomb and A. Yu. Lein, eds.). Wiley. pp. 3-19, 1989.

Schussler, A., Glomales SSU rRNA gene diversity. *New Phytologist* **144**, 205-207, 1999.

Schussler, A., H. Gehrig, D. Schwarzott and C. Walker, Analysis of partial *Glomales* SSU rRNA gene sequences: implications for primer design and phylogeny, *Mycological Research* **105** (1), 5-15, 2001.

Schwarzott, D. and A. Schübler, A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. *Mycorrhiza* **10**, 203-207, 2001.

Shear, W., The early development of terrestrial ecosystems, *Nature* **351**, 283-289, 1991.

Shen, Y., Buick, R., and Canfield, D.E. Isotopic evidence for microbial sulphate reduction in the early Archaean era, *Nature* **410**, 77-81, 2001.

Shock, E.L., Geochemical constraints on the origin of organic compounds in hydrothermal systems, *Orig. Life Evol. Biosphere*, **20**, 331-367, 1990.

Showman, A. P. and R. Malhotra, Tidal evolution into the Laplace resonance and the resurfacing of Ganymede, *Icarus*, **127**, 93-111, 1997.

Shuping, R. Y., Bally, J., Morris, M., \and Throop, H., Evidence for Grain Growth in the Protostellar Disks in Orion, *Astrophysical Journal Letters* (in press), 2003.

Smith, N., Bally, J. Thiel, J., and Morse, J. A. Discovery of Numerous Proplyd-Like Objects in the Harsh Environment of the Carina Nebula, *Astrophysical Journal* (in press), 2003.

Smith, S. E. and D. J. Read, Mycorrhizal Symbiosis. San Diego, Academic Press, 1997.

Spaun, N. A., R. T. Pappalardo, and J. W. Head, Evidence for shear failure in forming near-equatorial lineae on Europa, *J. Geophys. Res*., in press, 2003.

Spencer, R. J., H. P. Eugster, B. F. Jones, and S. L. Rettig, Geochemistry of Great Salt Lake, Utah I: Hydrochemistry since 1850. *Geochimica et Cosmochimica Acta*. **49**,727-737, 1985.

Stempel, M. M., and R. T. Pappalardo, Lineament orientations through time near Europa's leading point: Implications for stress mechanisms and rotation of the icy shell, in *Lunar Planet. Sci. Conf., XXXIII*, abstract #1661, Lunar and Planetary Institute, Houston (CD-ROM), 2002.

Stetter, K.O., and Gaag, G. Reduction of molecular sulphur by methanogenic bacteria, *Nature* **305**, 309-311, 1983.

Stetter, K.O. Hyperthermophiles in the History of Life, in Evolution of Hydrothermal Systems on Earth (and Mars?) (G.R. Bock and J.A. Goode), Wiley, pp. 1-10, 1996.

Strauss, H., and Moore, T.B. (1992) Abundances and isotopic compositions of carbon and sulfur species in whole rock and kerogen. in The Proterozoic Biosphere, (Schopf, J.W. and Klein, C, eds). Cambridge Univ. Press, Cambridge, pp. 709-798, 1992.

Strumer, S. L. and J. B. Morton, Developmental patterns defining morphological characters in spores of four species in *Glomus*, *Mycologia* **89** (1), 72-81, 1997.

Tanaka, K.L., N.K. Isbell, D.H. Scott, R. Greeley, and J.E. Grant, The resurfacing history of Mars: A synthesis of digitized, Viking-based geology, *Proc. 18th Lunar Planet. Sci. Conf*., 665-678, Cambridge Univ. Press, 1988.

Thiemens, M.H. Mass-independent isotope effects in planetary atmospheres and the early solar system, *Science* **283**, 341-345, 1999.

Thiemens, M.H., and Heidenreich, J.E. III. The mass-independent fractionation of oxygen: A novel effect and its possible cosmochemical implications, *Science* **219**, 1073-1075, 1983.

Thiemens, M.H., Savarino, J., Farquhar, J., and Bao, H. Mass-independent isotopic compositions in terrestrial and extraterrestrial solids and their implications, *Acc. Chem. Res.* **34**, 645-652, 2001.

Thode, H.G., Macnamara, J., and Collins, C.B. Natural variations in the isotopic content of sulphur and their significance, *Can. J. Res.* **27B**, 361, 1949.

Towe, K.M. Aerobic respiration in the Archaean, *Nature* **348**, 54-56, 1990.

Trendall, A.F., and Blockley, J.G. The iron formations of the Precambrian Hamersley Group, Western Australia, with special reference to the associated crocidolite, *Geol. Survey W. Australia Bull.* **119**, 366p, 1970.

Trendall, A.F., and Morris, R.C. Iron formations: Facts and problems, Elsevier, 1983.

Throop, H. B., Bally, J., Esposito, L. W., and McCaughrean, M. J., Large Large Grains in Young Circumstellar Disks, *Science*, **292**, 1686 -1689, 2001.

Throop, H., Bally, J., and Esposito, L. W., Dust Grain Growth in Young Circumstellar Disks in the Orion Nebula: Implications for Planetary Systems Architectures, *Astrophysical Journal* (submitted).

Toon, O. B., R.P. Turco, D. Westphal, R. Malone and M.S. Liu, A multi-dimensional model for aerosols: description of computational analogs, *J. Atmos. Sci.* **45**, 2123-2143, 1988.

Treiman, A.H., R.A. Barrett, and J.L. Gooding, Preterrestrial alteration of the Lafayette (SNC) meteorite, *Meteoritics*, **28**, 86-97, 1993.

Ungerechts, H., & Thaddeus, P., A CO Survey of Molecular Clouds in Taurus, Auriga, and Perseus, *Astrophysical Journal Supplements*, **63**, 645, 1987.

Urcelay, C., Co-occurrence of three fungal root symbionts in *Gaultheria poeppiggi* DC in Central Argentina, *Mycorrhiza* **12**, 89-92, 2002.

Vandenkoornhuyse, P. and C. Leyval, SSU rDNA sequencing and PCR-fingerprinting reveal genetic variation within *Glomus mosseae*, *Mycologia* **90**(5), 791-797, 1998.

Varnes, E.S., B.M. Jakosky, and T.M. McCollom, Biological potential of martian hydrothermal systems, submitted for publication, 2003.

Wainwright N.R. and A. Child, Use of amplified microbial detection assays in life detection and planetary protection studies, Abstract 2554, Workshop on Mars 2001, Abstract 2554, Lunar Planet. Inst., Houston, Oct. 2-4, 1999.

Walawender, J., Bally, J., & Reipurth, B., A narrow-band imaging survey of the Perseus molecular cloud, *Astronomical Journal*, in preparation.

Walker, J.C.G. Evolution of the atmosphere, Macmillan, 1977.

Walker, J.C.G., Klein, C., Schidlowski, M., Schopf, J.W., Stevenson, D.J., and Walter, M.R., Environmental evolution of the Archean-Early Proterozoic Earth, in Earth's Earliest Biosphere: Its Origin and Evolution, (J.W. Schopf, ed.), Princeton. pp. 260-290, 1983.

Walter, M.R. and D.J. Des Marais, Preservation of biological information in thermal spring deposits: Developing a strategy for the search for fossil life on Mars, *Icarus*, **101**, 129-143, 1993.

Watanabe, K., Naraoka, H., Wronkiewicz, D.J., Condie, K.C., and Ohmoto, H. Carbon, nitrogen, and sulfur geochemistry of the Archean and Proterozoic shales from the Kaapvaal Craton, South Africa, *Geochim. Cosmochim. Acta* **61**, 3441-3459, 1997.

Watson, L.L., I.D. Hutcheon, S. Epstein, and E.M. Stolper, Water on Mars: Clues from deuterium/hydrogen and water contents of hydrous phases in SNC meteorites, *Science*, **265**, 86-90, 1994.

Wolery, T.J. (1992) *EQ3/6, A Software Package for Geochemical Modeling of Aqueous Systems: Package Overview and Installation Guide (Version 7.0),* Lawrence Livermore Natl. Lab, 1992.

Wolery , T.J., EQ3NR, a Computer Program for Cheochemical Aqueous Speciation-Solubility Calculations: Theoretical Manual, User's Guide, and Related Documentation (Version 7.0), Lawrence Livermore Natl. Lab., Livermore, CA, 1992.

Wolery, T.J. and S.A. Daveler, EQ6, a Computer Program for Reaction Path Modeling of Aqueous Geochemical Systems: Theoretical Manual, User's Guide, and Related Documentation (Version 7.0), Lawrence Livermore Natl. Lab., Livermore, CA, 1992.

Yarus, M. and R. D. Knight, The Scope of Selection, in *The Genetic Code and the Origin of Life.* (L. Ribas de Pouplana, ed.), Landes Bioscience, Georgetown, TX, in press, 2002. Available at http://bayes.colorado.edu/Papers/scope_preprint.pdf or the publisher's site:
http://www.eurekah.com/chapter.php?chapid=771&bookid=67&catid=27

Yarus M., Primordial genetics: the phenotype of the ribocyte, *Annual Reviews of Genetics* **36,** 125-151, 2002.

Yung, Y.L., J.-S. Wen, J.P. Pinto, M. Allen, K.K. Pierce, and S. Paulsen, HDO in the martian atmosphere: Implications for the abundance of crustal water, *Icarus*, **76**, 146-159, 1988.

Zahnle, K., L. Dones, and H. F. Levison, Cratering rates on the Galilean satellites, *Icarus*, *136*, 202-222, 1998.

8. STRENGTHENING THE ASTROBIOLOGY COMMUNITY

8.1 Introduction

The NASA Astrobiology Institute has several distinct objectives as part of its mission, as expressed in its mission statement. First and foremost is research — both of the highest quality within the component disciplines and as collaboration between groups and disciplines as befits a research Institute. The other objectives include training astrobiologists, providing astrobiology leadership and input into the NASA flight programs, developing and using innovative approaches to collaborative research, and education and public outreach. These non-research goals fall under the heading of the title of this section, strengthening the astrobiology community, and are just as important as research in the long run. To some extent, it is our collective willingness to participate in these "non-research" activities that provides the impetus to be a part of an astrobiology institute; without this participation, members of the NAI easily could go to the individual Research and Analysis Programs for funding on the individual research tasks that are being proposed. Thus, first-rate research programs are a necessary starting place for NAI participation, and the efforts on the part of each team to strengthen the astrobiology community, individually and collectively, provide the added value that molds the teams into a program and an Institute.

The CU group has been a leader in developing the astrobiology community from the beginning. We have taken and will continue to take a leadership role in defining astrobiology, developing its formal and informal connections to the themes and programs within Space Sciences at NASA, and working to forge a single community out of a disparate group of disciplines.

Here, we list a few of the ways in which we have been leaders in developing the astrobiology community in the last few years, and then develop these further, along with our specific proposed activities, in the remainder of this section.

- Our group (through P.I. Jakosky) led the effort on the part of the NAI Executive Council to develop a series of white papers on the relationship between astrobiology and solar-system exploration. This was done as input into the NRC report on a Decadal Strategy for Solar System Exploration in 2001-2002, and resulted directly in that group integrating the two fields very effectively. This is exactly the type of synthesis that must be done in order to gain support for astrobiology from non-astrobiologists (who often, incorrectly, see astrobiology as a very narrow and non-scientific discipline).
- Our group (through Co-I Pace) is playing an important role in the Ecogenomics Focus Group, which is investigating the interplay of organisms in a hypersaline ecosystem in Baja California. This effort among a number of the existing NAI teams represents the type of collaborative effort that only the NAI can do, by bringing multiple approaches and techniques to bear on a single problem that spans different fields. The CU contribution to this effort, of determining the nature and diversity of organisms that exist in the ecosystem, is a fundamental component that is central to the overall program.
- Our group (through Co-I Mojzsis) is co-leading the Mission to Early Earth Focus Group. This Focus Group was formed when a consensus emerged within the astrobiological community that progress in the area of "bioenvironmental reconstruction" is fundamentally sample limited. To make informed decisions about where and what to look for in the astrobiological exploration of the solar system and beyond requires us to draw on the geological record provided by

the only planet that unequivocally has life — Earth. The group has organized field workshops and is central to the currently planned program of drilling in ancient rocks.

Our group of Co-Investigators at CU is built largely of researchers who excel both at their science and in providing programmatic and intellectual leadership to the community. While we could *propose* to help to develop and strengthen the astrobiology community, instead we *have played* leadership roles in these activities over the past five years and will continue to do so.

In addition, the University of Colorado has a strong commitment to developing astrobiology as a research endeavor and as a program. This commitment has included new faculty hires, commitment of funds, and other commitments, and is detailed below.

In the following sections, we outline the roles that we have played and our proposed efforts in areas where this is appropriate.

8.2 Education and Public Outreach (E and PO)

Astrobiology provides a marvelous hook on which to hang public outreach and education about science. Everybody has an interest in it, whether the topic is about microbes on Mars, habitable planets around other stars, or even UFOs and alien abductions. Thus, it provides an excellent opportunity to interact with the public at all levels, to teach about the nature of science, and to discuss its role in our society. We heartily embrace these goals, and are pleased to be able to propose and participate in a vigorous E and PO program.

No single approach to E and PO is valid for all programs or all groups. This is analogous to informing people about the news — some are satisfied with the sound bites on CNN Headline News, some like the slightly more detailed discussions on the national network news programs, others prefer the details at a level heard on National Public Radio, and still others prefer the greater detail in the New York Times. So it is with E and PO. We take the attitude that we wish to provide some information to a large number of people, and then to reach smaller numbers of people who desire larger amounts of information or greater involvement.

The E and PO program that we are proposing is substantially augmented over our previous efforts. We propose the following tasks, each of which is described below: (i) Public symposia held at CU to delve in depth into a given topic for the local University, Boulder, and Denver communities. (ii) Extramural public/university symposia (“travelling show”) to take this excitement to other institutions that do not normally have access to this information. (iii) Collaboration with the Denver Museum of Nature & Science to prepare a planetarium show and materials for dissemination to classrooms in Colorado that reach literally hundreds of thousands per year. [Note that our Co-Is participate in E and PO on an informal basis by giving public talks, presenting colloquia at other institutions, writing for the popular media, doing interviews with the media, etc. These are discussed elsewhere and are not a formal part of our E and PO effort. Also, we will discuss grades 13-16 (i.e., college level) along with graduate education in the section on training.]

To coordinate our efforts, LASP (the parent unit of the Center for Astrobiology) has hired an outreach coordinator, Dr. Emily CoBabe-Ammann. She will be a key person in implementing the proposed activities and in developing additional activities over the next five years.

8.2.1. Public symposia

The Center for Astrobiology has been running annual public symposia for more than four years that reach out to the local University, public community, and high-technology groups in the Boulder-Denver metropolitan area. For these, we have brought in nationally known experts and our own astrobiology faculty in exciting areas in astrobiology, and presented a coherent symposium on different aspects of a specific topic for an evening, and included visits to CU classrooms and interactions with students and faculty. Topics in the past have included the Mars exploration program, intelligent life in the universe, the nature of life, and the discipline of astrobiology. Each symposium has included a discussion of the science from multiple perspectives as well as discussion of the implications to the public or to society. Some of our speakers have been Don Brownlee, Ben Bova, Seth Shostak, Joel Achenbach, Norman Pace, William Calvin, Benton Clark, and Eugenie Scott. Public participation has been outstanding, with as many as 500 people attending each event.

We will continue these public symposia at an anticipated rate of 1-2 per year for the coming five years. These will allow us to continue to interact with the local community, something that fills the needs both of the University and the community. The emphasis of the continuing symposia will be on new and exciting topics. We will associate the symposia with spacecraft launches or arrivals (such as the Mars Exploration Rover missions to Mars or the Cassini mission to Saturn, both of which have strong astrobiology connections) or major discoveries that are announced. For example, CU presented a public symposium in 1996 on the potential for life in the martian meteorite ALH84001, held only three weeks following the public announcement, drawing on local scientists for analysis, and attended by over 600 people.

8.2.2. Extramural public/university symposia

The public symposia allow us to reach a large audience, but one that generally already is scientifically literate, technologically adept, and can (and will) take advantage of access to alternative sources of similar information. We also will develop a new program of extramural public symposia (informally called our “travelling show”) to visit places that generally are not able on their own to support this type of activity. We are targeting 2- and 4-year colleges and universities that do not have sufficient resources to support visits by leading researchers, and schools that serve historically underrepresented populations. Our goals are to take cutting-edge science to places that often do not have access to it, and to expose students and faculty to the excitement of astrobiology. We will use the CU Co-Is to create public symposia on the exciting new results and their astrobiological significance, typically spanning the breadth of topics and disciplines in astrobiology. We will visit other institutions at no expense to them, put on an evening public symposium for their faculty, students, and the local community, visit in small groups with students and faculty, and, where appropriate, give more-technical seminars to the upper-level students and faculty.

We are planning to visit three institutions per year, both in Colorado and around the country. We have committed to a “tune up” symposium as a demonstration of concept and to work out the bugs of the logistics and the program on April 8, 2003, at Ft. Lewis College in Durango, CO. Ft. Lewis College is a 4-year undergraduate school located in southwestern Colorado and having a substantial population of Native American students. This first symposium will involve our Co-Is William Friedman (talking about the origin and evolution of life on Earth), Bruce Jakosky (on the possibility of life in the rest of our solar system), and John Bally (on habitable planets outside of our solar system). In addition, we have tentatively planned our first travelling show following selection to be at Hampton University in Virginia; they are a four-year historically black school with which we have informal ties through other venues.

8.2.3. Science journalist Workshops

We will educate and inform the public via a series of workshops intended to provide background material to science journalists on issues in astrobiology. The goal is to inform the writers of the deep background of the science issues, so that they can better report on the issues. Our first workshop will be held in December 2003, and will deal with the Mars landings to take place in December and January. Emphasis will be on the missions, the science to be done, and the astrobiological connections.

8.3. Professional Community

The CU Center for Astrobiology has been and will continue to be at the intellectual center of forging the discipline of astrobiology. This has been done by participating in activities designed to turn the disparate disciplines into a single coherent field, by representing astrobiology on high-level national advisory committees, and by other activities on the part of the individual team members. Our participation and leadership is shown by example, using a listing of the recent activities with which we have been and are currently involved, both locally at CU and nationally within the astrobiology community:

NAI Focus Group active participation

Mission to Early Earth Focus Group (Mojzsis, co-chair)

Mars Focus Group (Jakosky and Mojzsis, members)

EcoGenomics Focus Group (Pace, member)

Europa Focus Group (Pappalardo and McCollom, members)

Astrophysics Focus Group (Bally, member)

Advisory Committees (recent and current)

NAI Executive Council working group to provide astrobiology input to NRC Decadal Solar System Exploration Survey group (Jakosky, Chair)

NASA Solar System Exploration Subcommittee (Jakosky and Pappalardo, members)

NASA Mars Exploration Program Analysis Group (MEPAG) (Mojzsis, member; Jakosky, member and incoming chair)

NASA MEPAG Astrobiology Sub-Group (Jakosky and Mojzsis, members)

NASA MEPAG Pathways Sub-Group (Jakosky, member)

NASA Astrobiology Roadmapping team (Jakosky, member)

NASA Astrobiology Shared Foundations Working Group (Jakosky, co-chair)

NASA ASTEP (Astrobiology Technology in Exploration Program) program review panel (Jakosky)

NRC Committee on Planetary and Lunar Exploration (Pappalardo)

NRC Solar System Exploration Strategy Committee, Steering Committee Member and Vice-Chair of Large Satellites Panel (Pappalardo)

NRC Committee on the Origin and Evolution of Life (Pace)

NRC Mars Sample Return Task Group (Pace, Jakosky)

NSF Molecular Biochemistry Review Panel (Copley)

Board of Directors of the Monterey Bay Aquarium Research Institute (MBARI) (Pace, member)

USRA Lunar and Planetary Science Institute Science Council (Jakosky, member)

Key invited talks

AAAS Symposium on “The search for life beyond the solar system” (Pace)

NSCORT Annual Symposium, Rensselaer Polytechnic Institute, keynote talk (Pace)

"The solar system: A user's guide", NAI primer talk, 2003 NAI biannual meeting (Jakosky)

First Annual McDonnell Distinguished Lecture, Washington Univ. St. Louis (Jakosky)

Origin of Life Gordon Conference invited talk, 2002 (Mojzsis)

Origin of Life Gordon Conference session chair, 2003 (Jakosky)

Journal editorial boards and associate editorships

"Astrobiology" (Jakosky, Mojzsis)

"International Journal of Astrobiology" (Jakosky)

"Geobiology" (Jakosky, Pace, and Mojzsis)

"Environmental Microbiology" (Pace)

"Geophysical Research Letters" (Pappalardo)

"Bioorganic Chemistry" (Copley)

Other professional activities relevant to astrobiology

Organizing and leading NAI Insight course in planetary science for non-planetary scientists, summer 2003, Flagstaff (Jakosky; Pappalardo, participating lecturer)

Denver Museum of Nature and Science, curator for astrobiology planetarium show (Bally)

American Geophys. Union, annual fall meeting, organizer for session on Mars rivers and climate (Toon)

Division of Planetary Sciences, Amer. Astron. Soc., annual meeting science program cmte. (Pappalardo)

Author of "The Search for Life on Other Planets" (Cambridge U. Press, 1998, used as textbook in numerous courses and sold to the public) (Jakosky)

Co-author of "Life in the Universe" (Addison Wesley, 2002, non-majors undergraduate textbook on astrobiology) (Jakosky)

Seminar series

CU Center for Astrobiology monthly colloquium series

CU Center for Astrobiology monthly journal club series

NAI journal club series (initiated by CU in 2002 prior to becoming an NAI-wide activity)

Regular participation as both speakers and audience in the NAI monthly seminar series

8.4. Training

The University of Colorado has a vigorous program of training for undergraduate students, graduate students, and post-doctoral fellows. Each is described below:

Undergraduate program. CU offers numerous undergraduate courses in astrobiology and astrobiology-related areas and plans to explore the utility of an undergraduate minor. Our two key courses are:

(i) Undergraduate non-majors course in astrobiology, titled "Extraterrestrial Life". This course has been offered each semester for the past five years. It draws typically 75 students per semester from all across campus, including students who are majoring in the sciences, engineering, the humanities, the social sciences, business, and so on. Topics include the entire range of sciences that comprise astrobiology, along with the philosophical and societal issues, and, perhaps most importantly, the nature of science and the role of science in society. Course has used the book by Jakosky, and currently uses the book by Bennett, Shostak, and Jakosky.

(ii) Senior-level/graduate course on astrobiology, science, and society. Intended for science majors, we explore the nature of science, the history of astrobiology, the role of science in society, and the philosophical significance of searching for life elsewhere. Course is offered in alternate years (was taught in 2002, and will be taught again in 2004).

Other relevant undergraduate courses include planetary atmospheres; planets, moons, and rings; evolution; microbial ecology and life in extreme environments.

We will explore the desire on the part of undergraduates for an undergraduate minor, as an adjunct to majors in any of the biological or physical sciences. We have no plans to offer an undergraduate major, as it is in the best interests of the students to focus on a single discipline; however, a minor will expose students to the breadth of astrobiology and encourage them to pursue it at the graduate level.

Graduate program. CU offers a graduate certificate in astrobiology and several related courses.

Our graduate certificate program was approved in 2002 and continues on an ongoing basis. It is not a formal degree in astrobiology, but instead provides additional coursework and recognition in astrobiology, and can be thought of as a graduate-level equivalent of an undergraduate minor. We have no plans to offer a formal degree, as it is in the students' best interests to remain in their home department and receive in-depth training in a single discipline. The certificate offers the opportunity to become exposed to the entire breadth of astrobiology. Required courses include a graduate course in astrobiology, a course in history and philosophy of science, and two additional science courses from outside of the student's home discipline (e.g., a biology student will take courses in physical sciences, and vice versa).

Graduate-level courses specific to astrobiology include:

(i) A one-semester graduate course in astrobiology, intended for students in all of the relevant disciplines. That course is offered in alternate years, has been taught twice now, and typically has about 20 students enrolled. Topics include the entire breadth of disciplines that comprise astrobiology and appropriate integration to address the questions of life in the universe. Reading consists of approximately 100 papers (original research papers, review papers, book chapters, etc.).

(ii) A course in the nature of life from the philosophy of science perspective, taught by a formally trained philosopher of science (Co-I Cleland). Topics include the logic of definition, critical evaluation of popular scientific definitions of 'life', problems with defining 'life', and the nature of the justification of astrobiological hypotheses.

(iii) A reading group for graduate students, postdoctorals, and faculty to explore the writings of a select group of evolutionists who can be viewed as the first astrobiologists (e.g., Robert Chambers, Alfred Russel Wallace, Baden Powell). Each of these individuals wrote explicitly about the origin (or origins) of life on Earth and considered the evidence for the origin and evolution of life on Earth within the context of 19th century cosmology (e.g., the nebular hypothesis), and anticipated many of the central questions associated with astrobiology.

We also offer research training for graduate students. Students do dissertation research on areas related to astrobiology, working with any of our Co-Investigators or Collaborators. Funding comes from the NASA support through the NAI or through individual NASA, NSF, or other grants held by the Co-Is or Collaborators.

Post-doctoral program. Through our individual research programs, we train post-doctoral research associates in astrobiology. Most of the Co-Is have supported and continue to support post-docs, and we are regularly contacted by researchers wishing to do post-docs with us. We currently have three NAI post-docs in our program at CU, working in very different parts of our program (Ruth Ley, microbial ecology; Sara Martinez-Alonso, Mars remote sensing; Alex Pavlov, early Earth atmospheric physics and chemistry).

8.5. Teaming with Minority Institutions

CU is an active participant in the NAI Minority Research Program that brings in summer faculty fellows. As described in our Education and Public Outreach section, we will be actively working to expose underrepresented groups to the excitement and science in astrobiology through our travelling symposia. And, we have informal connections with faculty at Hampton University in Virginia (a historically black college) that we are working to formalize.

8.6. Staff

Co-Investigators in the CU Center for Astrobiology contribute substantial time in support of this proposal in addition to that which is funded explicitly through the NAI. This time is supported via state funds, through the academic-year salaries of each Co-I. The estimated fractions of time integrated through the work year that each science Co-I will spend on activities connected to this proposal are as follows: Bally 20 %, Cleland 30 %, Copley 8.5 %, Friedman 10 %, Jakosky 25 %, McCollom 20 %, Mojzsis 20%, Pace 20%, Pappalardo 15 %, Toon 10 %, Yarus 15 %.

CU and LASP are contributing one work month per year of senior IT/Tech person in support of our virtual institute and other hardware activities.

Estimated dollar value of staff, faculty, and IT/Tech commitments are included in the section on “Summary of institutional commitment of resources”.

8.7. Flight missions

CU Center for Astrobiology Co-Is are actively involved in NASA flight missions that are pertinent to astrobiology, including participation in active missions, in missions currently under development (i.e., funded), and missions in advanced planning. Our participation includes (but not including participation on the part of our technology partners at Ball Aerospace and Lockheed Martin):

Active missions

Mars Global Surveyor (Jakosky, Interdisciplinary Scientist)

Mars Odyssey (Jakosky, Co-Investigator, Thermal Emission Spectrometer/THEMIS)

Galileo (Pappalardo, Affiliate member, Imaging science team)

Missions in development

Mars Airborne Regional Survey (ARES, one of four Scout proposals currently in Phase A development) (Jakosky, Co-Investigator)

MESSENGER (Mercury Discovery mission scheduled for 2004 launch) (McGrath)

SIRTF (Space Infrared Telescope Facility) Science Center Oversight Committee (Bally)

Ball Aerospace Terrestrial Planet Finder architecture study team (Bally)

Formal mission planning oversight

NASA/JPL Mars Science Laboratory Project Science Integration Group (Jakosky)

Europa Orbiter Science Definition Team (Pappalardo)

Europa Orbiter Analysis Team (Jakosky)

Mars Exploration Rover (MER) Landing Site Steering Group (Jakosky)

Mars Odyssey Project Science Group (Jakosky)

Mars Global Surveyor Project Science Group (Jakosky)

NASA Jupiter Icy Moons Orbiter Science Definition Team (Pappalardo)

8.8. Information Technology

The CU Center for Astrobiology will continue to participate in activities designed to take advantage of the “virtual institute” aspect of the NAI. We will participate in videoseminars, videoconferencing, development activities led by the NAI of desktop video capability, and development activities led by the NAI of new approaches for file sharing and exchange. In particular, we have been utilizing the videoconferencing equipment for point-to-point discussions with individuals on other NAI teams, and will continue to do so. One example of this is the ongoing collaboration between the teams at CU, Penn. State Univ., NASA/Ames, and NASA/JPL on the physics, chemistry, and climate of Earth-like planets and implications for the early solar system and for extrasolar planets (CU participation led by Co-I Toon); this group is ramping up its use of videoconferencing for science group meetings and beginning to make active use of the equipment.

8.9. Linkage to Other Agencies

CU Center for Astrobiology Co-Investigators have substantial research programs that extend well beyond the NAI. As a result, the NAI gets the benefit of and participation from a much larger research program. Here, we list astrobiology-related programs that are not funded through the NAI and have not been mentioned elsewhere, with full details being provided in Volume 2 under current and pending research grants:

NSF Petrology and Geochemistry (pending), Clues to the early crust in the chemistry of ancient (3900-4400 Ma) terrestrial zircons: Geochemical and geochronological studies of the oldest known terrestrial solids, up to 4.38 Ga zircon grains from Western Australia (Mojzsis).

NSF LExEn, Constraining the timing and nature of life's emergence (Mojzsis).

NSF Instruments and Facilities (pending), Acquisition of the ion microprobe SHRIMP for geological research: Creation of a new laboratory for geochemical studies of samples of astrobiological interest, geochronology, micro-scale geochemistry and isotopic biomarker development (Mojzsis).

NSF, Molecular Diversity and Structure of Photoendolithic Ecosystems, examining molecular (rRNA) diversity among endolithic ecosystems, Antarctic and Rocky Mountain (Pace).

NASA Exobiology Program (pending), Applying Geochemical Constraints to the RNA World (Mojzsis).

NASA Exobiology Program (pending), A Mission to Very Early Earth: Exploring the chemical record of habitability in ancient terranes and applying this knowledge to the search for life in the universe (Mojzsis).

NASA Exobiology Program, Astrobiological and Geological Implications of Convective Transport in Icy Outer Planet Satellites (Pappalardo).

NASA Planetary Geology and Geophysics Program, Remote sensing and geochemistry of planetary surfaces (Jakosky).

NSF, Division of Molecular and Cellular Bioscience (Molecular Biochemistry), MAA Isomerase to TCHQ Dehalogenase: Evolution in Action (Copley)

NSF Division of Molecular and Cellular Bioscience (Metabolic Biochemistry), Studies of Degradation of a Xenobiotic Compound: A Window on the Evolution of a Novel Metabolic Pathway at an Early Stage of Development (Copley)

NIH Division of General Medicine, Recruitment of Enzymes to Serve New Functions (Copley)

NIH Division of General Medicine, RNA structure and function studies via gene construction (Yarus)

NIH Division of General Medicine, RNA catalysis of translational reactions (Yarus)

8.10. Commitment of University of Colorado to Astrobiology

The University of Colorado has a strong commitment to astrobiology, both as a research endeavor and as an integrated program. It has made a substantial commitment of personnel and resources, both to the original proposal five years ago and to this proposal. The specific commitments are summarized here, with dollar values for the current proposal appearing in the next section:

Hiring of two new tenure-track faculty specifically in astrobiology (Pace, Mojzsis).

Hiring of two new faculty in areas relevant to astrobiology (Pappalardo [tenure track], McCollom [research faculty].

Providing start-up funds to support the astrobiology-relevant research program (Pappalardo).

Commitment of matching funds on equipment, in both the original proposal and in this proposal.

Providing a Graduate Teaching Assistant each semester to enhance interactions between the teaching and research programs in astrobiology.

Providing financial support out of its general funds (committed by each participating unit, through the Laboratory for Atmospheric and Space Physics).

Providing physical space to house an astrobiology program office and staff.

8.11. Summary of Institutional Commitments of Resources for this Proposal

The University of Colorado and its partner institutions are investing their own resources into our astrobiology program, allowing us to leverage the NASA funding to support a broader and larger program. Details of in-kind contributions are itemized below, although with the dollar value deleted.

Additional financial support for research, E and PO, colloquia, etc., from the University of Colorado through the Laboratory for Atmospheric and Space Physics.

Matching funds on purchase of equipment, from the University of Colorado.

Graduate Teaching Assistant support for astrobiology from CU Graduate School, total value over five years.

Contribution in kind from Ball Aerospace Corp. in support of our technology initiative over five years.

Contribution in kind from Lockheed Martin Aerospace in support of our technology initiative over five years.

Contribution of one month of senior IT/Tech support per year from CU LASP in support of the virtual institute and hardware aspects of our program.

Faculty contribution of time not supported directly through this grant.